The role of tryptophan dysregulation in a mouse model of migraine

Yara Mrad

UNIVERSITÉ CLERMONT AUVERGNE

ÉCOLE DOCTORALE DES SCIENCES DE LA VIE,
SANTÉ, AGRONOMIE, ET ENVIRONNEMENT

Année 2023

THÈSE

Présentée à Clermont-Ferrand pour l'obtention du grade de

DOCTEUR DE L'UNIVERSITÉ CLERMONT AUVERGNE

Spécialité : Neurosciences

The Role of Tryptophan Dysregulation in a Mouse Model of Migraine

Soutenue publiquement le 28 Septembre 2023 par

Yara MRAD

Directeurs : Dr Cristina ALBA-DELGADO et Pr Radhouane DALLEL

Neuro-Dol (INSERM U1107)

Membres du Jury

Pr Marc LANDRY	**Rapporteur**
Pr Dieudonnée TOGBE	**Rapportrice**
Dr Cyril RIVAT	**Rapporteur**
Pr Christine COURTEIX	**Président du jury**
Dr Michel LANTERI-MINET	**Examinateur**
Dr Cristina ALBA-DELGADO	**Directeur de thèse**

"All our dreams can come true if we have the courage to pursue them."

Walt Disney

"All it takes is faith and trust."

Peter Pan

To my Mama

To my Papa

To my Jado

To my Kas

I wouldn't be where I am without you

My love for you is endless

ACKNOWLEDGMENTS

First of all, I would like to thank all the members of the jury for their time and effort. Thank you, Pr. Marc Landry, Pr. Dieudonnée Togbe, and Dr. Cyril Rivat for agreeing to be reporters of my work. Thank you, Pr. Christine Courteix and Dr. Michel Lanteri-Minet for agreeing to be my thesis examiners.

To my thesis supervisor, Dr. Cristina Alba-Delgado. I remember clearly the first time I ever stepped foot in France and you were the one picking me up from the airport. From day one you were not only my supervisor but my friend. Cristina, I can't even begin to thank you for all the support, attentiveness, patience, and confidence you have given me. I consider myself lucky to have had the chance to go through this thesis with a supervisor like you and to be able to learn all that I have within these three years. Words can't express my gratitude to you for all your input that made this endeavor possible. Thank you for always being there with your optimism and ambition to answer my questions and discuss with me the ins and outs of our project. And also to discuss plans, food, experiences, and everything else. Forever a Tryptophan Team Member!

I would like to sincerely thank my second thesis supervisor, Pr. Radhouane Dallel. Professor, you have given me the opportunity to be a part of your lab and I am forever thankful for that. Thank you for being there to listen when I had questions and for all your great input into this project.

Isabelle, thank you for all the great advice and help you have given me through this journey. You were always someone who I can come to when I needed help. Thank you for always being there with your gentle ways and full of optimism.

François thank you for all the help in my project and the time you put into my data, but not only that thank you for all the conversations about France and learning more about this country and for all the advice and support. And thank you for the amazing game nights with the lab!

Karine, your sweet heart and care were always there. I can't begin to thank you for all your help. And of course a huge heart every time I see you!

Anne Marie & Amelie. Anne Marie, the adoptive mother of all of us. I am forever thankful for all your morning hugs and your affection. Amelie always with a smile, thank you for being there at times when I was feeling low. Plein des bisous!!

Sylviane, you have helped me a lot not only with the animals but also with listening and being a good friend. Thank you for all that!

Gisela, every time I was running around in the histology room I would always search for you for your input. You were always a great help to me. Thank you for being there and giving me great advice for the future. Next time I visit Porto I will for sure tell you!

Christelle and Anne. I can't imagine how many times we talked about teeth and dentists! Thank you for being so supportive, gentle, kind, and caring.

Lénaïc, Cedric, Myriam, Ahmad, Michael, and Philippe, thank you for the great working experience with all of you. It's been a real pleasure to know and work with you.

To all the students that I have helped mentor and who have added so much effort and input to this thesis. Anaïs, Vido, Manon, Robin, Tamara, Marion, Stephanie, Guillaume, Rayan, and Ilias. You guys have all been amazing and I am very thankful to have had the opportunity to teach you and learn from you. And for all the moments of laughter and joy, we shared, I am very thankful.

Ornella, thank you for being a friend and for all the moments we shared. I hope to always have reunions whenever I visit France!

Jenny, you were the first person I ever met when I came here, who helped me through every single step and was not only a friend but an older sister. I am so thankful for you Jenny!

To my girls! Manon, my petite Olaf, and stitch twin. I can't even begin to say how thankful I am to have met you during these couple of years. I am so happy to take with me a lot of our Disney memories and random moments. So much love for you monocle! The trio, my jeanouu, and pawpaw. I can't even begin to express how much I am going to miss you two! After spending three years in the same office together I for sure got used to our routine and our random shenanigans. I am so thankful for the huge bag of memories I will be carrying with me back home. From our outings, inside jokes, Channing Tatum, to Britney Spears. We have been through so much together both positive and negative but no matter what we were always each other's support. You

both have made this experience a thousand times brighter and I can't and don't want to imagine doing this whole thing without you. I love you, my girls!

Zazu and Hamada, to the two people that I call my best friends. Even with one of you being in Lebanon and the other in Germany, you both were a huge support system for me. I can't wait to celebrate more milestones with you by my side even from miles away!

To the people that matter the most my parents, brother, and fiancé.

Kas, my love, my fiancé, my baby, and my hubby-to-be. Even with the distance separating us and us being in two different continents and with our 10 hour time difference you always knew how to find ways to remind me that you are my support. Words will never be enough to explain how thankful I am for you and how much I love you. Thank you for always being my support and holding my hand through it all. I can't wait to spend the rest of our lives together and to share many more milestones. Hopefully our next step in Australia will be full of happiness and success. I love you my bubby!

To my Jado, thank you for not only being my brother and my support but my rock at times when I didn't know what to do. Thank you for always having my back and reminding me that all will be great in the end. To sharing many more successful moments. I love you more than anything my heart.

Mama and Papu, you are my biggest supporters. All the words of thanks and love that I have in my heart can't be written on paper. But if I had to say it in simple words, I wouldn't be the person that I am today without you. Thank you for being my rock when things got hard. For always pushing me to be the best I can be and to never give up on my dreams. Thank you for raising me to always dream and aim high, and that the sky is the limit. Packing my bags and leaving for this adventure wasn't easy at all but we know it's worth this moment right here. Your prayers and love are what brought me here and helped me to never give up on this dream that now became a reality. My love for you is endless. I love you!!

To the little girl who never gave up on her dreams , you did it ! Be proud of yourself for you are now a doctor and all this was the fruit of your hard work and perseverance.

ABSTRACT

Migraine is a neurological disease with an episodic nature. In 3% of patients, migraine progresses over time to reach a chronic state. Although the mechanisms underlying this chronification remain unknown, we hypothesize that a generalized inflammatory state coupled with impairment of tryptophan (TRP) metabolism could be a risk factor. TRP-derived metabolites are involved in neuronal modulation, regulate inflammation by activating the aryl hydrocarbon receptors (AhR), and play a pivotal role in migraine pathophysiology. Here, using biomolecular, immunohistochemistry, and behavioral approaches in a mouse model of migraine induced by systemic isosorbide dinitrate (ISDN) administration, we assessed the contribution of the TRP metabolism on the inflammatory responses during migraine progression. Two migraine-related regions were focused on, the noradrenergic locus coeruleus (LC) nucleus, and the spinal trigeminal nucleus caudalis (Sp5C). A single ISDN injection evoked cutaneous and light hypersensitivities, two prominent migraine-related symptoms, which were linked to enhanced systemic pro-inflammatory cytokine release. Repeated ISDN administrations also induced astrogliosis in the LC. Chronic TRP deficiency promoted microglial activation and noradrenergic dysfunction in the LC and worsened the intensity and duration of ISDN-induced cutaneous hypersensitivity in female mice. Interestingly, this process was concomitant with increased inflammation-related indoleamine 2,3-dioxygenase (IDO) activity and pro-nociceptive AhR action. TRP deficiency also blocked the efficacy of sumatriptan (an anti-migraine drug) in both male and female mice, coupled with an upregulation of the expression of subtype 1D of the serotoninergic receptors in the Sp5C. This project shows that migraine progression is associated with a systemic and local inflammation promoted by impaired TRP metabolism, which alters the descending pain modulatory system, and the abortive therapeutic efficacy of triptans.

RÉSUMÉ

La migraine est une maladie neurologique de caractère épisodique. Chez 3% des patients, elle progresse avec le temps vers un état chronique. Bien que les mécanismes sous-jacents à cette chronification restent inconnus, nous émettons l'hypothèse qu'un état inflammatoire généralisé, couplé à une dérégulation du métabolisme du tryptophane (TRP), pourrait être un facteur de risque. Les dérivés du TRP sont impliqués dans la modulation neuronale, régulent l'inflammation en activant les récepteurs d'aryl hydrocarbone (AhR), et jouent un rôle central dans la physiopathologie de la migraine. Ici, en utilisant des approches biomoléculaires, immunohistochimiques et comportementales dans un modèle murin de migraine induite par l'administration systémique de dinitrate d'isosorbide (ISDN), nous avons évalué la contribution du métabolisme TRP à la réponse inflammatoire au cours de la progression de la migraine. Deux régions liées à la migraine ont été ciblées, le locus coeruleus (LC) noradrénergique et le noyau caudal du trijumeau (Sp5C). L'injection aigüe de l'ISDN a provoqué une hypersensibilité cutanée et à la lumière, deux des symptômes migraineux les plus rependus, en parallèle d'une augmentation de la libération systémique de cytokines pro-inflammatoires. L'administration répétée de l'ISDN a induit en outre une astrogliose dans le LC. La déficience chronique en TRP a favorisé l'activation microgliale et déréglé la fonction noradrénergique dans le LC, et aggravé l'intensité et la durée de l'hypersensibilité cutanée induite par l'ISDN chez les souris femelles. Fait intéressant, ce processus était concomitant avec une activité accrue de l'indoleamine 2,3-dioxygénase (IDO) liée à l'inflammation et une action pro-nociceptive des AhR. La déficience en TRP a également bloqué l'efficacité du sumatriptan (un médicament antimigraineux) chez les souris mâles et femelles, couplée à une augmentation de l'expression du sous-type 1D des récepteurs sérotoninergiques dans le Sp5C. Ce projet montre que la progression de la migraine est associée à une inflammation systémique et locale favorisée par une altération du métabolisme du TRP, qui altère l'activité des contrôles descendants de la douleur, et l'efficacité thérapeutique des triptans.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

5-HIAA: 5-hydroxy indole acetic acid

5-HT: 5-hydroxytryptamine

5-HTP: 5-hydroxytryptophan

5-HTT: Serotonin transporter

ACh: Acetylcholine

AhR: Aryl hydrocarbon receptor

AhRR: Aryl hydrocarbon receptor repressor

AIP: Aryl hydrocarbon receptor-interacting protein

AR: Adrenoreceptors

ARNT: Aryl hydrocarbon receptor nuclear translocator

ATD: Acute tryptophan depletion

BBB: Blood-brain barrier

BDNF: Brain-derived neurotrophic factor

bHLH: basic helix-loop-helix

C3: Complement 3

CFB: Complement factor B

CGRP: Calcitonin gene-related peptide

CNS: Central nervous system

COX: Cyclooxygenase

cPLA2: Phospholipase A2

CSD: Cortical spreading depression

c-Src: Cellular-sarcoma

CTD: Chronic tryptophan depletion

CUL4B: Cullin 4B

CYP1A1: Cytochrome P450 family 1 subfamily A member 1

CYP1B1: Cytochrome P450 family 1 subfamily B member 1

DALY: Disability-adjusted life years

ERK1/2: Extracellular signal-regulated kinase 1/2

FAK: Focal adhesion kinase

FHM: Familial hemiplegic migraine

GABA: γ-Aminobutyric acid

GFAP: Glial fibrillary acidic protein

Hsp90: Heat shock protein 90

I3S: 3-indoxyl sulfate

IA: Anholocyclic acid

IAAld: Indole-3-acetaldehyde

IAld: Indole-3-aldehyde

IASP: International association for the study of pain

IB4: Isolectin B4

IBD: Inflammatory bowel disease

IBS: Irritable bowel syndrome

ICHD-3: International classification of headache disorders

IDO: Indoleamine 2,3-dioxygenase

IFN: Interferon

IHS: International headache society

IIi : Lamina II inner

IIo: Lamina II outer

IL: Interleukin

ILA : Indole-3-lactic acid

IPA : Indole-3-propionic acid

IPYA: Indole-3-pyruvic acid

ISDN: Isosorbide dinitrate

ITE: 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester

KCl: Potassium chloride

KLF6: Kruppel-like factor 6

KYNA: Kynurenic acid

Lat1: L-type amino acid transporter

LC: Locus coeruleus

LD: Lateral dorsal nucleus

LP: Lateral posterior nucleus

MAO: Monoamine oxidase

MAPK: Mitogen-activated protein kinase

MOH: Medication overuse headache

mRNA: Messenger RNA

MX1: Myxovirus resistance-1

NA: Noradrenaline

NAT: Noradrenaline transporter

NF-κB: Nuclear factor-kappa B

NMDA: N-methyl-D-aspartate

nNOS: Nitric oxide synthase

NO: Nitric oxide

NPY: Neuropeptide Y

NRM: Nucleus raphe magnus

NSAID: Non-steroidal anti-inflammatory drug

NTG: Nitroglycerine

PACAP: Pituitary adenylate cyclase-activating polypeptide

PAG: Periaqueductal gray

PGE-2: Prostaglandin E2

PKC: Protein kinase C

Po: Posterior nucleus

PVN: Paraventricular hypothalamic nucleus

RB : Retinoblastoma protein

RVM: Rostral ventromedial medulla

S1: Primary somatosensory cortex

S2: Secondary somatosensory cortex

SNP: Single nucleotide polymorphism

Sp5C: Spinal trigeminal nucleus caudalis

STAT3: Signal transducer and activator of transcription 3

TCC: Trigeminocervical complex

TCDD: 2,3,7,8-tetrachlorodibenzo-p-dioxin

TDO: 2,3-dioxygenase

TH: Tyrosine hydroxylase

TMF: 6,2',4'-trimethoxyflavone

TNF: Tumor necrosis factor

TRP: Tryptophan

TRPA1: Transient receptor potential ankyrin 1

V1: Ophthalmic

V2: Maxillary

V3: Mandibular

VIP: Vasoactive intestinal peptide

VPM: Ventral posteromedial nucleus

XAP2: X-associated protein 2

XRE: Xenobiotic response element

LIST OF TABLES

LIST OF FIGURES

PREFACE

This thesis is comprised of multiple sections, starting with an analysis of the background (Introduction), the hypotheses and objectives, the main results which are written in the format of a “manuscript to be sent for publication”, and a section with complementary results. Finally, it concludes with a general discussion of the main results and conclusions.

Moreover, this work was presented in multiple national and international conferences :

2023-Mrad Y et al. " Visualizing neuronal dynamics of the posterior thalamus in a mouse model of migraine " (Clermont-Ferrand, Doctoral School Day)

2022-Mrad Y et al. " Aryl hydrocarbon receptors are involved in the pathogenesis of migraine " (Vienna, 16th European Headache Congress)

2022-Mrad Y et al. " L'alimentation a-t-elle un rôle dans la chronicisation de la migraine?" (Lille, French Society for the Study and Treatment of Pain 2022)

2022-Mrad Y et al. "Dietary Tryptophan as a Potential Factor of Migraine Chronification" (Clermont-Ferrand, Pain Symposium 2022)

CONTRIBUTIONS AND COLLABORATIONS

Some of the procedures and techniques performed in this thesis have been carried out with the help and collaboration of other researchers. Their names and respective organizations are listed below.

International Collaborations

- Pr. Maria Angeles Moro, Dr. María Isabel Cuartero Desviat, and Dr. Alicia García Culebras from the Neurovascular Pathophysiology, Cardiovascular Risk Factor and Brain Function Program, Centro Nacional de Investigaciones Cardiovasculares (CNIC) (Madrid, Spain).
- Dr. Igor Horrillo and Mrs. Ines Erkizia Santamaría (Ph.D. student) from the Department of Pharmacology, University of the Basque Country (Biscay, Spain).

Local Contributions

From our Neuro-Dol Laboratory, Team II:

- Dr. François Gabrielli and Mrs. Karine Herault
- Mr. Vidosav Rajcic and Mrs. Anaïs Raoelina (Master 2 Students); Mr. Robin Lioutaud, Mrs. Manon Meunier (Master 1 Students); Mrs. Tamara Lucien, Mrs. Marion Ledevin, Mrs. Stéphanie Meritet, Mr. Guillaume Marsal, and Mr. Rayan Domet (Undergraduate Students).

INTRODUCTION

I. What is Migraine?

1.1. Definition, Epidemiology, and Diagnosis

Migraine is a frequent incapacitating neurobiological disorder characterized by severe, unilateral, pulsating headache attacks, often accompanied by nausea and/or vomiting as well as sensory and motor disturbances (IHS 2018). A recent epidemiological study estimated the global prevalence of migraine at 14-15%, ranked as the third most common disease in the world in both males and females (Steiner and Stovner 2023). Although migraine affects 3 to 10% of children (Borsook et al. 2014), incidence peaks between the ages of 20-24 years in women and 15-19 years in men, with 50% of first attacks occurring before the age of 25 years and in 75% before 35 years of age (Stewart et al. 2008). Worldwide prevalence data from the 2015 Global Burden of Disease Study show that migraine is two to three times more prevalent in women than in men between 30 and 50 years of age; after 50 years of age, the prevalence declines to ~5% in both men and women (Uthman 2016).

According to criteria defined by the International Classification of Headache Disorders (ICHD-3) established by the Headache Classification Committee of the International Headache Society (IHS), the two most common types of migraine are migraine without aura (formerly known as common or simple migraine), which affects approximately 70% of people (mainly women during reproductive years); and migraine with aura, in approximately 20% of cases. The remaining 10% of people suffer from both types of attacks (MacGregor, 2016). Migraine diagnosis is based on a set of guidelines, summarized in Table (1). Migraine without aura is characterized by headache attacks lasting 4-72 h (when untreated or unsuccessfully treated), unilateral with a pulsating quality ranging from moderate to severe in intensity, aggravated by routine physical activity, and associated with nausea, and/or photophobia (hypersensitivity to light) and/or phonophobia (hypersensitivity to sound). Migraine with aura encompasses a transitory-focused neurological symptom, termed aura, mostly consisting of visual disturbances but sometimes also concerning motor or speech impairments that frequently precede or occasionally coincide with the headache. Its prevalence increases with age, occurring in around 13% of attacks in migraineurs aged

18-29 years, 20.1% between 40-49 years, and 41% by age 70 years or older. Only 1% of patient experience migraine with aura without headache (Bigal and Lipton 2006).

Table 1-Diagnostic Criteria for Migraine as Formulated by the HIS

Without aura	With aura
≥5 attacks fulfilling the following criteria:	≥5 attacks, with at least 2 fulfilling the following criteria:
■ Headache lasting 4-72 h ■ ≥2 of the following characteristics: Unilateral location Pulsating quality Moderate or severe pain Exacerbation with routine activities ■ ≥1 of the following symptoms: Nausea and/or vomiting Photophobia and phonophobia	■ ≥1 of fully reversible aura symptoms: Visual Sensory Speech and/or language Motor Brainstem Retinal ■ ≥3 of the following characteristics: At least one aura spreads gradually ≥5 min ≥2 aura symptoms occur in succession Aura symptoms last 5-60 min ≥1 aura symptom is unilateral ≥1 aura symptom is positive Aura is accompanied, or followed by a headache

Adapted from (IHS 2018).

Although migraine is not life-threatening in itself, the severity and frequency of attacks can result in significant disability and reduced quality of life, even between attacks. According to the Global Burden of Disease Study migraine on its own is the sixth leading cause of disability-adjusted life years (DALY) score in the age group 25-39 years, tenth in Western Europe (Uthman 2016). Despite acute and/or prophylactic medications, 90% of patients report a negative impact of migraine on their overall quality of life (Estave et al. 2021), which limits their capacity for social and professional activities. Migraine controls one's life and/or makes life difficult. The fear of migraine attacks often creates anticipatory behaviors, amplifying migraine's effects with increased anxiety, pain catastrophizing, isolation, and hopelessness. Difficulty concentrating, communicating, and experiencing irritability are disabling ictal symptoms that affect functionality beyond pain and the typical associated migraine symptoms (e.g. photophobia, phonophobia, nausea, and vomiting). Migraine is also an expensive illness: in Europe, the mean per-person annual cost of migraine is estimated to be €1222 (M. Linde et al. 2012). A third of the financial burden is linked to the health

care system (including outpatient and inpatient care, prescriptions, and emergency care), while two-thirds is linked to indirect costs associated with absenteeism (full days of productive workforce loss) and/or presenteeism (days worked with diminished productivity) (Edmeads and Mackell 2002). No overall data for differences between men and women have been reported.

1.2. Clinical Phases of a Migraine Attack

Migraine is described as having distinct and discrete phases: premonitory, aura (in the case of migraine with aura), headache, and post-headache (Figure 1). In migraineurs, the median duration of an untreated headache attack is one day but can range from 4 h (2 h in children) to 3 days (Jensen and Stovner 2008). The duration is longer if premonitory and postdromal symptoms are included.

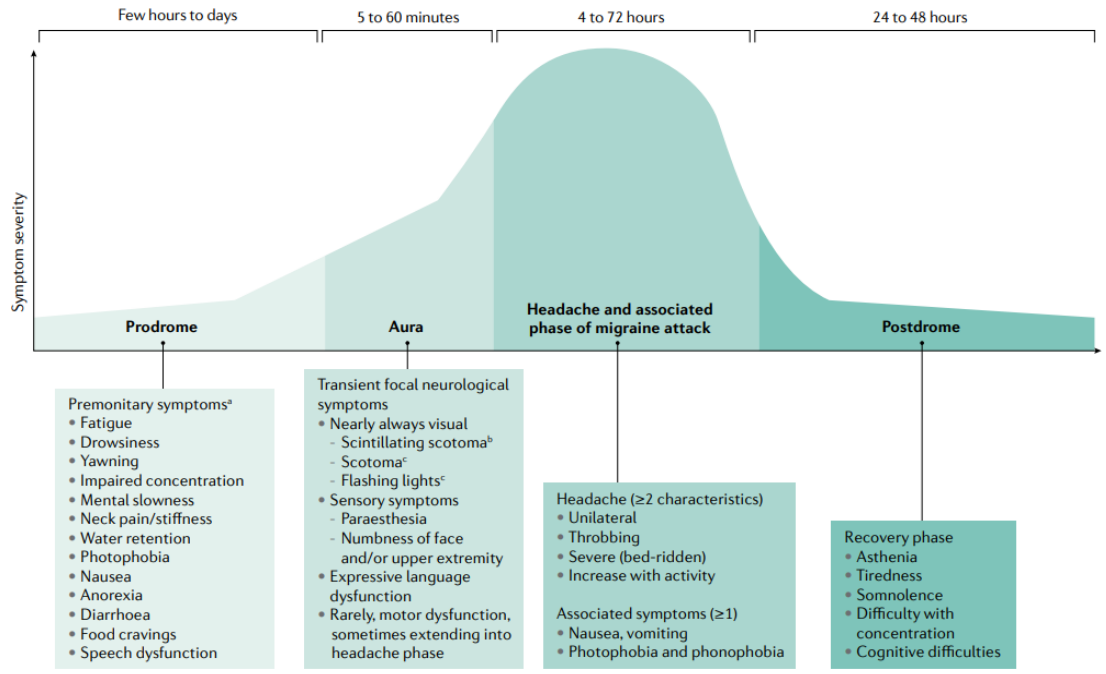


Figure 1-Stages of a Migraine Attack. Migraine attacks in patients with aura are developed in four distinct phases: prodrome, aura, headache, and postdrome. b, most common symptoms; c, less common symptoms. Adapted from (M. D. Ferrari et al. 2022).

The premonitory phase, also termed the prodromal phase, affects 77% of patients, being more prevalent in females (81%) than males (64%) (Laurell et al. 2016). This phase starts 24 to 48 h before the headache and is associated with a plethora of

symptoms, including fatigue, neck stiffness, mood and cognitive changes, photophobia, phonophobia, and osmophobia (hypersensitivity to odors). Other less frequent symptoms are yawning and food cravings (Eigenbrodt et al. 2022).

The aura phase occurs in over 90% of migraineurs suffering from migraine with aura (IHS 2018), and can precede the attack or be present simultaneously. The aura is typically experienced as visual symptoms characterized by reversible manifestations (such as flashes of light, blind spots, or visualizing 'zig zag' patterns), starting near the center of vision and spreading peripherally over 20-30 min. Sensory aura, such as pins and needles or numbness spreading up one arm into one side of the face and mouth, and transient speech disturbances, such as trouble in understanding words and/or word-finding difficulty, are less prevalent and, if present, are almost always accompanied by visual aura (IHS 2018).

The headache phase is characterized by moderate to severe frequently unilateral pulsatile, throbbing pain with an augmenting intensity within the first few hours lasting from 4 to 72 h in duration, without adequate treatment (IHS 2018). The throbbing aspect is often coupled with nausea, vomiting, photophobia, and/or phonophobia. Patients also report cutaneous hypersensitivity, also called cutaneous allodynia, which is defined as pain due to a non-noxious stimulus. Aggravation of the headache could occur due to mild physical activity hence patients may seek relief in dark places and require bedrest during the attack.

The postdrome phase encompasses persistent symptoms after headache cessation. The symptoms reflect those experienced during the premonitory stage, such as exhaustion, tiredness, difficulty concentrating, and neck stiffness (Nicola J. Giffin et al. 2016). It is still unclear however whether these symptoms were initiated during the headache phase or appeared after due to the effect of the abortive medication as reported by some patients.

1.3. Episodic Migraine *vs.* Chronic Migraine

Approximately 3% of people with episodic migraine progress gradually to chronic migraine each year (A. I. Scher et al. 2003). Chronic migraine is prevalent in 1.4-2.2% of the worldwide population (1.7-4% for females and 0.6-0.7% for males) (Natoli et al. 2010). According to the ICHD-3, chronic migraine is characterized by 15

or more headache days per month, for a duration exceeding three months, which on at least eight days per month meets the criteria for migraine with or without aura (IHS 2018). Chronic patients report an increase in headache frequency, more frequent cutaneous hypersensitivities, and more robust nausea and photophobia (Figure 2) (Rattanawong, Rapoport, and Srikiatkhachorn 2022).

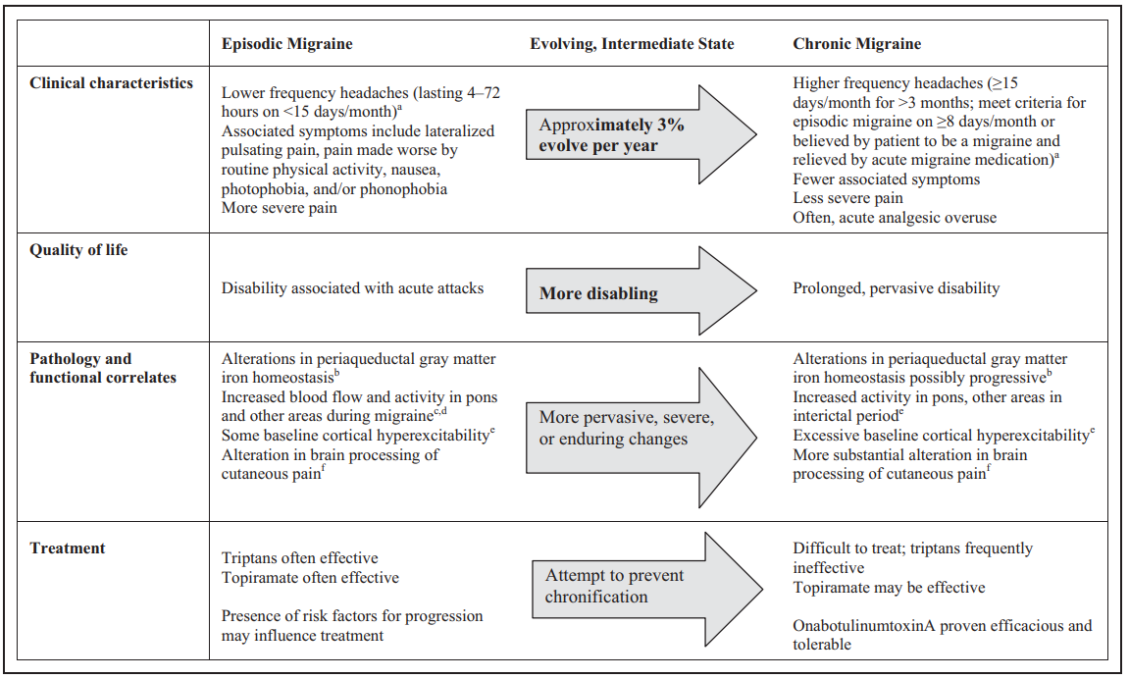


Figure 2-Features Associated with Episodic and Chronic Migraine. This representation summarizes the most important triggers and risk factors of migraine. Adapted from (Sheena K. Aurora and Brin 2017).

In Europe, the average medical costs of patients with chronic migraine are three times higher than those of episodic migraine as well as a twice-fold higher disability score (Bigal et al. 2003; Bloudek et al. 2012). The pathophysiological mechanisms by which migraine transforms from an episodic to a chronic state are complex and poorly understood (a special chapter within this thesis is focused on these mechanisms). Several factors have been associated with the risk of migraine progression, including non-modifiable (sex, hormones, and genetic background) to modifiable (acute headache medication use, obesity, other pain syndromes, and stress) factors (Ann I. Scher, Midgette, and Lipton 2008). Headache frequency is also an important risk factor for progression. Individuals with ≥4 headache days per month have an exponential risk of migraine transformation. Moreover, a negative impact comes from migraine on many

aspects of one's life with a higher burden among chronic migraineurs compared to those with episodic migraine (Buse et al. 2019).

1.4. Risk Factors, Comorbidities, and Triggers

More than 70% of migraineurs associate their attacks with certain triggers, and this figure exceeds 95% when subjects are responding to a specific list of triggers (Baldacci et al. 2013; Kelman 2007) (Figure 3). The most common triggers are stress (79.7%), hormones in women (65.1%), diet (57.3%), weather (53.2%), sleep disturbances (49.8%), and odors (43.7%) (Kelman 2007). Predisposing factors include also sex, stress susceptibility, and genetic background among others. In addition, the presence of concomitant chronic illnesses (e.g. mood, metabolic or inflammatory disorders) can play a bidirectional correlative link with migraine that brings additional elements to the table to further understand the pathophysiology of migraine.

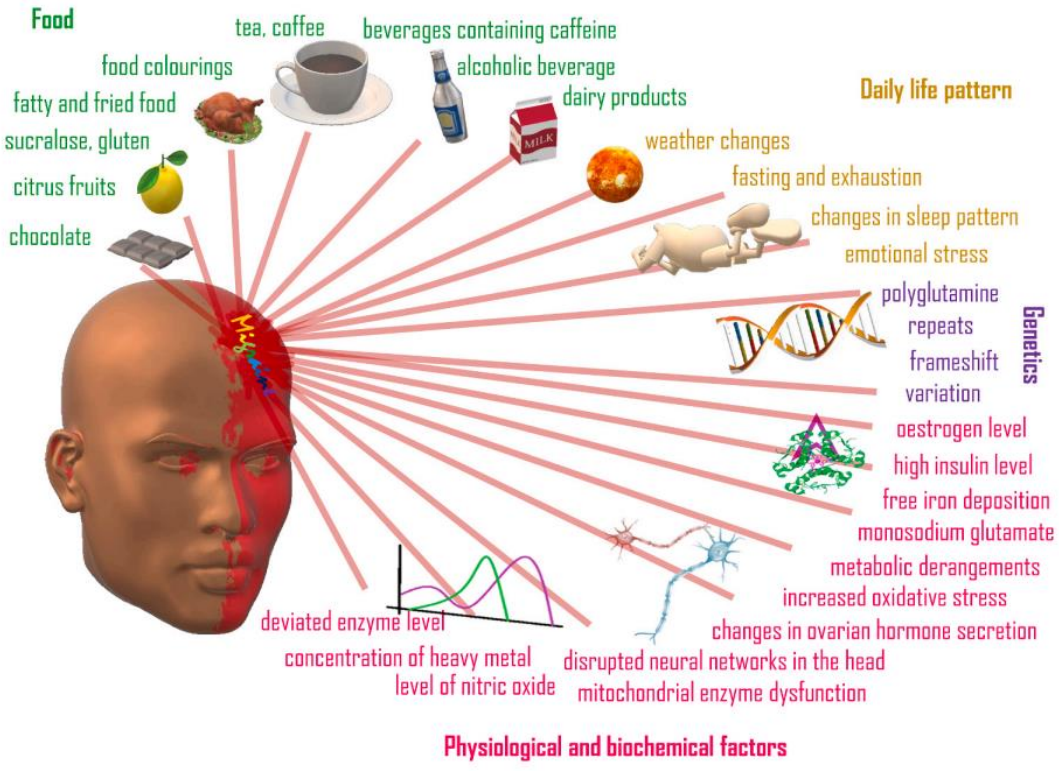


Figure 3-Triggers and Factors Affecting a Migraine Attack. This representation summarizes the most important triggers and risk factors of migraine. Adapted from (Khan et al. 2021).

1.4.1. Stress and Lifestyle

The stress response is generally associated with a subjective feeling of external or internal threat/demand and depends on the stressor quality, individual susceptibility, context, and previous experiences. It has been shown that psychological stress plays an important role not only before the onset of migraine but also in its maintenance and the frequency of attacks (Seng, Martin, and Houle 2022). Moreover, stressful life events may increase the risk of progression from episodic to chronic migraine (A. I. Scher et al. 2003). However, the distinction between stress as a trigger or as a migraine-associated symptom is not always clear for patients, and some longitudinal studies have revealed contradictory findings.

Lifestyle-related behavioral interventions, such as sleep, also impact migraine. Indeed, poor quality sleep (tiredness, excessive sleep, and restless sleep) has commonly been associated with next-day headaches in people with migraine (Peris et al. 2017). Like stress, the directionality of the sleep-migraine attack relationship is not clear, and poor sleep quality could be characterized as part of premonitory symptoms, such as fatigue and yawning. In addition, the surrounding environment (certain smells, strong lights, stroboscopic illuminations, blue LEDs, and alarming noises) can perturb lifestyle and sleep, and therefore, influence the frequency and severity of attacks (Hougaard et al. 2013).

1.4.2. Genetic Background

Genetic background has been found to play a vital role in the development of migraine (H. Zhao et al., 2016). In monozygotic twins, the concordance rate is higher compared to dizygotic twins indicating that migraine is of a heritable nature with 42% of heritability (Polderman et al. 2015). Genome-wide association studies have been able to identify 38 loci associated with a higher risk of migraine (Gormley et al. 2016), mainly localized in vascular tissue. Mutations have been also shown to affect the entire brain milieu (neurons, astrocytes, microglia, oligodendrocytes, and endothelial cells) indicating that all cell types play a role in migraine pathogenesis (Renthal 2018).

1.4.3. Sex Hormones

Ovarian hormones play a significant role in some adolescent girls and young adult women with migraine. The incidence of migraine is similar in both sexes until the age of 9 (2.5% of girls and 2.4% of boys) and then diverges to the disadvantage of girls (Pavlovic et al. 2017). During the female reproductive cycle, hormonal changes can influence or induce migraine. Onset of migraine frequently occurs around the time of menarche, as cyclic hormonal changes begin. The IHS defines "migraine attacks related to menstruation" as migraine without aura that occurs between days -2 and +3 of a menstrual cycle, with day 1 defined as the onset of menstrual flow (Nierenburg et al. 2015). Up to 70% of female patients suffering menstrual-related migraine report more painful and longer headaches, and higher resistance to treatment than non-menstrual-related migraines (Bushman, Varner, and Digre 2018; Calhoun and Ford 2008). Menstrual-related migraine frequently coincides with a drop in plasma estrogen, which contributes to disease propagation (Warnock et al. 2017). Adverse effects include decreased serotonergic and opioidergic tonus as well as increased excitability of trigeminal afferents, which, as we will see later, are involved in migraine initiation. (Aggarwal, Puri, and Puri 2012). Furthermore, estrogen decline is larger in migraine patients than in healthy controls (Reddy et al. 2021). Hence, this evidence indicates that migraine is associated with changes in circulating estrogen. Nevertheless, how estrogen might be protective and why its decrease triggers attacks remain unanswered.

1.4.4. Mood Disorders and Cognition

Some epidemiological studies have suggested that migraine is bidirectionally associated with several other disorders, including anxiety and depression, in both men and women (Breslau et al. 2000; Zwart et al. 2003). On one hand, the higher the headache frequency the more the levels of anxiety and depression (Oh et al. 2014). On the other hand, anxiety disorders are two to five times more frequent in patients with migraine (Buse et al., 2013), and migraineurs are 2.5 times more likely to suffer from depression compared to non-migraineurs (R. B. Lipton et al. 2000; Minen et al. 2016).

Migraine is also associated with poor cognitive performance (Pellegrino Baena et al., 2018). Patients frequently attend consultations for subjective cognitive complaints that are mainly attention- and memory-related and appear during or after migraine

attacks, and contribute to the disability of patients experiencing them. Cognitive symptoms came in second place to pain in terms of severity and attack-related impairment (Gil-Gouveia, Oliveira, and Martins 2016). Severe subjective cognitive decline is associated with worse pain severity, enhanced levels of anxiety and depression, poor sleep quality, and decreased sleep duration (Lee, Kang, and Cho 2017).

1.4.5. Gut-Brain Axis

The terminology “gut-brain axis” points out a bidirectional network between the gastrointestinal system and the CNS (central nervous system), which consists of multiple connections, including the immune system and bacterial metabolism. The dysregulation of the gut-brain axis is associated with altered permeability of the blood-brain barrier (BBB) and neuroinflammation and has been implicated in several neurological disorders such as multiple sclerosis, anxiety, depression, Alzheimer's, and Parkinson's diseases (Socała et al. 2021).

Evidence also supports the involvement of the gut-brain axis in migraine. During the attack, patients suffer gastrointestinal symptoms such as nausea and emesis, constipation, or diarrhea. Migraine is frequently comorbid with metabolic disorders [e.g. celiac disease (Zis et al., 2018)], gastrointestinal disorders [predominately inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS)], and chronic inflammatory diseases [rheumatoid arthritis and multiple sclerosis (X. Moisset et al. 2017; Xavier Moisset et al. 2013)]. Moreover, patients with migraine have been described to present a microbiota dysbiosis, which was positively correlated with attack severity and duration (Georgescu et al. 2019). Microbial alterations trigger gut barrier dysfunctions, that increase the permeability of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, and interleukins (IL) 1β and 6, affecting nociceptive responses in the trigeminal pathway during migraine initiation (as we will see later) (Arzani et al. 2020). In addition, microbiota dysbiosis contributes to chronicity in mice, since antibiotic treatment was shown to prolong the nitroglycerine (NTG)-induced acute migraine-like pain in wild-type mice, while probiotic administration significantly inhibited the pain prolongation (Yuanyuan Tang et al. 2020). Taken together, evidence supports the pivotal action of the microbiome and gut-brain axis in migraine, although more research is needed. The modulatory role of inflammation in migraine pathogenesis will be explained more in-depth in another chapter.

1.4.6. Diet

The impact of diet and nutrition on headaches has been extensively debated. Diet is believed to affect the modulation of neuroreceptors, neuropeptides, and CNS responses, and to play a role in causing and/or relieving inflammation (V. T. Martin and Vij 2016). Although long lists of potentially migraine-associated foods have been established (chocolate, citrus fruits, nuts, tomatoes, onions, caffeine, nitrites, gluten, red meat…) (Nazari and Eghbali 2012) controversy remains in the field: some dietary nutrients are considered as migraine triggers, while others may play a role in its prevention and prophylaxis. Moreover, exposure to a given food may not always trigger a headache, and the amount of food or the time of exposure might largely influence the outcome. Therefore, the identification of diet-associated modulators is challenging.

Food diaries and specific serological testing have been used to identify triggers in individual patients, with the aim of avoiding them and preventing migraine. This is the basis of elimination diet strategies. However, not only the diet content but also the meal pattern has been associated with migraine (Bakırhan, Yıldıran, and Uyar Cankay 2022). Several types of diets, including ketogenic, high-folate, low-fat, modified Atkins, and high omega-3/low omega-6 diets, have been proposed to be beneficial (Razeghi Jahromi, Ghorbani, et al. 2019). Many of these diets are not mechanism or evidence-driven, but have been proposed to act through a variety of mechanisms: on serotoninergic dysfunction, neuronal excitability, and/or concentration of migraine-related substances [such as calcitonin gene-related peptide (CGRP) (Fila et al. 2023) and nitric oxide (NO)], neuroinflammation, or hypothalamic function. Propositions have been done that dietary approaches with beneficial effects on the gut microbiota and gut-brain axis may improve migraine. For example, supplementation with vitamin D and omega-3 fatty acids, consumption of a low glycemic-index diet, probiotics, low-fat vegan diet, gluten-free diet, and weight-loss diets (Arzani et al. 2020). However, research on the microbiome and migraine is still novel, and future investigations are required before recommending a dietary intervention aiming to change the microbiome for migraine prevention.

II- Generalities of Pain Processing

According to the International Association for the Study of Pain (IASP), pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors. Pain and nociception are different phenomena. While pain is defined as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage" by the IASP, nociception is "the neural process of encoding noxious stimuli" (Raja et al. 2020).

Noxious stimuli are detected by nociceptors, which are subpopulations of peripheral nerve fibers (Basbaum et al. 2009). Mechanical, thermal, or chemical stimuli only stimulate when their intensities are in the noxious range. Based on their anatomical and functional characteristics, nociceptive fibers can be classified into three groups: (i) Aα and Aβ myelinated fibers that transmit information at a fast speed (with conduction velocities >30 m/s) to the spinal and medullar dorsal horn laminae III, IV, and V; (ii) Aδ fibers which are lightly myelinated and transmit nociceptive signals slowly (conduction velocities 2.5-3 m/s) to the laminae I and V; and (iii) C unmyelinated fibers have slow transmission (conduction velocities <2.5 m/s) and transmit to laminae I and II (Abraira and Ginty 2013) (Figure 4). C-fibers are also divided into two contingents according to their neurochemical nature: some contain peptides, such as substance P or CGRP, and are referred to as peptidergic fibers; while others are devoid of them, but are capable of binding to a specific lectin, isolectin B4 (IB4), and are referred to as non-peptidergic fibers (Dallel et al. 2003).

Nociceptors send noxious information to the dorsal horn of the spinal cord through the dorsal root ganglia and synapse with second-order neurons. The discrimination between noxious and non-noxious is done according to the gate control theory (Melzack and Wall 1965; Mendell 2014), which is based on the balance of three simultaneous inputs into the convergence neurons located in the posterior dorsal horn: nociceptive inputs from Aδ- and C-fibers; non-nociceptive inputs from Aα and Aβ-fibers; and inhibitory inputs from the descending inhibitory pathway. The selective stimulation of Aα/Aβ-fibers blocks Aδ/C fibers at the level of the substantia gelatinosa located in layer II. Like a gate, the cells of the substantia gelatinosa regulate the entry from the periphery to the convergence neurons and, therefore, control the access to the CNS. When the activity of the non-noxious fibers predominates, the activity of the

noxious fibers is inhibited. However, when the activity of Aδ/C-fibers predominates, the nociceptive message is sent by the second-order neurons.

From there, the message is transmitted to supraspinal areas by projection neurons located in layers I and V, by several ascending pathways, which connect the spinal cord to the thalamus direct or indirectly (Willis and Westlund 1997) (Figure 4). The message then reaches the cerebral cortex for integration purposes and emotional interpretation (Julius and Basbaum 2001).

Descending inhibitory supraspinal modulation occurs through the brainstem, and more specifically through three major structures: the monoaminergic periaqueductal gray (PAG), the serotoninergic nucleus raphe magnus (NRM), and the noradrenergic locus coeruleus (LC). The stimulation of these structures exerts an inhibitory control on convergence neurons in the dorsal horn by blocking the transmission of nociceptive messages (Ossipov, Dussor, and Porreca 2010).

III. Anatomical Substrates of Trigeminal Pain Pathways

3.1. The Trigeminovascular System

The trigeminovascular system integrates, in the brainstem, somatosensory information (mechanical, thermal, and proprioceptive) from the orofacial sphere and the meninges, and plays a pivotal role in the etiology of migraine and its associated symptoms. This system encompasses a rich plexus of small pseudo-unipolar sensory neurons that originate from the trigeminal ganglion and upper cervical dorsal nerve roots with axonal projections to meningeal blood vessels, including the superior sagittal sinus, middle meningeal artery, and large cerebral arteries (Ray and Wolff 1940). Stimulation of the dura mater with various stimuli (mechanical, chemical, or electrical) consequently leads to headache pain during a migraine attack (including the frontal, temporal, parietal, occipital, and high cervical regions) as well as other migraine symptoms such as nausea and photophobia (McNaughton and Feindel 1977; Penfield and McNaughton 1940).

3.1.1. The Meninges

Meninges envelop the CNS, the brain and the spinal cord, the intracranial spinal cord, the intracranial portion of cranial nerves, and the spinal nerve roots. Their primary function is to protect and anchor the brain and supply a support system for the blood vessels, lymphatics, nerves, and cerebrospinal fluid that surrounds the CNS. The meninges comprise two separate layers, the dura mater, and the leptomeninges. The dura mater or pachymeninx is a thick layer of connective tissue while the leptomeninges can be further separated into the arachnoid and pia mater (Haines 1991). Recently a fourth layer, named a subarachnoid lymphatic-like membrane, that compartmentalizes the subarachnoid space has been detected (Møllgård et al. 2023).

Pain molecules have shown a robust expression at the level of the cranial dura, with the sensory peptides, CGRP, and substance P, being expressed in axons innervating the cranial dura, cranial arteries, and venous sinuses (L. Edvinsson, Rosendal-Helgesen, and Uddman 1983; Sampaolo et al. 2017; Andrew M. Strassman and Levy 2004). The nociceptive Pituitary Adenylate Cyclase-Activating Polypeptide (PACAP), expressed by autonomic fibers, also innervate the dura mater, in addition to other fibers expressing neuronal nitric oxide synthase (nNOS), tyrosine hydroxylase (TH), acetylcholine (ACh), and neuropeptide Y (NPY) (Csati et al., 2012; Meßlinger et al., 1993). The other meningeal layers, primarily the pia matter, also play a role in sensory processing (Dallel et al. 2003; Fontaine et al. 2018). Pial arteries are the main site of sensory (mediates conscious activities) and autonomic (mediates unconscious activities) fiber innervations originating from sympathetic, parasympathetic, and sensory trigeminal ganglia (Levy, Labastida-Ramirez, and MaassenVanDenBrink 2019).

3.1.2. The Peripheral Trigeminal Afferents

Intracranial and meningeal vasculature inputs come from the trigeminal nerve's three extracranial divisions (V1: Ophthalmic, V2: Maxillary, and V3: Mandibular). The V1 branch is a relay of sensory information coming from facial regions located above the orbit of the eyes; the V2 branch is responsible for sensory information between the mouth and the orbit; and the V3 branch has both a motor and sensory component with only the latter projecting to the spinal trigeminal nucleus (Figure 4). These three divisions fuse at the trigeminal ganglion, and then extend as sensory and motor rootlets

towards the different trigeminal nuclei, including the motor, principal sensory, spinal, and mesencephalic nuclei, afterward projecting towards the cortex (Erzurumlu, Murakami, and Rijli 2010).

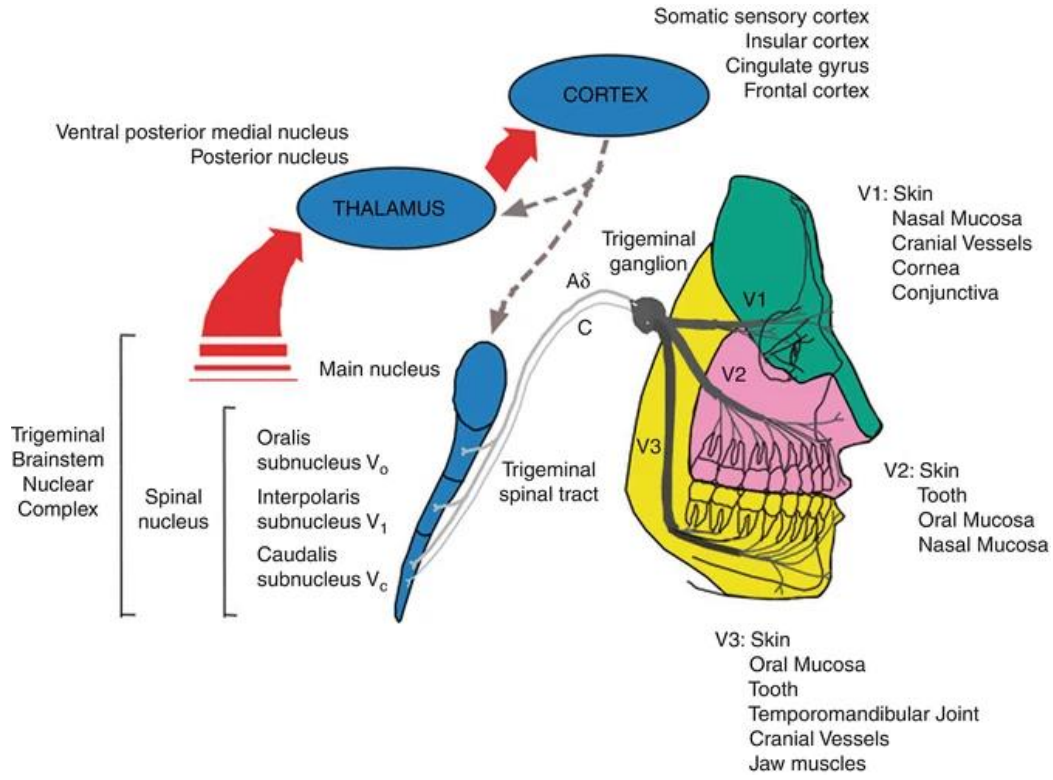


Figure 4-Nociceptive Somatosensory Organization of the Orofacial Area. The three divisions of the trigeminal nerve supply a variety of tissues including meninges. The primary afferent neurons mediating nociceptive messages (Aδ and C) project to the spinal tract nucleus. Ascending pathways arise from the spinal tract nucleus to reach higher-order areas such as the thalamus and cortex. Descending pathways can also modulate the activity of the thalamus and spinal nucleus. Oralis subnucleus (Vo), Interpolaris subnucleus (V_1), Caudalis subnucleus (V_c), ophthalmic (V1), maxillary (V2), and mandibular (V3). Adapted from (Hu and Woda 2013).

Trigeminal axons are of two natures, non-myelinated C-fibers, and thinly myelinated Aδ-fibers (Pennisi et al., 1991). Additionally, Aβ-fibers have been found. Since no other sensation can be triggered at the meningeal level, they most likely also serve a nociceptive function (Schueler et al. 2014). Both peptidergic and nonpeptidergic C-fibers are expressed, just like at the level of the skin. As they are suspected to be implicated in the neurogenic inflammation thought to occur during migraine attacks, the former has received considerable attention.

3.2. The Spinal Trigeminal Nucleus

Located in the lateral medulla of the brainstem, the spinal trigeminal nucleus is a relay of multiple sensory modalities including pain, touch, and temperature from the ipsilateral facial side in addition to relaying nociceptive information from the supratentorial dura mater (T. Bartsch and Goadsby 2003). The spinal trigeminal nucleus is divided into three nuclei, the pars oralis, pars interporalis, and pars caudalis. The pars oralis is involved in stem reflexes, while the pars interpolaris receives input from the ipsilateral face, and the pars caudalis constitutes the ascending anterior trigeminothalamic tract (Bradnam and Barry 2013). We primarily focus on the pars caudalis or the spinal trigeminal nucleus caudalis (Sp5c) that receives input from the face, cheek, forehead, and jaw and is a primary relay of nociceptive and thermal information (Barry J. Sessle 2000).

3.2.1. Organization of the Sp5c

The Sp5c is a portion of the spinal trigeminal nucleus that extends from the obex, the termination of the ependymal duct in the fourth ventricle, to the first cervical segments where it enters in continuity with the dorsal horn of the spinal cord. This structure shares various cytoarchitectural similarities with the posterior horn, hence named the medullary posterior horn. It is divided into five distinct laminae according to the size and morphology of neurons (Rexed 1952) (Figure 5). *Layer I* termed the marginal layer, is the most superficial layer of the Sp5c (Olszewski 1950). It includes both small glutamatergic/glycinergic interneurons and larger projection neurons. Neurons of lamina I are crucial in relaying nociceptive information from the peripheral nervous system toward higher-order regions of the brain. The latter being primarily the thalamus, hypothalamus, and parabrachial nucleus (Saito et al. 2017). *Layer II* is also referred to as the substantia gelatinosa due to its translucent appearance courtesy of the low amount of myelinated fibers (Olszewski 1950). It is further divided into lamina II outer (IIo) and lamina II inner (IIi) with different roles in somatosensory processing. This has been shown as the Aδ-fibers terminate in IIo only while the C-fibers terminate in both laminae IIo and IIi (L. Li et al. 2011) (Figure 5). It also demonstrated the existence of substance P receptors in the postsynaptic neurons of both laminae I and II (D. Wang et al. 2000). In addition, the marker protein kinase C (PKC)γ, is primarily

expressed at the level of the lamina IIi and when increased in the Sp5C it is associated with orofacial thermal and mechanical allodynia (Alba-Delgado et al. 2015, 2018; Mermet-Joret et al. 2022; Xie et al. 2015)

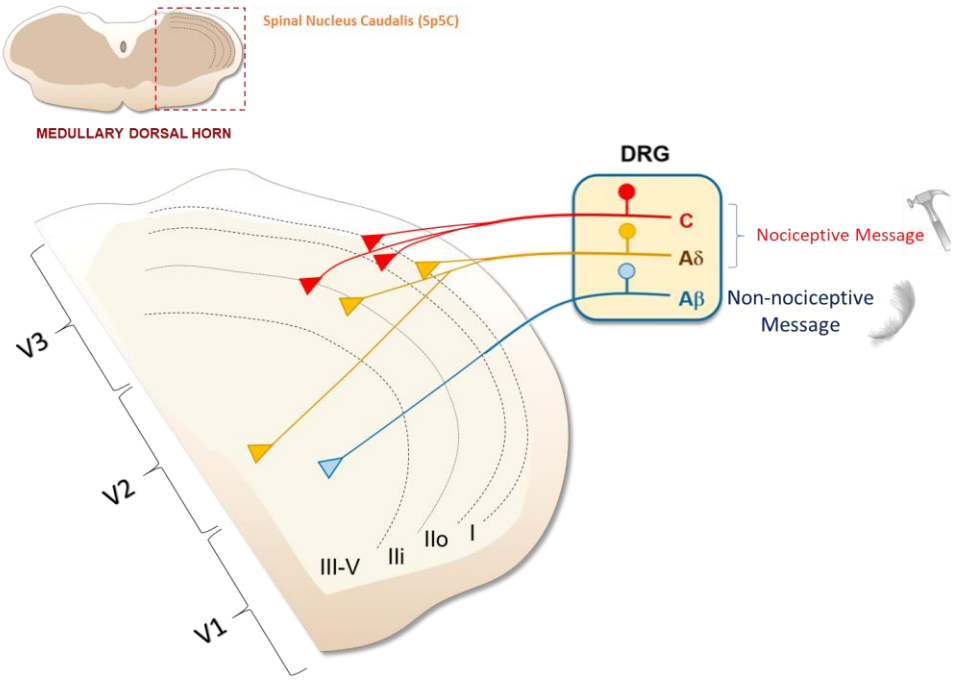


Figure 5-Organization of the Sp5c. The distinct laminae of the Sp5c with the corresponding innervations of the branches of the trigeminal nerve (V1, V2, and V3). Following nociceptive stimuli messages are transmitted through C fibers (Toward laminae I, IIo, and IIi) and Aδ-fibers (Toward laminae I, IIo, and V). Non-nociceptive messages are transmitted by Aβ-fibers (Toward laminae III, IV, and V). Lamina I (I), lamina II outer (IIo), lamina II inner (IIi), lamina III-V (III-V), ophthalmic (V1), maxillary (V2), and mandibular (V3), DRG: Dorsal Root Ganglion. (Created with Biorender.com).

Layer III encompasses a high density of both interneurons and projection neurons larger in size compared to lamina II. Neurons of lamina III are activated by vibrissae and/or guard hair deflection (Renehan et al. 1986). *Layers IV-V* are separated from lamina II by the heterogeneity in the neuronal size of lamina IV neurons compared to the smaller cells of lamina III. In addition, there is extensive evidence that identified lamina V neurons as important in pain pathways (Amano, Hu, and Sessle 1986).

3.3. Central Afferent Projections

The trigeminocervical complex (TCC) encompasses the Sp5C and the C1 and C2 regions of the cervical spinal cord. The stimulation of the meningeal vasculature has

been shown to result in the activation of the TCC (Goadsby & Hoskin, 1999). Such activated neural pathways have been pivotal to further deciphering the pathophysiology of migraine and finding potential therapeutic targets. From the TCC, nociceptive messages are transmitted to the cerebral cortex, the amygdala, or the hypothalamus via relays located in the thalamus, the bulbar reticular formation, the parabrachial nucleus, or the nucleus of the solitary tract (Figure 6). These structures participate in the integration of the different aspects of pain.

In the thalamus, the nature of the message is defined by the thalamic nuclei of origin. The ventral posteromedial (VPM) thalamic nucleus for instance contains dura-sensitive neurons having a role in the sensory-discriminative components of migraine (location, intensity, and quality of pain), which project towards the insula as well as primary and secondary somatosensory cortices (S1/S2) (Noseda et al. 2011). The dura-sensitive neurons of the posterior (Po), lateral posterior/dorsal (LP/LD) thalamic nuclei have a role in motor, focus, cutaneous hypersensitivity (allodynia), phonophobia, and photophobia and hence project to motor, somatosensory, auditory, visual, olfactory, and retrosplenial cortices.

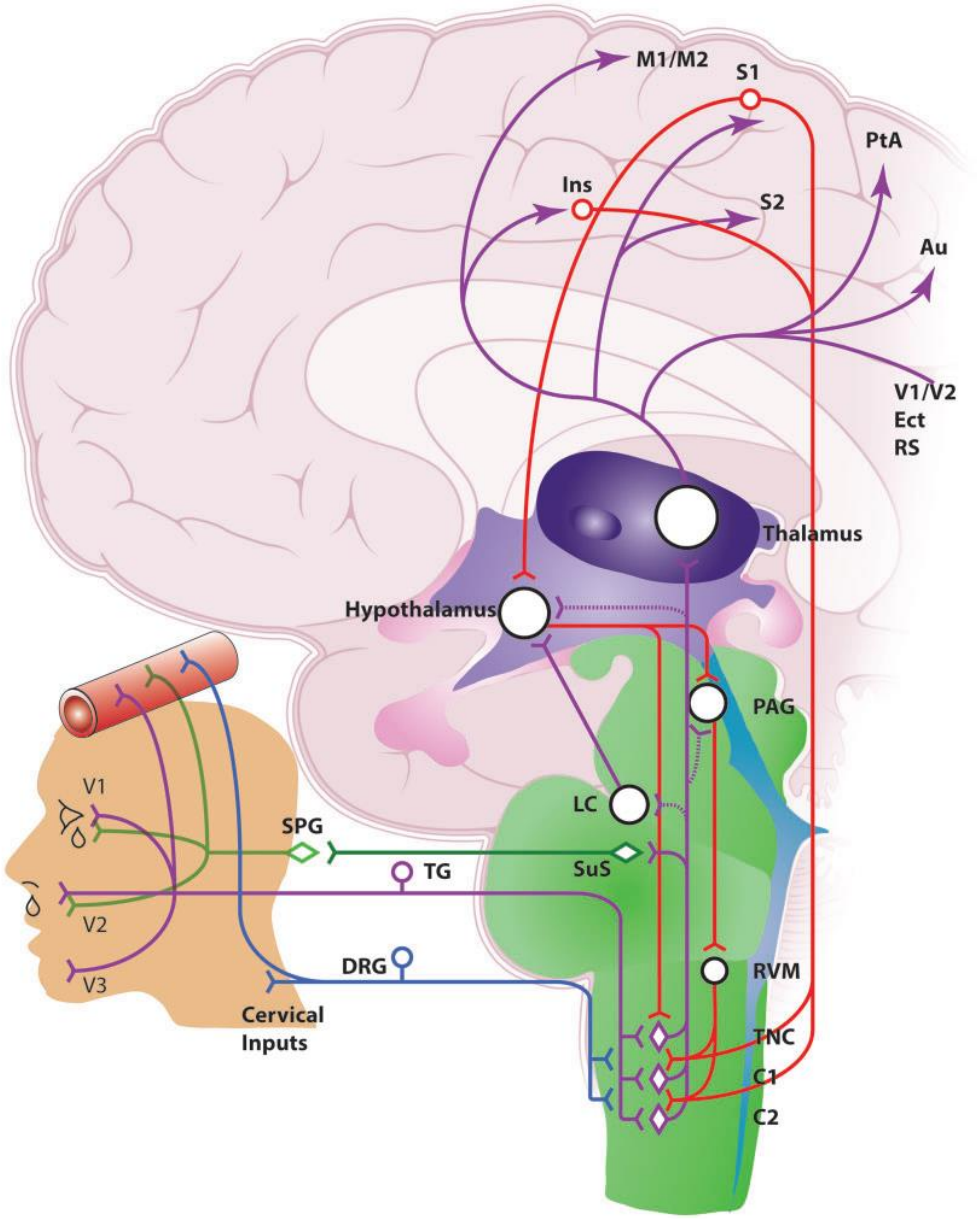


Figure 6-Anatomy of Neurovascular Pathways Modulating the Trigeminal Neurotransmission. Schematic representation of ascending and descending neuronal pathways in the medullary and upper cervical dorsal horn, which are involved in aspects of migraine and cluster headache. Au: auditory association cortex; DRG: dorsal root ganglion; Ect: entorhinal cortex; Ins: insula; LC: locus coeruleus; M1/M2: primary and secondary motor cortex; PtA: parietal cortex; PAG: periaqueductal gray; RS: retrosplenial cortex; RVM: rostral ventromedial medulla; S1/S2: primary and secondary somatosensory cortex; SPG: sphenopalatine ganglion; SuS: superior salivatory nucleus; TNC: trigeminal nucleus caudalis; TG: trigeminal ganglion; V1/V2: primary and secondary visual cortex. Adapted from (Hoffmann, Baca, and Akerman 2019).

IV. Modulation of the Trigeminal Pain Pathways

Several descending pathways modulate trigeminovascular nociception processing (Figure 6). Starting with the brainstem modulation, the PAG and the LC, located in the midbrain, makes direct projections with the trigeminovascular system. For the LC modulation, a separate section will be addressed. The PAG, could either augment the neuronal activity facilitating trigeminovascular pain transmission or suppress the activity of neurons in the spinal and medullary dorsal horn that inhibit trigeminovascular pain transmission (Porreca, Ossipov, and Gebhart 2002). The RVM (rostral ventromedial medulla) is a cluster of neurons in the medulla that receives bidirectional projections from the PAG and the spinal dorsal horn. Descending modulatory cells of the RVM either augments or blocks the responses of ascending nociceptive neurons to noxious stimulation of their respective receptive fields (Edelmayer et al. 2009).

The hypothalamus, a key player in autonomic and endocrine control, has been implicated in migraine, precisely in the premonitory symptoms experienced before an attack (sleep-wake cycle disturbances and mood changes) (Marie Denuelle et al. 2007; N. J. Giffin et al. 2003). There is a reciprocal anatomical connection between the hypothalamus and Sp5c (Malick, Strassman, and Burstein 2000). Direct projections toward the spinal and trigeminal dorsal horns and indirect projections are received by nociceptive brainstem structures (PAG, LC, and RVM) from various hypothalamic areas (Holstege 1987; Robert et al. 2013). The hypothalamus has a role in several aspects of migraine, according to structural connections between it and Sp5c and the existence of neurons that produce c-Fos in numerous hypothalamic nuclei following dural stimulation (Rami Burstein and Jakubowski 2005). Hypothalamic activation during migraine is also consistent with a role in pain modulation and hence may contribute to the development of central sensitization of trigeminovascular neurons. A recent study in rodents has shown experimental evidence for this scenario whereby the paraventricular hypothalamic nucleus (PVN) directly controls both spontaneous and evoked activities of the Sp5c (Robert et al. 2013). Therefore, PVN neurons play a role as either modulators or triggers of migraine through the integration of nociceptive, autonomic, and stress responses. Moreover, the contribution to the parasympathetic autonomic symptoms observed in migraine is done by the circuit projecting from the

hypothalamus to the superior salivatory nuclei in the brainstem (Lai, Fuh, and Wang 2009).

The thalamus is a major relay of sensory nociceptive information to be processed in respective cortical structures. Its role in migraine goes beyond the relay part and extends toward the modulation of trigeminovascular and other spinal nociceptive inputs. The thalamic nuclei VPM, Po, and LP/LD receive nociceptive inputs from the dura mater and hence can modulate the perception of pain (Rami Burstein et al. 2010; Younis et al. 2019). Sensitization, which is an increase in the responsiveness of nociceptive neurons to their normal afferent input, of the thalamic neurons (precisely the VPM and lateral nuclei) leads to the spreading of cutaneous hypersensitivity (or cutaneous allodynia) (Rami Burstein et al. 2010). It affects 60-80% of migraineurs (Rami Burstein, Collins, and Jakubowski 2004), in both cephalic and extracephalic (hands, feet) areas. The successional sensitization of first, second, and third, trigeminovascular neurons through the signals coming from the peripheral dural trigeminal innervations is thought to be the reason for the sensitization of the thalamic nuclei (Rami Burstein et al. 1998, 2010; A. M. Strassman, Raymond, and Burstein 1996). The thalamus's involvement in migraine, precisely photophobia is clear. Photophobia is one of the principal non-head pain symptoms of migraine. Primarily defined as an abnormal sensitivity to light, other definitions arose as well, such as ocular discomfort (oculodynia), exacerbation of headache by light, and a general aversion to light (Fine and Digre 1995; Lebensohn 1951; Noseda, Kainz, et al. 2010; Noseda, Borsook, and Burstein 2017). In a recent mouse model, the stimulation of the Po nucleus was sufficient to induce light-aversive behavior (Sowers et al. 2020). It is important to note that the neural pathways that mediate the aversion to light are different than the ones mediating the exacerbation of headache by light or the ones mediating ocular discomfort (Noseda, Copenhagen, and Burstein 2019).

Trigeminal nociceptive modulation originates from the cortex. The mechanisms by which alterations in cortical excitability contribute to migraine pathophysiology are largely unknown; however, through cortico-trigeminal connections, distinct cortical regions and their level of excitability may be implicated in the regulation of migraine pain. Direct descending projections have been described from the cerebral cortex to the Sp5c in both rodents and humans (Kuypers 1958; Noseda, Constandil, et al. 2010) (Figure 7). The cortico-trigeminal projections originate mainly from the contralateral

primary somatosensory and insular cortices and innervate both deep and superficial layers of the Sp5c. Anatomical positioning of these organized cortico-trigeminal networks aims at influencing meningeal nociception as what was demonstrated by S1-mediated inhibition and insula-mediated facilitation of the excitability of Sp5c dura-sensitive neurons (Noseda, Constandil, et al. 2010; B J Sessle et al. 1981).

V. Migraine Mechanisms and Pathophysiology

Although understanding migraine attack pathophysiology has substantially improved, the pathogenesis of this disease (that is, why and how attacks are repeatedly triggered or occur spontaneously) remains poorly understood. Clinical and preclinical models (which are listed in a separate section below) have provided critically important insights into migraine mechanisms, but results from these studies must always be validated against those in spontaneous attacks.

5.1. The Premonitory Phase and Triggering Mechanisms

5.1.1. The Vascular Theory

Suggestions that migraine may have a vascular origin came from its pulsatile properties. Galen of Pergamon was the first to postulate the vascular theory in the second century (Isler 1992), which took hold when Ray and Wolf noted during the 1940s that the origin of migraine attacks was located in cerebral blood vessels (Ray and Wolff 1940). More studies showed that vasodilatory peptides, such as CGRP, NO, and PACAP, are all potent contributors to migraine pathophysiology (Kuburas and Russo 2023; Pradhan, Bertels, and Akerman 2018). However, not all data fit this hypothesis. In a cohort of 20 migraine patients, Ahn did not find a temporal relationship between arterial pulsation and throbbing migraine pain (Ahn 2010). Moreover, studies using NTG, a potent NO donor, have reported a lack of vasodilation during the headache phase (Thomsen et al. 1994). CGRP-evoked vasodilation couldn’t induce a nociceptive effect on meningeal nociceptors (Levy, Burstein, and Strassman 2005). In addition, although Olesen mentioned in 1981 that the initial vasodilatory phase could be important in the pain process, pain ensued only after the subsidence of vasodilation (Olesen, Larsen, and Lauritzen 1981). The inducer of arterial vasodilation, Vasoactive

intestinal peptide (VIP) also did not trigger a migraine attack in patients (Rahmann et al. 2008). Amin et al. showed using MR-angiography that middle cerebral arterial dilation during spontaneous attacks persisted after relief with sumatriptan (an abortive drug; further discussed in another section), whereas extracranial arteries constricted (Amin et al. 2013). These data suggest that cerebral arterial dilation is an epiphenomenon reflecting activation of perivascular afferents in response to vasodilator neurotransmitters and support that vasculature is important in migraine but not the only player.

5.1.2. The Neuronal Theory

5.1.2.1. Dysfunction of the Brainstem

Studies conducted over the past decade suggest that the triggering of migraine attacks may be based on dysfunction of brainstem nuclei involved in modulatory control (Afridi et al. 2005; Moulton et al. 2008). Indeed, migraine attacks are accompanied by increased blood flow in various regions of the brainstem, including the PAG, LC, and NRM (Afridi et al. 2005; Bahra et al. 2001). Interestingly, a persistence of brain stem activity was documented in humans even after cessation of the headache and its confounding symptoms post-sumatriptan administration (Weiller et al. 1995). Hence, suggestions came about that there are headache-generating centers located in the brainstem.

5.1.2.2. Involvement of the PAG

The involvement of the PAG in the pathophysiology of migraine stems from clinical observations showing that in humans, the stimulation of the ventrolateral PAG in the treatment of chronic pain triggers migraine-like headaches (Veloso, Kumar, and Toth 1998). Furthermore, the connections shared between the PAG and several brain areas involved in pain modulation, such as the prefrontal and anterior cingulate cortex, as well as the amygdala, are diminished, even between attacks, in migraine patients (Z. Chen et al. 2017; Mainero, Boshyan, and Hadjikhani 2011). At the same time, stronger increased connections are formed with other areas such as the insula and other frontal and temporal regions (Schwedt et al. 2014). The involvement of PAG in migraine is also demonstrated through animal studies. Indeed, in various animal models, electrical stimulation (Knight and Goadsby 2001) or microinjection of a triptan (anti-migraine

medication) (T. Bartsch, Knight, and Goadsby 2004) into the PAG is sufficient to inhibit meningeal nociception.

5.1.2.3. Involvement of the Hypothalamus

Migraine's premonitory symptoms are highly associated with homeostatic functions that are regulated by the hypothalamus (feeding, sleep, and arousal). Through brain imaging studies, using positron emission tomography, the hypothalamus shows increased blood flow during the early stages of sudden migraine attacks (M. Denuelle et al. 2011), and in NTG-induced migraine attacks, changes are seen during the premonitory phase (Maniyar et al. 2014). In animals, stimulation of the rat dura mater has been shown to induce c-Fos protein expression in the ventromedial, dorsomedial, and paraventricular nuclei of the hypothalamus (Malick et al. 2001), while stimulation of the cat's superior longitudinal sinus induces expression of this marker in the supraoptic and posterior nuclei of the hypothalamus (Benjamin et al. 2004). Dopaminergic areas in the hypothalamus precisely the A11 nucleus has shown to project toward the TCC and hence modulate the ascending trigeminovascular pathway (Charbit, Akerman, and Goadsby 2011). In addition to dopamine, the hypothalamus synthesizes orexins, neuropeptides involved in arousal, appetite, and wakefulness, and controls the release of orexins from the pituitary gland. In a rat model of migraine, intravenous injections of an orexinergic receptor antagonist decrease meningeal vasodilation and TCC activation (Hoffmann et al. 2015), indicating a pronociceptive role for orexins in this pathology. Thus, dysfunction of this inhibitory control could have a pro-nociceptive effect on the TCC, leading to the nociceptive phase of migraine (Charbit, Akerman, and Goadsby 2011).

5.1.2.4. Involvement of the LC

Several recent studies have confirmed the involvement of LC in the pathophysiology of migraine. This will be further explained in a separate chapter specified for the LC.

5.1.2.5. Cortical Hyperexcitability

The hypothesis of hyperexcitability of the cerebral cortex arose from the vulnerability of migraine sufferers to certain factors, such as stress or intense sensory stimuli, particularly visual, which trigger attacks. Generally speaking, the sensory

cortex of migraine sufferers responds excessively to repeated stimuli, and this sensitivity sometimes persists permanently (G. Coppola, Pierelli, and Schoenen 2007). Transcranial magnetic stimulation, suggests that there are alterations in the cortical activity precisely at the level of the occipital lobe which appears hyperactive in migraineurs (S. K. Aurora et al. 1999). Moreover, visual cortex hyperexcitability was linked to photophobia during spontaneous migraine attacks (M. Denuelle et al. 2011). These observations suggest the existence of periodic changes in cortical excitability, which can lead to a migraine attack when the change in the cortical excitability threshold coincides with a triggering stimulus. These changes also touch the motor cortex (Brighina et al. 2011) and the parietal cortex (Andreatta et al. 2012). The mechanisms underlying altered cortical excitability remain largely unknown and are probably multifactorial. The possibility of excessive excitation caused by an abnormal release of excitatory neurotransmitters is suggested by the finding of higher cerebrospinal fluid glutamate concentrations in chronic migraine sufferers (M. Peres et al. 2004).

5.1.3. Cortical Spreading Depression and Migraine with Aura

Cortical Spreading Depression (CSD) or spreading depolarization is a transient wave of cortical neuronal depolarization. It is known to be the pathophysiological brain mechanism underlying the clinical phenomenon of migraine with aura (A. C. Charles & Baca, 2013). The majority of cells in the brain undergo an almost full membrane depolarization during CSD, whereby a massive efflux of potassium and glutamate, and other neurotransmitters and neuromodulators in addition to an influx of H_2O, sodium, and calcium coupled with neuronal swelling (Somjen 2001). Metabolic and electrocortical changes during CSD are accompanied by changes in perfusion resulting in cortical blood flow changes (Kevin C. Brennan et al. 2007). The actual mechanism by which CSD starts and ends are unknown. During a migraine aura in humans, there is no demonstration of an electrophysiological correlate of a CSD probably due to the difficulty in capturing a CSD in a non-invasive manner in such an unpredictable disorder. However, it has been profoundly shown in animal models and human brain direct recordings after trauma and ischemia (Dreier et al. 2009; Lauritzen et al. 2011). The long debate about whether CSD is a migraine attack trigger is still ongoing without consensus. In terms of connection, it has been reported that CSD activates the trigeminal nociceptive pathways hence triggering a migraine attack (Noseda,

Constandil, et al. 2010; X. Zhang et al. 2011). A CSD has been shown to activate both pial and dural macrophages and dendritic cells (Schain et al. 2018), and is associated with the release of nociceptive mediators (Schain et al. 2017). Multiple exogenous and endogenous factors such as hormones (estrogen and testosterone), and medications (anti-migraine preventive drugs) also modulate brain tissue susceptibility in animal models of CSD (Bogdanov et al. 2011; A. C. Charles and Baca 2013).

5.2. Mechanisms of the Headache Phase

The headache phase of migraine involves the ascending trigemino-thalamic pathway's activation. Based on early observations in humans who underwent awake brain surgery, the idea that pain during a migraine attack is perceived to be felt on intracranial structures (dura matter and intracranial vasculature) became well established (Ray and Wolff 1940).

5.2.1. Neurogenic Inflammation

Data from Ray and Wolff added to consolidate the theory that the activation of nociceptive nerve fibers (C- and Aδ-fibers) innervating blood vessels could lead to a migraine attack (Ray and Wolff 1940). The activation of these fibers was an enigma until the theory of dural inflammation came into place (Moskowitz 1984). The latter is based on the local dural release of endogenous inflammatory mediators (CGRP, substance P, prostaglandins, and neurokinin A) hence inducing an increase in the blood flow (as described before), mast cell degranulation, leakage of plasma proteins, and aggregation of platelets. Mast cells, in turn, release inflammatory molecules, including histamine known to activate C-fibers and promote the release of CGRP (Lassen et al. 2002).

Substance P, mainly from C-fibers, is a potent vasodilator and provokes the release of bradykinin, histamine, and serotonin (also called 5-hydroxytryptamine (5-HT)), contributing to inflammation. CGRP, also released by C-fibers, is a neuropeptide able to trigger headaches in migraineurs (Lassen et al. 2002) It targets the CGRP receptor present in the blood vessel muscles, A-fibers, and glial cells, and induces brain vessel vasodilation. At the level of the trigeminal ganglion, 70% of CGRP-positive neurons co-localize with the TRPV1 receptors, more than in the dorsal horn, which could explain the pivotal role of CGRP in the trigeminovascular system signaling (Price

and Flores 2007). Regarding prostaglandins, they have a vasodilator activity and can evoke CGRP release. Their intravenous injection in controlled individuals triggers headaches. The sympathetic fibers will release 5-HT, which promotes the nociceptor excitation at the level of meninges and their respective sensitization (A. M. Strassman, Raymond, and Burstein 1996), and NO, increases the synthesis and release of CGRP, in addition to its vasodilator activity. Thus, all of these pro-inflammatory molecules act synergistically to lower the response threshold of nociceptors to mechanical stimuli.

5.2.2. Peripheral and Central Sensitization

Following all that has been postulated about migraine, migraine is now considered a neurovascular disorder. This theory is based on the sensitization of peripheral and central trigeminovascular neurons. The throbbing perception of the headache is mediated by peripheral sensitization (A. M. Strassman, Raymond, and Burstein 1996), while the sensitization of second-order neurons in the Sp5C mediates cephalic cutaneous hypersensitivity as well as muscle tenderness (R. Burstein, Cutrer, and Yarnitsky 2000; Rami Burstein et al. 2010) (Figure 7). Indeed, systemic administration of NTG (S. Wu et al. 2022) or ISDN (Isosorbide dinitrate) (Flores Ramos et al. 2017) in rats, induced Sp5C sensitization associated with migraine-like pain. Moreover, in a mouse model of inflammatory soup, the astrocytic activation of the Sp5c contributed to the central sensitization in chronic migraine (L. Zhang et al. 2022).

The expansion of the dura and cutaneous receptive fields, as well as enhanced responsiveness to mechanical and thermal stimulation of the skin and the dura, are all signs of this neuronal sensitization (Rami Burstein et al. 1998). Trigemino-cervical neurons receiving inputs from both intracranial dura and extracranial periorbital skin are subjected to long-lasting sensitization following the topical application of inflammatory agents to the rat dura (Ebersberger et al. 1997; Schepelmann et al. 1997). A malfunction in the pain-modulating systems may also be a factor in the development of central sensitization and the vulnerability to developing chronic migraine. The onset or maintenance of sensitization and the emergence of chronic migraine may both be influenced by an imbalance of pain facilitation and inhibition (Schwedt 2014; Welch et al. 2001). What's intriguing is that medications like the recently developed monoclonal antibodies against the CGRP system and botulinum toxin, which act peripherally at least on trigeminal fibers, can block chronic migraine in at least 60–70% of individuals. As

peripheral sensory inputs are crucial for maintaining central drive in chronic migraine, this further supports the trigeminal system's crucial function.

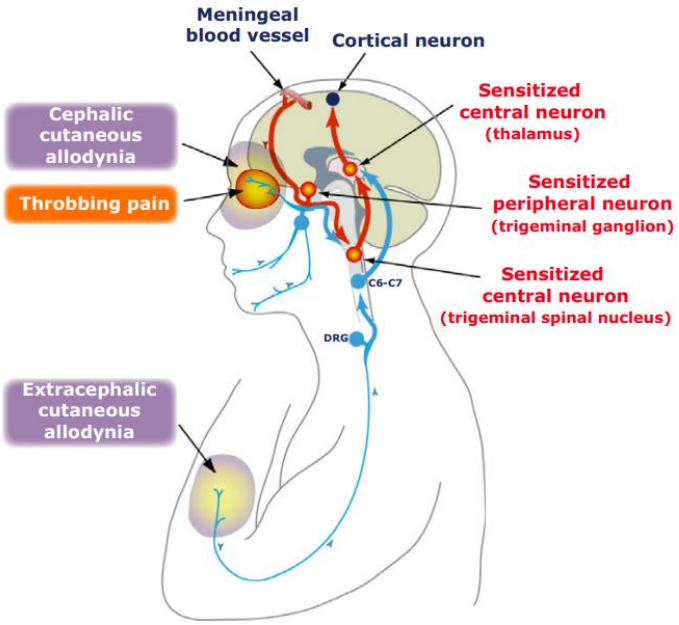


Figure 7-Neuronal Sensitization of Trigeminovascular system during migraine. Schematic representation of the peripheral sensitization of the trigeminal ganglion neurons (mediator of periorbital throbbing pain), and the central sensitization of the spinal trigeminal nucleus caudalis (Sp5c) (mediator of cephalic cutaneous hypersensitivity), and thalamic trigeminovascular neurons (responsible of extracephalic cutaneous hypersensitivity) during migraine attack. Adapted from (Noseda and Burstein 2013).

The proposition for a neural substrate for extracephalic cutaneous hypersensitivity during migraine wasn't done until recently. A recent study demonstrated that innocuous heat and brush stimuli induce a heightened activity in the pulvinar thalamic nucleus of patients showcasing signs of whole-body allodynia (cannot wear tight clothing, cannot use a heavy blanket, and cannot take a shower) during migraine, as compared to the pain-free state (Rami Burstein et al. 2010). In addition, sensitization of thalamic trigeminovascular neurons located in VPM, Po, and LP nuclei occurred after the topical application of inflammatory molecules on the rat meninges (Rami Burstein et al. 2010) (Figure 7). Altogether, it is suggested that the rostral subdivision of the pulvinar in the posterior thalamus of human beings and the most dorsal and posterior part of the thalamus (i.e., Po) in animals mediate to a certain extent whole-body cutaneous hypersensitivity.

5.3. The Interictal Migraine “Brain State”

Between migraine attacks, the brain of migraineurs exhibits structural and functional differences compared with the brain of healthy controls. Altered morphologies include changes in the grey and white matter volume and integrity, which are associated with functional interictal alterations including enhanced visual, auditory, somatosensory, and motor-evoked responses, enhanced nociceptive processing, and abnormal pain processing in the brainstem. Such alterations offer an alternative hypothesis that considers migraine as a “brain state” (Goadsby et al. 2017). The brainstem is in close relations with other regions and nuclei of the brain and hence any dysfunction in said regions alters the descending pain modulatory pathways. The latter leads to a false perception of pain under normal conditions and ongoing dysfunction further leads to the central sensitization of trigeminovascular neurons in addition to increasing both cutaneous extracephalic and cephalic allodynia (Goadsby et al. 2017). The same dysfunction could affect the hypothalamus and hence the sleep cycle, and the thalamus in terms of the hypersensitivity to smells, sounds, and light as it projects towards the cortex. Determining whether these structural and functional abnormalities are a result or a cause of the repetitive migraine attacks is critical to improving understanding of migraine pathophysiology.

5.4. Migraine as a Chronic Evolutive Condition

5.4.1. Genetic and Epigenetic Components

Although genome-wide association studies failed to shed light on the molecular alterations that are responsible for migraine’s evolutive nature, one can postulate that knowledge from multiple variants will highlight which molecular pathways that could be involved in migraine pathophysiology (Van Den Maagdenberg, Nyholt, and Anttila 2019). 44 SNPs (single nucleotide polymorphism) were linked to migraine without aura in the most recent genome-wide association study, which involved samples from more than 300,000 controls and approximately 60,000 patients (Gormley et al. 2016). This implicated 38 different genetic locations. Most of them were discovered to be involved in vascular function-related molecular pathways. Other loci found in this study were connected to metal ion homeostasis pathways, which led to the fairly unexpected conclusion that metal ion homeostasis might contribute to migraine susceptibility. Ion

channel activity was found to be restricted to a small number of loci, with substantially weaker signals (Van Den Maagdenberg, Nyholt, and Anttila 2019). The relative importance of those to the vascular function is still up for debate since one study showed that vascular dysfunction has a significant impact on migraine susceptibility whereas neuronal dysfunction has a much less significant impact (Van Den Maagdenberg, Nyholt, and Anttila 2019). On the other hand, genetic research on FHM (Familial hemiplegic migraine) (Michel D. Ferrari et al. 2015) has provided information on specific genes that encode proteins essential for ion channel and transporter function. In-depth research of specific mutations in these genes has shown that they can cause either function loss or gain in cellular assays or mutant murine. In summary, mutations in the genes CACNA1A, ATP1A2, and SCN1A, which each encode a subunit of the voltage-gated CaV2.1 Ca2+, NaV1.1 Na+, and glial Na+ K+ ATPases, were linked to FHM. It's interesting to note that these mutations frequently result in increased glutamate availability at the synaptic cleft of cells. CACNA1A gene mutations may result in increased glutamate production due to augmented calcium flux at the level of the presynaptic terminal (Schneggenburger and Neher 2005). A lower electrochemical gradient for Na+ is the outcome of ATP1A2 gene mutations. One result of this is a decrease in or inactivity of astrocytic glutamate transporters, which causes synaptic glutamate to accumulate (De Fusco et al. 2003). High-frequency discharges may be facilitated by SCN1A mutations, which may also raise glutamate levels in the synapses (Dichgans et al. 2005). In this way, glutamatergic synapses' neurons can fire more often than they would under normal circumstances, which could account for why migraine aura sufferers are more susceptible to CSD (Van Den Maagdenberg, Nyholt, and Anttila 2019). It's interesting to note that mice with the CACNA1A mutation have an altered CGRP expression and attenuated trigeminovascular nociceptive responses (Mathew 2011; Park et al. 2014).

Only a few studies have been conducted on migraine patients when it comes to the emerging field of epigenetics. A small pilot study compared the genome-wide DNA methylation levels in episodic migraineurs and chronic migraineurs with medication overuse headache (MOH) before and after a detox program to identify changes in DNA methylation associated with headache chronification. Although there were no statistically significant differences between the groups at the various time points, specific CpG sites of interest were detected and are believed to be implicated in the

comorbidity of neuropsychiatric disorder and drug addiction pathways (Terlizzi et al. 2018). These preliminary findings suggest that epigenetic processes play a role in migraine, and they may also play a role in the mechanisms underlying brain plasticity and other processes specific to migraine. All these preliminary results need to be replicated, though, because migraine, in its episodic and chronic forms, is a complex and multidimensional condition.

5.4.2. Brain Alterations

Beyond functional alterations, other investigations comparing migraine patients and controls have found variations in the structural integrity of the brain, affecting both the white and gray matter, that change over time. White-matter abnormalities were discovered in the brainstem and cerebellum of chronic migraine patients (Bilgiç et al. 2016). Various brain regions known to have a role in multisensory integration, nociception/anti-nociception, and analgesic reliance were shown to exhibit minor changes in gray matter volume in association with chronic migraine, according to other research (Gianluca Coppola et al. 2017). Gray matter alterations have reportedly been linked to headache frequency measurements in both episodic and chronic migraine sufferers (Neeb et al. 2017). It is unknown what causes these anatomical alterations in the migraine brain. Some changes might be brought on by a genetic predisposition to migraine attacks.

Repeated occurrences of head pain may lead to other alterations. Such anatomical changes may be the result of brain plasticity, which is the brain's capacity to alter its structure and function in response to changes in the body or the surrounding environment. A variety of CNS modifications, including angiogenesis, synaptogenesis, gliogenesis, neurogenesis, enhancement in cell size, increase in myelin size, and augmentation in blood flow or interstitial fluid, can lead to changes in gray matter. Axonal remodeling and variations in blood flow are typically the causes of alterations in white matter (May 2011). Although there have been several reports of structural alterations in migraine sufferers' brains, it is still unclear how important these changes are to the biology of migraine. However, the presence of structural modifications suggests that migraine causes a progressive anatomical transformation in the brain, which may play an evolving role in the development of the condition and any accompanying disability.

VI. Migraine Treatments

The treatment of migraine aims at limiting the triggers, finding the appropriate abortive treatment, and applying an effective long-term preventive treatment (S. D. Silberstein et al. 2012). Pharmacological treatments are divided into two categories: abortive treatments which must be taken at the onset of the migraine attack to be effective, and preventive treatments whose objective is to reduce the frequency and intensity of attacks. Non-pharmacological approaches include behavioral therapy and biofeedback, education, relaxation, mindfulness, and diet adaptation among others.

6.1. Abortive Drugs

The goals of acute migraine treatment (with or without aura) are to obtain freedom of pain 2 h after medication intake (significant pain relief is also acceptable) with 24 h sustained response and without (or with minimal) adverse events. According to the revised guidelines of the French Headache Society (Ducros et al. 2021) abortive drugs can be classed into two categories:

- *Non-specific abortive drugs* include the use of non-steroidal anti-inflammatory drugs (NSAIDs: diclofenac, ibuprofen, ketoprofen, naproxen) and analgesic drugs (paracetamol, aspirin). They are the cyclooxygenase (COX) inhibitors currently used in the management and treatment of pain, with analgesic, anti-inflammatory, and antipyretic properties. Evidence shows that aspirin (acetylsalicylic acid) with or without metoclopramide (an anti-emetic drug), and most NSAIDs are effective acute migraine treatments (Kirthi, Derry, and Moore 2013). Paracetamol (acetaminophen), alone or in combination with codeine, caffeine, or aspirin is also effective in reducing migraine pain, but only in attacks of mild-to-moderate intensity with few bothersome symptoms (Richard B. Lipton et al. 2000).

- *Specific abortive drugs* include triptans and gepants (see below).

Opioids are not recommended to treat migraine attacks as they exacerbate nausea and increase the risk of MOH. Ditans are serotonin receptor agonists like triptans but with specificity for the subtype 1F (5-HT_{1F}). These receptors are very little present in the periphery and almost absent at the vascular level, but they are found in abundance in the CNS (Vila-Pueyo 2018). The only ditan currently being used to treat

acute migraine attacks is lasmiditan (Lamb 2019), which is primarily used in cases of patients with acute migraine and cardiovascular risks.

6.1.1. Triptans

Triptans are recommended as first-line drugs for patients suffering from moderate to severe migraine, associated with disability, who do not respond to non-specific abortive drugs. They are agonists of serotonin 1B and 1D (5-$HT_{1B/D}$) receptors, with a lower affinity for other serotonergic receptors, such as 1A, 1E, and 1F. Seven triptans are available in France: almotriptan, eletriptan, frovatriptan, naratriptan, rizatriptan, sumatriptan , and zolmitriptan (Ducros et al. 2021). Sumatriptan was the first to be marketed, at the beginning of the 1990s. Even if it is absorbed orally at a fast rate, its bioavailability is only 14% and it has a short half-life of about 2 h. The other six triptans were later introduced, which have greater oral bioavailability, longer plasma half-life, active metabolites, higher lipophilicity, and greater potency and affinity for 5-$HT_{1B/D}$ receptors (P. Tfelt-Hansen, De Vries, and Saxena 2000).

Clinical trials show that triptans have superior or equal effectiveness at relieving acute migraine pain to NSAIDs and paracetamol (Cameron et al. 2015). A multiple treatment comparison meta-analysis done in 2013 ranked eletriptan as the most effective triptan at relieving pain at 2 h and 24 h. Rizatriptan was the second most effective but did not have the same efficacy at 24 h, followed by sumatriptan (Thorlund et al. 2014). The administration route and dose can also modify the effectiveness of triptans. For example, subcutaneous administration of sumatriptan (6 mg) shows great efficacy in terms of complete pain relief at 2 h, although adverse events were also high (Derry, Derry, and Moore 2014).

6.1.1.1. Mechanisms of Action

Triptans inhibit the release of vasoactive and pro-inflammatory neuropeptides (including CGRP), having mainly a vasoconstrictive effect. Studies have also shown an action on trigeminal transmission, by acting on 5-HT_{1D} presynaptic receptors (Ho et al. 2014). Sumatriptan, one of the most notable triptans uses its vasoconstrictive action, and hence its ability to reduce the release of CGRP from nociceptive fiber terminals of the trigeminal nerve, and to modulate 5-HT synthesis at the cortical level (Humphrey and Goadsby 1994; Sakai et al. 2008). The majority of 5-$HT_{1B/D}$ receptors are localized at

axon terminals in the presynaptic region of the Sp5c and trigeminal ganglion (Ma 2001). It is also important to note that 5HT receptors also have a central effect and reside at the central level of other migraine-related regions. The LC, hippocampus, and cortex, all contain low densities of central 5-HT_{1D} receptors with similar distributions among humans and rodents (Tricklebank and Daly 2019). While with respect to 5-HT_{1B} receptors, in humans, high densities have been found in the substantia nigra and globus pallidus (Bonaventure et al. 1997; Varnäs et al. 2001). The dorsal raphe nucleus and the cortex show intermediate densities and lower expressions are in the hippocampus, amygdala, and thalamus (Varnäs et al. 2001; Varnäs, Halldin, and Hall 2004). This distribution is homologous with the distribution in rodents (Bruinvels, Palacios, and Hoyer 1993).

6.1.1.2. Triptan Non-responders

Triptans are highly effective if taken early within the first few minutes of the start of the headache attack. When the attack is established, and there is the presence of cutaneous cephalic hypersensitivity (indicative of ongoing central sensitization) patients are no longer responsive to triptans (Rami Burstein, Collins, and Jakubowski 2004). Although triptans are recommended as a first-line treatment, approximately 30-40 % of migraineurs suffer from insufficient efficacy or tolerability (Lipton et al., 2013; Viana et al., 2013). Migraine patients can be divided into triptan responders or non-responders. In the first category of patients, triptan is effective against an acute attack in at least three out of four migraine attacks (Sacco, Amin, et al. 2022), while in the second group of patients, triptan fails to abort the headache. However, triptan non-responders are within a larger group of triptan failures that further includes; triptan resistant (failure of at least 2 different triptans); triptan refractory (failure of at least 3 different triptans, including subcutaneous formulation); and triptan ineligibility (presence of a contraindication) (Sacco, Lampl, et al. 2022). In the case of sumatriptan, for example, non-responders show lower and slower absorption after oral administrations compared to responders (A. Ferrari et al. 2008). Moreover, in women, the efficacy rate of triptans is the same as in men, however, their risk of a headache recurrence is higher (Casteren et al. 2021). It is important also to add that inadequate efficacy and/or tolerability with one triptan does not always predict outcomes with another triptan and switching to another triptan might benefit patients (Viana et al. 2013).

6.1.2. Gepants

Gepants were the primary oral agents designed for both migraine treatment and prevention. They are CGRP receptor antagonists, and include rimegepant, ubrogepant, and olcegepant (Croop et al. 2021; Dodick et al. 2020; Recober and Russo 2007). The functionality of gepants is through their binding to CGRP receptors and inhibiting their activation. They hence block the amplification and progression of the pain process from the trigeminal ganglion, trigeminal nerve fibers and, dura. Ubrogepant, an FDA-approved gepant used for the acute treatment of migraine has shown a highly selective efficacy and is considered a first class of drugs in the class of oral CGRP antagonists (Scott 2020). Gepants are a reasonable alternative for migraineurs that are triptan non-responders and for those with triptan contraindications (Moreno-Ajona, Villar-Martínez, and Goadsby 2022). For instance, the efficacy and tolerability of ubrogepant showed no difference compared to that of groups using triptans (Blumenfeld et al. 2021). Moreover, the coadministration of ubrogepant with other triptans such as sumatriptan is well tolerated by patients with minimal side effects (Jakate et al. 2020).

6.2. Prophylactic Treatments

Preventive migraine treatments aim at reducing monthly migraine days by at least 50% in episodic migraine and by at least 30% in chronic migraine. Prophylaxis also aims at reducing the consumption of acute treatments, intensity and duration of attacks, and improving the quality of life (Ducros et al. 2021). Most patients under prophylaxis will still have attacks, which will be treated by abortive approaches. In Europe, commonly prescribed prophylactic agents include:

- *Anticonvulsant drugs* (venlafaxine, valproate, and topiramate). Their effect is exerted by the inhibition of calcium channels that limit the release of excitatory molecules or increase the release of GABA (γ-Aminobutyric acid)(Mattias Linde et al. 2013). Topiramate is the main potent anticonvulsant drug against episodic migraine with high efficacy (Stephen D. Silberstein 2015).

- *Antihypertensives*, block both β1 and β2 receptor subtypes, and therefore, the action of the endogenous catecholamines, adrenaline, and noradrenaline (NA). Propranolol is the most common and one of the most effective first-line medications

used for migraine prophylaxis, which presents high effectiveness in cases of episodic migraine (J. L. Jackson et al. 2019). Other β-blockers that can be used are timolol, atenolol, and metoprolol, which should be considered in patients with underlying cardiovascular diseases.

- *Antidepressant*. Evidence shows that amitriptyline, a tricyclic antidepressant, is superior to a placebo to reduce headaches by 50% in episodic migraine, with an efficacy comparable to that of propranolol (Ziegler et al. 1987). A systematic review concluded that venlafaxine, a dual reuptake inhibitor, had antimigraine effectiveness (F. Wang et al. 2020) while no evidence supports the prophylactic effect of selective serotonin reuptake inhibitors.

- *Onabotulinumtoxin A* (Botox) has been pivotal in chronic migraine through its efficacy in reducing monthly headache days and improving the quality of life in addition to being well tolerated by patients (Escher et al. 2017). However, it has not shown any superiority over the placebo in cases of episodic migraine (Herd et al. 2018).

- *Monoclonal anti-CGRP molecules*, targeting CGRP or its receptor, represent the first specific and selective migraine prophylactic treatment (Garland, Smith, and Gums 2019). Anti-CGRP antibodies are effective and well tolerated in patients with chronic migraine even in the presence of prior failed therapies or medication overuse (Caronna et al. 2021). In one recent study, eptinezumab, erenumab, fremanezumab, and galcanezumab significantly reduced the monthly migraine days compared to placebo (X. Wang et al. 2021). However, safety-wise, galcanezumab solely increased the incidences of treatment-emergent adverse events.

6.3. Non-pharmacological Interventions

Changing one's daily dietary intake and designing a systematic, and mechanism-driven diet where dietary supplements are introduced such as vitamin B2 and food rich in magnesium and omega-3 can provide both medicinal and health benefits for migraine (Puledda and Shields 2018). In addition, the usage of behavioral techniques (such as cognitive behavioral therapy, and relaxation techniques), acupuncture, and hypnosis have aimed at helping patients to further cope with their symptoms. They have evidence of positive functionality to a certain degree in migraineurs where the treatments are no longer functional (Penzien et al. 2015). Applying changes to one's lifestyle can also

improve the effectiveness of the treatment, the usage of stress reduction methods and the introduction of physical activity can enhance treatment efficacy and one's quality of life (Wells et al. 2021).

Non-invasive neuromodulation is also used by utilizing techniques that stimulate the central and peripheral nervous systems through either an electric current or magnetic field through the skin such as transcutaneous cranial nerve stimulation (Schoenen et al. 2013). This field is backed up by evidence of functionality in migraine however it is only located in specialized centers (Puledda and Shields 2018). Moreover, invasive neuromodulation techniques also exist such as occipital nerve stimulation but those are limited to the highest cases of refractory migraineurs with multiple failed interventions (Thorsten Bartsch and Goadsby 2002).

VII. Animal Models of Migraine

Various animal models have been designed to understand the pathophysiology of migraine. So far there has not been an animal model that can replicate all the features of this complex disorder. Currently, the focus of animal model designs is to stimulate the TCC. Dura mater, large cerebral arteries, and meningeal vasculature are pain-sensitive areas innervated by nociceptors located on meningeal and trigeminovascular afferents derived from the trigeminal nerve. Stimulation of the nerves innervating these structures leads to the induction of headaches similar to migraine (Olesen et al. 2009).

The most investigated animal models are the ones based on chemical stimulations using different vasodilating agents. The "inflammatory soup" model using a mix of inflammatory mediators [prostaglandin E2 (PGE-2), histamine, 5-HT, and bradykinin] administered through a micro-catheter at the level of the meninges is used for meningeal and trigeminovascular stimulation hence inducing cephalic mechanical sensitivity (Boyer et al. 2014; Munro, Jansen-Olesen, and Olesen 2017). One of the limitations of this technique is that the usage of such chemicals can compromise the functionality of the BBB.

Other models use systemic administration of NO donors, such as NTG or ISDN. Typically, NTG infusion induces headache (often with premonitory symptoms) in up to 83% of patients with migraine without aura, and in 67% of those with migraine with

aura (Goadsby and Lipton 1997). Attacks are not provoked in controls who do not have migraine and NTG does not induce aura. In mice, NTG induces mechanical and thermal extracephalic hypersensitivity which are symptoms reversed by sumatriptan (Bates et al. 2010). In addition, NTG-injected mice experience light aversion (as a surrogate of clinical photophobia) and augmentation of meningeal blood flow (K. C. Brennan et al. 2013; Markovics et al. 2012). Intermittent injections of NTG were also used to assess the progression of migraine from an acute to a chronic state, in which extracephalic mechanical hypersensitivity became progressive and sustained (Pradhan et al. 2014). Even with its positive aspects, this model has some limitations. First, NTG is hypotensive and requires a vehicle consisting of alcohol and propylene glycol hence having non-specific effects (Ramachandran et al. 2012). Second, repetitive administrations of NTG induce anxiety in rats (Taheri et al. 2020) which could limit the study of light avoidance as a migraine-associated symptom. Finally, in most of the studies using NTG in rodents there were no differences between males and females (Targowska-Duda et al. 2020), and the data were often pooled (Jalgaonkar et al. 2023).

ISDN has been already validated to trigger headaches in humans (Bellantonio et al. 1997; Castellano et al. 1998). In male rats, a single systemic ISDN injection induces reversible cephalic mechanical hypersensitivity that can be aborted by sumatriptan and olcegepant (Dallel, Descheemaeker, and Luccarini 2018). Furthermore, repeated ISDN administrations provoke persistent cephalic and transitory extracephalic mechanical hypersensitivities, both of which are prevented by propranolol (Dallel, Descheemaeker, and Luccarini 2018). ISDN also induces central sensitization of trigeminal pathways (Flores Ramos et al. 2017) that is correlated to migraine progression into chronic forms. Compared to NTG, ISDN has its pros, starting with being less hypotensive (Manabe et al. 2001). Moreover, it comes in an injectable form hence being easily administered to both humans and animals allowing for a mimic of what occurs in clinical settings. Last but not least, it does not require a vehicle such as alcohol or propylene glycol to be dissolved in, hence this removes the non-specific variables (Ramachandran et al. 2012).

A similar migraine provocation pattern as with NTG has been seen with CGRP. In humans, an intravenous administration of CGRP is capable of inducing headaches (Asghar et al. 2010). When it comes to animals, subcutaneous injections of CGRP in male rodents induced periorbital mechanical hypersensitivity (De Logu et al. 2019). In

addition, dural administrations of CGRP, induced periorbital touch hypersensitivity in mice (Avona et al. 2019).

CSD, a slow wave of depolarization of neuronal and glial cells in the cortex, forms another migraine model that can be induced by injections of potassium chloride (KCl) (Andreou et al. 2010). It can also be induced by electrical and mechanical stimulations of the cortex in rodents (Eikermann-Haerter and Moskowitz 2008). This technique does remain invasive and behavioral assessments can't be done since the animal is anesthetized.

VIII. The Noradrenergic System

8.1. Synthesis, Release, and Reuptake of Noradrenaline

NA is a monoamine that, like adrenaline and dopamine, belongs to the catecholamine group and is synthesized from the amino acid L-tyrosine. Each stage in the synthesis of NA (Figure 8) is dependent on a distinct enzyme that acts as a catalyst, with the rate-limiting enzyme being TH.

NA is deposited in synaptic vesicles, and released into the synaptic cleft following an electrical input to the noradrenergic terminal. The release is regulated by a negative feedback that involves the presynaptic α2- adrenoreceptors (AR) (Bukharaeva et al. 2021). NA then binds to presynaptic and postsynaptic AR and the signal is either propagated or inhibited depending on the AR subtype. NA can either be taken up by the NA transporter (NAT) or degraded by the monoamine oxidase (MAO) A and catechol-O-methyltransferase, enzymes located within the synaptic cleft and nerve terminal.

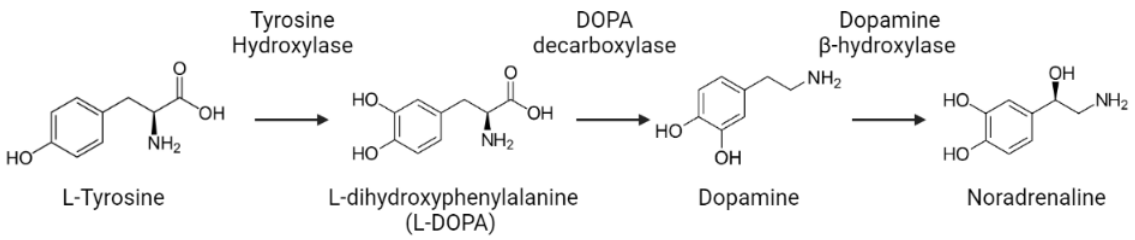


Figure 8-Synthesis of Noradrenaline. The rate-limiting enzyme tyrosine hydroxylase converts the amino acid L-tyrosine into L-dihydroxyphenylalanine. L-DOPA is then decarboxylated to become

dopamine. NA is produced by the enzyme dopamine-hydroxylase's subsequent metabolic alterations of dopamine. (Created with Biorender.com).

8.2. The Noradrenergic Regions in the CNS

In rodents and primates, the noradrenergic system is primarily composed of seven separate clusters of cell bodies that are located in the brainstem. The pontine's lateral and ventral reticular formations, respectively, are home to the A1 and A7 cell groups. The nucleus of the solitary tract contains A2 neurons. A3 and A4 are located in the roof of the fourth ventricle, ventral to the cerebellar nuclei. A5 is located close to the superior olivary complex in the pontine tegmentum while the LC (A6) is located in the dorsal wall of the rostral pons on the lateral floor of the fourth ventricle (Dahlström and Fuxe 1964; Felten and Sladek 1983). These noradrenergic areas send axonal projections to different nuclei of the CNS, but the target regions from each nucleus differ considerably.

8.3. The Locus Coeruleus

The noradrenergic LC has been implicated in a variety of physiological and neurobiological functions such as cardiovascular regulation, arousal, vigilance, decision-making, attention, learning, memory, sleep-wake cycle, motor activity, and cognitive activation (Clayton et al. 2004; James et al. 2021; Maness et al. 2022). Moreover, it has been also established to be intimately related to the etiology of neuropathological and psychiatric states, for example, stress, Alzheimer’s disease, depression, and anxiety disorders (Giorgi et al., 2021; Itoi & Sugimoto, 2010). The involvement of the LC in pain modulation and specifically in migraine will be detailed below.

8.3.1. Anatomical and Molecular Organization

The LC is considered the principal noradrenergic source in the CNS (Dahlstroem and Fuxe 1964) in all mammalian species (Russell 1955), with an elaborate network of ascending and descending projections. It consists of two bilateral nuclei located on either side of the dorsal wall of the rostral pons on the lateral floor of the fourth ventricle (Amaral and Sinnamon 1977). Its size is reduced with an average

diameter of ~300 µm in mice, and only ~1,500 neurons in mice against 20,000 in humans in each hemisphere (Sara & Bouret, 2012; Schwarz & Luo, 2015). The cluster of NA neurons located in the LC constitutes about half of the noradrenergic neurons of the CNS. Studies have shown that most LC neurons synthesize the enzyme dopamine β-hydroxylase which is responsible for the conversion of dopamine to NA hence deducing that NA is the primary neurotransmitter released (Grzanna and Molliver 1980; Mateo, Pineda, and Meana 1998). In addition to NA, other neuropeptides and neuromodulators are expressed and released by the LC, such as galanin, which is expressed in up to 80% of neurons and plays a role in wake/sleep states, and nociception, NPY, vasopressin, somatostatin, and enkephalin (Holets et al. 1988; Lang et al. 2015; Sutin and Jacobowitz 1991; Tanaka et al. 1989). Moreover, recent studies showed a GABAergic population of neurons that could modulate the activity of noradrenergic neurons (Breton-Provencher & Sur, 2019).

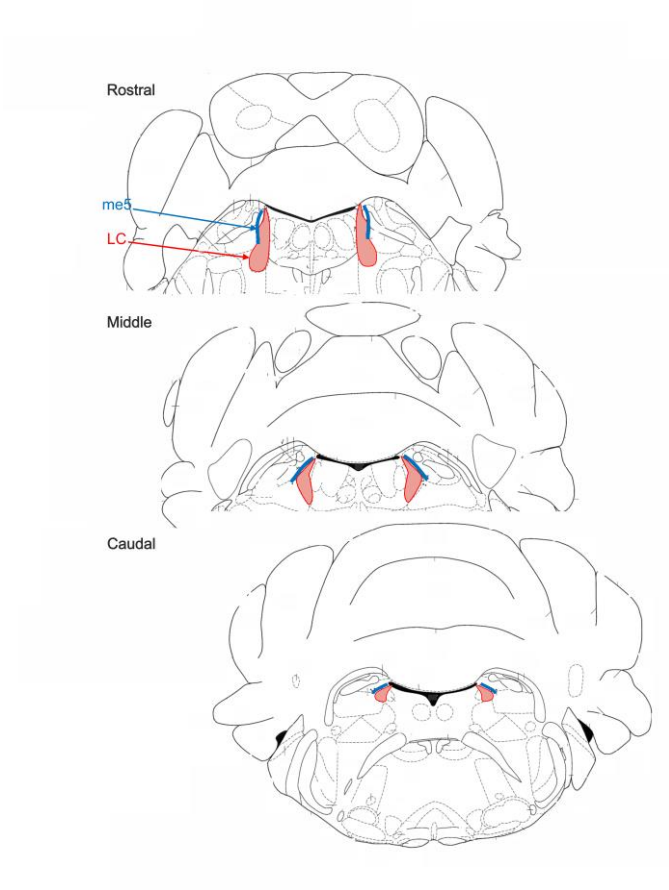


Figure 9-The Localization of the LC. The location and shape of the LC according to its orientation. rostral, middle, or caudal according to the mouse brain atlas. The rostral section starts from AP -5.2 to AP -5.34, relative to Bregma, followed by the middle portion (from AP -5.34 to AP -5.52) and finished in the

most caudal slide (from AP -5.52 to AP -5.8 me5: mesencephalic trigeminal nucleus; LC: locus coeruleus. Edited and Adapted from (Paxinos & Franklin, 2019).

Noradrenergic cells in the LC exhibit different well-organized morphologies with rostrocaudal (Figure 9) and dorsoventral orientations (Figure 10). At least two subpopulations have been described: large multipolar cells (approx. 35 μm) located more ventrally, and small fusiform cells (approx. 20 μm) located more dorsally in rats (Grzanna and Molliver 1980; Swanson 1976). There also exists a third group of scattered noradrenergic neurons not specified in terms of structure. Noradrenergic neurons can be also described according to their molecular composition, including numerous AR subtypes, with α2 being the most prevalent and α1 being less prevalent (W. S. Young and Kuhar 1980). α2-ARs are heavily located at the level of the posterior LC while α1-ARs are more abundant in the anterior LC (Chamba et al. 1991). Neurons express also nicotinic ACh receptors, GABA, orexin/hypocretin, and opioid receptors (Luque, Malherbe, and Richards 1994; Mansour et al. 1994; Marcus et al. 2001).

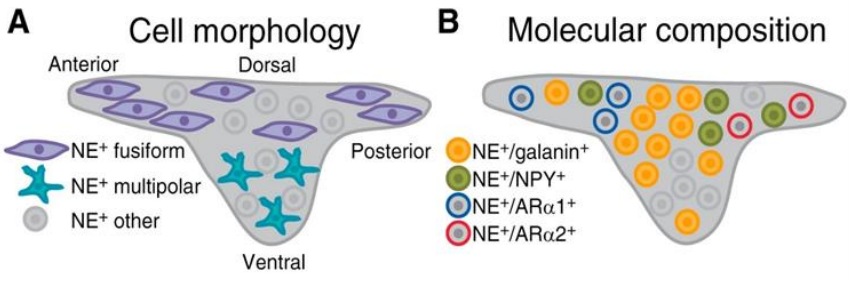


Figure 10-The Heterogeneous Cellular and Molecular Composition of the LC. (A) Differing locations of the morphologically different LC NE+ cells along the dorsal-ventral axis. **(B)** Expression of different sets of molecules in NE+ cells, with subsets co-releasing other molecules and expressing different neurotransmitters and receptors. NE: Norepinephrine; ARa1: adrenoceptor a1 subtype; ARa2: adrenoceptor a2 subtype; NPY: Neuropeptide Y; me5: mesencephalic trigeminal nucleus; LC: locus coeruleus. Edited and Adapted from (Schwarz and Luo 2015).

8.3.2. Afferent and Efferent Networks

Through retrograde and anterograde tracing studies, the LC was shown to be composed of various specialized noradrenergic modules each comprising distinct populations of NA neurons with wide projection targets and receiving inputs from a

diverse array of brain regions (Figure 11A) (Totah, Logothetis, and Eschenko 2019; Uematsu et al. 2017).

At the level of the brainstem and midbrain, the LC receives projections of a nociceptive nature from PAG, reticular formation, nucleus tractus solitarius, and vestibular nucleus, in addition to information about both the visceral and sympathetic nervous system (Aston-Jones et al., 1986; Van Bockstaele et al., 1998). The LC is also a recipient of complex emotional, cognitive, and homeostatic information from the hypothalamus (dorsomedial, lateral, and paraventricular nuclei), prefrontal cortex, insular cortex, and the bed nucleus of the stria terminalis (Luppi et al., 1995; Reyes et al., 2005; Van Bockstaele et al., 1998). Other afferent connections are between the LC and other neuromodulatory brain regions such as the serotoninergic dorsal raphe nucleus and the dopaminergic ventral tegmental area (Deutch et al., 1986; Ornstein et al., 1987). The aforementioned afferent connections, pave the way for the LC's neural processing and regulation of sensory and visceral experiences, through the conveyance of highly processed cognitive and emotional information from higher-order structures.

The LC is a broad projector toward most forebrain regions in addition to the cerebellum, spinal cord, and some midbrain and brainstem nuclei (Aston-Jones & Cohen, 2005; Berridge & Waterhouse, 2003; Valentino & Van Bockstaele, 2014). It sends projections related to learning and memory toward the amygdala and the medial prefrontal cortex (Amy F. T. Arnsten 2009). The heterogeneous specific nature of the LC neurons in terms of their efferent connectivity was assessed using a combinatorial strategy using two or more retrograde tracers. Interestingly, Chandler and Waterhouse were able to detect three non-overlapping cell populations after injecting different retrograde tracers at the level of the medial prefrontal cortex, orbitofrontal cortex, and anterior cingulate cortex (Figure 11B) (D. Chandler and Waterhouse 2012). The same team was also able to find another population, distinct from the others already found with a lower excitatory rate, projecting at the level of the motor cortex (D. J. Chandler, Gao, and Waterhouse 2014). It has been also mentioned that the efferent connectivity of the LC may be governed by some form of functional demand. LC neurons have been shown to send more prominent axon collaterals toward the somatosensory cortex while less toward the visual cortical/thalamic brain regions (Simpson et al. 1997). All of this adds to the idea that the functional demand could be the underlying player in the degree of connectivity and collateralization exhibited by sole populations of LC neurons. In

addition, there exists a topographical connection between the LC according to its rostrocaudal orientation within the other areas of the CNS. Within the caudal LC, noradrenergic neurons project toward the thalamus, cortex, and amygdala, while the rostral LC projects to the hippocampus, septum, and hypothalamus mainly. As for the middle portion of the LC it sends its projections towards the hippocampus, cerebellum, and spinal cord (Schwarz and Luo 2015). The LC also sends NA projections toward the Sp5c, whereby electrophysiological studies have shown that the stimulation of the LC inhibits the spontaneous neuronal activity and responses of the Sp5c neurons (Tsuruoka et al. 2003).

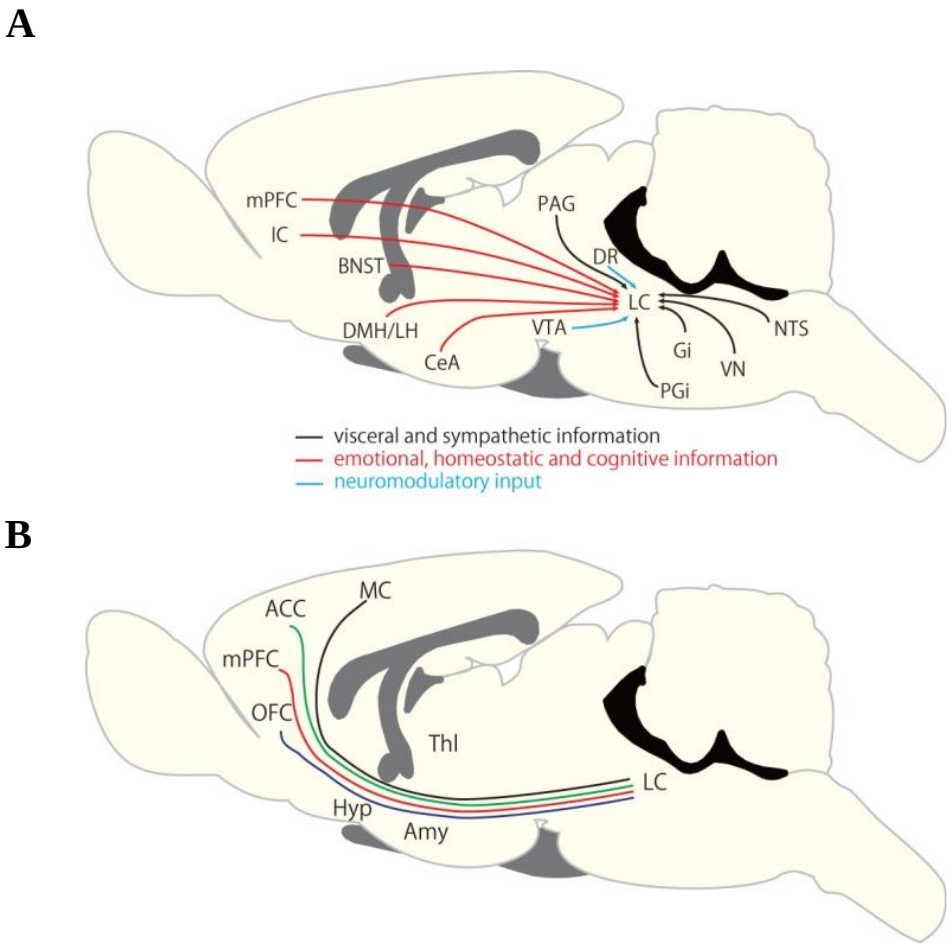


Figure 11-The Afferent and Efferent Connections of the LC. (A) The different projections from brain regions towards the LC, starting with those encoding visceral and sympathetic information from the brainstem and midbrain in black, the emotional, homeostatic, and cognitive information from the forebrain regions in red, and the neuromodulatory inputs in blue. BNST: bed nucleus of the stria terminalis; CeA: central nucleus of the amygdala; DMH/LH: dorsomedial and lateral hypothalamus; DR: dorsal raphe; Gi: nucleus gigantocellularis; IC: insular cortex; LC: Locus coeruleus; mPFC: medial prefrontal cortex; NTS: nucleus tractus solitarius; PAG: periaqueductal gray; PGi: nucleus

paragigantocellularis; VN: vestibular nucleus; VTA: ventral tegmental area. **(B)** The different projections from the LC toward various brain regions, with an emphasis on the heterogeneity of the individual populations of LC neurons shown in colors projecting to their specific region of interest. ACC: anterior cingulate cortex; Amy: amygdala; Hyp: hypothalamus; LC: Locus coeruleus; IC: insular cortex; mPFC: medial prefrontal cortex; MC: motor cortex; OFC: orbitofrontal cortex; Thalamus: Thl. Adapted from (Uematsu, Tan, and Johansen 2015).

8.3.3. Modulation of the LC Neuronal Activity

AR are plasma membrane receptors belonging to the heterotrimeric GTP-binding protein-coupled receptors family. They have seven transmembrane domains and mediate the biological effects of endogenous catecholamines. Nine AR subtypes have been cloned and grouped into three different functional classes: α_1-AR (α_{1A}, α_{1B}, α_{1D}) located primarily in postsynaptic sites and coupled to intracellular $G_{q/11}$-dependent signals; α2-AR ($\alpha2_A$, $\alpha2_B$, $\alpha2_C$), placed pre- and postsynaptically, activate $G_{i/o}$ proteins; and β-AR (β_1, β_2, β_3), which stimulate G_s proteins in the postsynaptic membrane (Bylund et al. 1994). In the LC, α2-ARs are primarily $\alpha2_A$ and $\alpha2_C$ and are located pre- and postsynaptically in noradrenergic neurons or presynaptically on non-noradrenergic terminals (Bücheler, Hadamek, and Hein 2002; Gyires et al. 2009).

The main function of presynaptic α2-AR is acting as an autoinhibitory feedback to control the NA release from the neuron itself and terminal areas (Starke 2001). The NA binding to α2-AR triggers the activation of a $G_{i/o}$ protein-coupled cascade: inhibition of adenylyl cyclase and reduction of cAMP levels, a decrease of Ca^{2+} channel opening, activation of K^+ channels, and phosphorylation of the mitogen-activated protein kinase (MAPK) pathway (Gilsbach and Hein 2008). These mechanisms modulated by regulators of G-protein signaling, result in presynaptic NA release inhibition.

As described above, the LC receives numerous innervations from the CNS and even, there is evidence of local non-adrenergic circuits. Several studies have described the presence of GABA, *N*-methyl-D-aspartate (NMDA), nicotinic, purine, and corticotropin-releasing factor receptors in the LC (Breton-Provencher and Sur 2019; Karolewicz, Stockmeier, and Ordway 2005; Léna et al. 1999; Qiong Wang et al. 2020; Yao and Lawrence 2005). For example, glutamatergic responses of LC neurons are mediated largely via ionotropic receptors (NMDA receptors) present in noradrenergic

neurons, while GABA responses are mediated by $GABA_A$ receptors (Chandley et al. 2014; Koga et al. 2005).

The serotoninergic system also plays a role in the functionality of the LC. The LC is a recipient of a robust innervation from serotoninergic nuclei, including the dorsal raphe nucleus (Pudovkina, Cremers, and Westerink 2002). The dorsal raphe nucleus through the activation of the 5-HT_{1A} and 5-HT_{2A} receptors is capable of exerting an effect on the LC (Haddjeri, De Montigny, and Blier 1997). Reductions in the spontaneous and pain-evoked activity of the LC occur post the stimulation of serotoninergic receptors (Segal 1979). While the application of serotonin in the LC attenuates the sensorial, neurochemical, and electrical evoked excitatory responses of the LC (G. Aston-Jones et al. 1991; Bobker and Williams 1989). Moreover, further validating the modulatory role of serotonin, studies have shown that serotonin modulates the release of NA. Interestingly, through a study using *in vivo* microdialysis in rats, the infusion of the 5-HT_{1A} receptor agonist flesinoxan into the LC decreased the release of NA in this structure (Pudovkina et al. 2001).

8.3.4. Involvement of the LC in Pain

Numerous studies have also shown that the LC aids in the sensory and emotional integration of pain. Indeed, it is activated by various stimuli (such as tail pinch, foot shock, and heat), which increases the firing rate and NA turnover, and suggests that this nucleus plays a role in pain perception (Taylor and Westlund 2017). Although some studies suggest that LC has pain-facilitating properties, its function has traditionally been understood as a source of pain inhibition (Brightwell and Taylor 2009). Indeed, descending noradrenergic projections to the spinal cord were described some time ago and characterized as pain inhibitory (S. L. Jones and Gebhart 1986; Kwiat and Basbaum 1992; Westlund and Coulter 1980). Subsequent studies in awake animals often conclude that tonic activation of LC reduces acute nociception(W. J. Martin et al. 1999). The LC is also capable to promote the feedback inhibition of the persistent nociception that arises after tissue or nerve injury. For example, molecular genetic silencing of the LC produced cutaneous hypersensitivity and increased the heat hypersensitivity associated with inflammation (Howorth, Teschemacher, and Pickering 2009). The LC promotes analgesia by activating α2-AR, in particular the subtype 2A, that is expressed on the primary afferents in the spinal cord (Hentall et al. 2003). Spinal

delivery of α2-AR agonists reduces signs of pain in both animals and humans(Baba, Shimoji, and Yoshimura 2000; Kawasaki et al. 2003), while intrathecal injection α2-AR antagonist produces hyperalgesia as well as Fos expression in the dorsal horn (Hughes et al. 2013). Gabapentin (an anticonvulsant drug used to treat chronic pain) activates the LC to induce spinal NA release that in turn stimulates α2-AR, leading to analgesia (Hayashida et al. 2008).

Under certain conditions, intense or long-term sensory stimulation invokes mechanisms that produce brain nociceptive facilitation that can persist long after the injury has healed, contributing to central sensitization that drives the neural and behavioral manifestations of chronic pain. Hence, both descending pain modulatory outflows from the LC to the spinal cord and trigeminal dorsal horns, as well as ascending pain modulatory outflows to the cortex become predominantly facilitatory. Pronociceptive and antinociceptive actions are mediated by distinct subpopulations of LC neurons: the antinociceptive effect is originated from the ventral region and subcoeruleus that project to the dorsal horn of the spinal cord (Bruinstroop et al. 2012; Howorth, Teschemacher, and Pickering 2009) while pronociceptive effects would originate from neurons localized more dorsally.

In addition to controlling sensory pain, the LC modulates the cortico-thalamic loop, which processes the emotional aspect of pain, by sending ascending signals to cortical regions like the prefrontal cortex (Gary Aston-Jones and Cohen 2005). Additionally, several findings evidence the pivotal role of the LC on the antalgic effect of antidepressants in chronic pain (Seki, Yoshida, and Jaiswal 2018). This effect seems to be related to the delay of the onset of the action of antidepressants.

8.3.5. Involvement of the LC in Migraine

Both preclinical and clinical evidence support the potential involvement of the LC in migraine pathophysiology. Altered LC activity and functional connectivity were recorded through neuroimaging studies in migraineurs, with changes in the dorsal pontine activation and connectivity with the hypothalamus (Maniyar et al. 2014; Moulton et al. 2014). Migraine-involved pain molecules including CGRP and PACAP were also located at the level of the LC (Tajti, Uddman, and Edvinsson 2001). Moreover, the LC has been shown to respond to trigeminovascular activation, as shown by its increased activation following the administration of NTG in rats (Tassorelli and

Joseph 1995). Also, it has been shown that the prophylactic effect of propranolol in a migraine model of inflammatory soup in rats was exerted through blockage of the chronic sensitization of descending controls of pain arising from the LC (Boyer et al. 2017). In addition, in a conscious rat model of trigeminal nociception, the LC and the dorsal raphe nucleus were both activated, both being participants in antinociceptive mechanisms (Ter Horst et al. 2001).

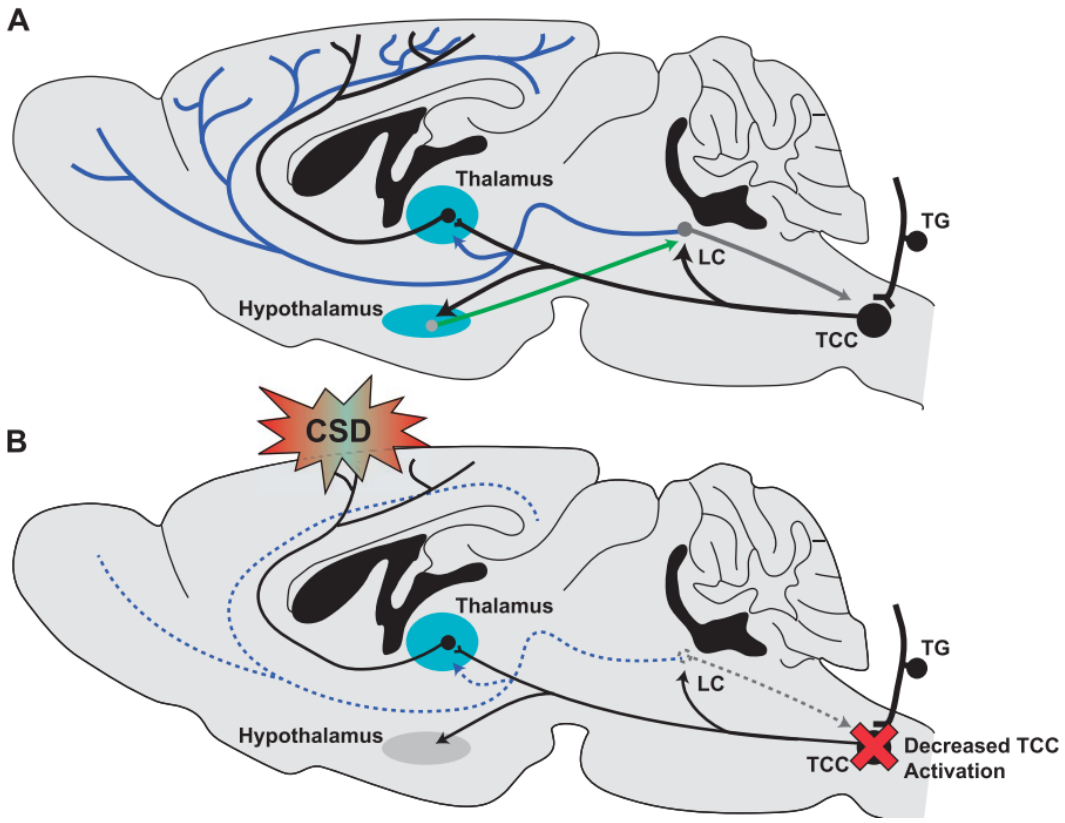


Figure 12-The LC in Migraine. (A) Nociceptive input from the trigeminal ganglia (TG) is mediated by primary afferents that synapse centrally on the trigeminocervical complex (TCC). Ascending projections from the TCC mostly target the sensory thalamic nuclei; however, excitatory projections also target the LC, activating it. The LC receives descending excitatory inputs from the hypothalamus during wakefulness, which enhances its activity (green arrow). The LC, in turn, sends noradrenergic projections to the majority of the CNS, including descending projections to the TCC and spinal cord as well as ascending projections to the thalamus and cortex, which play roles in arousal and nociceptive processing. **(B)** Loss of LC noradrenergic signaling impairs trigeminal nociceptive signaling at the cellular level. Adapted from (Vila-Pueyo et al. 2019).

A recent study assessed the impact of LC dysregulation in two validated preclinical models of migraine (Figure 12A) (Vila-Pueyo et al. 2019). They highlighted the antinociceptive role of LC inhibition in migraine-related pain. When LC signaling is

decreased, there is an inhibition of dural-evoked trigeminal activation at the level of the TCC and hence nociceptive inhibition. Moreover, they showed a reduction in the threshold of CSD induction due to the decreased cortical LC noradrenergic signaling, impacting the occurrence of migraine aura (Figure 12B). Further showcasing of the role of the LC came about through the transcutaneous auricular vagus nerve stimulation in migraine patients, which lead to the modulatory decrease in the activity of the LC while increasing its connectivity with other regions (hippocampus, somatosensory cortex, and amygdala) (Y. Zhang et al. 2019). All that has been done so far to showcase the role of the LC in migraine, highlights the pivotal role of the LC and its noradrenergic projections in the pathophysiology of migraine.

IX. Neuroinflammation

9.1. Definition and its Role in Migraine Pathophysiology

Neuroinflammation is the reaction of the nervous system to noxious stimuli such as trauma, toxins, or injury that affect homeostasis leading to progressive neuronal death and carries a primary role in the pathophysiology of a plethora of neurological diseases (Gilhus and Deuschl 2019). The hallmark of neuroinflammation is attributed to the activation of adaptive immunity (the body learns to recognize the unique antigens of a pathogen, and thus formulates an antigen-specific response against it), microglia, and astrocytes (Ransohoff and Perry 2009).

Over the last decades, the role of neuroinflammation in primary headache pathophysiology has been increasingly recognized. Indeed, data from pre-clinical models support the involvement of pro-inflammatory cytokines and immune cells, both in the induction and maintenance of migraine.

According to the current neurovascular theory described before, migraine attacks are related to hypothalamic activation, which is responsible for the stimulation of the Sp5C that, in turn, stimulates TG that releases CGRP. CGRP induces plasma protein extravasation together with dural mast cell activation, which releases histamine, 5-HT, NO, and pro-inflammatory cytokines, including IL-1, TNF-α, and IL-6. These inflammatory mediators contribute to the activation of dural trigeminal fibers, and

enhance CGRP release, creating a positive feedback loop within the ganglion, therefore contributing to trigeminal pain transmission.

Besides mast cells, other cells are believed to contribute to meningeal neurovascular inflammation. For instance, activated macrophages can release pro-inflammatory cytokines. Also, dendritic cells along with T lymphocytes (for the most part memory CD4+ and CD3 T cells) in the dural vessels, and the subarachnoid space, have been reported as putative players in the pathophysiology of migraine (McMenamin et al. 2003). This is supported by evidence from experimental models of their role in mediating inflammatory pain (Kashem et al. 2015). Resident glial cells also possess CGRP receptors, whose activation stimulates the production of TNF-α and IL-1, both of which amplify trigeminal nociception (Lars Edvinsson, Haanes, and Warfvinge 2019).

Based on the putative role of neuroinflammation in migraine coming from experimental animal models, several researchers investigated changes in cytokine levels and lymphocyte subsets in migraineurs, both in headache-free periods and during attacks. For instance, TNFα plays a role in the sensitization of trigeminal nerve fibers, the regulation of pain threshold, and the modulation of migraine perception (Bruno et al. 2007). TNFα has also shown an increase after the onset of migraine pain and a progressive decrease over time post-attack onset in migraineurs (Perini et al. 2005). Changes of IL-6 and IL-10 in the blood of migraineurs were also shown with those of IL-6 being significantly higher during and between the attacks compared to healthy controls (Aydın et al. 2015). As for IL-10, they were the highest only during the attacks compared to the controls. Hence, migraine headache pathogenesis is affected by the contribution of cytokines including TNF-α, IL-6, IL-10, and IL-1β.

In migraine with aura, CSD may trigger neuroinflammation via the pannexin-1 channel opening and subsequent caspase-1 activation, which is responsible for the cleavage of IL-1β and IL-18. These cytokines, in turn, activate parenchymal neuro-inflammatory signaling and nuclear factor-kappa B (NF-κB) activation in astrocytes (Karatas et al. 2013). In addition, CSD rodent models using KCl cortical application, optogenetics, pinprick, and electrical stimulation show the induction of neuroinflammatory responses (Takizawa et al., 2016, 2020). Although there is a debate surrounding the permeability of the BBB during migraine, CSD reportedly transiently activates the matrix metalloproteinase-9 temporarily breaking down the BBB,

potentially showing that the vasoactive and intracellular nociceptive molecules induce inflammation and sneak their way through the BBB to reach the dura mater nociceptors (S.-P. Chen et al., 2017; Hougaard et al., 2017).

9.2. The Glial Cells

Glia is a non-neuronal, immune-like cell population that constitutes the vast majority of cells within the CNS. They comprise satellite glial cells in the ganglia, microglia, astrocytes, and oligodendrocytes within the spinal cord and brain. The anatomical co-localization of astrocytes and microglia with pre- and postsynaptic neurons forms a key site of interaction termed the “tetrapartite synapse” (De Leo, Tawfik, and LaCroix-Fralish 2006). Each cell within this functional unit reciprocally signals to another, contributing to a neuroimmune communication that allows glia to respond rapidly to disruptions in neuronal signaling. The reactivity state and control of astrocytes and microglia is therefore critical in maintaining healthy CNS activity. Within this part, we will primarily focus on the role of astrocytes and microglia in migraine.

9.2.1. Astrocytes

The most abundant glial cells in the mammalian brain, astrocytes, play a central role in the maintenance of the function of the CNS (Liddelow and Barres 2017). They are broadly involved in water homeostasis, neuronal metabolic support, and BBB maintenance (Parpura and Verkhratsky 2012; Verkhratsky et al. 2018). Furthermore, through astrocyte-microglia crosstalk, astrocytes modulate microglial phenotypes and phagocytosis and through astrocyte-neuron interactions, they regulate excitatory synaptic transmission (Jha et al. 2019). In terms of structure, astrocytes are smaller in size compared to neurons, with a smaller soma, surrounded by various cytoplasmic extensions capable of connecting with up to 4 to 8 neurons for every single astrocyte (Bushong et al. 2002; Vasile, Dossi, and Rouach 2017). Identification of astrocytes is commonly done using the glial fibrillary acidic protein (GFAP) as a marker, which is an intermediate filament protein. Another marker used is the S100B (Ca^{2+} binding protein) (Brozzi et al. 2009).

In a process known as reactive astrogliosis, astrocytes undergo a series of phenotypic and functional alterations in response to painful stimulation and nerve injury. During this process, naïve astrocytes transform into reactive ones with specific phenotypic changes including (1) morphological changes (e.g. hypertrophy), (2) proliferation, (3) gene expression changes, (4) significant molecular changes, and (5) functional changes. Afterward, reactive astrocytes proliferate, migrate, and transform into scar-forming astrocytes (Hara et al. 2017). High expressions of the astrocyte marker proteins (GFAP, β-catenin, nestin, and N-cadherin), are found in both reactive and scar-forming astrocytes however they do also have their personal marker genes (Figure 13).

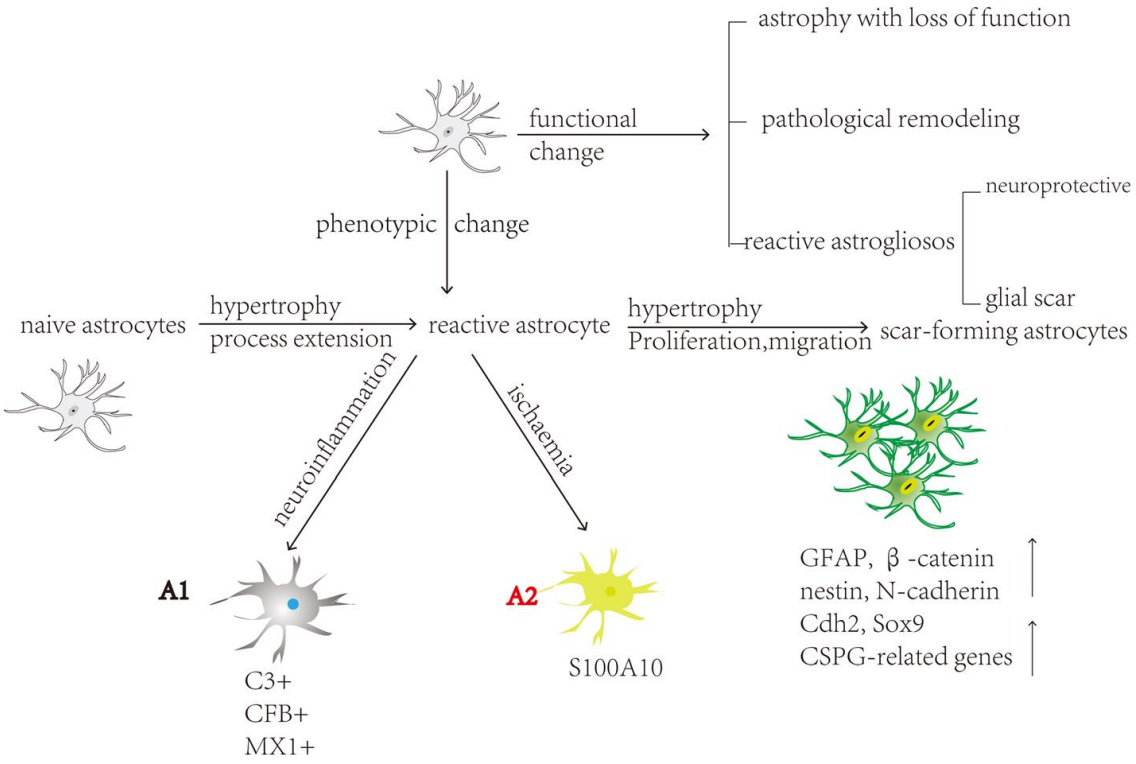


Figure 13-Morphological and Functional Astrocytic Changes After Noxious Stimulation and Nerve Injury. Based on the phenotypic changes of astrocytes, they can be divided into reactive and scar-forming astrocytes. Reactive astrocytes can be further classified by A1 and A2 subtypes. GFAP: Glial fibrillary acidic protein; Cdh2: Cadherin-2; Sox9: SRY-Box Transcription Factor 9; CSPG: Chondroitin Sulfate Proteoglycan 4; C3: Complement 3; CFB: Complement factor B; MX1: myxovirus resistance-1. Adapted from (T. Li et al. 2019).

Reactive astrocytes can be further divided into toxic A1 astrocytes which induce rapid death of neurons and oligodendrocytes, and A2 astrocytes, which are neuroprotective and promote neuronal survival and tissue repair (Liddelow and Barres

2017). Both types can be identified according to their genetic expressions. In A1 astrocytes Complement 3 (C3), Complement factor B (CFB), and myxovirus resistance-1 (MX1) are the most characteristic and predominately upregulated genes that are not expressed in A2, while the S100 protein family member S100A10 has been identified as a specific marker of A2 astrocytes (Liddelow et al. 2017). Reactive astrogliosis can increase neuroprotection and nutritional support for damaged neurons. Furthermore, activated astrocytes can reconstruct the damaged BBB and limit the infiltration of peripheral leukocytes. Thus, astrogliosis is an initial defense mechanism for repairing damage.

The transformation of astrocytes from their normal state to reactive phenotypes encompasses multiple intercellular and intracellular signaling processes, which can release various molecules by multiple cell types, including neurons and other glial cells. Released signaling molecules include gene transcription factors [extracellular signal-regulated kinase 1/2 (ERK1/2) and signal transducer and activator of transcription 3 (STAT3)], pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6), and proteins (GFAP and connexins) (T. Li et al. 2019).

9.2.2. Microglia

Microglia are cells with macrophage-like abilities residing in 5-10% of the parenchyma of the brain and spinal cord. They continuously survey the environment, clearing debris and dead cells caused by potential infection or damage (Gomez Perdiguero et al., 2015), and form interactions with neurons, astrocytes, and oligodendrocytes to modulate synaptic neurotransmission (Bachiller et al. 2018; Matejuk and Ransohoff 2020). When activated under neuroinflammatory conditions, microglia migrate toward the site of damage exerting beneficial or detrimental effects through the release of inflammatory mediators, neurotrophic factors, and reactive oxygen species (Wolf, Boddeke, and Kettenmann 2017).

Changes in the expression of cell surface receptors, certain polarization reactions, and the release of several inflammatory mediators are all linked to microglial activation and can either play a beneficial neuroprotective role in tissue repair or a harmful neurotoxic response (Wendimu and Hooks 2022). A traditionally activated M1-like phenotype or an alternatively activated M2-like phenotype are two broad

polarization states that are typically used to characterize microglial activity. The triggering stimuli, phenotypic marker expression, and mediator secretion of these polarization states vary, which all affect the outcome. Generally speaking, pro-inflammatory and neurotoxic responses are linked to the classically activated M1 phenotype, whereas anti-inflammatory and neuroprotective actions are mostly mediated by the M2 phenotype (Yu Tang and Le 2016). Interferon (IFN) and/or the Gram-negative bacterial endotoxin lipopolysaccharide both promote the M1-like microglia phenotype in vitro. M1-like microglia are crucial for inducing the adaptive immune response and innate immunological responses to fight invading invaders (Lehnardt 2010). However, neuroinflammation, oxidative stress, and neurotoxicity are all exacerbated by prolonged activation under pathological situations (Lehnardt 2010; G. J. Song and Suk 2017). Through the release of neurotrophic and anti-inflammatory substances (including IL-4 and IL-10), M2 polarized microglia are frequently linked to tasks like immunological resolution and tissue repair. They may also enter an "alternatively activated" or "acquired deactivation" state. A common link between microglia ad astrocytes exists, whereby by releasing Il-1α, TNF, and C1q cytokines (essential for A1 astrocytes), activated microglia induce the transformation of naïve astrocytes into A1 astrocytes (Liddelow et al. 2017).

9.2.3. Their Dual Role and Contribution in Persistent Pain

Following injury or nociceptive events, compounds released from injured tissue and neuronal neurotransmitters stimulate microglia and astrocytes, which increase their expression and secretion of various pro-inflammatory cytokines and chemokines. This pro-inflammatory response is important in protecting against challenges that disrupt the homeostatic balance of the CNS. However, under certain conditions, glial reactivity is not advantageous and can instead be detrimental to neuronal function, such as during the manifestation of persistent pain.

Astrogliosis is a double-edged sword. Although the present evidence suggests that there exists an important neuroprotective and reparative role for both reactive astrocytes and microglia in the initial stage of nerve injuries, however, their inhibitory effects on functional recovery after injury are undeniable. In particular, they may encourage the development and maintenance of chronic pain. Their activation has been observed in neuropathic pain models and spinal cord infection models (Cao and Zhang

2008; Raghavendra, Tanga, and DeLeo 2003). Moreover, intrathecal transfer of highly reactive microglia alone, or injection of their pro-inflammatory products (such as IL-1β and TNFα) into naive animals, can induce symptoms of neuropathic pain (Tsuda et al. 2003). IL-1β is upregulated in spinal microglia and astrocytes following peripheral nerve injury, accumulating evidence of its involvement in pain sensitization (Kiguchi, Kobayashi, and Kishioka 2012). TNF-α plays an important role in the initiation and development of persistent pain and central sensitization and is primarily produced by microglia (Ji, Berta, and Nedergaard 2013). Both mechanical and thermal hypersensitivities were induced after an intrathecal injection of exogenous TNF, while prevention of the development of neuropathic pain is done after an intrathecal injection of anti-TNF antibodies prior to a peripheral nerve injury (Clark, Old, and Malcangio 2013).

During the development of pain, the responses of microglia are typically early and transient, while the activation of astrocytes is later and remains longer than microglia. At the level of the dorsal horn, glial-derived pro-inflammatory mediators enhance nociceptive signaling by facilitating glutamatergic neurotransmission (Figure 14). IL-1β has been shown to increase presynaptic release of glutamate and together with TNFα and IFNγ, increases postsynaptic N-methyl-D-aspartic (NMDA) and AMPA receptor currents. The aberrant uptake and/or release of glutamate, as well as the enhanced activity of its postsynaptic receptors, can contribute to excessive nociceptive signaling reaching the brain (Dodds et al. 2016):

To prevent the persistence of pathological pain, minocycline can be used as a microglia inhibitor, although it does not reverse the hyperalgesia that is established after nerve injury/inflammation (Raghavendra, Tanga, and DeLeo 2003) However, intrathecal injections of astrocyte inhibitors (such as valerine, fluorocitrate, and l-1-amino-hexanedioic acid), could reverse mechanical cutaneous hypersensitivity and reduce the maintenance of abnormal pain (Cao and Zhang 2008). These studies indicate the role of microglia as a contributor to the initiation of mechanical allodynia, while astrocytes could be responsible for its maintenance.

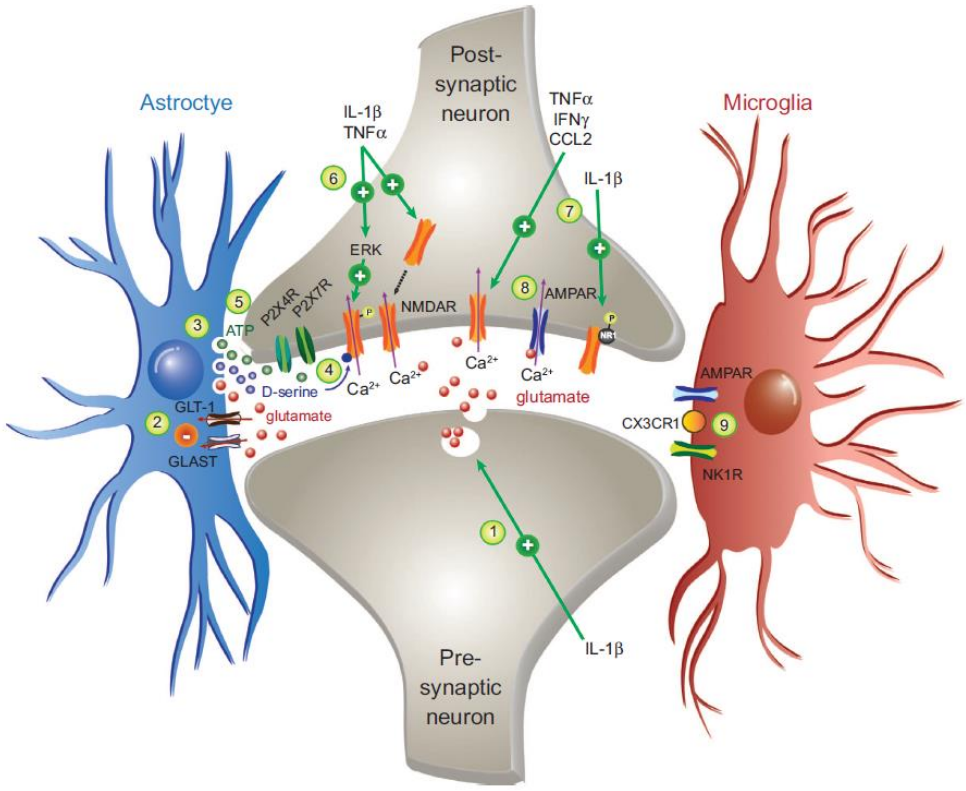


Figure 14-Major pro-inflammatory glial-mediated alterations to excitatory synapses that contribute to Central Sensitization. Noxious activation of glial cells can lead to the aberrant release of pro-inflammatory mediators, such as TNFα and IL-1β. The overarching effect on excitatory synapses contributes to central sensitization and facilitates the transmission of nociceptive signals to the brain. Some of the major known adaptations include the increased release of glutamate from presynaptic terminals; suppression of astrocytic glutamate reuptake via downregulation of GLT-1 and GLAST activity; release of glutamate from astrocytes, which increases the neuronal excitability, and of D-serine that enhances Ca^{2+} influx on postsynaptic neurons. Astrocytic release of ATP also increases postsynaptic excitability via the activation of P2X4R and P2X7R, while IL-1β, TNFα, IFNγ, and CCL2 increase NMDA receptor-mediated excitatory signaling. ERK, extracellular signal-regulated kinase; IFN, interferon; IL, interleukin; TNFα, tumor necrosis factor-α. Adapted from (Dodds et al. 2016).

9.2.4. Their Role in Migraine Pathophysiology

Unfortunately, few studies have focused on the role of astrocytes and microglia in migraine pathogenesis. FHM type 2 is caused by the Atp1a2 gene, encoding the α2 subunits of the Na+/K+-dependent adenosine triphosphatase (De Fusco et al. 2003), that is exclusively expressed in astrocytes (Melone et al. 2019). This data provide evidence that astrocytes may have a role in the diffuse alterations in brain function that occur during a migraine attack. Moreover, in a mouse model of migraine, astrocyte

dysfunction in the cingulate gyrus leads to prominent hypersensitivity and could potentially be involved in sensitization in familial migraine (Romanos et al. 2020). Interestingly, astrocytes have shown waves of activity with similar patterns to those of CSD in terms of temporal and spatial characteristics, postulating that these waves could be initiating a propagation of changes in the vascular changes and brain activity observed in migraineurs (A. Charles and Brennan 2009). In addition, the dysfunction of glutamate clearance by cortical astrocytes also leads to the potential facilitation of CSD initiation (Capuani et al. 2016). From here, growing evidence is emerging to further elucidate the contribution of astrocytes in migraine pathogenesis, and further work has to be put into the potential comprehension of their role.

Regarding microglia, recurrent administrations of NTG induce central sensitization by activation of microglial cells (Long et al. 2020). Precisely through the microglial P2X4R/BDNF (Brain-derived neurotrophic factor) pathway whereby the blockade of P2X4Rs, which are a part of the purinergic P2 receptors that have been widely studied in neuropathic pain, prevented both hyperalgesia and the release of CGRP (Tsuda et al. 2013). CSD is one of the stimuli for microglial activation, as it is a dysregulated slow propagating wave of neuronal and glial activation that leads to the release of a plethora of microglia-activating molecules (Borgdorff 2018; Shibata and Suzuki 2017). Post-activation microglia take on two different phenotypes (M1 and M2) depending on their activating agent (S. Zhao et al. 2017). Linked with the production of proinflammatory cytokines, the M1 phenotype, releases IL-1, TNF-α, NO, and ROS. On the other hand, the M2 phenotype is the one responsible for the repair and regeneration of tissue through the release of the anti-inflammatory cytokines, IL-13, IL-4, and IL-10 (Kraft and Harry 2011). The activation of the microglia for prolonged durations has shown adverse effects in other neurodegenerative and neoplastic diseases, and potentially in migraine as well (Taipa et al., 2018). So far, we have concrete evidence of the contribution of microglia in migraine precisely chronic migraine and its central sensitization, however further work is to be undertaken to further understand its mechanistic contribution

X. Aryl Hydrocarbon Receptors

Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor of the basic helix-loop-helix (bHLH)-PER/ARNT(AhR Nuclear Translocator)/SIM homology family. They are involved in many signaling pathways such as the response to environmental pollutants (xenobiotics), hypoxia, the circadian rhythm, and neural development. Moreover, AhR mediates the regulation of immunity, maintains gut homeostasis, and ameliorates inflammation.

The original discovery of AhR was in hepatocytes in the 1970s as the mediator of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD/dioxin) toxicity (Poland, Glover, and Kende 1976). Overt dioxin exposure in humans leads to a chronic inflammatory condition characterized by persistent painful skin lesions.

10.1. Protein Structure

Murine AhR is encoded by a gene located on chromosome 12, while human AhR is located on chromosome 7. The mouse AhR protein consists of 805 amino acids and its molecular weight is 90 kDa. Its structure consists of an amino (N-) terminal bHLH domain, a PAS domain in the center made up of two degenerate repeats (A and B), and a carboxy (C-) terminal transactivation domain (Figure 15). The N-terminal bHLH domain helps in dimerization and forms the DNA-binding domain. PAS domains control DNA recognition, chaperone interactions, and binding of ligands. In addition, the PAS coupled with the N-terminal bHLH domain, are supporters of the heterodimerization of AhR with ARNT. The transactivation domain can be divided into a first part rich in acid residues, a second part rich in glutamine necessary for the binding of cofactors, and a final part rich in proline/serine/threonine. The sequence of the transactivation domain is very different between mice and humans with only 58% homology. This difference raises various hypotheses such as the divergence in the coactivator recruitment or the different levels of gene induction.

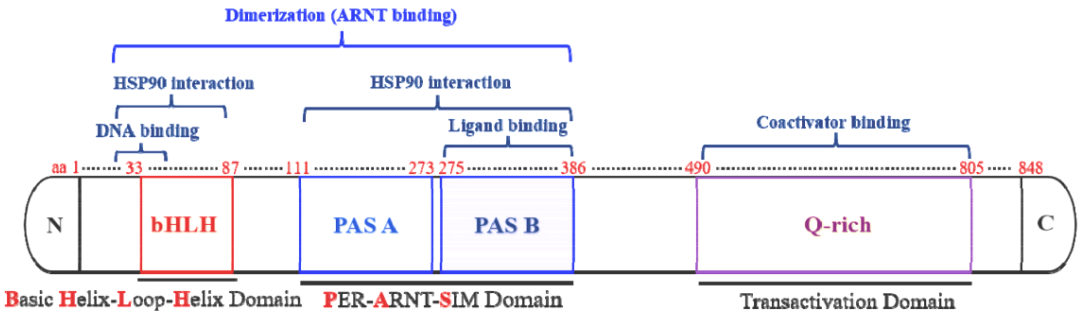


Figure 15-The Structure of the Aryl Hydrocarbon Receptor. N: Amino-terminal; bHLH: basic helix-loop-helix; PAS A/PAS B: period aryl hydrocarbon receptor nuclear translocator single-minded A/B; Q-rich: Glutamine Rich; C: Carboxy-terminal; Hsp90: heat shock protein 90. Adapted from (Lin, Dai, and Xia 2022).

AhR structure is highly conserved between species and expressed in vertebrates with different isoforms. In mammals, a unique AhR isoform, AhR1, has been identified (Hahn et al. 2006).

10.2. AhR Expression

Many studies have shown that AhR is expressed ubiquitously, however, variations exist depending on the tissue. Its expression begins during embryonic development, primarily in the neuroepithelium and the heart; then in the liver, and finally in other tissues including kidneys, lungs, muscles, and epidermis. In adult humans, the highest levels of expression are found in the lung and liver (Dolwick et al. 1993).

In the brain, AhR messenger RNA (mRNA) (together with ARNT mRNA) shows specific temporal and spatial patterns of expression in the mouse cortex, hippocampus, cerebellum, and olfactory bulb (E. Kimura and Tohyama 2017). The brainstem including the LC (E. Kimura et al. 2021), the thalamus (including Po), and several nuclei of the hypothalamus have markedly higher AhR amounts than other regions of the brain. These regions include the ventromedial hypothalamus, paraventricular nucleus, suprachiasmatic nucleus, and dorsal and median raphe nuclei (Petersen et al. 2000). Interestingly, AhR mRNA levels were low in the spinal trigeminal tract, amygdala, septal region, and forebrain; and undetectable in the

basomedial nucleus, superior olivary complex, vestibular nuclei, NRM, and lateral preoptic area (Petersen et al. 2000).

AhR protein is present in neurons, astrocytes, oligodendrocytes, and microglia (Barroso et al. 2021), and is involved in the regulation of neurogenesis, neuronal migration, and neurite elongation during development in mice and humans (Dever et al. 2016; E. Kimura and Tohyama 2017). The regulation of its expression depends not only on internal stimuli, but also on traumatic injury, exposure to xenobiotics, and endocrine disruptors such as bisphenol A.

10.3. The AhR Signaling Pathways

AhR activation results in its translocation to the cell nucleus and control of the expression of target genes harboring AhR-responsive DNA elements [known as xenobiotic response elements (XRE)] in their regulatory regions. Additional nongenomic AhR signaling pathways have also been described.

10.3.1. XRE-dependent Control of Gene Expression by AhR

When inactive, AhR is complexed with the cellular-sarcoma (c-Src) kinase, cochaperone p23, two heat shock protein 90 (Hsp90) chaperons, and X-associated protein 2 (XAP2) also known as the AhR-interacting protein (AIP)/ARA9) at the level of the cytoplasm. This chaperone complex keeps AhR inactive, prevents proteasomal degradation, and keeps it in a high-affinity state for its ligands. Upon agonist binding, AhR and some components of the chaperone complex translocate to the nucleus, whereby further dissociation of the other parties (p23, XAP2, and the second Hsp90) allows for AhR to dimerize with ARNT (Figure 16). The AhR/ARNT heterodimer then binds to XREs and hence regulates the transcription of target genes. AhR is a regulator of a plethora of prototypic genes such as Cytochrome P450 Family 1 Subfamily A Member 1 (CYP1A1), Cytochrome P450 Family 1 Subfamily B Member 1 (CYP1B1), and the AhR repressor (AhRR). AhRR competes with the AhR-ligand complex for its interaction with ARNT, limiting the availability of ARNT for AHR-ligand binding and consequently providing an additional negative regulatory mechanism of AhR-driven gene expression (Sakurai, Shimizu, and Ohto 2017).

10.3.2. XRE-independent Control of Gene Expression by AhR

AhR can also interact with additional transcription factors to target their specific binding sites (Rothhammer and Quintana 2019). For example, AhR controls nuclear NF-κB and its dependent transcriptional programs indirectly through the suppression of cytokine signaling 2 -dependent mechanisms and directly by interacting with RELA, RELB, and other members of the NF-κB signaling complex (Salisbury and Sulentic 2015; Vogel et al. 2014). Similarly, AhR has been reported to regulate the activation of STAT proteins that play central roles in the regulation of immune responses (A. Kimura et al. 2008; Quintana et al. 2010). This interaction leads to the modulation of several outcomes including inflammation, proliferation, differentiation, and endocrine disruption (Kim et al. 2000; Ohtake et al. 2003; Procházková et al. 2011).

10.3.3. Additional AhR Signaling Mechanisms

In addition, when a ligand binds to AhR, this modulates AhR's ability to form interactions with different proteins, such as kinases, Kruppel-like factor 6 (KLF6), retinoblastoma protein (RB), and Cullin 4B (CUL4B) (D. P. Jackson et al. 2014; Tomkiewicz et al. 2013). The interactions with those molecules activate nongenomic pathways. For instance, exposure to TCDD in rats, leads to a rapid increase in the intracellular concentration of Ca^{2+}, activating PKC (Hanneman et al. 1996). PKC then phosphorylates phospholipase A2 (cPLA2) leading to the production of arachidonic acid. Following the dissociation of the cytoplasmic AhR complex, c-Src is released, activating the MAPKs, and leads to the transcription of the COX2 mRNA (Figure 16). The latter's protein uses arachidonic acid to produce prostaglandins and hence causes inflammation (Matsumura 2009). Moreover, the activated c-Src induces the activation of focal adhesion kinase (FAK) leading to the modification of cellular adhesion (Tomkiewicz et al. 2013).

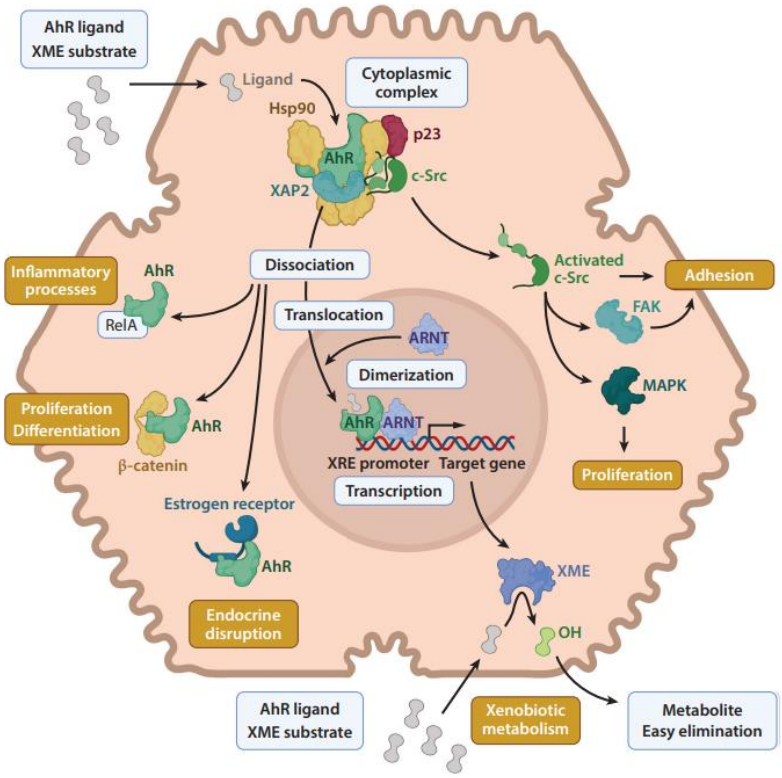


Figure 16-Aryl Hydrocarbon Receptor Signaling Pathways. Upon binding of the ligand, the complex dissociates activating multiple pathways (the genomic pathway and pathways regulating the transcription of target genes). Other parts of the complex such as c-Src trigger different pathways, activating FAK or MAPK with modulation of proliferation and adhesion. AIP: AhR-interacting protein; AhR: aryl hydrocarbon receptor; ARNT: aryl hydrocarbon receptor nuclear translocator; c-Src: cellular-sarcoma; FAK: focal adhesion kinase; Hsp90: heat shock protein 90; MAPK: mitogen-activated protein kinase; NF-κB: nuclear factor-kappa B; XAP2: X-associated protein 2; XME: xenobiotic metabolizing enzyme; XRE: xenobiotic response element. Adapted from (Larigot et al. 2022).

10.4. AhR Ligands

A multitude of structurally diverse compounds constitute the ligands of AhR, including endogenous substances such as intermediary and microbial metabolites of tryptophan (TRP), and exogenous compounds such as drugs, alkaloids, and synthetic compounds. Differences in the binding affinity exist between these ligands, in addition to differences in chemical structures. In terms of their intrinsic activity, AhR ligands are either agonists or antagonists.

10.4.1. AhR Agonists

AhR agonists are multiple and can be classified into exogenous and endogenous agonists. The main source of exposure in animals and humans to exogenous ligands comes from their diet (90%). They can be grouped according to their origin: “synthetic” or natural (Denison and Nagy 2003). Synthetic exogenous agonists include environmental pollutants (xenobiotics) from halogenated aromatic hydrocarbons (e.g. TCDD) and polycyclic aromatic hydrocarbon families. Ligands from the halogenated aromatic hydrocarbons family have a stronger affinity for AhR than polycyclic aromatic hydrocarbons. Most of these xenobiotics cause numerous toxic effects. For example, TCDD exposure in rats reduces food intake, influences body composition, and alters the function of the hypothalamus (Lindén, Lensu, and Pohjanvirta 2014). In humans, TCDD induces chronic malformations that manifest with time including, hypertension, diabetes, neural damage, and atherosclerosis (Pelclová et al. 2006).

Non-xenobiotic exogenous AhR agonists, provided by food, have also been identified. Vegetables are a source of indole-3-acetonitrile, indole-3-carbinol, and 3,3.-diindolylmethane (Table 2). The resveratrol (3,5,4'trihydroxystilbene), which belongs to the polyphenol family, is present in certain fruits and has recognized antioxidant and anti-inflammatory activity. Several phytochemicals, such as flavones, isoflavones, flavonols, and flavanones also act as AhR modulators (Goya-Jorge et al. 2021; Xue et al. 2017).

To date, the endogenous (physiological) AhR agonists are not yet known with certainty. However, many candidates have been identified and are regulators of the expression of the AhR-dependent gene cluster. They include TRP derivatives and indoles, arachidonic acid, certain leukotrienes (lipoxin A4), and heme metabolites (bilirubin and biliverdin) (Table 2). Among these ligands, TRP and its derivatives have been the most studied. TRP is one of the essential amino acids, naturally synthesized by microorganisms and plants, and cannot be synthesized by the human body. Its derivatives are formed in three main ways: kynurenine, serotonin, and indole pathways (a specific section will be dedicated to TRP metabolism below). Reports have shown that kynurenine is a highly potent agonist of AhR (Seok et al. 2018). For instance, when binding to AhR, kynurenine, considered a high-affinity ligand, stimulates the receptor which enhances immunosuppression (Salminen 2022).

Indole produced by gut bacteria has demonstrated AhR agonist activity in yeast assay (Bansal et al. 2010), although, in certain specific conditions, it also behaves as an AhR antagonist (Hubbard et al. 2015; U.-H. Jin et al. 2014). Other compounds derived from TRP such as indole-3-acetic acid, tryptamine, and 3-indoxyl sulfate (I3S) have also shown an agonistic role (Zelante et al. 2013). Furthermore, 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) is considered an endogenous AhR ligand (J. Song et al. 2002).

Table 2-Selected AhR Agonists and Antagonists

Agonists	**Biochemical pathway**
L-Kynurenine	Tryptophan Metabolism
Indole-3-aldehyde/IAld	Microbial metabolism
Indole-3-acetic acid	Microbial metabolism
Indole-3-acetaldehyde/IAAld	Microbial metabolism
Tryptamine	Microbial metabolism
Indoxyl-3-sulfate	Microbial and host metabolism
2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester/ITE	Generated from tryptophan and cysteine
Indole-3-acetonitrile	Derived from cruciferous vegetables
Indole-3-carbinole	Derived from cruciferous vegetables
3,3'-diindolylmethane	Derived from cruciferous vegetables
Bilirubin	Heme metabolism
Antagonists	
6,2',4'-trimethoxyflavone/TMF	Flavonoid
Quercitrin	Flavonoid
Galangin	Flavonoid
BAY-2416964	Polygonum capitatum

Edited and Adapted from (Rothhammer and Quintana 2019).

10.4.2. AhR Antagonists

AhR is bound by structurally different phytochemicals, one of which being brevifolincarboxylic acid which inhibits their activity (Table 2) (Amakura et al. 2003). Moreover, green tea is a potential inhibitor of the expression of AhR-regulated genes (Fukuda et al. 2015; Han et al. 2012). Through further comprehension of the role of AhR and their respective pathways, the interest in constructing novel AhR antagonists arose through structural modifications intended to improve both pharmacokinetics and pharmacological properties. For instance, a potent oral AhR antagonist, BAY-2416964

was able to inhibit the receptor's activity in both humans and mice by preventing AhR-mediated signaling (L. Sun 2021). Another antagonist, 6,2',4'-trimethoxyflavone (TMF) was found to be a competing antagonist with other agonists of AhR possessing the characteristics of allowing further dissection of the role of AhR (Murray et al. 2010).

10.5. The Physiological Significance of AhR

Several studies have indicated that AhR activation can regulate the homeostasis and development of physiological barriers, in several tissues, and organs, immune activity, intestinal homeostasis, and carcinogenesis (Figure 17). AhR integrates into both innate and adaptive immunity through diverse mechanisms; including repression of acute phase response genes, differentiation of regulatory T cells, lymphocytes, and B-cell differentiation. Its activity and role depend on ligands and the timespan of receptor activation. The dysfunction of AhR or a lack of functionality is a trigger for pathophysiological processes. For instance, in AhR knockout mice, there is the development of vascular and cardiac hypertrophies (G. P. Lahvis et al. 2000; Garet P. Lahvis et al. 2005). In addition, AhR deficiency alters mice growth and fertility (B. D. Abbott et al. 1999) and accelerates the aging of mice (Bravo-Ferrer et al. 2019). Disruption of any of the homeostatic functions can disrupt other organs and from there the cascade continues potentially leading to a communicational impairment between organs.

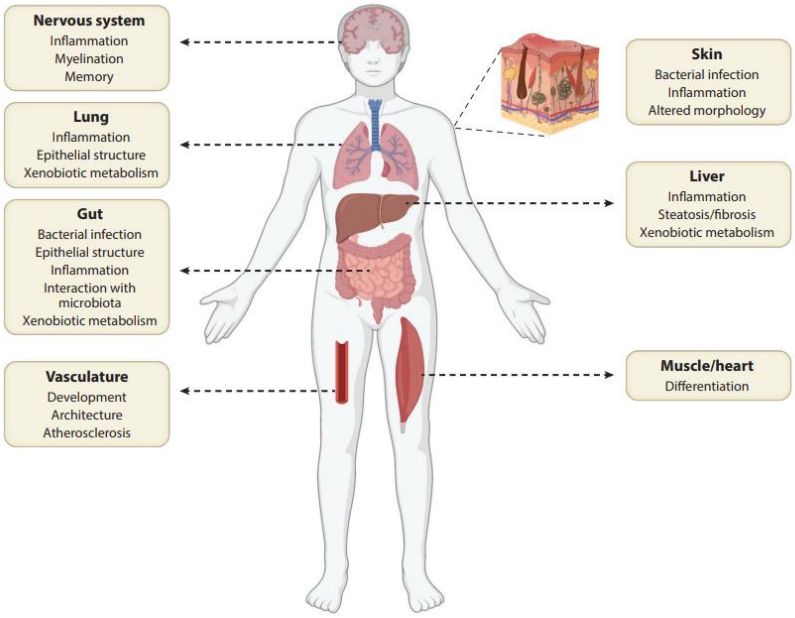


Figure 17-Roles of Aryl Hydrocarbon Receptors. AhR are regulators of a plethora of specific processes at the level of different physiological barriers, organs, and tissues. Any form of disturbance or deficiency in the function of this could lead to pathophysiological states. Adapted from (Larigot et al. 2022).

AhR has shown its involvement in a plethora of inflammatory disorders with its role being either of a pro or anti-inflammatory nature depending on the tissue and cell type (Juricek and Coumoul 2018). In rheumatoid arthritis, activation of AhR contributes to disease progression and severity (Fu et al. 2018). Furthermore, in AhR knockout mice, low levels of pro-inflammatory cytokines were detected in the serum, concomitant with a decrease in disease severity (Nakahama et al. 2011).

Cases of IBD, such as Crohn's Disease, have shown low expression levels of AhR (Qiu and Zhou 2013). Moreover, in IBD patients, decreased levels of AhR ligands compared to their controls have also been reported (Rothhammer et al. 2016). The administration of AhR ligands such as TCDD can ameliorate the symptoms of certain IBD with a decrease in the production of pro-inflammatory cytokines and increasing anti-inflammatory cytokine productions (such as IL-22) (Q. Wang et al., 2018).

As previously mentioned, AhR is expressed at the level of different CNS cellular populations. However, their roles differ according to at which cells they are expressed. Activation of astrocytic AhR by endogenous indoles reduces inflammation and has a neuroprotective action (Rothhammer et al. 2016). However, the activation of neuronal

AhR plays a rather pro-nociceptive role in facilitating an inflammatory state (Ntranos et al. 2022). The role of AhR in pain has been mildly studied, whereby in one animal model of chronic constrictive nerve injury there was an augmentation of AhR expression, and the deletion of AhR worsened the damage, while the administration of an AhR agonist counteracted such changes (Sheu et al. 2022). So far concerning migraine, AhR's influence on the pathophysiology of migraine has never been addressed and therefore remains to be identified.

XI- The Metabolism of Tryptophan

11.1. Tryptophan, an Essential Amino Acid

TRP is an essential amino acid, that can only be obtained through the daily diet ([tuna, cheese, milk, turkey, oats, seeds, and nuts (Shabbir et al., 2013)], necessary for various metabolic reactions, and for the production and maintenance of proteins, enzymes, muscles, and neurotransmitters. It is an aromatic amino acid from the erythrose 4-phosphate and phosphoenolpyruvate family (Palego et al. 2016). Levels of circulating plasma TRP are heavily influenced by geographic and cultural diet practices, physical activity, and stress (K. Gao et al. 2020; Yu et al. 2017). The majority of ingested TRP is absorbed by the small intestine, and its availability is affected by the simultaneous absorption of other neutral amino acids (valine, leucine, isoleucine, serine, histidine, glutamine, asparagines, phenylalanine, tyrosine) (Agus, Planchais, and Sokol 2018). Dietary carbohydrates induce an influence on TRP uptake since there is a preferable capture of non-TRP neutral amino acids due to the induction of insulin release (Keszthelyi, Troost, and Masclee 2009). When insulin is elevated, this promotes the uptake of long neutral amino acids except for TRP to the skeletal muscles, hence increasing the plasma TRP to long neutral amino acid ratio and hence the influx of TRP to the brain. In peripheral blood circulation, TRP is either albumin-bound (80-90%) or freely solubilized. The differential uptake of amino acids by the skeletal muscles is due to the fact that TRP is bound to albumin in the plasma. Free TRP proceeds to the CNS via the L-type amino acid transporter (Lat1) which is the chaperon responsible for the transportation of large neutral amino acids across the BBB (Pardridge and Fierer 1990).

Typically, individuals consume between 900 to 1000 mg of TRP daily even though the recommended daily allowance of TRP is between 250 to 425 mg for adults which corresponds to 3.5 to 6.0 mg/kg of body weight per day (Richard et al. 2009). A TRP deficiency is not quite common naturally but can be attributed to insufficient dietary protein consumption (veganism, vitamin B6, high sugar intake, or excessive consumption of alcohol). There are other cases involving the excretion of TRP in the urine. For example, the Hartnup disease, a recessive genetic disorder in which the renal and intestinal transport of neutral amino acids is defective, patients are likely to have TRP deficiency due to increased urinary excretion (Ganapathy, Gupta, and Martindale 2006). Moreover, in certain cases, TRP might not be fully catabolized by the intestines. Patients with histories of inflammatory gut disorders such as IBD, have impairments in their intestinal permeability (Chang et al. 2017). This malfunctioning creates a cascade of release of pro-inflammatory mediators that modifies the microbiota profile (Al Bander et al. 2020). Hence, potentially modifying the catabolism of TRP by the gut.

11.2. Metabolic Pathways of Tryptophan

TRP is metabolized by three major metabolic pathways: 95% is metabolized along the kynurenine pathway; approximately 1-2 % are used for serotonin synthesis, and the rest are metabolized by the gut microbiota to obtain indole and its respective derivatives (Agus, Planchais, and Sokol 2018; Muneer 2020; Richard et al. 2009).

11.2.1. Kynurenine Pathway

Approximately 95% of TRP is metabolized by 2,3-dioxygenase (TDO), giving rise to kynurenine while the remainder of TRP is metabolized in the brain, liver, and gastrointestinal tract by indoleamine 2,3-dioxygenase (IDO) (Figure 18). Kynurenine is then catabolized giving rise to two neuroactive inflammatory molecules: quinolinic acid and kynurenic acid (KYNA). Kynurenine catabolites acting centrally are normally produced locally and rarely cross the BBB. KYNA is an NMDA receptor antagonist at the glycine binding site acting as a neuroprotective factor, while quinolinic acid acts as an NMDA agonist and is considered neurotoxic (Guillemin et al. 2005). Further catabolizing of quinolinic acid gives NAD+ and niacin which are two active molecules in other primary cellular metabolic processes. The kynurenine pathway and its derived

metabolites exert their function at the level of the nervous, endocrine, and immune systems, with anti-inflammatory, anti-oxidative, and/or neuroprotective/neurotoxic properties (Tsuji et al. 2023).

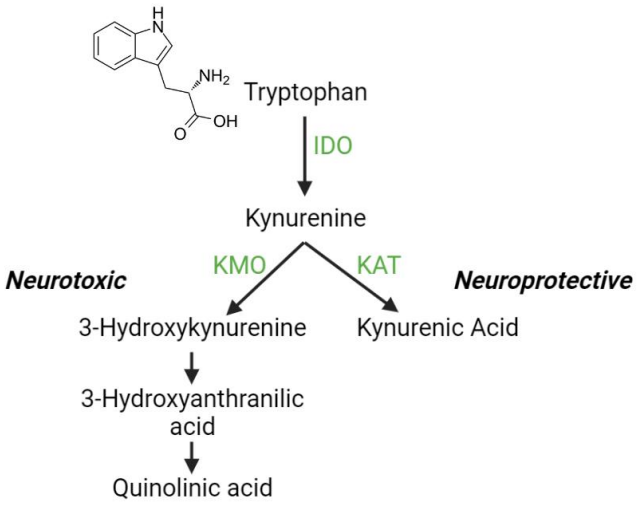


Figure 18-The Tryptophan/Kynurenine Pathway. Tryptophan is converted to kynurenine by IDO. Kynurenine is then further catabolized to give either the neuroprotective target kynurenic acid (through the action of KAT) or the neurotoxic one, quinolinic acid (through the action of KMP). IDO: indoleamine 2,3-dioxygenase; KMO: kynurenine-3-monooxygenase; KAT: kynurenine aminotransferase. (Created with BioRender.com)

11.2.2. Serotonin Pathway

The 5-HT synthesis from TRP occurs predominantly at the level of the gastrointestinal tract (90%) and at a minor level in the CNS (10%) (Boadle-Biber 1993). The initial rate-limiting step is catalyzed by tryptophan hydroxylase (Figure 19), which presents two isoforms, isoform 1 expressed by the enterochromaffin cells within the gut, and isoform 2 expressed by the serotoninergic neurons of the CNS located in the raphe nuclei mainly. These enzymes convert TRP to 5-hydroxytryptophan (5-HTP) which is then converted to serotonin by the L-amino acid decarboxylase. Serotonin can be further converted to melatonin, a primary regulator of circadian rhythms and sleep initiation within the pineal gland (Cassone 1990), or catabolized to 5-hydroxy indole acetaldehyde by MAO, and from there to 5-hydroxyindoleaceticacid (5-HIAA) by aldehyde dehydrogenase (O'Mahony et al. 2015). The serotonin receptors, spread out in different areas of the CNS, all of which are GTP binding protein-coupled receptors are classified into seven families, further subdivided into 14 subtypes except the 5HT-3

receptor (Nichols and Nichols 2008). In terms of function, serotonin is important for mood, pain, appetite, and sleep regulation, in addition to its role in cognition (memory and learning), and feelings of happiness and well-being (L. A. Jones et al. 2020).

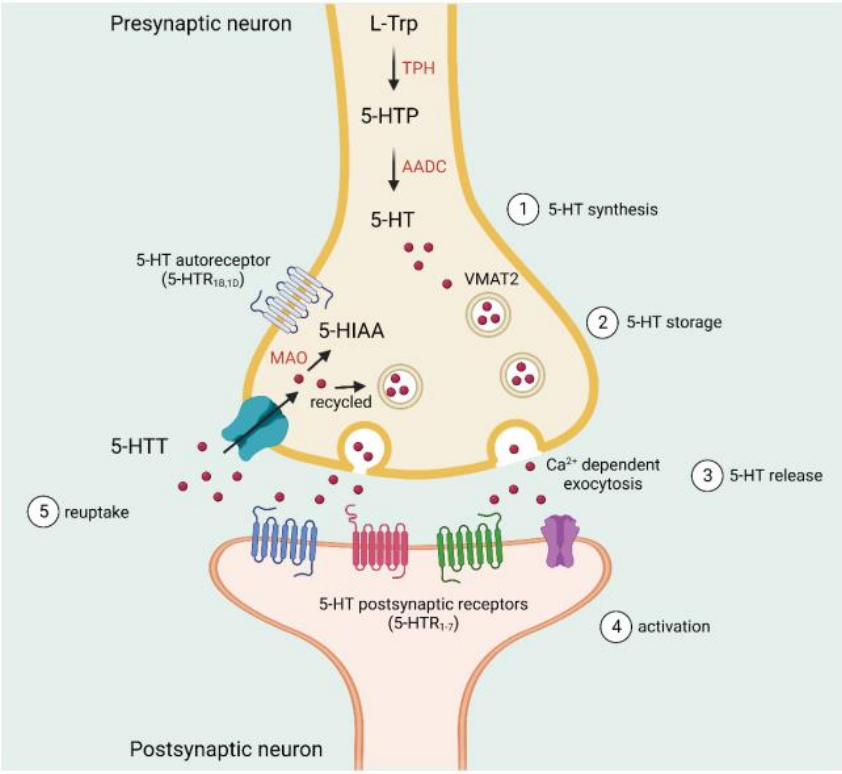


Figure 19-The Tryptophan/Serotonin Pathway. 1- Tryptophan is converted to serotonin by TPH and AADC enzymes. **2-** Serotonin is moved into the vesicles located in the axon terminal through the VMAT2. **3-** Following an action potential, serotonin is released into the synapse. **4-** Activation of autoreceptors and post-synaptic receptors. **5-** Free-5HT is taken from the synapse by the 5-HTT and the rest can be metabolized giving rise to 5-HIAA by MAO and aldehyde dehydrogenase. 5-HTP: 5-hydroxytryptophan; 5-HT: serotonin; 5-HTT: serotonin transporter; 5-HIAA: 5-hydroxyindoleaceticacid; AADC: L-aromatic amino acid decarboxylase; MAO: monoamine oxidase; Trp: Tryptophan; TPH: tryptophan hydroxylase; VMAT2: vesicular monoamine transporter isoform 2. Adapted from (Körtési, Spekker, and Vécsei 2022).

11.2.3. Indole Pathway

To obtain indole, several bacterial clusters use TRP through the action of the enzyme tryptophanase. The cluster consists of bacteria involved in the development of various neuropsychiatric disorders. Some of the bacteria involved in TRP metabolism include the phyla Actinobacteria, Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria (Kaur, Bose, and Mande 2019; Roager and Licht 2018). TRP is converted

to indole-3-propionic acid (IPA) through the cascade involving the conversion of TRP to indole-3-pyruvic acid (IPYA) which is then converted to indole-3-lactic acid (ILA) and anholocyclic acid (IA) before being converted to IPA (Figure 20). In addition, using other TRP degrading enzymes, different metabolic end products are created by microbes including indole-3-acetaldehyde (IAAld) which is formed from tryptamine, and indole-3-aldehyde (IAld) which is derived from IAAld. Furthermore, TRP can be directly converted to indole. In terms of their function, indoles regulate gut insulin secretion, regulate inflammatory processes, drug resistance, and toxicity, and maintain the health and youth of animals (Chimerel et al. 2014; Powell et al. 2020).

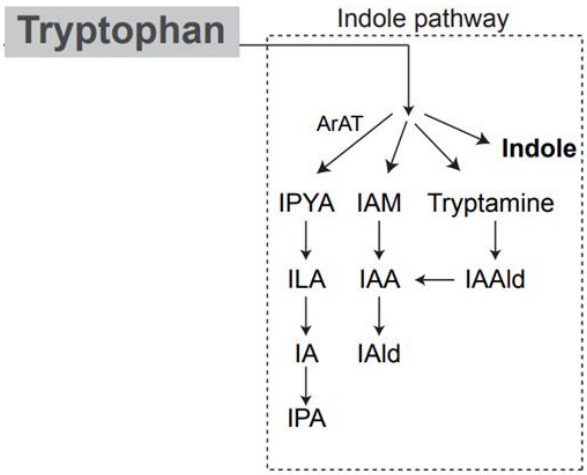


Figure 20-The Tryptophan/Indole Pathway. At the level of gut microbes; tryptophan is metabolized into indole and other indole derivatives. ArAT: aromatic amino acid aminotransferase; IA: anholocyclic acid; IAA: indole-3-acetic acid; IAAld: indole-3-acetaldehyde; IAld: indole-3-aldehyde; IAM: indole-3-acetamide; ILA: indole-3-lactic acid; IPA: indole-3-propionic acid; IPYA: indole-3-pyurvic acid. Edited and adapted from (Roth et al. 2021).

11.3. The Metabolism of TRP and its Role in the Inflammation

The metabolism of TRP is linked to aging and generates metabolites that modulate inflammation, maintain energy homeostasis, and influence behavior (Cervenka, Agudelo, and Ruas 2017). TRP metabolites also have a role in the regulation of tissue damage and inflammation. Serotonin is one example, which has been linked to intestinal inflammation (Spohn and Mawe 2017); KYNA, which induces anti-inflammatory changes in adipose tissue (Agudelo et al. 2018); 3-hydroxy anthranilic

acid and cinnabarinic acid, (two other Kynurenine metabolites), which have been linked to vascular inflammation (P. Song et al. 2017) and autoimmune encephalomyelitis (Fazio et al. 2014); NAD^+, which protects from renal kidney damage (Poyan Mehr et al. 2018), and indoles, which are a pivotal player in neuronal and gastro-intestinal inflammation (Roager and Licht 2018). Modulations in the levels of TRP, such as its deficiency has been shown to compromise immune responses and impair disease resistance in teleost fish (Machado et al. 2019). Furthermore, gut health and intestinal immunity require sufficient dietary TRP for efficient immunological response and intestinal homeostasis (J. Gao et al. 2018). TRP metabolites are known to support gut microbiota, microbial metabolism, the host's immune system, and host–microbiota synergy (Lamas et al. 2016). Indeed, intestinal inflammation and colitis could occur due to disruptions in the gut lactobacillus strains responsible for TRP metabolism (Lamas et al. 2016).

11.4. The TRP Metabolism in the Migraine Pathophysiology

Disturbances in the levels of TRP can lead to imbalances in the synthesis of 5-HT and melatonin in the brain, potentially playing a role in the pathophysiology of a plethora of neurodegenerative and neuropsychiatric disorders (Comai et al. 2020). Animal models of TRP depletion (acute and chronic) have demonstrated the pivotal role of TRP in different pathologies.

Acute TRP depletion (ATD), uses a combination of a low TRP diet and a TRP-deficient protein load containing large amounts of the other large neutral amino acids to produce maximal CNS TRP depletion. It is attained using a mixture 7-amino-acid ATD mixture (L-phenylalanine, L-leucine, L-isoleucine, L-methionine, L-valine, L-threonine, and L-lysine) as described by Moja et al., which is associated with a marked reduction in plasma TRP levels (Moja et al. 1988). This protocol was later modified for administration to young people (termed Moja-De) with a body-weight-adapted dosing scheme, and a lower amount of methionine relative to conventional mixtures (to reduce unwanted side effects). TRP competes with the other large neutral amino acids for uptake by the Lat1 from the plasma to the brain (Fernstrom et al. 2013). The ATD model has been well established in mice and humans showing a robust decrease in the peripheral and central levels of both TRP and serotonin (Biskup et al. 2012; Wong et al. 2018).

Chronic TRP depletion (CTD) has previously and reproducibly induced neurochemical and behavioral alterations in rats (Cahir et al. 2007) and mice (Browne et al. 2012). It relies on the same concept as the ATD amino acid drink, but rather administering dietary regimens (food) lacking in TRP for a longer period of time. CTD has shown stronger effects, reducing peripheral and central levels of both TRP and serotonin approximately 30 days post-diet (Browne et al. 2012). Moreover, long-term TRP-depleting diets lead to changes in serotonergic receptors in animals, for instance, increasing serotonin 5- HT2A receptor density (Cahir et al. 2007).

When it comes to migraine, changes in the levels of TRP are documented with a prominent decrease in the serum and plasma levels of TRP migraineurs compared to healthy controls (D'Andrea et al. 2014; Ren et al. 2018). However, other studies have shown that there is an increase in the level of TRP during the headache phase of the attack while a decrease is noted between the attacks (Deen et al. 2017; S. N. Young 2013). The fluctuations in the levels of TRP and its depletion are not a migraine trigger but rather an enhancer of the symptoms of migraine (Carpenter et al. 1998; van der Stelt et al. 2004; Williams et al. 1999). Drummond was able to show that under Trp-depleted conditions, migraineurs suffer from an increase in headache and photophobia as well as an increase in nausea and dizziness (P. Drummond 2006). Adding to what he found, Jahromi et al, presented interesting data showing a 54-60% decrease in the risk of a migraine attack due to a daily dietary intake of TRP (Razeghi Jahromi, Togha, et al. 2019). The entirety of the data obtained from the clinical studies opens the door that since TRP is the precursor of multiple compounds possibly involved in the pathogenesis of migraine (serotonin, kynurenine, and indoles), this could explain the link between TRP and migraine pathogenesis.

11.3.1.1. The Role of the Serotoninergic Pathway in Migraine

Serotonin is a vasoconstrictor located in the blood with a potential role in the modulation of nociception (Taylor and Basbaum 1995). It directly activates the nociceptive afferents in order to augment the number of nociceptive impulses that are sent to the spinal cord. Its receptors are found within locations in migraine-related regions such as the thalamus, cortex, striatum, raphe nuclei, and cerebellum (Barnes and Sharp 1999; Varnäs, Halldin, and Hall 2004). The important role of serotonin in migraine came about when Sicuteri et al., in addition to other researchers after found

that the amount of 5-HIAA increased in the urine during the migraine attack, while the platelet concentration of serotonin decreased (Curran et al., 1965; Sicuteri et al., 1961). In addition, reports of low levels of serum serotonin were found in migraineurs which were consistent with prior data (Ren et al. 2018). Also, in the case of reserpine-induced headaches, the infusion of serotonin was able to inhibit the spontaneous attack (Kimball, Friedman, and Vallejo 1960).

The serotonin transporter (5-HTT) occurring mainly at the level of raphe nuclei and serotonergic projection areas such as the thalamus, uptakes the serotonin located in the synaptic cleft to reduce the effect of serotonin through a process controlled by a Na+/Cl- ion gradient (Lesch et al. 1993; Murphy et al. 1989). Interestingly, the distribution of 5-HTT is increased in migraine patients at the level of the brain stem (Schuh-Hofer et al. 2007). Not only that but in the case of FHM, there exists a low level of serotonin in platelets in addition to a low transport capacity of 5-HTT coupled with reduced cerebrospinal fluid metabolite levels (Horvath et al. 2011).

In addition to the transporter, serotonin receptors play a distinguished role in migraine. It is shown that the neurons of the trigeminal ganglia and the dorsal raphe are serotonergic (Berman et al. 2006; Lambert 2010). Moreover, the $5HT_{1B}$ and $5HT_{1D}$ subtypes, where triptans act, are located at the level of trigeminal neurons, with their mRNA and protein detections in the trigeminal ganglia (J. C. A. Edvinsson et al. 2022). They are also located at the level of the axon of presynaptic neurons in the basal ganglia and frontal cortex. Furthermore, they are colocalized with the pain molecules, substance P, CGRP, and nNOS (Hou et al. 2001). Interestingly, the acute depletion of TRP in healthy human subjects (using seventy capsules of an amino acid mixture that doesn't contain TRP), induced changes in the expression and availability of 5HT1B in the cortex and raphe nuclei indicating a major link between TRP depletion and serotonin (Baldassarri et al. 2020).

One of serotonin's derivatives, melatonin, also has a role in migraine, where it can be an advantage for migraine through antagonizing glutamate-induced excitotoxicity, blocking NO synthesis, inhibiting the release of dopamine, and other functions (M. F. P. Peres et al. 2006).

11.3.1.2. The Role of the Kynurenine Pathway in Migraine

The contribution of kynurenine as a therapeutic potential in migraine came from the idea that KYNA is a competitive antagonist of the NMDA receptors that when inhibited protects against excitotoxicity induced by glutamate (Kessler et al. 1989). Curto et al. observed a decrease in the levels of KYNA in addition to other metabolites in the serum of migraineurs suffering from chronic migraine compared to their controls in addition to having higher concentrations of TRP (Curto et al. 2015). The reduction of KYNA during migraine supports the theory of NMDA hyperactivity since KYNA is an antagonist of NMDA (Hoffmann and Charles 2018). Kynurenine's involvement was also shown in a model of CSD whereby the intake of kynurenine inhibited CSD probably through the increase of KYNA in the cortex (Chauvel et al. 2012). From what has been found so far there is a positive link between migraine, TRP, kynurenine, glutamate, and NMDA that could explain the contribution of this pathway in the pathophysiology of migraine. Kynurenine metabolites also modulate migraine-related regions such as the LC. The administration of quinolinic acid intracerebroventricularly increased the activity of LC neurons, while the administration of KYNA inhibited the noxious stimulation evoked by central noradrenergic neurons in the LC (S. B. Abbott et al. 2012; Ping, Wu, and Liu 1990). A recent study shows that the depletion of TRP in cell culture increased the expression of AhR (Solvay et al. 2023). In addition, they found kynurenine, an endogenous ligand of AhR, whose cellular uptake was also increased through the augmentation of the expression of Lat1. As we know kynurenine is the metabolite of TRP, and TRP is involved in migraine pathogenesis (P. Drummond 2006). All of this adds to the activation of AhR by kynurenine under TRP-depleted conditions and potentially a link between TRP, AhRs, and migraine.

11.3.1.3. The Role of the Indole Pathway in Migraine

Indole and some of its downstream metabolites tryptamine and ILA have a role in the attenuation of inflammation at the level of the macrophages and intestinal epithelial cells (Ehrlich et al. 2020). In addition, indole, tryptamine, and IPA exert a plethora of effects by either activating or inhibiting the intestinal epithelial AhRs. Furthermore, ILA and IPA are scavengers of free radicals and reducers of oxidative stress in systemic tissue (Romani et al. 2014). IPA also exerts a neuroprotective effect

and prevents weight gain in animal models and is an inhibitor of gut dysbiosis (Konopelski et al. 2019; Z.-H. Zhao et al. 2019).

Indoles have shown a contribution in nociception whereby through the direct activation of transient receptor potential ankyrin 1 (TRPA1) nociceptors, they induce a painful response and a role in pain signaling (Chung et al. 2022). However, currently, there are no concrete links between indoles and migraine. The actual mechanism by which indoles contribute to migraine pathogenesis is quite vague. One study measured the levels of tryptamine in the plasma of chronic migraineurs, which are lower than their respective controls (D'Andrea et al. 2014). As a function of time, more work should be done to further understand the contribution of this pathway to migraine.

HYPOTHESIS & OBJECTIVES

Migraine is a neurobiological disorder with episodic manifestations. However, in approximately 3% of patients, these episodic patterns take on a chronic form. Although the mechanisms of this progression are unclear, the neuroinflammatory state could play a key role. During headaches, migraineurs report cutaneous cephalic hypersensitivity (correlated with central sensitization) and dysfunctional descending pain modulatory pathways (at the level of the brainstem among others). These neuronal alterations are concomitant with increased plasmatic levels of pro-inflammatory cytokines during and between attacks. Moreover, migraine is comorbid with other chronic inflammatory disorders, and it has been described that under nociceptive conditions, glial cell function is dysregulated. Hence, we first hypothesized that migraine is coupled with an enhanced inflammatory response, which modulates both the central sensitization and the brainstem function, contributing to the progression of migraine toward a chronic state. The inflammatory signaling can be initiated by TRP-derived metabolites through the activation of AhR. Both players have already been involved in comorbid inflammatory diseases, and TRP metabolism is impaired in migraineurs. Therefore, our second hypothesis came that the dysregulation of TRP metabolism and AhR activity promote an abnormal neuroinflammatory state during migraine that contributes to disease progression (Figure 21).

Thus, we studied the involvement of TRP and its metabolites in the inflammatory-related processes during migraine initiation and chronification, and in the therapeutic efficacy of triptans. We chronically dysregulated the metabolism of TRP by dietary TRP depletion and examined the potential neuronal changes at the level of the LC and the Sp5C in an experimental mouse model of migraine-like pain induced by systemic ISDN administration. We focused our study on these two regions for several reasons: (1) the Sp5C is a key player in the nociceptive integration at the level of the trigeminovascular system, and its activation contributes to central sensitization, and hence, to cutaneous hypersensitivity; (2) the LC has been clinically and preclinically implicated in migraine, it modulates the trigeminal system by its descending noradrenergic inputs, and it is involved in pain chronification. Our experimental approach combined behavioral assessment (pain, light aversion, and anxiety), immunohistochemistry, and biomolecular techniques (RT-PCR, HPLC, western blot, and ELISA).

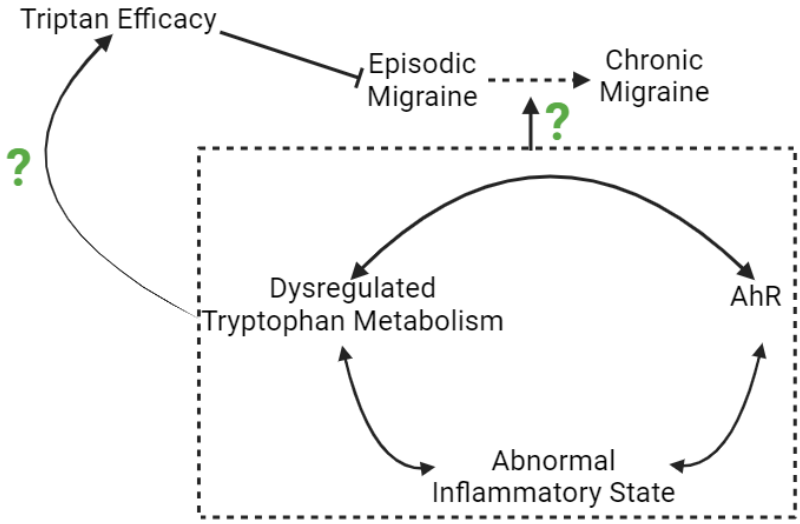


Figure 21-Schematic Representation of the Experimental Hypotheses. Migraine could be linked to an abnormal systemic and neuronal inflammatory state, which can increase the risk of spontaneous headache triggering, and hence, contribute to progression from an episodic to a chronic state. Inflammation is modulated by tryptophan-derived metabolites, which are implicated in both episodic and chronic migraine, through the aryl hydrocarbon receptor (AhR). Hence, TRP and neuroinflammation could be key players in migraine chronification, and in the therapeutic efficacy of abortive drugs. (Created with Biorender.com).

To address our hypotheses, this project was mainly divided into two studies, with some complementary results. The specific objectives for each study were:

. ***Study I:***

To investigate the consequences of TRP dysregulation on:

- the onset of migraine-like pain induced by a single ISDN administration in male and female mice through the assessment of the two most prevalent sensory symptoms: cephalic mechanical hypersensitivity and light hypersensitivity.

- the effectiveness of sumatriptan to block cephalic mechanical and light hypersensitivities induced by ISDN administration in both sexes.

. Study II:

To investigate the consequences of TRP dysregulation on:

- the migraine progression after repeated ISDN administration in female mice, through the behavioral assessment of cephalic mechanical sensitivity, and the evaluation of the LC function.

- the systemic (plasmatic) and local (in the LC and the Sp5C) inflammatory state in female mice after repeated ISDN administration.

To characterize the contribution of AhR on migraine progression through behavioral assessment of cephalic mechanical sensitivity after AhR agonist/antagonist drug administration, and their activity at the level of the LC and the Sp5C after repeated ISDN administrations.

STUDY I

Dietary Tryptophan Deficiency Reduces the Effectiveness of Sumatriptan in a Mouse Model of Migraine

Yara Mrad[1], Vidosav Rajcic[1], Robin Lioutaud[1], Ines Erkizia-Santamaria[2], Igor Horrillo[2,3,4], Radhouane Dallel[1], Cristina Alba-Delgado[1*]

[1]Université Clermont Auvergne, CHU Clermont-Ferrand, Inserm, Neuro-Dol, F-63000 Clermont-Ferrand, France; [2]Department of Pharmacology, University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain; [3]Centro de Investigacion Biomedica en Red de Salud Mental, Instituto de Salud Carlos III, Spain; [4]Biocruces Bizkaia Health Research Institute, Barakaldo, Bizkaia, Spain

***Correspondance to**: Dr. C. Alba-Delgado; Université Clermont Auvergne, Neuro-Dol Inserm/UCA U1107, Faculté de Chirurgie Dentaire, 2 Rue de Braga, Clermont-Ferrand, 63100, France. Phone: +33473177978. Email: cristina.alba_delgado@uca.fr

ABSTRACT

Sumatriptan is considered a specific acute treatment for migraine attacks. However, approximately 30% of migraineurs do not respond to it or only partially. The mechanism of this inefficacy is unclear. Tryptophan (TRP), the precursor of 5-HT, has been also involved in migraine pathophysiology. Its acute depletion aggravates ictal episodes and can alter the brain expression of 5-HTR_{1B}. Hence, we aimed to study the contribution of TRP on sumatriptan efficacy in a migraine-like pain model induced by isosorbide dinitrate (ISDN). Two of the most prevalent clinical symptoms (cephalic mechanical hypersensitivity -CMH- and light hypersensitivity -LH-) were evaluated in male and female mice subjected to a TRP-deficient diet for one week. In addition, 5-HT levels and the expression of 5-$HTR_{1B/D}$ were measured. ISDN-induced CMH in both sexes and LH in females only. The TRP-deficient diet reduced plasmatic 5-HT, and significantly prolonged ISDN-induced CMH, but did not worsen the LH in females. Interestingly, TRP deficiency also blocked the antinociceptive efficacy of sumatriptan on both CMH and LH. It was concomitant with an increased 5-HTR_{1D} expression in the trigeminal nucleus caudalis, without alteration of local 5-HTR_{1B} nor 5-HT levels. Our results suggest that the inability of sumatriptan to block nociception could be associated with an alteration of 5-HTR_{1D} functionality in the trigeminal system, induced by impaired TRP metabolism. It remains to be determined whether these alterations are exclusive to non-responders and whether the action of other triptans is also compromised.

INTRODUCTION

Migraine is a prevalent incapacitating neurological disorder ranked as the third most common disease in the world (Vos et al. 2016). It is characterized by unilateral, pulsating headache attacks, accompanied by sensory disturbances, such as cephalic mechanical (CMH) and light (LH) hypersensitivities. Sumatriptan, a 5-hydroxytryptamine (5-HT) 1B/1D receptor (5-$HTR_{1B/1D}$) agonist, is recommended as a first-line abortive drug for patients suffering from moderate to severe migraine attacks. Nevertheless, ~30% of migraine patients are non-responders to sumatriptan, and individual responsiveness to triptans is variable (J.-W. Wu et al. 2022). To date, the response variability is not fully understood, and only a few studies have identified the predictors for triptan response in migraine. Current evidence showed that a lower pain severity prior to treatment and a higher polygenic risk score was associated with a better response to triptans (Diener et al. 2004; Kogelman et al. 2019). An early study suggested that triptan efficacy is less optimal after a patient develops cutaneous hypersensitivity, but new controlled studies have shown conflicting results (Rami Burstein, Collins, and Jakubowski 2004; Cady et al. 2009). Differences in pharmacokinetic parameters and bioavailability of sumatriptan (Peer Tfelt-Hansen 2007) or genetic diversity in 5-HTR_{1B} have also been suggested (MaassenVanDenBrink et al. 1998).

Evidence supports 5-HT transmission as a main factor involved in migraine pathogenesis. Migraineurs present decreased 5-HT levels between attacks and increased levels during the ictal period (Deen et al. 2017). Moreover, changes in the sensitivity to 5-HT agonists (triptans) might occur during attacks probably due to a defect in 5-HT metabolism (Deen et al. 2018; Proietti-Cecchini, Afra, and Schoenen 1997; Sakai et al. 2008). The changes in 5-HT turnover or the sensitivity of its receptors might participate in the disturbances of sensory processes (Deen et al. 2017). Thus, it is proposed that migraine headaches might develop during a period of attenuation in 5-HT activity and consequent stimulation of 5-HT receptors (Gupta, Nahas, and Peterlin 2011).

Tryptophan (TRP) is an essential precursor required for different metabolic reactions including 5-HT production (Höglund, Øverli, and Winberg 2019). Dietary TRP has been shown to have an inverse relationship with migraine risk (Razeghi Jahromi, Ghorbani, et al. 2019), suggesting that low brain concentrations of TRP itself or its metabolites enhance the risk of a migraine attack (Biringer 2023). Short-term reduction

of TRP intake induces nausea, dizziness, and motion sickness (P. D. Drummond 2005), boosts the intensity of headaches and LH in migraineurs (P. Drummond 2006), and modulates the brain availability of 5-HTR_{1B} in healthy individuals (Baldassarri et al. 2020). Hence, we sought to study the contribution of TRP on the abortive effect of sumatriptan in a mouse model of migraine-like pain induced by a single systemic administration of isosorbide dinitrate (ISDN) (Dallel, Descheemaeker, and Luccarini 2018) in which CMH and LH were used as surrogates for headache-associated symptoms.

MATERIALS AND METHODS

1. Animals

Adult male and female C57BL/6 mice (7 to 12 weeks old) were obtained from Charles River (L'Arbresle, France) or born in our amenities. Mice were housed under a 12 h light/dark cycle with *ad libitum* access to food and water. Environmental enrichment was provided for nest building and shelter and transferred with mice when cages were cleaned. Procedures were conducted according to the experimental design (Figure 1A) during the dark phase (between 8 AM and 3 PM). At the end of the experiments, mice were sacrificed using a lethal dose of sodium pentobarbital (150 mg/kg, i.p., CEVA Santé Animale, Libourne, France). Death was confirmed by permanent cessation of cardiorespiratory functions.

All efforts were made to minimize the number of animals used. A total of 224 animals were used to complete this study: 87 for the von Frey test, including weight and food consumption assessments; 72 for the modified elevated plus maze (mEPM) test; 40 for ELISA and HPLC; and 25 for the western blots.

2. Study Approval

All procedures and analyses were performed following ARRIVE (Animals in Research: reporting of in vivo experiments) guidelines. Experiments were following the ethical guidelines of Directive 2010/63/UE of the European Parliament and the Council and French Decree 2013-118 on the protection of animals used for scientific purposes. Protocols were approved by the French Ministry of Higher Education and Research (Ref. 2020012716162262).

3. Dietary Tryptophan Depletion

Mice were randomly assigned to one of two dietary conditions: the control diet (balanced diet, BAL) contained 2.3 g of TRP per kg of food, which is considered a normal intake in mice (John and Bell 1976); or the TRP-deficient diet (TRP^{-}) contained 0.3 g/Kg of TRP. Both diets were manufactured by SAFE® Custom Diets company (Augy, France) and formulated as described in Table 1, with the required levels of amino acids necessary for optimal health. The form of the pellets was similar between both diets, with a 10-12 mm diameter and a crushing resistance of ~5 kgf/cm^2. The body weight of mice (Figures S1A and S1A2) and their food consumption (Figures S1B1 and S1B2) were measured on a daily basis as an indication of the well-being of animals.

4. Drug administration

A single intraperitoneal (i.p.) injection of ISDN (Risordan®, Sanofi-Aventis, Paris, France; 10 mg/kg in a 10 ml/kg volume) or vehicle (0.9% NaCl) was administrated as previously described (Dallel, Descheemaeker, and Luccarini 2018). Sumatriptan succinate (S1198, Sigma-Aldrich, St. Quentin Fallavier, France) was injected i.p. (1 mg/kg in 10 ml/kg volume, dissolved in saline) 5 min after ISDN/vehicle injection.

5. Behavioral Tests

Briefly, mice were first allowed to acclimatize to the room used for behavioral testing as well as to innocuous mechanical stimulation for 3 days before the start of the behavioral sensory testing. Test sessions took place during the light phase, between 11 a.m. and 7 p.m.

5.1. Cephalic Mechanical Sensitivity

Periorbital sensitivity to non-nociceptive mechanical stimuli was measured following the modified up-down method described previously (Alba-Delgado et al. 2018). A series of eight von Frey filaments (0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6, and 1.0 g; 1.0 g cut-off; Bioseb, Vitrolles, France) were presented perpendicularly to the left periorbital surface of the forehead. Testing began with the 0.4 g filament. After drug administration, the last weak von Frey filament was used. The stimuli were presented using an up-down sequence five times at 5 s intervals. Detection of a positive response (eye winking, turning the head away, attacking filament, and/or body freezing) three or more times lead to choosing the next weaker stimulus. In the absence of a positive

response, a stronger stimulus was presented. The cephalic mechanical sensitivity threshold (in g) was determined as the weakest filament used which obtained three or more positive responses. The cephalic sensitivity threshold was measured before (baseline) and every hour for 3 h after ISDN/vehicle administration. Analysis exclusion was done for mice showing distress signs after drug injection, or those that did not habituate to the 0.6 g filament before the testing day.

5.2. Light Sensitivity

Light avoidance behavior was evaluated in a modified version of the elevated plus maze test (mEPM) according to (Chanda et al. 2013). An LED lighting system (5000 K; Greenice SA, Toledo, Spain) was placed on top of the closed compartments (termed "light sides") while the open compartments (termed "dark sides") were not modified. The left closed arm was set at 300 lux while the right closed arm was set at 50 lux (the brightness was checked with a lux meter at the level of the mouse's eye). The test lasted 10 min (Figure 1B): during the first 5 min (LED-Off condition), the whole device was in darkness (<20 lux); then, only the light sides (closed arms) were lit for 5 min (LED-On condition). The animal's free exploratory behavior was filmed by an infrared camera connected to a video tracking analysis software (EthoVision XT v13.0; Noldus Information Tech., Wageningen, Netherlands). The light sensitivity threshold was calculated as a light aversion index (LAI) (Matynia et al. 2012). Mice with negative scores or around zero were considered to have no light avoidance. Positive scores were considered to be light aversive. The percentage of time spent in the closed arms during the LED-Off condition was used to evaluate the anxiety-like behavior.

6. Blood and Tissue Preparation

Sample collection was performed 1 h after ISDN/vehicle injection (Figure 1A). Mice were fasted for 1 h and then anesthetized with an i.p. administration of 25% urethane (1.5 g/kg i.p.). Blood was collected after decapitation and stored in 2 ml tubes without anticoagulant at room temperature. After complete coagulation, samples were centrifuged at 15000 x g for 10 min at room temperature, and the serum was carefully collected. At the same time, the brain was removed from the skull and the two spinal trigeminal nucleus caudalis (Sp5c) were manually dissected. Blood and brain samples were immediately frozen in liquid nitrogen and then stored at -80 ℃ for later use.

7. Detection of Metabolites

TRP or 5-HT levels in serum were measured by using the Enzyme-Linked Immunosorbent Assay (ELISA) kits (BA E-2700 or BA E-8900 respectively, ImmuSmol, Bordeaux, France). Provider instructions were followed.

High-Performance Liquid Chromatography (HPLC) with electrochemical detection was used to quantify the levels of TRP in the Sp5C. Corresponding tissue was first homogenized using an Ultra-Turrax T8 homogenizer in 15 µl/mg homogenization buffer (0.1 M HClO4 and 100 µl EDTA). The crude homogenate was centrifuged at 21,000 x g for 15 min at 4 ºC, and the supernatant was diluted 1:5. Samples were then filtered by centrifugation at 15,000 x g for 15 min using Costar® Spin-X® Centrifuge Tubes (0.22 µm Pore CA Membrane; Merck Life Science S.L.U., Madrid, Spain). The concentration of TRP was estimated with reference to standards prepared and injected on the same day as described before (Unzueta-Larrinaga et al. 2023). The mobile phase composition was 150 mM H2NaPO4, 0.2 mM EDTA, 4.3 mM octyl sodium sulfate (pH 6.3), and 8% (vol/vol) methanol. The filtered mobile phase was delivered at a flow rate of 0.2 ml/min by a Hewlett-Packard model 1200 pump. Sample separation was carried out at 30 °C on a Zorbax Eclipse Plus column (3.5 µm C18, 2.1×150 mm, Agilent Technologies, Madrid, Spain). TRP amperometric detection was carried out using a Hewlett-Packard model 1049A detector (Agilent Technologies Deutschland GmbH, Waldbronn, Germany) at an oxidizing potential of +950 mV.

8. Western Blot

The Sp5C samples were homogenized using a lysis buffer containing EDTA (0.5 M), Trizma base (50 mM), sodium chloride (10 mM), Triton 100% (0.25%), and supplemented with a cocktail of protease and phosphatase inhibitors (Thermo Fisher Scientific, Illkirch-Graffenstaden, France). An equal amount of total protein (20 µg/each sample) was resolved in each lane of 10% SDS-PAGE gels using Mini-PROTEAN Tetra Vertical Electrophoresis Cell (Bio-Rad Laboratories) at 80 V for 15 min followed by 200V for approximately 30 min. The resolved proteins were then transferred onto a PVDF membrane (Merck Chimie SAS Île-de-France, France) using a Mini Trans-Blot® Cell (Bio-Rad Laboratories) at 100 mA for 1 h. Thereafter, membranes were incubated at 25 °C for 1 h with a solution containing 1% Casein in tris-buffered saline (TBS) to block non-specific bindings. Membranes were then incubated overnight at 4 °C with the

primary antibodies against 5-HTR_{1B} or 5-HTR_{1D} (1:500; OSS00185W or OSS00182W respectively, Osenses, Keswick, Australia). Subsequently, they were washed with TBS 1X and incubated with the StarBright Blue 700 goat anti-rabbit secondary antibody (1:2,500; 64297093, Bio-rad Laboratories, Marnes-la-Coquette, France) at room temperature for 2 h. Blots were visualized with the ChemiDoc XRS system (Bio-Rad Laboratories) and analyzed using the software Image Lab (v6.1, Bio-Rad Laboratories).

9. Statistical Analysis

Data are presented as mean ± SEM. The number (n) of mice utilized for each analysis was shown in Figures or Tables. Normality and homogeneity of variance were determined using Shapiro–Wilk test, and F or Levene tests, respectively. Unpaired Student's t-test was used to compare mean values between two groups with equal variances. Comparisons of more than two means were performed using repeated (RM) or non-repeated 2 or 3-way ANOVA including Geisser-Greenhouse correction to account for possible unequal variances between groups. Post hoc comparisons between groups were analyzed with the Tukey HSD posttest. T-values, F-values, their associated degrees of freedom, and p-values were summarized in Table 2, and in Table S1. The level of significance was set to $p < 0.05$. All statistical analyses and graphs were performed using GraphPad Prism (v8.4.0, GraphPad Inc., La Jolla, CA, USA). Figures were made using CorelDraw Graphics software (v12.0, Ottawa, Canada).

RESULTS

TRP deficiency reduces serum TRP and 5-HT concentrations in both sexes

First, we examined the serum concentration of TRP in both male and female mice subjected to one week of a balanced (BAL) or TRP-deficient diet (TRP^{-}) (Table 3). In the BAL diet group, vehicle-injected females showed significantly higher TRP levels (176.0 ± 16.0 µM) than vehicle-injected males (90.2 ± 10.9 µM). After dietary TRP depletion, the serum TRP concentration decreased by 68.5 % and 66.7 % in female and male mice, respectively (Table 3). No differences were found between the sexes (Table 3).

The basal serum concentration of 5-HT was similar in both sexes in the BAL diet group (1.63 ± 0.05 µM *vs.* 1.68 ± 0.08 µg/ml respectively; Table 3). After dietary TRP depletion, the serum 5-HT concentrations decreased by 12.9 % in vehicle-injected females (1.42 ± 0.04 µM) and 22 % in males (1.31 ± 0.03 µM) (Table 3). ISDN-injected males under a TRP^{-} diet did not show reduced 5-HT levels (1.56 ± 0.04 µM), which were higher than vehicle-injected males (1.31 ± 0.03 µM) and similar to groups under a BAL diet (Table 3).

TRP deficiency worsens ISDN-induced CMH in both sexes

Basal values were similar in BAL (female: 0.93 ± 0.07 g, male: 0.93 ± 0.07g) and TRP^{-} diet groups (female: 0.87 ± 0.08 g, male: 0.93 ± 0.07 g) of both sexes (Figures 2A1 and 2A2).

A single ISDN administration induced CMH in both sexes, peaking at 1 h and lasting for at least 3 h in the BAL diet group (Figures 2A1 and 2A2). Time-course of CMH was similar between sexes (female AUC: 0.70 ± 0.13 AU *vs.* male AUC: 0.65 ± 0.16 AU; Figure 2B). Interestingly, under TRP-deficient conditions, ISDN also induced CMH with a similar peak at 1 h, but with a significant lack of recovery at 3 h (females: 0.04 ± 0.02 g; males: 0.10 ± 0.03 g) compared to their BAL counterparts (females: 0.48 ± 0.04 g; males: 0.45 ± 0.13 g; Figures 2A1 and 2A2). Indeed, the AUC measured between 1 to 3 h post-injection confirmed these significant differences between both diets, in females and males (Figure 2B). No differences were observed between the sexes.

TRP deficiency blocks the efficacy of sumatriptan to prevent the ISDN-induced CMH

In the BAL diet group, sumatriptan (1 mg/kg i.p.) delivered 5 min after ISDN was able to inhibit ISDN-induced CMH in both sexes (Figures 2C1 and 2C2). The therapeutic efficacy of sumatriptan was similar between the sexes, as shown by their similar AUC (female: 1.22 ± 0.22 AU, male: 1.44 ± 0.24 AU; Figure 2D).

After dietary TRP deficiency, sumatriptan did not affect ISDN-induced CMH in both sexes (Figures 2C1 and 2C2). One hour post-administration, the cephalic mechanical thresholds were not different between the vehicle (females: 0.02 ± 0.01 g; males: 0.11 ± 0.07 g) and sumatriptan (females: 0.12 ± 0.07 g; males: 0.01 ± 0.01 g) injected animals. This was confirmed by the significant differences in the AUC between TRP^- ISDN groups (females: 0.22 ± 0.14 g; males: 0.03 ± 0.01 g) compared to BAL groups (females: 1.22 ± 0.22 g; males: 1.44 ± 0.24 g; Figure 2D).

ISDN induces LH exclusively in females, which is not altered by TRP deficiency

In the BAL diet group, vehicle-injected female mice did not show LH neither at 50 lux nor 300 lux, as shown by the negative LAI (-0.20 ± 0.15 and -0.27 ± 0.13 respectively; Figure 3A). However, vehicle-injected males show a positive LAI at 300 lux (0.29 ± 0.12; Figure 3B), indicating an innate LH. ISDN administration evoked a positive LAI in females at 300 lux (0.35 ± 0.08; Figure 3A) but did not modify the innate LH in males (0.32 ± 0.10; Figure 3B). TRP deficiency did not affect light sensitivity neither in vehicle-injected nor in ISDN-injected female or male mice, at both 50 lux and 300 lux (Figures 3A and 3B).

To verify that LH measured in the mEPM was not linked to anxiety, the time spent in the closed arms during the LED-Off condition was measured (Figure S2). Animals did not present anxiety-like behavior neither after BAL or TRP^- diets, nor after the ISDN injection.

TRP deficiency blocks the efficacy of sumatriptan to prevent the ISDN-induced LH

In the BAL diet group, sumatriptan prevents ISDN-induced LH in female mice in the mEPM (Figure 3B), as shown by the negative LAI at 300 lux in the sumatriptan-treated ISDN group (-0.14 ± 0.07). Interestingly, in the TRP^- diet group, the efficacy of

sumatriptan was affected by the TRP deficiency. ISDN-injected females showed a positive LAI at 300 lux (0.36 ± 0.08), similar to ISDN-injected mice (0.54 ± 0.08; Figure 3A). Sumatriptan did not affect the innate LH in males.

TRP deficiency does not change the TRP concentration in the Sp5C

To evaluate if the sumatriptan inefficacy was linked to the dysregulation of TRP metabolism in the Sp5C following one week of a TRP-deficient diet, we quantified the TRP concentration using HPLC in this region (Table 3). No differences between sexes were observed, hence the data were pooled to increase the power of the statistical analysis. Surprisingly, the TRP-deficient diet did not reduce TRP levels (2.7 ± 0.5 nM) which were similar to the BAL condition (3.0 ± 0.3 nM). Systemic ISDN administration also did not modify TRP levels in the SP5C (at 1 h post-injection), in neither BAL nor TRP-deficient diets.

TRP deficiency increases the expression of 5-HTR$_{1D}$ in the Sp5C

We assessed the expression of the 5HT$_{1D}$ and 5HT$_{1B}$ receptors in the Sp5C through western blot. Since no difference was observed in the 5HT$_{1D}$ and 5HT$_{1B}$ receptors expression between males and females, we pooled the data. In the BAL diet group, ISDN does not affect the 5HT$_{1D}$ and 5HT$_{1B}$ receptor expression in the Sp5C. Dietary TRP deficiency alone also did not induce changes in the 5HT$_{1D}$ and 5HT$_{1B}$ receptors in the Sp5C (Figures 4A2 and 4B2). Interestingly, ISDN administration in the TRP^{-} diet group produced a dramatic increase of 5HTR$_{1D}$ expression (Figure 4A2), but not 5HTR$_{1B}$ in the Sp5C (Figure 4B2), compared to vehicle-injected mice under the same diet.

DISCUSSION

The present study demonstrated that chronic dietary TRP depletion reduces the availability of plasmatic TRP which (i) worsens ISDN-induced CMH in both sexes and (ii) reduces the efficacy of sumatriptan on both ISDN-induced CMH and LH. These changes were associated with increased 5-HTR_{1D} expression in the Sp5C and reduced serum levels of 5-HT.

ISDN as a preclinical model of migraine

The headache research field has well-established animal models that improved our understanding of the peripheral and central headache mechanisms and their interaction. Several models exist simulating different aspects of migraine with certain limitations (Harriott et al. 2019). Here, we used ISDN as an animal model of migraine because it reliably produces headaches in migraineurs, though less often in healthy subjects (Bellantonio et al. 1997; Castellano et al. 1998), and results in CMH associated with sensitization of the trigemino-cervical complex in rats (Dallel, Descheemaeker, and Luccarini 2018; Flores Ramos et al. 2017). Moreover, sumatriptan and olcegepant, two specific acute antimigraine drugs, block ISDN-induced CMH (Dallel, Descheemaeker, and Luccarini 2018). As in rats, we found that a single ISDN injection induced reversible CMH that was similar in mice in both sexes.

Moreover, ISDN evoked LH exclusively in females. LH is the most bothersome symptom associated with a migraine attack but is also reported in headache-free periods in patients (Pinheiro et al. 2021). Light aversive behavior has also been previously reported after an acute NTG injection, in both male (Markovics et al. 2012) and female mice (Eller et al. 2021). However, no differences were detected after NTG (Targowska-Duda et al. 2020) or CGRP administrations (Mason et al. 2017). But, our results were in accordance with clinical studies that reported a higher incidence of LH during migraine attacks in women than men (Boardman et al. 2003; Bolay et al. 2015). The lack of LH in males could be attributed to the fact that females are more sensitive to nitric oxide (NO) donors than males, as shown by the higher activity of the NO synthase in females compared to males (Oydanich et al. 2019). Moreover, males showed a higher innate sensibility to light than females, which could support different behavioral and neuronal mechanisms for light adaptation (Chellappa 2021).

Both CMH and LH were blocked by systemic sumatriptan administration, which was consistent with previous studies in ISDN-injected rats (Dallel, Descheemaeker, and Luccarini 2018) and after an acute NTG administration in rats (Farajdokht et al. 2017) and mice (Casili et al. 2020).

Dietary deficit in TRP dysregulates systemic TRP metabolism and prolongs cephalic pain

Prior to any change in the availability of TRP, we saw a higher level of baseline TRP in females than in males which adds up to the higher circulating levels of TRP in females compared to males in animal studies (Jans, Lieben, and Blokland 2007). Here, we used a TRP-deficient diet for one week before inducing migraine-like pain, which reduced serum TRP and 5-HT levels in male and female mice. Following the implementation of a one-week TRP-deficient diet a 70% decrease in the serum levels of TRP was attained following one week coupled with a significant decrease in its metabolite serotonin. During this decrease, we have shown no sign of a deterioration in the well-being of the animals represented by no robust decrease in their weights, change in the amount of food consumed, and no anxiogenic behavior. This decrease has been priorly shown in rats on low TRP-containing diets for periods of up to one week (Franklin, Cowen, and Craven 1995) and in humans on low-calorie TRP-restricting diets for three weeks (Walsh et al. 1995). Deficiency in TRP and 5-HT levels was correlated with an augmented duration of the ISDN-induced CMH. Our findings are in agreement with the clinical studies using acute TRP depletion. They showed that TRP depletion augments the manifestations of migraine including headaches, LH, and nausea, in addition to boosting motion sickness (P. Drummond 2006; P. D. Drummond 2005).

However, a TRP-deficient diet did not aggravate the ISDN-induced LH in females. This contradicts what the literature has shown as to an increase in LH in migraineurs post-TRP depletion (P. Drummond 2006). This could be due to the pathway targeted by TRP. Since CMH and LH are two distinct pathological pathways, the effect of a TRP-deficient diet could be solely targeting CMH. Moreover, it could be attributed to the timing of the assessment of light hypersensitivity post-ISDN administration, which was one-hour post-ISDN, perhaps assessment at a different time point would showcase an effect on LH.

Sumatriptan is no longer efficacious under TRP deficiency

Even with the efficacy shown by sumatriptan, 30-40% of migraineurs report total or partial inefficacy, alone or combined with other analgesic drugs (Viana et al. 2013). Changes in serotonin levels can be induced through dietary acute depletion of TRP, which is its precursor and is a potent inducer of migraine manifestations including LH and pain (P. D. Drummond 2005).

Quantification of the expression of 5-$HTR_{1B/1D}$ shows a significant increase in the protein expression in the Sp5C, specifically that of 5-HTR_{1D}. Changes solely done at the level of 5-HTR_{1D} could be attributed to its location at the level of primary migraine-related regions including the LC, hippocampus, and cortex (Tricklebank and Daly 2019). This increase in the expression coupled with the low concentration of 5-HT could contribute to migraine episodes (Panconesi 2008). Moreover, the loss of the efficacy of sumatriptan could be related to receptor functionality as well whereby it is sensitized, rather than expressed. Since sumatriptan acts at the level of the 5-$HTR_{1B/1D}$, impaired serotonergic neurotransmission, which was reported in migraineurs (Aggarwal, Puri, and Puri 2012), and hence the 5-$HTR_{1B/1D}$ functionality, could impact the triptan efficacy.

Changes in 5-HTR_{1D} expression could be associated with altered functionality, as shown for other 5-HTR_1 (Yücel et al. 2016). In this study, we did not examine the sensitivity of receptors to sumatriptan agonism. However, this could be further assessed through the plasma extravasation method which has priorly demonstrated the efficacy of sumatriptan and $5HTR_{1B}$ in inhibiting the latter following the electrical stimulation of the trigeminal ganglion and hence can be used for $5HTR_{1D}$ as well (Buzzi and Moskowitz 1990; Moskowitz and Waeber 1996).

CONCLUSION

We showed the pivotal role of TRP metabolism on two of the most prevalent migraine-related sensory symptoms, the CMH and LH, in mice, as well as on sumatriptan's effectiveness. Our results open a new way of understanding triptan resistance, and how altering one's daily diet could potentially ameliorate the migraine phenotype.

AUTHOR CONTRIBUTION

YM and CAD designed the research study; YM and RL performed and analyzed behavioral experiments; YM and CAD collected samples for biomolecular experiences, and performed the ELISA essays; VR performed and analyzed western blot experiments; RL performed behavioral experiments (LH); IH and IES performed and analyzed sample by HPLC; CAD supervised technical performance of experiences, and analyzed the final data; RD provided the funding; YM, RD, and CAD evaluated the results; YM and CAD wrote the manuscript, which was reviewed and approved by all authors.

ACKNOWLEDGEMENTS

We would like to thank Ms. Karine Herault, Ms. Anne-Marie Gaydier, and Ms. Sylviane Rousselin for their technical help. All authors declared no conflicts of interest. This work was supported by funding from Institut National de la Santé et de la Recherche Médicale (Inserm), Université Clermont Auvergne (France), CHU Clermont-Ferrand, and Région Auvergne-Rhône-Alpes (CPER DEPICURE2019).

REFERENCES

Aggarwal, Milan, Veena Puri, and Sanjeev Puri. 2012. "Serotonin and CGRP in Migraine." *Annals of Neurosciences* 19(2): 88–94.

Alba-Delgado, Cristina et al. 2018. "5-HT2A Receptor-Induced Morphological Reorganization of PKCγ-Expressing Interneurons Gates Inflammatory Mechanical Allodynia in Rat." *Journal of Neuroscience* 38(49): 10489–504.

Baldassarri, Stephen R. et al. 2020. "Inverse Changes in Raphe and Cortical 5-HT1B Receptor Availability after Acute Tryptophan Depletion in Healthy Human Subjects." *Synapse* 74(10): e22159.

Bellantonio, P et al. 1997. "Haemodynamic Correlates of Early and Delayed Responses to Sublingual Administration of Isosorbide Dinitrate in Migraine Patients: A Transcranial Doppler Study." *Cephalalgia* 17(3): 183–87.

Biringer, Roger Gregory. 2023. "Migraine Signaling Pathways: Purine Metabolites That Regulate Migraine and Predispose Migraineurs to Headache." *Molecular and Cellular Biochemistry*. https://doi.org/10.1007/s11010-023-04701-7 (August 2, 2023).

Boardman, HF, E Thomas, PR Croft, and DS Millson. 2003. "Epidemiology of Headache in an English District." *Cephalalgia* 23(2): 129–37.

Bolay, Hayrunnisa et al. 2015. "Gender Influences Headache Characteristics with Increasing Age in Migraine Patients." *Cephalalgia* 35(9): 792–800.

Burstein, Rami, Beth Collins, and Moshe Jakubowski. 2004. "Defeating Migraine Pain with Triptans: A Race against the Development of Cutaneous Allodynia." *Annals of Neurology* 55(1): 19–26.

Buzzi, M. G., and M. A. Moskowitz. 1990. "The Antimigraine Drug, Sumatriptan (GR43175), Selectively Blocks Neurogenic Plasma Extravasation from Blood Vessels in Dura Mater." *British Journal of Pharmacology* 99(1): 202–6.

Cady, Roger K. et al. 2009. "Elevated Saliva Calcitonin Gene-Related Peptide Levels during Acute Migraine Predict Therapeutic Response to Rizatriptan." *Headache: The Journal of Head and Face Pain* 49(9): 1258–66.

Casili, Giovanna et al. 2020. "Dimethyl Fumarate Alleviates the Nitroglycerin (NTG)-Induced Migraine in Mice." *Journal of Neuroinflammation* 17(1): 59.

Castellano, A. E. et al. 1998. "Indomethacin Increases the Effect of Isosorbide Dinitrate on Cerebral Hemodynamic in Migraine Patients: Pathogenetic and Therapeutic Implications." *Cephalalgia* 18(9): 622–30.

Chanda, Mona Lisa et al. 2013. "Behavioral Evidence for Photophobia and Stress-Related Ipsilateral Head Pain in Transgenic Cacna1a Mutant Mice." *PAIN*® 154(8): 1254–62.

Chellappa, Sarah L. 2021. “Individual Differences in Light Sensitivity Affect Sleep and Circadian Rhythms.” *Sleep* 44(2): zsaa214.

Dallel, Radhouane, Amélie Descheemaeker, and Philippe Luccarini. 2018. “Recurrent Administration of the Nitric Oxide Donor, Isosorbide Dinitrate, Induces a Persistent Cephalic Cutaneous Hypersensitivity: A Model for Migraine Progression.” *Cephalalgia: An International Journal of Headache* 38(4): 776–85.

Deen, Marie et al. 2017. “Serotonergic Mechanisms in the Migraine Brain - a Systematic Review.” *Cephalalgia: An International Journal of Headache* 37(3): 251–64.

Deen et al. 2018. “High Brain Serotonin Levels in Migraine between Attacks: A 5-HT4 Receptor Binding PET Study.” *NeuroImage : Clinical* 18: 97–102.

Diener, Hans-Christoph et al. 2004. “Topiramate in Migraine Prophylaxis--Results from a Placebo-Controlled Trial with Propranolol as an Active Control.” *Journal of Neurology* 251(8): 943–50.

Drummond, PD. 2006. “Tryptophan Depletion Increases Nausea, Headache and Photophobia in Migraine Sufferers.” *Cephalalgia* 26(10): 1225–33.

Drummond, Peter D. 2005. “Effect of Tryptophan Depletion on Symptoms of Motion Sickness in Migraineurs.” *Neurology* 65(4): 620–22.

Eller, Olivia C. et al. 2021. “Voluntary Wheel Running Partially Attenuates Early Life Stress-Induced Neuroimmune Measures in the Dura and Evoked Migraine-Like Behaviors in Female Mice.” *Frontiers in Physiology* 12. https://www.frontiersin.org/articles/10.3389/fphys.2021.665732 (July 28, 2023).

Farajdokht, Fereshteh et al. 2017. “Chronic Ghrelin Treatment Reduced Photophobia and Anxiety-like Behaviors in Nitroglycerin- Induced Migraine: Role of Pituitary Adenylate Cyclase-Activating Polypeptide.” *The European Journal of Neuroscience* 45(6): 763–72.

Flores Ramos, José María et al. 2017. “The Nitric Oxide Donor, Isosorbide Dinitrate, Induces a Cephalic Cutaneous Hypersensitivity, Associated with Sensitization of the Medullary Dorsal Horn.” *Neuroscience* 344: 157–66.

Franklin, M., P.J. Cowen, and R.D. Craven. 1995. “The Effects of a Low Tryptophan Diet on Brain 5 -HT Metabolism and 5-HT-Mediated Neuroendocrine Responses in the Male Rat.” *Journal of Psychopharmacology* 9(4): 336–41.

Gupta, Saurabh, Stephanie J. Nahas, and B. Lee Peterlin. 2011. “Chemical Mediators of Migraine: Preclinical and Clinical Observations.” *Headache* 51(6): 1029–45.

Harriott, Andrea M., Lauren C. Strother, Marta Vila-Pueyo, and Philip R. Holland. 2019. “Animal Models of Migraine and Experimental Techniques Used to Examine Trigeminal Sensory Processing.” *The Journal of Headache and Pain* 20(1): 91.

Höglund, Erik, Øyvind Øverli, and Svante Winberg. 2019. "Tryptophan Metabolic Pathways and Brain Serotonergic Activity: A Comparative Review." *Frontiers in Endocrinology* 10. https://www.frontiersin.org/articles/10.3389/fendo.2019.00158 (August 12, 2023).

Jans, L. A. W., C. K. J. Lieben, and A. Blokland. 2007. "Influence of Sex and Estrous Cycle on the Effects of Acute Tryptophan Depletion Induced by a Gelatin-Based Mixture in Adult Wistar Rats." *Neuroscience* 147(2): 304–17.

John, Ann-Marie, and J. Milton Bell. 1976. "Amino Acid Requirements of the Growing Mouse." *The Journal of Nutrition* 106(9): 1361–67.

Kogelman, Lisette J. A. et al. 2019. "Migraine Polygenic Risk Score Associates with Efficacy of Migraine-Specific Drugs." *Neurology Genetics* 5(6). https://ng.neurology.org/content/5/6/e364 (August 2, 2023).

MaassenVanDenBrink, A. et al. 1998. "5-HT1B Receptor Polymorphism and Clinical Response to Sumatriptan." *Headache* 38(4): 288–91.

Markovics, Adrienn et al. 2012. "Pituitary Adenylate Cyclase-Activating Polypeptide Plays a Key Role in Nitroglycerol-Induced Trigeminovascular Activation in Mice." *Neurobiology of Disease* 45(1): 633–44.

Mason, Bianca N. et al. 2017. "Induction of Migraine-Like Photophobic Behavior in Mice by Both Peripheral and Central CGRP Mechanisms." *The Journal of Neuroscience* 37(1): 204–16.

Matynia, Anna et al. 2012. "Intrinsically Photosensitive Retinal Ganglion Cells Are the Primary but Not Exclusive Circuit for Light Aversion." *Experimental Eye Research* 105: 60–69.

Moskowitz, Michael A., and Christian Waeber. 1996. "Migraine Enters the Molecular Era." *The Neuroscientist* 2(3): 191–200.

Oydanich, Marko et al. 2019. "Mechanisms of Sex Differences in Exercise Capacity." *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 316(6): R832–38.

Panconesi, Alessandro. 2008. "Serotonin and Migraine: A Reconsideration of the Central Theory." *The Journal of Headache and Pain* 9(5): 267–76.

Pinheiro, Carina F. et al. 2021. "Interictal Photophobia and Phonophobia Are Related to the Presence of Aura and High Frequency of Attacks in Patients with Migraine." *Applied Sciences* 11(6): 2474.

Proietti-Cecchini, A., J. Afra, and J. Schoenen. 1997. "Intensity Dependence of the Cortical Auditory Evoked Potentials as a Surrogate Marker of Central Nervous System Serotonin Transmission in Man: Demonstration of a Central Effect for the 5HT1B/1D Agonist Zolmitriptan (311C90, Zomig)." *Cephalalgia: An International Journal of Headache* 17(8): 849–54; discussion 799.

Razeghi Jahromi, Soodeh et al. 2019. “Association of Diet and Headache.” *The Journal of Headache and Pain* 20(1): 106.

Sakai, Y. et al. 2008. “Sumatriptan Normalizes the Migraine Attack-Related Increase in Brain Serotonin Synthesis.” *Neurology* 70(6): 431–39.

Targowska-Duda, Katarzyna M. et al. 2020. “NOP Receptor Agonist Attenuates Nitroglycerin-Induced Migraine-like Symptoms in Mice.” *Neuropharmacology* 170: 108029.

Tfelt-Hansen, Peer. 2007. “Parenteral vs. Oral Sumatriptan and Naratriptan: Plasma Levels and Efficacy in Migraine. A Comment.” *The Journal of Headache and Pain* 8(5): 273–76.

Tricklebank, Mark, and Eileen Daly. 2019. *The Serotonin System: History, Neuropharmacology, and Pathology*. Academic Press.

Unzueta-Larrinaga, Paula et al. 2023. “Isolation and Differentiation of Neurons and Glial Cells from Olfactory Epithelium in Living Subjects.” *Molecular Neurobiology* 60(8): 4472–87.

Viana, Michele et al. 2013. “Triptan Nonresponders: Do They Exist and Who Are They?” *Cephalalgia: An International Journal of Headache* 33(11): 891–96.

Vos, Theo et al. 2016. “Global, Regional, and National Incidence, Prevalence, and Years Lived with Disability for 310 Diseases and Injuries, 1990–2015: A Systematic Analysis for the Global Burden of Disease Study 2015.” *The Lancet* 388(10053): 1545–1602.

Walsh, A. E. S. et al. 1995. “Dieting Decreases Plasma Tryptophan and Increases the Prolactin Response to D-Fenfluramine in Women but Not Men.” *Journal of Affective Disorders* 33(2): 89–97.

Wu, Jr-Wei et al. 2022. “The Use of Neuroimaging for Predicting Sumatriptan Treatment Response in Patients With Migraine.” *Frontiers in Neurology* 13. https://www.frontiersin.org/articles/10.3389/fneur.2022.798695 (August 2, 2023).

Yücel, Yavuz et al. 2016. “Association of Polymorphisms within the Serotonin Receptor Genes 5-HTR1A, 5-HTR1B, 5-HTR2A and 5-HTR2C and Migraine Susceptibility in a Turkish Population.” *Clinical Psychopharmacology and Neuroscience* 14(3): 250–55

FIGURES

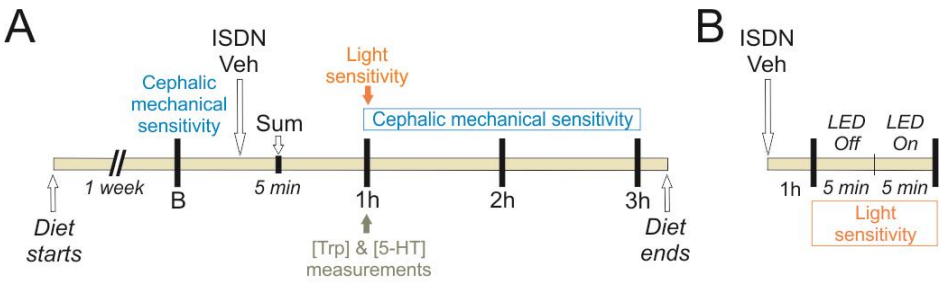


Figure 1. Experimental Paradigm. **A-** After one week of a balanced or a TRP-deficiency diet, a single intraperitoneal injection of ISDN (10 mg/kg, i.p.) or vehicle (Veh; 0.9% NaCl) was administered. Sumatriptan (Sum; 1mg/kg, i.p.) or its vehicle (0.9% NaCl) was injected 5 min after ISDN. Cephalic mechanical sensitivity was measured before (baseline, B), and one, two, and three hours after the ISDN/vehicle injection. Light sensitivity was assessed 1 h after the ISDN/vehicle injection using the modified elevated plus maze (mEPM). Blood and brain samples for serotonin (5-HT) and tryptophan (TRP) measurements were collected at 1h after drug administration. **B-** The testing period in the mEPM was divided into two parts: a 5 min LED-Off period (with the LED system turned off) followed by a 5 min LED-On (with the LED system turned on in the closed arms).

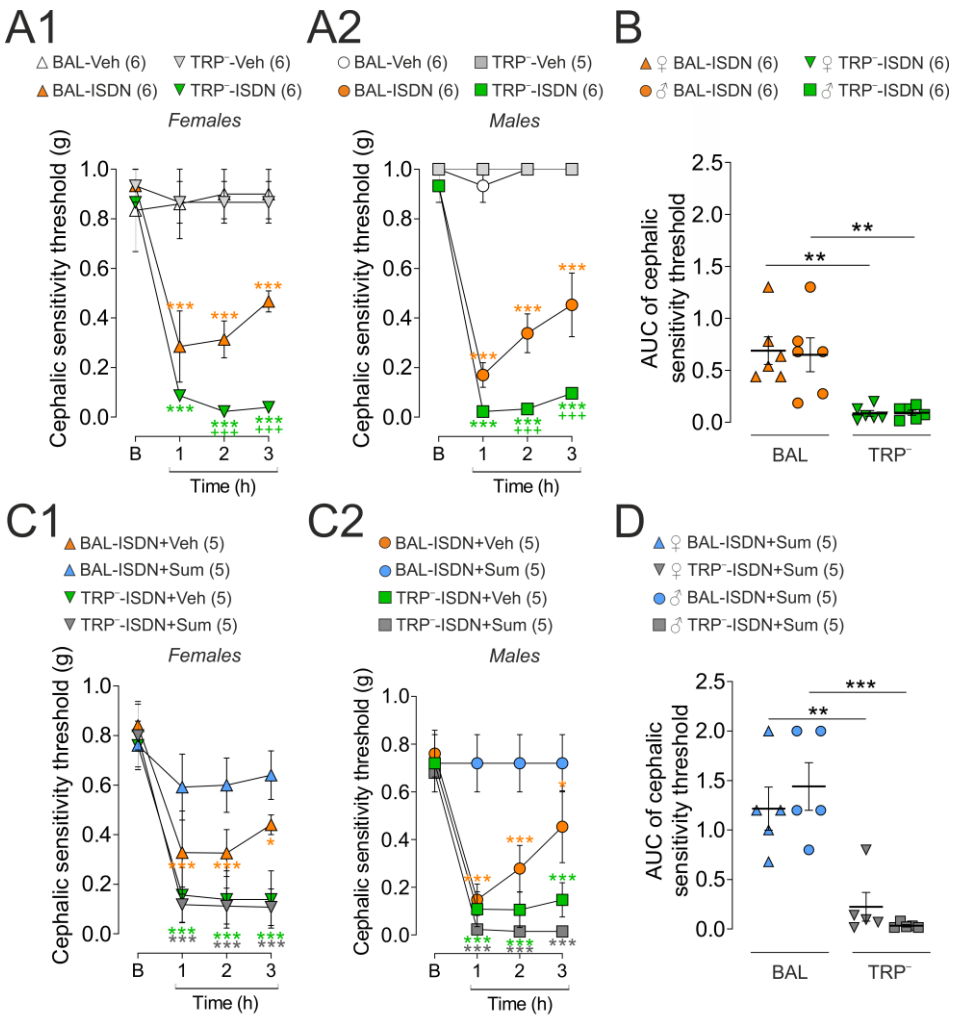


Figure 2. Effect of TRP Deficiency on Cephalic Mechanical Sensitivity and Sumatriptan Efficacy. A- Mechanical sensitivity threshold of female (**A1**) and male (**A2**) mice in response to von Frey stimulation after ISDN or vehicle administration at one week of balanced (BAL) or TRP-deficient (TRP^{-}) groups. Data are presented as mean ± S.E.M of (n) mice per group. ***$p < 0.001$ vs. baseline, $^{+++}p < 0.001$ vs. BAL-ISDN group by Tukey HSD posttest following RM 3-way ANOVA. **B-** Scatter plot shows the area under the curve (AUC) of the cephalic sensitivity threshold for both females and males. The horizontal line represents the mean ± S.E.M of (n) mice per group. Symbols represent individual values. **$p < 0.01$ by Tukey HSD following 2-way ANOVA. **C-** Sumatriptan or its vehicle was injected 5 min after the ISDN injection in female **(C1)** and male **(C2)** mice in BAL and TRP^{-} groups. *$p < 0.05$, ***$p < 0.001$ vs. baseline by Tukey HSD following RM 3-way ANOVA. **D-** Scatter plot shows the AUC of the cephalic sensitivity threshold after sumatriptan treatment. **$p < 0.01$, ***$p < 0.001$ by Tukey HSD following 2-way ANOVA.

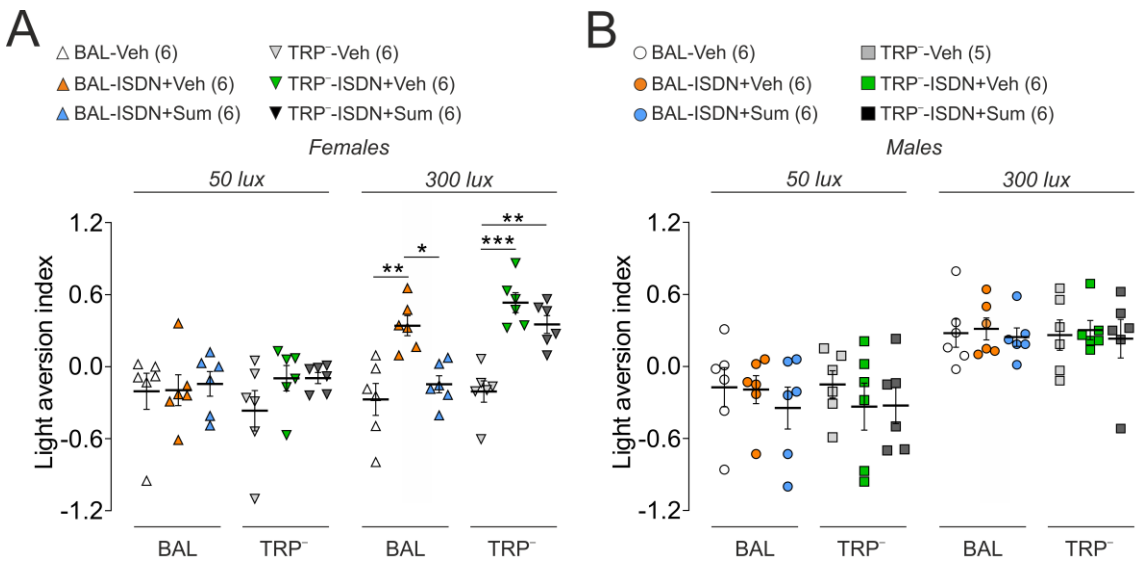


Figure 3. Effect of TRP-Deficiency on Light Sensitivity and Sumatriptan Efficacy. Light aversion index (LAI) obtained from female **(A)** and male **(B)** mice in the mEPM test after ISDN or vehicle administration at one week of balanced (BAL) or TRP-deficient (TRP^{-}) diet. Two light stimulations were tested: 50 and 300 lux. Sumatriptan or its vehicle was injected 5 min after ISDN. The horizontal line represents the mean ± S.E.M of (n) mice per group. Symbols correspond to individual values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ by Tukey HSD posttest following 2-way ANOVA.

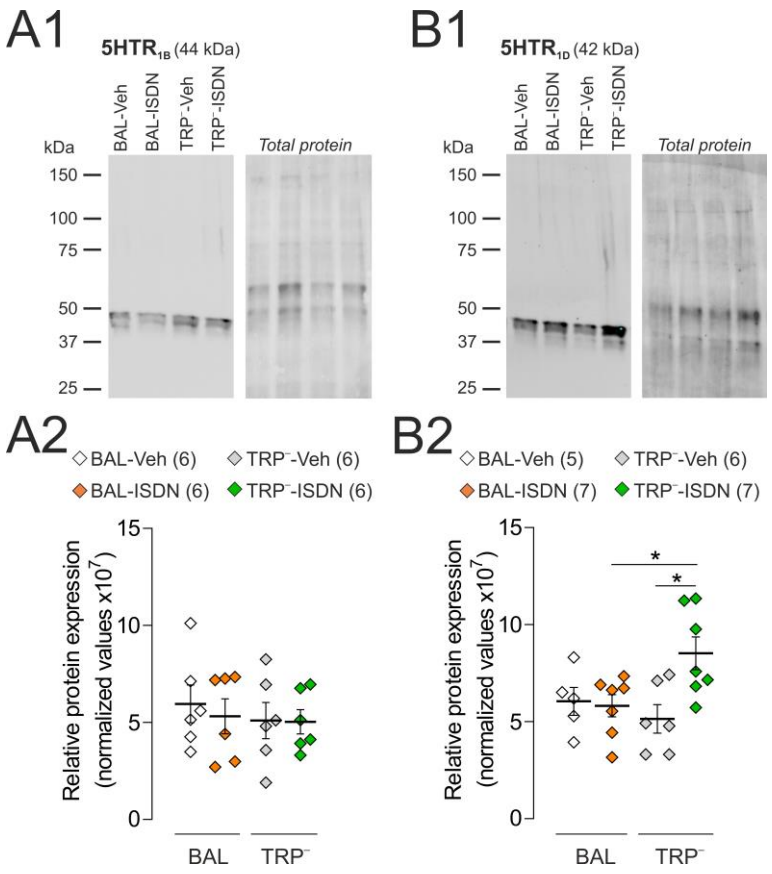


Figure 4. Expression of 5HTR$_{1B/1D}$ in the Sp5C. The effect of ISDN or vehicle administration on (**A)** 5HT$_{1B}$ and (**B)** 5HT$_{1D}$ receptor expression in the Sp5C was evaluated by western blot in balanced (BAL) or TRP-deficient (TRP^{-}) groups. Representative blot for each receptor (**A1** and **B1**) with the total protein used for normalization. Scatter plots show the relative intensity of 5HTR$_{1B}$ (**A2**) and 5HTR$_{1D}$ (**B2**). The horizontal line represents the mean ± SEM of (n) mice per group (males and females were pooled). Symbols correspond to individual values obtained from the average of 3 replicates. * $p < 0.05$ by Tukey HSD posttest following 2-way ANOVA.

TABLES

Table 1. Diet Composition

	BAL diet	**TRP-deficient diet**
Component	*Content*	*Content*
Cereals	60.4%	60.6%
Vegetal Proteins	14.0%	14.0%
Vitamins and Minerals	4.8%	4.8%
Carbohydrates	10.0%	10.0%
Oils and Fats	10.0%	10.0%
Amino Acids	0.9%	0.7%
Arginine	13.0 g/kg	13.0 g/kg
Cystine	3.2 g/kg	3.2 g/kg
Glycine	34.1 g/kg	34.1 g/kg
Lysine	7.6 g/kg	7.6 g/kg
Methionine	4.1 g/kg	4.1 g/kg
Tryptophan	**2.3 g/kg**	**0.3 g/kg**

Both balanced (BAL) and TRP-deficient diets were formulated with the following basic ingredients: maize, gelatin, corn oil, sucrose, pre-mixture of minerals (calcium, phosphorus, sodium, potassium, magnesium, manganese, iron, copper, zinc, and chlorine), pre-mixture of vitamins (vitamin A, D3, E, K3, B1, B2, B3, B5, B6, B9, B12, biotin, and choline), and an amino acid mixture.

Table 2. Summary of Statistical Analyses Corresponding to Figures 2 to 4, and Table 3

Figure	ANOVA	$F_{[DFn, DFd]}$	*p-value*	$F_{[DFn, DFd]}$	*p-value*	$F_{[DFn, DFd]}$	*p-value*	$F_{interaction\ [DFn, DFd]}$	*p-value*
2A1	RM 3-way	$F_{Time[3,60]}$=46.6	*<0.0001*	$F_{ISDN[1,20]}$= 39.7	*<0.0001*	$F_{Diet[1,20]}$=2.2	*0.15*	$F_{[3,60]}$=2.6	*0.12*
2A2	RM 3-way	$F_{Time[3,57]}$=106.9	*<0.0001*	$F_{ISDN[1,19]}$= 230.2	*<0.0001*	$F_{Diet[1,19]}$=5.2	*0.03*	$F_{[3,57]}$=4.4	*0.01*
2B	2-way	$F_{Diet[1,20]}$=29.5	*<0.0001*	$F_{Sex[1,20]}$<0.1	*0.88*			$F_{[1,20]}$<0.1	*0.83*
2C1	RM 3-way	$F_{Time[3,48]}$=50.7	*<0.0001*	$F_{Sumatriptan[1,16]}$= 0.7	*0.40*	$F_{Diet[1,16]}$=9.8	*0.006*	$F_{[3,48]}$=2.2	*0.09*
2C2	RM 3-way	$F_{Time[3,48]}$=65.1	*<0.0001*	$F_{Sumatriptan[1,16]}$= 1.9	*0.19*	$F_{Diet[1,16]}$=17.4	*0.0007*	$F_{[3,48]}$=6.8	*0.0006*
2D	2-way	$F_{Diet[1,16]}$=45.5	*<0.0001*	$F_{Sex[1,16]}$<0.1	*0.93*			$F_{[1,16]}$=1.4	*0.26*
3A-50 lux	2-way	$F_{ISDN[2,30]}$=1.1	*0.36*	$F_{Diet[1,30]}$<0.1	*0.96*			$F_{[2,30]}$=0.6	*0.54*
3A-300 lux	2-way	$F_{ISDN[2,30]}$=27.6	*<0.0001*	$F_{Diet[1,30]}$=11.5	*0.002*			$F_{[2,30]}$=3.0	*0.07*
3B-50 lux	2-way	$F_{ISDN[2,30]}$=0.6	*0.54*	$F_{Diet[1,30]}$<0.1	*0.80*			$F_{[2,30]}$=0.2	*0.83*
3B-300 lux	2-way	$F_{ISDN[2,30]}$=0.2	*0.83*	$F_{Diet[1,30]}$<0.1	*0.89*			$F_{[2,30]}$<0.1	*0.99*
4A	2-way	$F_{ISDN[1,20]}$=0.2	*0.69*	$F_{Diet[1,20]}$=0.4	*0.52*			$F_{[1,20]}$=0.1	*0.74*
4B	2-way	$F_{ISDN[1,21]}$=4.5	*0.04*	$F_{Diet[1,21]}$=1.5	*0.24*			$F_{[1,21]}$=6.0	*0.02*
Table 3									
$[5\text{-HT}]_{serum}$ Females	2-way	$F_{ISDN[1,16]}$=4.7	*0.045*	$F_{Diet[1,16]}$=40.7	*<0.0001*			$F_{[1,16]}$=2.3	*0.15*
$[5\text{-HT}]_{serum}$ Males	2-way	$F_{ISDN[1,16]}$=7.8	*0.01*	$F_{Diet[1,16]}$=27.1	*<0.0001*			$F_{[1,16]}$=3.6	*0.08*
$[TRP]_{serum}$ Females	2-way	$F_{ISDN[1,13]}$=38.2	*<0.0001*	$F_{Diet[1,13]}$=50.5	*<0.0001*			$F_{[1,13]}$=12.4	*0.004*
$[TRP]_{serum}$ Males	2-way	$F_{ISDN[1,13]}$=7.9	*0.01*	$F_{Diet[1,13]}$=28.1	*<0.0001*			$F_{[1,13]}$=4.6	*0.05*
$[TRP]_{Sp5c}$	2-way	$F_{ISDN[1,19]}$=1.2	*0.29*	$F_{Diet[1,19]}$=1.4	*0.25*			$F_{[1,19]}$=0.2	*0.63*

Data represent F values for repeated measures (RM) or non-repeated measures 2 or 3-way ANOVA with corresponding degrees of freedom (DFn, DFd), and p-values. ANOVA factors were designed as followed: *Time*, for comparisons between different time points; *Diet*, for comparisons between balanced (BAL) and TRP-deficient diet (TRP–); *ISDN*, for comparisons between vehicle and ISDN groups; *Sumatriptan* for comparisons between vehicle and sumatriptan groups; *Sex*, for comparisons between female and male mice; and *Interaction*, for the effect between factors on the dependent variable. p <0.05 was considered statistically significant.

Table 3. Levels of Tryptophan and Serotonin

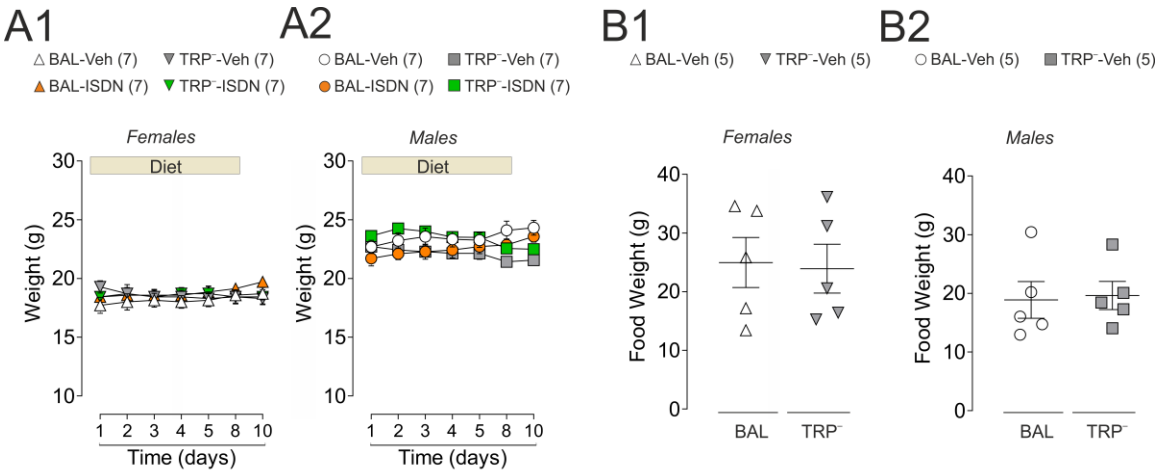
	Female	Male
Serum TRP concentration (µM)		
BAL-Vehicle	176.0 ± 16.0 (5)	90.2 ± 10.9$^{\$\$}$ (5)
BAL-ISDN	66.0 ± 7.5 (4)	50.7 ± 6.8 (4)
TRP^{-}-Vehicle	55.5 ± 5.6*** (5)	30.1 ± 2.3*** (4)
TRP^{-}-ISDN	25.3 ± 7.8 (3)	25.6 ± 6.3 (4)
Serum 5-HT concentration (µM)		
BAL-Vehicle	1.63 ± 0.05 (5)	1.68 ± 0.08 (5)
BAL-ISDN	1.79 ± 0.05 (5)	1.73 ± 0.05 (5)
TRP^{-}-Vehicle	1.42 ± 0.04* (5)	1.31 ± 0.03*** (5)
TRP^{-}-ISDN	1.45 ± 0.03$^{\#\#\#}$ (5)	1.56 ± 0.04$^{\&}$ (5)
TRP concentration in the Sp5C (nM)		
BAL-Vehicle	3.0 ± 0.3 (5)	
BAL-ISDN	3.6 ± 0.3 (6)	
TRP^{-}-Vehicle	2.7 ± 0.5 (6)	
TRP^{-}-ISDN	2.9 ± 0.4 (6)	

Data represent mean ± SEM of (n) mice per group. Serum levels of tryptophan (TRP) and serotonin (5-HT) in both sexes were measured after one week of a balanced (BAL) or Trp-deficient (TRP^{-}) diet by ELISA. Trp levels in the Sp5C were measured one week after diets by HPLC (males and females were pooled). Samples were collected 1 h after ISDN or vehicle i.p. administration. *p <0.05 and *** p <0.001 *vs.* BAL-Vehicle group; $^{\#\#\#}$ p <0.05 *vs.* BAL-ISDN group; $^{\&}$ p <0.05 *vs.* TRP^{-}-Vehicle group by Tukey's HSD post-hoc test following 2-way ANOVA. $^{\$\$}$ p <0.01 female *vs.* male by Student-t test.

SUPPLEMENTARY DATA

TRP deficiency does not affect the animal well being

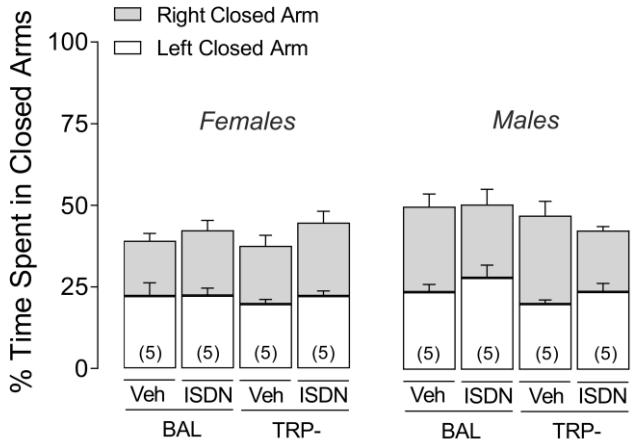
Mice well-being under balanced or TRP-deficient food conditions coupled or not with an ISDN/vehicle administration was evaluated on a daily basis through the measurement of the body weight over 10 days. All mice showed no overall robust change in their weight with similar progression in TRP-deficient and control animals, in both ISDN or vehicle-injected groups (Figures S1A1 and S1A2). In addition, no change was seen in the amount of food consumed during the period of deficiency (Figures S1B1 and S1B2).



Supplementary Figure S1. A- Body weight of female (**A1**) and male **(A2**) mice after ISDN or vehicle administration, in balanced (BAL) or TRP-deficient (TRP^{-}) groups. P>0.05 by Tukey HSD posttest following RM 3-way ANOVA. **B-** Scatter plots showing the food consumption in female (**B1**) and male **(B2**) mice in BAL and TRP^{-} groups. Data represent mean ± SEM of (n) mice per group. $p > 0.05$ by Student's t-test.

Neither TRP-deficiency nor ISDN induces an anxiety-like behavior

Assessment of anxiety-like behavior was done through measurement of the percentage of time spent in the closed arms during the 5 min of LED-off condition in the mEPM under a balanced and TRP-deficient diet, and/or after an ISDN/vehicle administration (Figure S2). Both male and female mice spent an equal amount of time inside both arms (right and left) with no preference for any one of them. Hence, neither ISDN nor TRP-deficiency induced an anxiogenic effect.



Supplementary Figure S2. Assessment of the Anxiety-like Behavior. The percentage of time spent in the left and right closed arms in the mEPM was evaluated in both male and female mice after ISDN or vehicle administration, and one week of a balanced (BAL, control) or TRP-deficient (TRP^{-}) diet. Data represent mean ± SEM of (n) mice per group. $p > 0.05$ by Tukey HSD posttest following 3-way ANOVA.

Table S1. Summary of Statistical Analyses Corresponding to Figures S1 and S2

Figure	**Statistical analysis**	**$F_{[DFn, DFd]}$**	***p-value***	**$F_{[DFn, DFd]}$**	***p-value***	**$F_{[DFn, DFd]}$**	***p-value***	**$F_{interaction\ [DFn, DFd]}$**	***p-value***
S1A1	RM 3-way	$F_{Time[6,168]}$=0.20	*0.96*	$F_{ISDN[1,168]}$= 2.40	*0.13*	$F_{Diet[1,168]}$<0.1	*0.85*	$F_{[1,168]}$=0.20	*0.99*
S1A2	RM 3-way	$F_{Time[6,168]}$=0.30	*0.96*	$F_{ISDN[1,168]}$= 0.70	*0.40*	$F_{Diet[1,168]}$=1.50	*0.22*	$F_{[1,168]}$=0.20	*0.96*
S1B1	Student t-test	$t_{4,4}$=1.10	*0.96*						
S1B2	Student t-test	$t_{4,4}$=1.70	*0.61*						
S2 Female	3-way	$F_{Diet[1,32]}$<0.1	*0.92*	$F_{ISDN[1,32]}$=1.70	*0.20*	$F_{Side[1,32]}$=1.30	*0.26*	$F_{[1,32]}$<0.10	*0.92*
S2 Males	3-way	$F_{Diet[1,32]}$=0.70	*0.41*	$F_{ISDN[1,32]}$=0.70	*0.42*	$F_{Side[1,32]}$<0.1	*0.84*	$F_{[1,32]}$=0.70	*0.42*

Data represent F values for repeated measures(RM) or non-repeated measures 2 or 3-way ANOVA, and t values for Student t-test, with corresponding degrees of freedom (DFn, DFd), and p-values. ANOVA factors were designed as followed: *Time*, for comparisons between different time points; *Diet*, for comparisons between balanced (BAL) and TRP-deficiency (TRP^{-}) diets; *ISDN*, for comparisons between vehicle and ISDN groups; *Side*, for comparisons between left and right closed arms in the mEPM; and *Interaction*, for the effect between factors on the dependent variable. p <0.05 was considered statistically significant.

STUDY II

Migraine-like Pain is Concomitant with Enhanced Neuroinflammation and Pro-nociceptive AhR Activity in the Locus Coeruleus

Yara Mrad[1], Anais Raoelina[1], Vidosav Rajcic[1], Ines Erkizia-Santamaria[2], Alicia García Culebras[5,6,7], María Isabel Cuartero Desviat[5,6,7], Igor Horrillo[2,3,4], Maria Angeles Moro[5,6,7], Radhouane Dallel[1], Cristina Alba-Delgado[1*]

[1]Université Clermont Auvergne, CHU Clermont-Ferrand, Inserm, Neuro-Dol, F-63000 Clermont-Ferrand, France; [2]Department of Pharmacology, University of the Basque Country UPV/EHU, Leioa, Bizkaia, Spain; [3]Centro de Investigación Biomédica en Red de Salud Mental, Instituto de Salud Carlos III, Spain; [4]Biocruces Bizkaia Health Research Institute, Barakaldo, Bizkaia, Spain; [5]Neurovascular Pathophysiology, Cardiovascular Risk Factor and Brain Function Programme, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain; [6]Unidad de Investigación Neurovascular, Departamento de Farmacología, Facultad de Medicina, Universidad Complutense de Madrid (UCM), Madrid, Spain; [7]Instituto de Investigación Hospital 12 de Octubre (i+12), Madrid, Spain.

***Correspondance to**: Dr. Cristina Alba-Delgado; Université Clermont Auvergne, INSERM/UCA U1107 Neuro-Dol, Faculté de Chirurgie Dentaire, 2 Rue de Braga, Clermont-Ferrand, 63100, France. Phone: +33473177978. Email: cristina.alba_delgado@uca.fr

ABSTRACT

Chronic migraine (CM) is a debilitating neurological condition that affects more than 2% of the worldwide population. The underlying mechanisms of progression from episodic to CM remain unclear. Enhanced inflammatory responses have been described in migraineurs, which could modulate the central sensitization of the trigeminal system, and hence, the initiation and persistence of cutaneous mechanical hypersensitivity (CMH). Factors triggering this abnormal inflammatory signaling are unknown. However, tryptophan (TRP)-derived metabolites, which are involved in migraine pathophysiology, have been demonstrated to modulate inflammatory responses by aryl hydrocarbon receptor (AhR) activation. Here, we evaluated the contribution of the dysregulation of TRP metabolism and neuroinflammatory responses in the progression of migraine-like pain induced by the systemic administration of isosorbide dinitrate (ISDN) in female mice. A single ISDN injection induced transitory CMH while repeated injections evoked persistent CMH. The pain was concomitant with increased systemic release of pro-inflammatory cytokines (IL-6, IL-1β, and TNFα), indoleamine 2,3-dioxygenase (IDO) activity, and astrogliosis at the level of the locus coeruleus (LC) nucleus (a noradrenergic descending modulator of trigeminal nociceptive responses). Chronic TRP deficiency induced by a TRP-deficient diet, prolonged ISDN-induced CMH, and contributed to an abnormal pro-inflammatory state by stimulating microglial activation and noradrenergic impairment (tyrosine hydroxylase increase coupled with decreased noradrenaline release) in the LC. 2-(1′H-indole-3′-carbonyl)thiazole-4-carboxylic acid methyl ester (ITE)-induced AhR activation potentiated the ISDN-evoked CMH while 6,2′,4′-trimethoxyflavone (TMF)-induced AhR inhibition had an analgesic effect. Our findings support the pro-nociceptive action of AhR and dietary TRP deficiency in migraine progression and highlight the role of the LC in CM.

INTRODUCTION

Chronic migraine (CM) is a disabling neurological condition with a global prevalence of 2%, 2.5 to 6.5-fold higher in women than in men (Natoli et al. 2010). It is characterized by recurrent headache attacks (≥15 days/month) (IHS 2018), accompanied by cutaneous hypersensitivity in more than 40% of cases (Louter et al. 2013). Each year, ~3% of episodic migraineurs suffer from increasing attack frequencies and progress gradually to a CM state (A. I. Scher et al. 2003). Although underlying physiological mechanisms of this progression remain unclear, altered modulation of pain pathways and trigeminal central sensitization represent physiological correlates of chronification (Bigal and Lipton 2008).

Impaired descending inhibition from the brainstem is a contributor to this progression (Akerman, Holland, and Goadsby 2011). Among the brainstem nuclei, the locus coeruleus (LC) has been involved in the pathophysiology of migraine. It is activated by intracranial trigeminal stimulation in rats (Ter Horst et al. 2001). As the main source of noradrenaline (NA) descending inputs to the trigeminal complex, it can modulate nociceptive responses (Sasa et al. 1974; Vila-Pueyo et al. 2019). In addition, neuroimaging studies highlighted altered LC functional connectivity in migraineurs (Moulton et al. 2014), and LC activation following nitroglycerin (NTG) infusion (Maniyar et al. 2014) and during spontaneous headaches (Weiller et al. 1995).

Emerging data also suggest that neuroinflammation is a modulator of central sensitization. Increased levels of pro-inflammatory chemokines and cytokines have been detected during ictal and interictal periods in migraine sufferers (Biscetti et al. 2022). In rats, NTG-induced CM was correlated with increased transcriptional activity of interleukin (IL)-6 and tumor necrosis factor (TNF)α in the trigeminal ganglia and pons (including the LC) (Greco et al. 2017), and with microglial activation in the spinal trigeminal nucleus caudalis (Sp5C), contributing to central sensitization (He et al. 2019).

Together, these data place both the LC and neuroinflammation as potential key players in migraine progression. However, how neuroinflammatory signaling is initiated and the exact role of the LC in this process are unknown. Recently, tryptophan (TRP) and its metabolites (mainly kynurenine -KYN- and serotonin derivatives) have been demonstrated to be associated with neuroinflammation (Rothhammer et al. 2016) and migraine pathophysiology (Deen et al. 2019; P. Drummond 2006; Körtési, Spekker, and

Vécsei 2022). TRP regulates inflammatory processes through the activation of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor involved in neural and glial functions (Juricek and Coumoul 2018), and linked to various chronic diseases (Rothhammer and Quintana 2019). Although its contribution to migraine has not yet been studied, evidence supports it: (i) TRP-derived AhR agonists are key regulators of migraine pathophysiology (Deen et al. 2019; Körtési, Spekker, and Vécsei 2022); AhR is expressed in migraine-related-regions (Petersen et al. 2000) including the LC (E. Kimura et al. 2021); (ii) its activation directly regulates the tyrosine hydroxylase (TH) expression (Akahoshi et al. 2009), and hence the production of the analgesic NA; while its depletion increases cutaneous sensitivity in chronic neuropathic mice (Sheu et al. 2022).

Here, we evaluate the contribution of TRP metabolism and AhR activity in regulating migraine-like pain induced by the systemic isosorbide dinitrate (ISDN) administration (Dallel, Descheemaeker, and Luccarini 2018): indirectly by submitting female mice to a chronic TRP deficient diet (that reduces endogenous AhR agonists); and directly by pharmacological AhR activation/inhibition. We also investigated the neuroinflammatory state, including glial activation and cytokine release. Our findings establish a critical link between perturbed TRP metabolism, dysregulated neuroinflammation in the LC, and AhR in migraine progression.

MATERIAL AND METHODS

1. Animals

Adult female C57BL/6 mice (7 to 12 weeks old) were obtained from Charles River (L'Arbresle, France) or born in our amenities. Mice were housed in the same room under standard conditions (12h light/dark cycle, *ad libitum* access to food and water, environmental enrichment). Procedures were performed according to the experimental design (Figure 1A) between 8 AM and 3 PM (dark phase). At the end of the experiments, mice were sacrificed (150 mg/kg, i.p. of sodium pentobarbital; CEVA Santé Animale, Libourne, France) and death was confirmed by permanent cessation of cardiorespiratory functions. All efforts were made to minimize the number of animals used.

2. Study approval

Experiences were performed following ARRIVE (Animals in Research: reporting of in vivo experiments) guidelines, and the ethical guidelines of the Directive 2010/63/UE of

the European Parliament and the Council and French Decree 2013-118 on the protection of animals used for scientific purposes. Protocols were approved by the French Ministry of Higher Education and Research (Ref. 2020012716162262).

3. Diets

Mice were randomly assigned to one of two dietary conditions: the balanced diet (BAL; control diet) that contained 2.3 g of TRP per kg of food; or the TRP deficient diet (TRP^{-}) that contained 0.3 g of TRP/kg. Diets were formulated with 60.4% of cereals, 14.0% of vegetal proteins, 4.8% of vitamins and minerals mixture, 10.0% of carbohydrates, 10.0% of oils and fats, and 0.9% of amino acid mixture, which contains (in g per kg of food): 13.0 arginine, 3.2 cystine, 34.1 glycine, 7.6 lysine, 4.1 methionine, and the corresponding amount of TRP (SAFE® Custom Diets company, Augy, France). Both diets have similar pellets (10-12 mm diameter) and crushing resistance (~5 kgf/cm^2). Each diet was started at 7-8 weeks of age and continued from 7 to 30 days according to the experimental design (Figure 1A). Body weight was measured on a daily basis during the observation period.

4. Drug administration

Single (ISDN-1 or Veh-1) or repeated (ISDN-3 or Veh-3; twice per day for two days) intraperitoneal (i.p.) injections of ISDN (10 mg/kg in a 10 ml/kg volume; Risordan®, Sanofi-Aventis, Paris, France) or vehicle (0.9% NaCl) were administrated as described in (Dallel, Descheemaeker, and Luccarini 2018). 6,2′,4′-trimethoxyflavone (TMF; T4080, Sigma-Aldrich, St. Quentin Fallavier, France), a potent AhR antagonist, was injected i.p. (5 mg/kg in 10 ml/kg volume, dissolved in 2% DMSO in saline) 10 min after every repeated ISDN/vehicle injection. 2-(1′H-Indole-3′-carbonyl) thiazole-4-carboxylic acid methyl ester (ITE; SML3139, Sigma-Aldrich, St. Quentin Fallavier, France), an AhR agonist, was injected i.p. (10 mg/kg in 10 ml/kg volume in saline) 5 h before the single ISDN/vehicle injection.

5. Behavioral assessment

5.1. Cephalic mechanical sensitivity

The periorbital mechanical sensitivity to non-noxious stimuli was measured by using a series of eight von Frey filaments (from 0.008, 0.02, 0.04, 0.07, 0.16, 0.4, 0.6, and 1.0 g; 1.0-g cut-off; Bioseb, Vitrolles, France) as described previously (Alba-Delgado et al. 2018). For the first three days, habituation was done using the 0.6 g filament. Testing

began with the 0.4 g filament for baseline measurement and with the last weak filament used for measurements after drug administration. Stimuli were presented perpendicularly to the left periorbital surface of the forehead, using an up-down sequence five times at intervals of at least 5 s. The detection of a positive response three or more times lead to choosing the next weaker stimulus. In the absence of a positive response, a stronger stimulus was presented. Eye winking, turning the head away, attacking the tip of the filament, and/or body freezing were considered positive detection responses. The cephalic mechanical sensitivity threshold (in g) was determined as the weakest von Frey filament used which obtained three or more positive responses.

Measurements were performed before (baseline), every hour for 3 h after single or repetitive ISDN/vehicle administrations, and prolonged to 30 days after the last repeated ISDN/vehicle injection (Figure 1A). The effect of ITE or TMF was also measured (Figure 1A). Mice showing distress signs not habituated to the 0.6 g filament before the testing day were excluded.

5.2. Extra-cephalic Mechanical Sensitivity

The mechanical sensitivity of the hind paw to non-noxious stimuli was also measured by von Frey filaments (from 0.008 to 1.4 g; 1.4 g cut-off; Bioseb, Vitrolles, France). Habituation was done using the 1.0 g filament. Stimuli were presented perpendicularly to the bottom of the left or right hind paw, using the up-down sequence five times at intervals of at least 5 s. Like cephalic sensitivity, the hind-paw mechanical sensitivity threshold (in g) was determined as the weakest von Frey filament used which obtained three or more positive responses. Paw shaking or withdrawal, attacking the filament tip, and/or freezing were considered positive responses. As no differences between the left and right paw were found, an average of values was done. Measurements were performed before (baseline), every hour for 3 h after repetitive ISDN/vehicle administrations, and prolonged to 3 days after the last injection, according to the experimental protocol (Figure 1A).

5.3. Anxiety-like behavior

The assessment of anxiety-like behavior was done using the open-field test (OFT). The free movement of mice was recorded in an open square box (50x50x30 cm high) for a 5-min duration using a digital infrared camera (DMK 21AF04, The Imaging Source

Europe GmbH, Bremen, Germany) connected to an analysis software (EthoVision XT, version 13.0; Noldus Information Technology, Wageningen, Netherlands). Two virtual zones were delimited: center (in the middle of the arena; 30x30 cm) and periphery (surrounding border zone with 20 cm in width). The time spent in the peripheral zone (in %) and the total distance covered in the open area (in m) were measured 30 min after the last repeated ISDN/vehicle injection and 30 days after the start of the diet (Figure 1A).

6. Quantification of Metabolites and Cytokine Levels

6.1. Blood and tissue preparation

Sample collection was performed 1 h after ISDN/vehicle injection (Figure 1A). Mice were fasted for 1 h and then anesthetized with 25% urethane (1.5 g/kg i.p.). Blood was collected after decapitation in tubes with cold heparin (50 µl/tube; Heparine Choay, Vidal, France) and centrifuged at 5000 x g for 12 min at 4 °C. Plasma was carefully collected and immediately frozen in liquid nitrogen. At the same time, the brain was also collected, and the two LC manually dissected and immediately frozen in liquid nitrogen. All samples were finally stored at -80 °C for later use.

6.2. Detection by HPLC

The quantification of plasmatic and cerebral TRP, KYN, and NA concentrations was performed by high-performance liquid chromatography (HPLC) with electrochemical detection according to (Unzueta-Larrinaga et al. 2023). Samples were first homogenized using an Ultra-Turrax T8 homogenizer in 15 µL/mg homogenization buffer (0.1 M HClO4 and 100 µl EDTA). The crude homogenate was centrifuged at 21,000 x g for 15 min at 4 ºC, and the supernatant was diluted 1:5. All samples were then filtered by centrifugation at 15,000 x g for 15 min using Costar® Spin-X® Centrifuge Tubes (0.22 µm Pore CA Membrane, Merck Life Science S.L.U., Madrid, Spain). The concentration of TRP, KYN, and NA was estimated with reference to standards prepared and injected on the same day. The mobile phase composition was 150 mM H2NaPO4, 0.2 mM EDTA, 4.3 mM octyl sodium sulfate (pH 6.3), and 8% (vol/vol) methanol. The filtered mobile phase was delivered at a flow rate of 0.2 ml/min by a Hewlett-Packard model 1200 pump (Agilent Technologies Deutschland GmbH, Waldbronn, Germany). Sample separation was carried out at 30 °C on a Zorbax Eclipse Plus column (3.5 µm C18, 2.1×150 mm, Agilent Technologies, Madrid, Spain). Metabolites' amperometric

detection was carried out using a Hewlett-Packard model 1049 A detector (Agilent Technologies Deutschland GmbH, Waldbronn, Germany) at an oxidizing potential of + 950 mV.

6.3. Detection by ELISA

The quantification of the plasmatic concentration of pro-inflammatory cytokines (IL-1β, IL-6, interferon-gamma -IFNγ- and TNFα) was performed by a Multiplex ELISA kit (ARG82842, ArigoPLEX®, Arigo Biolaboratories, Hsinchu City, Taiwan) according to the manufacturer's protocol. The intensity of sample color was measured at 450 nm by a microplate reader (BioTek Epoch Microplate Spectrophotometer, BioTek Instruments SAS, Colmar, France). The final concentration of cytokines was determined through comparisons with the standard curve.

7. Quantification of Protein and mRNA Relative Expression

7.1. Tissue preparation

Sample collection was performed 1 h after ISDN/vehicle injection (Figure 1A). Mice were fasted for 1 h and then anesthetized with 25% urethane (1.5 g/kg i.p.). Brains were collected after decapitation, and both right and left LC were manually dissected and immediately frozen together in liquid nitrogen. All samples were finally stored at -80 °C for later use.

7.2. Western blot

LC samples were homogenized using a lysis buffer containing EDTA (0.5 M), Trizma base (50 mM), sodium chloride (10 mM), Triton 100% (0.25%), and supplemented with a cocktail of protease and phosphatase inhibitors (Thermo Fisher Scientific, France). An equal amount of total protein (20 µg/each sample) was resolved in each lane of 10% SDS-PAGE gels using Mini-PROTEAN Tetra Vertical Electrophoresis Cell (Bio-Rad Laboratories) at 80 V for 15 min followed by 200V for approximately 30 min. The resolved proteins were then transferred onto a PVDF membrane (Merck Chimie SAS Île-de-France, France) using a Mini Trans-Blot® Cell (Bio-Rad Laboratories) at 100 mA for 1 h. Thereafter, membranes were incubated at 25 °C for 1 h with a solution containing 1% Casein in tris-buffered saline (TBS) to block non-specific bindings. Membranes were then incubated overnight at 4 °C with the primary antibody rabbit anti-TH (1:1000; ab152, Merck Millipore SAS, Île-de-France, France). Subsequently,

they were washed with TBS 1X and incubated with the StarBright Blue 700 goat anti-rabbit secondary antibody (1:2,500; 1200416, Bio-rad Laboratories, Marnes-la-Coquette, France) at room temperature for 2 h. Blots were visualized with the ChemiDoc XRS system (Bio-Rad Laboratories) and analyzed using the software Image Lab (v6.1, Bio-Rad Laboratories).

7.3. Real-time PCR

LC samples were prepared with TRIzol Reagent (15596026, Thermofisher Scientific, Massachusetts, United States) and mRNA was reverse-transcribed with the high-capacity cDNA Reverse Transcription kit (Applied Biosystems; Carlsbad, CA, USA) according to the manufacturer's protocol. Real-time quantitative PCR assays (RT-PCR) were performed with an Applied Biosystems 7900HT Fast RT- PCR System sequencer detector (4329001, Thermofisher Scientific). The mRNA expression was normalized to that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and expressed as the fold difference relative to the vehicle-injected group. All primers and mouse probes were from Applied Biosystems (Thermofisher scientific): AhR (Mm00478932_m1), Cyp1a1 (Mm00487218_m1), Cyp1b1 (Mm00487229_m1), AhRR (Mm00477443_m1), and GAPDH (Mm99999915_g1).

8. Quantification of Immunolabeled Cell Number and Morphometric Analysis

8.1. Preparation of sections

Sample collection was performed 1 h after ISDN/vehicle injection (Figure 1A). Under anesthesia (25% urethane, 1.5 g/kg i.p.), mice were perfused intracardially, first with saline (0.9% NaCl), then with a paraformaldehyde (PFA) solution (DiaPath Antigenfix P0014, DiaPath, Martinengo, Italy). Brains were then harvested and post-fixed in 4% PFA for 2 h at 4 °C before being cryoprotected in phosphate-buffered saline 0.1 M (PBS, pH 7.4) containing 30% sucrose and 0.05% sodium azide for at least 2 days at 4 °C. Slices (40 µm thickness) containing the LC region (from -1.54 and -1.40 mm interaural and -5.34 and -5.20 mm relative to the Bregma towards -2.00 and -1.88 mm interaural and -5.80 and -5.68 mm from the Bregma) (Paxinos and Franklin 2019) were cut using a microtome (MICROM GmbH, Neuss, Germany) and preserved in a solution containing 0.1 M PBS, 40% glycerol, and 0.05% sodium azide at -20 °C until usage.

8.2. Immunofluorescence assays

Sections were washed with 0.1 M PBS + 0.3% Triton X100 (0.3% PBST) and held for 1 h in a blocking solution containing 0.15M Glycine, 10% normal horse serum (NHS), and 0.3% PBST at room temperature. They were then incubated with the primary antibodies: rabbit anti-AhR (1:150; BML-SA210-0100, Enzo Life Sciences, Villeurbanne, France), mouse anti-GFAP (1:500; G3893, Sigma Aldrich Chimie S.a.r.l, Saint-Quentin-Fallavier, France), guinea pig anti-IBA1 (1:250; 234308, Synaptic Systems GmbH, Goettingen, Germany) and sheep anti-TH (1:300; ab113, Merck Millipore SAS, Île-de-France, France) for 4 days at 4 °C. Afterward, sections were rinsed with 0.3% PBST, and incubated with the secondary antibodies (from Jackson ImmunoResearch Europe Ltd, Ely, United Kingdom) for 2 h at room temperature: DyLight 405 donkey anti-rabbit (1:200; 711-475-152), Alexa 488 goat anti-mouse (1:200; 715-545-003), Cy5 donkey anti-guinea pig (1:200; 706-175-148) and Cy3 donkey anti-sheep (1:200; 713-165-003) respectively. Finally, sections were mounted on gelatinized slides in a DPX mounting medium, coverslipped, and conserved at 4 °C.

8.3. Image acquisition and analysis

LC immunofluorescence sections were examined using an epifluorescence microscope Zeiss LSM 800 (Carl Zeiss, Aalen, Germany) equipped with ZEISS Microscope Software ZEN lite software. Images were acquired at x10 and x40 magnifications under the same conditions. Images were post-processed using the Fiji software (ImageJ). Counting of immunoreactive (IR) neurons to different target proteins was initiated at the most rostral section of the LC (from AP -5.2 to AP -5.34, relative to Bregma), continued through the middle portion (from AP -5.34 to AP -5.52) and finished in the most caudal slide (from AP -5.52 to AP -5.8; Figure S1) (Paxinos and Franklin 2019). On average, counting was conducted on three to ten sections per animal, and in all cases, at least one rostral, one middle, and one caudal section were included for all analyses.

8.4. Quantification of immunoreactive (IR) cells

Quantifications of the number of TH-IR cells and the TH-positive area (that included TH-IR body cells and their labeled ramifications) were done by using the Fiji Count plugin from images taken at a 40X magnification. The number of neurons that co-expressed AhR and TH labeling was evaluated manually and expressed as the percentage of TH-IR cells expressing AhR (AhR^+). In addition, the morphological

characteristics (including the body and nuclear size) of these neurons were analyzed. Microglia or astrocyte density and body cell sizes were quantified by IBA1 or GFAP immunolabelling respectively at the level of the TH-IR body cells and projection regions of the LC by using images taken at a 10X magnification.

8.5. Morphological and structural analysis of astrocytes

Reconstructions of three-dimensional (3D) GFAP-labeled astrocytes were manually performed from x40 images using the Simple Neurite Tracer plugin of Fiji software. Cell complexity was analyzed using the fractal dimension approach by the box-counting method obtained from the Fractal Box count tool. Quantifications of the number and the total length of astrocyte branches were performed automatically after 3D reconstruction. A branch was defined as a continuous projection from the cell body or at a branch point ending. Branch organization around the cell bodies was assessed by using the Sholl analysis tool. Concentric circles around the soma were traced with radii increasing by 2.0 μm. Afterward, the global number of interactions for every astrocyte is added up and graphed as a function of the radial distance from the cell body.

9. Statistical analysis

The data were presented as mean ± SEM. The number (n) of mice utilized for each analysis was shown in Figures or Tables. The normality distribution of each data set was measured by the Shapiro-Wilk test. Unpaired Student's t-test alone or with Welch's correction was used to compare mean values between two groups with equal or unequal variances respectively. Comparisons of more than two means were performed using repeated (RM) or non-repeated one, 2, or 3-way measure analysis of variance (ANOVA) including Geisser-Greenhouse correction to account for possible unequal variances between groups. Then, post hoc comparisons between groups were analyzed with the Tukey HSD post-hoc test. T-values, F-values, their associated degrees of freedom, and p-values, and the factors were summarized in Table 1 and Table S1 from Supplementary Results. The level of significance was set at a p-value below 0.05. All statistical analyses and graphs were performed using GraphPad Prism (v8.4.0, GraphPad Inc., La Jolla, CA, USA). Figures were made using the CorelDraw Graphics software (v12.0, Ottawa, Canada).

RESULTS

The TRP-deficient diet reduces TRP but not KYN levels in the plasma

Mice subjected to one week of a TRP-deficient diet showed a significant decrease in TRP concentration compared to physiological levels observed after one week of the BAL diet (Table 2). The percentage of this reduction was similar between groups: ~70.5% for vehicle-injected mice, ~62.0% 1 h after the first ISDN injection (ISDN-1), and ~56.9% 1 h after the third ISDN injection (ISDN-3). Plasma levels of KYN, which is the main endogenous metabolic pathway of TRP (Badawy 2017), were not altered after one week of a TRP-deficient diet consumption in either group compared to vehicle-injected mice subjected to the BAL diet (Table 2). However, the KYN/TRP ratio was augmented in all TRP^{-} groups.

In the LC, mice subjected to one week of the TRP deficient diet also showed reduced TRP concentration (Table 2), with a similar reduction percentage in the three groups: ~79.1% for vehicle-injected mice, ~62.0% 1 h after the ISDN-1, and ~48.1% 1 h after ISDN-3 administrations. KYN levels were also globally reduced as shown by the significant 2-way ANOVA (Table 1), although no individual post-hoc test differences were detected (Table 2). The KYN/TRP ratio was not augmented in all TRP^{-} groups

Metabolic reduction after a TRP-deficient diet did not reduce the body weight in any experimental group (Figure S2). Moreover, it did not produce any anxiety-like behavior in the OFT; no differences were found between TRP^{-} and BAL conditions, in the total distance travelled (Figure S3A) or the total time spent in the periphery (Figure S3B). Moreover, repeated ISDN administrations did not evoke an anxiety-like behavior when compared to vehicle-injected mice.

Repeated ISDN induces persistent CMH which was prolonged by TRP deficiency

The effect of ISDN and/or a TRP-deficient diet on cephalic mechanical sensitivity was measured using the von Frey test (Figure 1). The baseline threshold in mice subjected to the BAL diet was established at 0.93 ± 0.50 g (Figure 1B Left Panel). One hour after ISDN-1, it significantly decreased (0.15 ± 0.02 g), with partial recovery at 3 h (0.47 ± 0.04 g). The baseline threshold before the third ISDN injection was still lower (0.33 ± 0.08 g) than the baseline before ISDN-1, indicating persistent interictal CMH. ISDN-3 also reduced the cephalic threshold that peaked at 1 h post-injection (0.04 ± 0.00 g;

Figure 1B Left Panel) and was similar to the ictal threshold after ISDN-1. The cephalic mechanical threshold returned to baseline level 18 days later (0.32 ± 0.05 g; Figure 1C).

Mice subjected to one week of TRP deficiency showed a baseline threshold (0.87 ± 0.08 g; Figure 1B Right Panel) similar to BAL groups, which was reduced 1 h after the first ISDN injection (0.07 ± 0.02 g). This time, no partial recovery was observed at 3 h (0.08 ± 0.03 g). CMH was also interictally observed before the third ISDN-3 injection (0.16 ± 0.07 g). Repeated ISDN-3 administration kept the threshold lowered (Figure 1B Right Panel), which needed 30 days to achieve total recovery (Figure 1C).

The effect of both diets on ISDN-induced CMH was compared by calculating the AUC of cephalic mechanical thresholds between 1 h and 3 h after ISDN administration (ictal period; Figure 1D1), and between 2 and 30 days after the third ISDN injection (interictal period; Figure 1D2). Indeed, the AUC of both ISDN-1 and ISDN-3 groups submitted to the TRP deficient diet (0.11 ±0.03 AU and 0.10 ± 0.03 AU respectively) were significantly lower than those from the BAL diet (0.55 ± 0.06 AU and 0.36 ± 0.06 AU) during the ictal period (Figure 1D1). The reduced AUC was also obtained from ISDN-3-injected mice subjected to a TRP-deficient diet for 30 days (16.07 ± 2.29 AU) compared to those following the BAL diet for the same time (6.40 ± 0.98 AU) (Figure 1D2). Moreover, when the time needed to obtain 50% cephalic threshold recovery was estimated for both groups, a marked difference was observed; it took twice as long for mice suffering from a TRP deficiency to reach the same level of recovery (Figure 1E). Hence, TRP deficiency augments ictal and interictal migraine-like pain induced by single as well as repeated ISDN injections.

The TMF-induced AhR inhibition partially blocks the ISDN-induced CMH

In order to investigate whether the effect of TRP deficiency on cephalic mechanical sensitivity was mediated by AhR, TMF, a potent AhR antagonist was administered 10 min after each repeated ISDN injection. As TMF was injected intraperitoneally, we first validated that it crossed the blood-brain barrier. Therefore, we measured the mRNA level of Cyp1A1, a target gene transcribed after AhR activation. It was significantly reduced after i.p. TMF administration in naïve mice (data not shown), as described before (Cuartero et al. 2014).

TMF alone did not induce pain in vehicle-injected mice subjected to the BAL diet, who showed a similar cephalic threshold throughout the 3 h testing period (Figure 1F1).

TMF injected after the ISDN-1 administration under a BAL regimen has a potential antalgic effect whereby the cephalic sensitivity threshold showed a plateau at ~0.6 g between 1 h and 3 h post-ISDN (Figure 1F1), and the AUC was significantly higher (1.12 ± 0.08 AU) than the group receiving ISDN-1+vehicle (0.44 ± 0.14 AU; Figure 1F1). A similar effect was also shown after repetitive ISDN-3 administrations, although in this case the analgesic effect of TMF was less marked and the cephalic threshold remained ~0.4 g (Figure 1G1), without a significant difference between the AUC of each ISDN-3 group (TMF: 0.73 ± 0.22 AU *vs.* vehicle: 0.37 ± 0.12 AU; Figure 1F2).

ISDN-1-injected mice subjected to a TRP-deficient diet and receiving TMF showed a reduced cephalic threshold from 1 h to 3 h post-ISDN, although this reduction was less important than that observed in vehicle-injected ISDN mice and ISDN-1+TMF under a BAL diet (Figure S4A). Comparison of the AUC from the groups revealed a significant difference (TMF: 0.55 ± 0.15 AU *vs.* vehicle: 0.11 ± 0.03 AU; Figure S4B), indicating that TMF did not maintain its analgesic effect under TRP deficiency.

The ITE-induced AhR activation potentiates the ISDN-induced CMH

The systemic administration of ITE, a selective nontoxic AhR agonist that crosses the BBB (Neelamegam et al. 2021) on cephalic mechanical sensitivity was evaluated in mice subjected to BAL or TRP deficient diets. The number of genes differentially regulated by ITE decreases over time, with maximal transcription 6 h post-AhR activation (Yan et al. 2019). Hence, we administered ITE 5 h before the first ISDN injection. Under BAL diet conditions, ITE on its own showed no sign of inducing nociception in vehicle-injected mice (Figure 1G1). However, when ITE was followed by a single ISDN administration, the cephalic sensitivity threshold was lower than the threshold from vehicle-injected ISDN mice (Figure 1G1), as confirmed by the reduced AUC (ITE: 0.12± 0.07 AU *vs.* vehicle: 0.70± 0.12 AU; Figure 1G2). Hence, results indicate that ITE potentiates the nociceptive action of ISDN. No effect was shown when ITE was coupled with the TRP deficient diet which was further verified by the non-significant AUC (data not shown). This could be due to the fact that mice had an initial threshold close to the detection the limit of von Frey technique.

ISDN induces transitory extracephalic mechanical hypersensitivity, which was not altered by TRP deficiency

The effect of ISDN on the mechanical sensitivity of extracephalic regions (left and right hind paws) was evaluated using the von Frey test. The baseline threshold was established at 1.20 ± 0.01 g in mice subjected to the BAL diet (Figure 2A Left Panel). A single ISDN injection reduced the extracephalic threshold from 1 h post-administration, with recovery as a function of time (Figure 2A Left Panel). The baseline threshold before the ISDN-3 injection was still lower (0.92 ± 0.08 g) than the baseline before ISDN-1, indicating persistent interictal hypersensitivity. ISDN-3 also reduced the mechanical threshold at 1 h post-injection (0.29 ±0.09 g; Figure 2A Left Panel) and was similar to the ictal threshold after ISDN-1. Extracephalic mechanical threshold returned to baseline level after 3 days.

The implementation of a TRP-deficient diet shows no difference compared to the BAL groups (Figure 2A Right Panel) as shown by the similar AUC values (Figure 2B). Hence, a TRP deficiency doesn't influence the ISDN capacity to induce transitory extracephalic mechanical hypersensitivity.

Both repeated ISDN administration and TRP deficiency provoke functional noradrenergic impairment in the LC

Under pathological conditions, impaired descending inhibition from the LC could contribute to ISDN-induced CMH. Thus, the possible changes after ISDN related to the noradrenergic system were examined by immunohistochemistry, western blot, and HPLC at 1 h after drug administration and one week after the diet started (Figure 1A). Under a BAL diet, staining with an antibody against TH revealed an increase in the TH-positive area (that includes cell bodies and their noradrenergic projections) in the total LC after the third ISDN injection (24.46 ± 2.00 $x10^4$ μm^2) but not after ISDN-1 (20.1 ± 1.60 $x10^4$ μm^2) (Figures 3A1 and 3A2). When the LC was divided into rostral, middle, and caudal sections, no differences were observed between groups (Table S2). The increased TH surface in ISDN-3-injected females was not correlated neither with an increase in the number of TH-IR cells, which was similar between all BAL-diet groups (Figures 3A3), nor with an increase of TH expression measured by western blot (Figure 3B). NA levels measured by HPLC were also similar between ISDN and vehicle-injected mice (Figure 3C).

One week after the TRP-deficient diet did not induce any change in the number of detected TH-IR cells (Figure 3A3) nor in their projection area (Figure 3A2). However, TRP deficiency increased TH expression in both vehicle and ISDN-injected groups (vehicle: 8.4 ± 1.0 x10^7, ISDN-1: 8.0 ± 1.1 x10^7, ISDN-3: 8.3 ± 1.3 x10^7; Figure 3B) compared to the BAL-diet groups (vehicle: 5.6 ± 0.7 x10^7, ISDN-1: 5.4 ± 0.8 x10^7, ISDN-3: 4.6 ± 1.1 x10^7; Figure 3B). TRP deficiency also altered the production of NA, which was significantly lower in ISDN-1 (141.7 ± 8.8 nM) and ISDN-3 (132.3± 12.4 nM) than in vehicle-injected mice (186.7 ± 17.4 nM; Figure 3C)

Both repeated ISDN and TRP deficiency provoke morphological changes in noradrenergic LC neurons

It has been described that the LC neurons express AhR (E. Kimura et al. 2021). Therefore, the effect of ISDN alone or coupled with a TRP-deficient diet on AhR function in noradrenergic LC neurons was evaluated. First, LC cells that expressed AhR were identified by immunohistochemistry (Figure 4A). Under a BAL diet, 50.4 ± 1.9 % of TH-IR cells co-expressed AhR in vehicle-injected mice (Figure 4B). A similar cell proportion was observed in the three LC sections (Table S3). A total of 253 AhR^+ TH-IR neurons (pooled from 5 vehicle-injected mice under a BAL diet) were characterized by a cell body area of 160.5 ± 3.4 μm^2 (Figure 4C1) with a nuclear area of 47.9 ± 1.1 μm^2 (Figure 4C2).

Second, the effect of ISDN and/or TRP deficiency on TH-IR cells was analyzed. Under a BAL-diet, ISDN-3 increased the global size of AhR^+ TH-IR, both the cell body (189.3 ± 6.3 μm^2; Figure 4C1) and nuclear areas (57.8 ±2.1 μm^2; Figure 4C2), compared to the vehicle-injected mice (160.5 ±3.4 μm^2 and 47.9 ± 1.1 μm^2 respectively). This change was predominately at the level of the middle and caudal LC (Table S3). No changes were observed after ISDN-1.

AhR expression and activation are altered after ISDN administration in the LC

AhR expression in the total LC, including TH-IR neurons and glial cells that also expressed AhR, was quantified by RT-PCR. Reduced mRNA expression was observed in ISDN-3 groups (BAL diet: 69.8 ± 13.0 and TRP deficient diet: 65.9 ± 17.5, as shown by the significant 2-way ANOVA (Table 1), although Tukey post-hoc test did not reveal significant intergroup differences (Figure 4D). TRP deficiency did not modify AhR

expression in vehicle-injected mice and does not have a cumulative effect in mice receiving an ISDN administration.

The activation of AhR induces its translocation to the nucleus, where it activates the transcription of target genes (Hubbard et al. 2015). Thus, the expression of AhR immunolabelling in the soma and the nucleus of AhR^+ TH-IR cells was quantified. The ratio (in %) of labeling in the nucleus to that of the cytoplasm was calculated following the method described by (E. Kimura et al. 2021) and used as an indirect measure of AhR activation. A decrease in the percentage of nuclear intensity of AhR was observed in ISDN-3-injected mice subjected to the BAL diet (31.4 ± 0.6 %) compared to the vehicle group (32.3 ± 0.78 %; Figure 4E). This reduction was mainly detected at the caudal level of the LC (Table S3). No differences were observed in mice subjected to a TRP-deficient diet.

The AhR activity is blocked by the AhR repressor (AhRR), which is transcribed after AhR activation to induce a negative control (Mimura et al. 1999). Indeed, a significant effect of ISDN on AhRR expression was detected by 2-way ANOVA (Table 1), as shown by increased mRNA levels in the ISDN-3 groups (Figure 4F), although the Tukey post-hoc test did not reveal significant intergroup differences. TRP deficiency did not modify AhRR expression in vehicle-injected mice and also does not have a cumulative effect in the ISDN-injected groups.

Finally, the mRNA expression of two main target genes of AhR, Cyp1A1, and Cyp1B1, were also examined by RT-PCR (Figure 4G Left Panel). Cyp1A1 expression was unaltered in any of the conditions showing similar levels in all groups (vehicle and ISDN), in both BAL and TRP deficient conditions. However, Cyp1B1 showed a significant ISDN effect by 2-way ANOVA (Table 1), which reduced mRNA levels in ISDN-3 groups without Tukey post-hoc test intergroup differences (Figure 4G Right Panel). Vehicle-injected mice under a TRP-deficient diet showed comparable Cyp1B1 expression to that of the BAL group, indicating that a TRP-deficient diet does not induce changes in the expression of Cyp1B1.

Repeated ISDN coupled with TRP deficiency increases microglial reactivity in the LC

AhR activity is linked to various immunological responses, which can contribute to central sensitization and cephalic and extracephalic hypersensitivities (Ji et al. 2018). At the level of glial cells, analysis of the morphology and quantification of cell number are commonly used as single measures to report on the activation state of microglia and astrocytes. Thus, we examined the possible glial changes at 1 h after ISDN administration and/or one week of TRP deficiency by immunohistochemistry (staining with an antibody against IBA1 for microglia and against GFAP for astrocytes) at the level of the LC.

Analysis of IBA1 labeling was separately done in the area occupied by noradrenergic cell bodies and in the TH-IR branching area. As no differences were observed between both LC areas, data were added together. Under a BAL diet, no change was seen in the number of quantified IBA1-IR cells in the total TH-IR region (Figure 5A and 5B), neither in any of the three portions nor after ISDN-1 or ISDN-3 groups. TRP deficiency also did not modify the microglia density. However, concerning the average size of the microglia, the sole change could be seen with a significant increase in the body cell size 1 h after the third ISDN injection in mice subjected to a TRP deficient diet (109.20 ± 2.30 μm^2) compared to its respective vehicle-injected mice (90.10 ± 2.00 μm^2) (Figure 5C). Morphological changes were also found at the level of the middle (vehicle: 88.00 ± 1.87 μm^2 *vs.* ISDN-3: 110.27 ± 3.00 μm^2) and caudal (Vehicle: 91.46 ± 2.80 vs. ISDN-3: 106.87 ± 3.21) portions of the LC (Table S6). Hence, signs of microglial activation occur under a combined effect of both TRP deficiency and repetitive ISDN administrations.

Repetitive ISDN administrations induce astrogliosis in the LC

Like microglia, analyses of GFAP labeling included the area occupied by noradrenergic cell bodies and the TH-IR branch area. A significant increase in the GFAP immunoreactivity was detected after both the first (4.56 ± 0.37) and the third ISDN injections (3.75 ± 0.29) compared to vehicle-injected mice under a BAL diet (2.15 ± 0.28; Figure 6A and 6B). The same increase in GFAP-IR was found after ISDN-1 (4.66 ± 0.21) and ISDN-3 (4.17 ± 0.34) with respect to the vehicle (2.59 ± 0.42) under the TRP deficient diet (Figure 6B). No changes were found in the reactivity of GFAP under TRP-deficient conditions compared to their BAL food counterparts. Looking at the

three portions of the LC, the same reactivity seen in the total LC can be seen primarily at the level of the rostral and middle portions (Table S5).

The density of the astrocytes showed no changes neither with ISDN nor with a TRP-deficient regimen in the total LC (Figure 6C) and as well in every portion of it (Table S5). However, the cell body size of the astrocytes showed a significant change after three ISDN injections in mice subjected to a BAL regimen that could be seen at the level of the total LC (Veh: 29.70 ± 1.35; ISDN: 40.19 ± 0.90) (Figure 5D) and also at the level of all rostrocaudal subregions (Table S5). Hence, ISDN provokes astrocytic reactivity under a BAL food regimen, with the TRP deficiency showing no specific effect on this reactivity. Moreover, the effect of ISDN is interestingly limited to the rostral and middle portions of the LC, with a slight change at the level of the caudal LC.

After a noxious stimulation, astrocytes undergo a series of phenotypic and functional changes such as process extensions and hypertrophy, which form the phenotypic characteristics of reactive astrocytes (T. Li et al. 2019). Our results showed increased cell body size of astrocytes after ISDN-3. Hence, we reconstructed a total of 166 GFAP-IR astrocytes to analyze their 3D morphology (Figure 7A). The morphometric assessment gave rise to the following four parameters for the total LC and the three rostrocaudal regions from each group: the fractal dimension, the number of total branches, the average branch length, and the area covered for each astrocyte. Vehicle-injected mice subjected to the BAL diet showed astrocytes with a fractal dimension of 1.11 ± 0.01 (Figure 7B), a number of branches of 18.8 ± 1.0 (Figure 7C), and an average length of 11.1 ± 0.7 µm (Figure 7D). The maximal number of intersections (peak of the Sholl plot) was at an average distance of 10 µm to the cell body (Figure 7F1 Left Panel). One ISDN injection induced some morphological changes, including a reduction of the branch number (14.3 ± 1.0; Figure 7C) and therefore, of their area (0.6 ± 0.1 μm^2; Figure 7E), and a remodeling of cell process distribution: the number of intersections was reduced, in particular at distances between 10 to 30 µm around the body cell. The peak of the Sholl plot reduced in amplitude and shifted to small distances (~5 µm; Figure 7F1 Left Panel). After the third ISDN injection, astrocytes were again remodeled: they presented reduced cell complexity, as shown by the reduced fractal dimension (1.05 ± 0.01; Figure 7B) compared to vehicle-injected mice (1.11 ± 0.01). Changes in the morphological organization were associated with a reduced number of branches (14.20 ± 0.80; Figure 7C), which were longer (13.10 ± 0.50 µm; Figure 7D).

Hence, the area covered by each astrocyte also increased (Figure 7E). Moreover, they showed fewer interactions of vehicle-injected mice (Figure 7F2), with a Sholl peak around 10 µm of distance (Figure 7F1 Left Panel).

TRP deficiency reduced the number of cell processes (14.90 ± 0.80; Figure 7C) in vehicle-injected mice, with no significant change in the number of intersections (Figure 7F1 Right Panel) compared to vehicle-injected mice under a BAL diet (Figure 7F2). TRP deficiency also did not potentiate the morphological remodeling induced by ISDN-1 or ISDN-3, as shown by similar morphometric values as those of astrocytes from ISDN groups under the BAL diet. Together, these data indicate that ISDN alone or coupled with TRP deficiency induces rapid astrocytic activation in the LC, which was observed in the three rostrocaudal subregions (Table S6).

Both ISDN administration and TRP deficiency increase plasma levels of pro-inflammatory cytokines

Reactive microglia and astrocytes are associated with the release of pro-inflammatory cytokines, whose levels have been described to be increased in both episodic and chronic migraineurs (Aydın et al. 2015). Therefore, the plasma concentrations of four pro-inflammatory cytokines were measured: IL-1β and IL-6, TNFα which are mainly released by activated astrocytes and microglia (Liddelow and Barres 2017), and IFNγ, which is released by activated microglia (Lehnardt 2010). Following one ISDN administration, a significant increase was shown in the levels of IL-1β (67.28 ± 2.84 pg/mL), TNFα (447.5 ± 11.7), and IL-6 (95.4 ± 3.54 pg/mL) compared to vehicle-injected mice (26.75 ± 2.09 pg/mL, 257.82 ± 15.21 pg/mL, 46.76 ± 3.91 pg/mL respectively; Table 3). The same increase in the plasma concentration was significantly shown after repetitive ISDN administrations with respect to IL-1β (76.55 ± 6.24 pg/mL), TNFα (439.45 ± 11.24 pg/mL), and IL-6 (89.98 ± 3.08 pg/mL), with an effect not more robust than that of one ISDN administration (Table 3). Interestingly, TRP deficiency induced the same inflammatory increase of all three cytokines IL-1β in vehicle-injected mice (Table 3). However, it did not augment the effect of ISDN as mice showed comparable cytokine levels under a TRP-deficient diet compared to the BAL diet (Table 3). No changes were seen with respect to IFNγ either after ISDN or under all the remaining conditions (Table 3). Hence, based on the prominent increase in the levels of pro-inflammatory cytokines, both ISDN and TRP deficiency induce a state of general inflammation.

DISCUSSION

In this study, we demonstrated that ISDN induces a pro-inflammatory state shown by the systemic pro-inflammatory cytokine release, enhanced astrogliosis, and altered AhR function in the LC. TRP deficiency also increased plasmatic pro-inflammatory cytokines. Combined, ISDN and TRP deficiency worsen the pro-inflammatory state and impaired the noradrenergic function of the LC, which was concomitant with a long-lasting CMH. Moreover, we highlight that the inflammatory-related pain facilitation during migraine progression was linked to the pro-nociceptive AhR activity.

CMH is concomitant with an abnormal systemic and local inflammatory state

Systemic administration of ISDN induced a robust acute CMH and EMH in females, in accordance with our published work showing that ISDN elicits both cephalic and extracephalic hypersensitivities in rats (Dallel, Descheemaeker, and Luccarini 2018). In our results, ISDN-induced CMH lasted for approximately 14 days while EMH was reversed after 4 days. This has priorly been shown in an inflammatory soup model of migraine-like pain whereby repetitive stimulations induce a persistent CMH but a reversible EMH probably due to their mechanistic differences in reference to sensitization (Boyer et al. 2014). This distinction is due to the different mediators of sensitization being central sensitization of the Sp5c as a mediator of cephalic cutaneous hypersensitivity while the thalamic trigeminovascular neurons are responsible for extracephalic cutaneous hypersensitivity.

Enhanced inflammatory activity under ISDN conditions was shown by significant astrogliosis in the LC coupled with increased levels of pro-inflammatory cytokines in the plasma. Several features have been described to be associated with astrogliosis, such as the up-regulation of GFAP and cellular hypertrophy (Sofroniew 2015). Our analyses showed that single and repeated ISDN administrations increased GFAP cell reactivity, as described before after nitroglycerine (NTG) administration in mice (Filippone et al. 2022). Repeated ISDN also altered the morphology and reduced the arbor complexity of the astrocytes in the LC, matching with the peak of CMH. Morphological changes in astrocytes haven’t been shown under migraine-like conditions, however, in conditions of harmful insults astrocytes become more reactive and change their shape through hypertrophying and processes extension towards the area of insult to perform their role of protection under chronic pain conditions (D. Sun and Jakobs 2012, 20; Y. Wang et al.

2018). Although microglial activation has been described to be related to astrogliosis (D. Zhang et al. 2010), our results did not reveal increased microglial reactivity after ISDN (as far as size or cell number are concerned). However, changes in gene expression or the release of inflammatory molecules by microglia without morphological alterations cannot be excluded. Indeed, after both single and repeated ISDN administrations, increased plasmatic levels of certain pro-inflammatory mediators including IL-6, TNFα, and IL-1β were measured, which are mainly released by activated astrocytes but also microglial cells (Liddelow and Barres 2017). NO has been also described to stimulate cytokine release (Soufli et al. 2016). Since both astrogliosis and cytokine levels were measured at the same time point after ISDN administration, it is difficult to determine the temporal sequence of events, and further studies will be necessary in order to analyze if systemic cytokine release came from glial activation, ISDN-derived NO, or from a combination of both.

Dysregulated TRP metabolism promotes systemic inflammation and worsens CMH

Acute dietary TRP depletion has been used as a tool for clinical and preclinical research about the role of TRP-derived metabolites in migraine pathophysiology. In humans, TRP depletion augmented clinical manifestations of migraine, such as nausea, headache intensity, and photophobia (P. Drummond 2006). In rodents, TRP depletion has previously and reproducibly induced neurochemical and behavioral alterations in both rats (Cahir et al. 2007) and mice (Browne et al. 2012). TRP is the precursor of serotonin and KYN, which have a prominent role in migraine (Berman et al. 2006; K. Gao et al. 2020). These metabolites play a role in the pathophysiology of migraine and are altered in migraineurs. To mimic this clinically persistent TRP dysregulation in mice, we used a chronic dietary TRP depletion, which reduced approximately 70% of the plasmatic TRP concentration after one week, without affecting anxiety or the welfare state of animals. It has been described that TRP metabolism modulates peripheral inflammatory responses, at the level of the gut-brain axis (Bosi et al. 2020), through AhR activation. We observed that TRP deficiency increased IL-6, TNFα, and IL-1β levels in the blood, as described before (van Wissen et al. 2002), maybe by activating AhR. Pro-inflammatory cytokines, mainly TNFα (Gostner et al. 2020), activate the enzyme indoleamine 2,3-dioxygenase (IDO) in peripheral dendritic cells, which transforms TRP to KYN. IDO plays a minor role under physiological circumstances. However, it is

strongly activated upon inflammation (Yeung et al. 2015). IDO activity is often measured by the blood KYN/TRP ratio, which is increased in diseases characterized by excessive or chronic inflammation (Schröcksnadel et al. 2006). Our data showed that ISDN also increased peripheral IDO activity thus shifting TRP metabolism toward the production of KYN, leading to low TRP levels, and increased KYN/TRP ratio. This effect could be explained by an indirect action of ISDN on IDO activity: NO produced from ISDN could stimulate TNFα release, which in turn, activates IDO that metabolizes TRP to KYN (Figure 8A). Impaired turnover of KYN and TRP has been described in migraineurs, who presented low levels of both metabolites compared to healthy controls (Tuka et al. 2021).

Both levels of TRP and KYN were reduced after ISDN and/or TRP-depleted diet in the LC. IDO expression in the brain is low and restricted to specific brain regions (Kwidzinski and Bechmann 2007). Cerebral TRP metabolism is therefore largely dependent on the transport of TRP and KYN across the blood-brain barrier. Both have access to brain tissue through the large neutral amino acid transporter, hence entering through a competition with other large neutral amino acids leading to their marginally lower levels.

KYN is a ligand of AhR and regulates inflammatory processes through the promotion of cytokine release and hence through the further activation of the IDO creating a cyclic event (Figure 8A). This process included the activation of astrocytes and microglia, which progress to a reactive morphological state when both TRP deficiency and repetition of ISDN were combined. NO also controls the activity and expression of AhR by decreasing it (Niedbala et al. 2011). Thus, the final balance between KYN and NO actions on AhR could be a partial reduction of AhR expression after ISDN, leaving enough active AhR to promote the pronociceptive effect observed after its direct activation by ITE. This enhanced pro-inflammatory state could be a potential inducer of pain facilitation and persistence, as observed by long-lasting CMH in mice after TRP deficiency. It should be noted that TRP deficiency did not affect ISDN-induced extracephalic hypersensitivity since the targeted pathway is different. This difference could be due to the different mechanisms of both hypersensitivities considering in episodic migraine cephalic hypersensitivity is mostly mechanical, whereas extracephalic is mostly thermal (Guy et al. 2010).

AhRs have a pro-nociceptive action in migraine pathophysiology

AhRs are prominent transcription factors of either pro-or anti-inflammatory capacities, that are located at the level of the astrocytes, microglia, and noradrenergic neurons. In most inflammatory diseases, AhR has been described as neuroprotective by regulating the neuroinflammatory responses in the brain (Ntranos et al. 2022). For instance in an animal model of chronic constrictive nerve injury the deletion of AhR worsened the damage, while the administration of an AhR agonist counteracted such changes (Sheu et al. 2022).

Interestingly, our data showed that AhR has a pro-nociceptive action during migraine initiation and progression. On the one hand, systemic activation of AhR by the non-toxic agonist ITE, prior to ISDN administration, enhanced the intensity and duration of ictal CMH. On the other hand, AhR inhibition by non-toxic TMF prevents both ictal and interictal ISDN-induced CMH. Our hypothesis is that AhR activation promotes the sensitization of the inflammatory signaling pathways, which respond inordinately after ISDN administration, facilitating nociceptive response and central sensitization of the trigeminal system (Figure 8B). Since AhR is blocked by TMF, KYN can no longer execute its function as a ligand, and hence the IDO/AhR axis is potentially hindered (Figure 8C). Supporting this idea, the combination of TMF with a TRP-deficient diet (that prior activates AhR and hence sensitizes the peripheral inflammatory system) leads to a lower TMF-induced antalgic effect.

Impaired noradrenergic function contributes to migraine progression

Multiple players have an important role in the pathophysiology of migraine including the LC. This nucleus plays an important role in the descending control of pain during migraine. Moreover, its role in migraine has been shown in neuroimaging studies showcasing altered LC functional connectivity in migraineurs (Moulton et al. 2014), and LC activation following the infusion of NTG infusion (Maniyar et al. 2014). In our study, we observed that the noradrenergic function of the LC neurons was altered under TRP deficiency. Indeed, TH expression was increased in ISDN-injected mice coupled with reduced local NA release. TRP depletion has been described to reduce NA levels in rodents (Ardis et al. 2009), although the exact mechanism is not clear. It could involve altered serotoninergic modulation of LC activity (Deen et al. 2018) or enhanced turnover of NA, as described in other chronic pain models (Alba-Delgado et al. 2016). In any case, NA has an endogenous analgesic function in the spinal cord (Pertovaara

2006), and low release could contribute to long-lasting CMH induced by TRP deficiency in ISDN-injected mice.

The effect of TRP metabolism disruption also alters the noradrenergic cell morphology. Indeed, after repeated ISDN administrations, an increase in the cell volume of noradrenergic neurons and more TH-IR projections were observed. However, TRP deficiency blocked these structural alterations, leading to a concomitant long-lasting CMH. These results suggest that morphological changes of LC neurons are necessary to modulate nociceptive responses. Indeed, several studies have described morphological changes in LC neurons in response to stressful stimuli (Borodovitsyna, Joshi, and Chandler 2018). These alterations include an increase in the complexity of the dendritic arborization associated with the expression of genes involved in cell structure and synaptic plasticity with the primary aim of pain amelioration and modulation.

CONCLUSION

This study demonstrates that migraine-associated CMH is linked to an enhanced neuroinflammatory state promoted by impaired TRP metabolism and comorbid with noradrenergic dysfunction. Moreover, we propose AhR as a pivotal player in TRP-mediated inflammatory signaling with a potential pro-nociceptive role. Other studies will be necessary to dive into the underlying circuit of AhR activation during migraine initiation and progression, and thus propose future therapeutic targets.

AUTHOR CONTRIBUTION

YM and CAD designed the research study; YM performed and analyzed behavioral experiments; YM and CAD collected samples for biomolecular experiences, and performed the ELISA essays; YM, AR, and CAD performed and analyzed the immunohistochemistry experiments; VR performed and analyzed western blot experiments; IH and IES performed and analyzed samples by HPLC; MAM, MICD, and AGC performed and analyzed the RT-PCR data; CAD supervised technical performance of experiences, and analyzed the final data; RD provided the funding; YM, RD, and CAD evaluated the results; YM and CAD wrote the manuscript, which was reviewed and approved by all authors.

ACKNOWLEDGEMENTS

The authors thank Ms. Tamara Lucien, Ms. Marion Ledevin, and Mr. Rayan Domet for their help with the astrocyte reconstructions; Ms. Manon Meunier for their contribution to behavioral testing; and Ms. Anne-Marie Gaydier and Ms. Sylviane Rousselin for their technical help. All authors reviewed and approved the final manuscript and declared no conflicts of interest. This work was supported by funding from the “Institut National de la Santé et de la Recherche Médicale” (INSERM) and the Clermont Auvergne University (France).

REFERENCES

Akahoshi, Eiichi, Seiko Yoshimura, Saeko Uruno, and Mitsuko Ishihara-Sugano. 2009. "Effect of Dioxins on Regulation of Tyrosine Hydroxylase Gene Expression by Aryl Hydrocarbon Receptor: A Neurotoxicology Study." *Environmental Health* 8(1): 24.

Akerman, Simon, Philip R. Holland, and Peter J. Goadsby. 2011. "Diencephalic and Brainstem Mechanisms in Migraine." *Nature Reviews. Neuroscience* 12(10): 570–84.

Alba-Delgado, Cristina et al. 2018. "5-HT2A Receptor-Induced Morphological Reorganization of PKCγ-Expressing Interneurons Gates Inflammatory Mechanical Allodynia in Rat." *Journal of Neuroscience* 38(49): 10489–504.

Alba-Delgado, Cristina, Alberto Cebada-Aleu, Juan Antonio Mico, and Esther Berrocoso. 2016. "Comorbid Anxiety-like Behavior and Locus Coeruleus Impairment in Diabetic Peripheral Neuropathy: A Comparative Study with the Chronic Constriction Injury Model." *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 71: 45–56.

Ardis, T. C. et al. 2009. "Effect of Acute Tryptophan Depletion on Noradrenaline and Dopamine in the Rat Brain." *Journal of Psychopharmacology (Oxford, England)* 23(1): 51–55.

Aydın, Meliha et al. 2015. "Plasma Cytokine Levels in Migraineurs During and Outside of Attacks." *Electronic Journal of General Medicine* 12(4): 307–12.

Badawy, Abdulla A-B. 2017. "Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects." *International Journal of Tryptophan Research* 10: 1178646917691938.

Berman, Nancy E.J. et al. 2006. "Serotonin in Trigeminal Ganglia of Female Rodents: Relevance to Menstrual Migraine." *Headache: The Journal of Head and Face Pain* 46(8): 1230–45.

Bigal, Marcelo E., and Richard B. Lipton. 2008. "Concepts and Mechanisms of Migraine Chronification." *Headache: The Journal of Head and Face Pain* 48(1): 7–15.

Biscetti, Leonardo et al. 2022. "Immunological Findings in Patients with Migraine and Other Primary Headaches: A Narrative Review." *Clinical and Experimental Immunology* 207(1): 11–26.

Borodovitsyna, Olga, Neal Joshi, and Daniel Chandler. 2018. "Persistent Stress-Induced Neuroplastic Changes in the Locus Coeruleus/Norepinephrine System." *Neural Plasticity* 2018: 1892570.

Bosi, Annalisa et al. 2020. "Tryptophan Metabolites Along the Microbiota-Gut-Brain Axis: An Interkingdom Communication System Influencing the Gut in Health and Disease." *International Journal of Tryptophan Research : IJTR* 13: 1178646920928984.

Boyer, Nelly, Radhouane Dallel, Alain Artola, and Lénaïc Monconduit. 2014. “General Trigeminospinal Central Sensitization and Impaired Descending Pain Inhibitory Controls Contribute to Migraine Progression.” *Pain* 155(7): 1196–1205.

Browne, Caroline A., Gerard Clarke, Timothy G. Dinan, and John F. Cryan. 2012. “An Effective Dietary Method for Chronic Tryptophan Depletion in Two Mouse Strains Illuminates a Role for 5-HT in Nesting Behaviour.” *Neuropharmacology* 62(5–6): 1903–15.

Cahir, Marie, Tara Ardis, Gavin P. Reynolds, and Stephen J. Cooper. 2007. “Acute and Chronic Tryptophan Depletion Differentially Regulate Central 5-HT1A and 5-HT2A Receptor Binding in the Rat.” *Psychopharmacology* 190(4): 497–506.

Cuartero, María I. et al. 2014. “L-Kynurenine/Aryl Hydrocarbon Receptor Pathway Mediates Brain Damage After Experimental Stroke.” *Circulation* 130(23): 2040–51.

Dallel, Radhouane, Amélie Descheemaeker, and Philippe Luccarini. 2018. “Recurrent Administration of the Nitric Oxide Donor, Isosorbide Dinitrate, Induces a Persistent Cephalic Cutaneous Hypersensitivity: A Model for Migraine Progression.” *Cephalalgia: An International Journal of Headache* 38(4): 776–85.

Deen, Marie et al. 2018. “High Brain Serotonin Levels in Migraine between Attacks: A 5-HT4 Receptor Binding PET Study.” *NeuroImage : Clinical* 18: 97–102.

Deen et al. 2019. “Migraine Is Associated with High Brain 5-HT Levels as Indexed by 5-HT4 Receptor Binding.” *Cephalalgia* 39(4): 526–32.

Drummond, PD. 2006. “Tryptophan Depletion Increases Nausea, Headache and Photophobia in Migraine Sufferers.” *Cephalalgia* 26(10): 1225–33.

Filippone, Alessia et al. 2022. “BAY-117082-Driven NLRP3 Inflammasome Inhibition Resolves Nitro-Glycerine (NTG) Neuronal Damage in in Vivo Model of Migraine.” *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* 156: 113851.

Gao, Kan, Chun-long Mu, Aitak Farzi, and Wei-yun Zhu. 2020. “Tryptophan Metabolism: A Link Between the Gut Microbiota and Brain.” *Advances in Nutrition* 11(3): 709–23.

Gostner, Johanna M. et al. 2020. “Tryptophan Metabolism and Related Pathways in Psychoneuroimmunology: The Impact of Nutrition and Lifestyle.” *Neuropsychobiology* 79(1): 89–99.

Greco, Rosaria et al. 2017. “Effects of Kynurenic Acid Analogue 1 (KYNA-A1) in Nitroglycerin-Induced Hyperalgesia: Targets and Anti-Migraine Mechanisms.” *Cephalalgia* 37(13): 1272–84.

Guy, N. et al. 2010. “Are There Differences between Cephalic and Extracephalic Cutaneous Allodynia in Migraine Patients?” *Cephalalgia: An International Journal of Headache* 30(7): 881–86.

He, Wei et al. 2019. “Microglial NLRP3 Inflammasome Activation Mediates IL-1β Release and Contributes to Central Sensitization in a Recurrent Nitroglycerin-Induced Migraine Model.” *Journal of Neuroinflammation* 16(1): 78.

Hubbard, Troy D. et al. 2015. “Adaptation of the Human Aryl Hydrocarbon Receptor to Sense Microbiota-Derived Indoles.” *Scientific Reports* 5: 12689.

IHS. 2018. “Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd Edition.” *Cephalalgia: An International Journal of Headache* 38(1): 1–211.

Ji, Ru-Rong et al. 2018. “Neuroinflammation and Central Sensitization in Chronic and Widespread Pain.” *Anesthesiology* 129(2): 343–66.

Juricek, Ludmila, and Xavier Coumoul. 2018. “The Aryl Hydrocarbon Receptor and the Nervous System.” *International Journal of Molecular Sciences* 19(9): 2504.

Kimura, Eiki et al. 2021. “Neurons Expressing the Aryl Hydrocarbon Receptor in the Locus Coeruleus and Island of Calleja Major Are Novel Targets of Dioxin in the Mouse Brain.” *Histochemistry and Cell Biology* 156(2): 147–63.

Körtési, Tamás, Eleonóra Spekker, and László Vécsei. 2022. “Exploring the Tryptophan Metabolic Pathways in Migraine-Related Mechanisms.” *Cells* 11(23): 3795.

Kwidzinski, Erik, and Ingo Bechmann. 2007. “IDO Expression in the Brain: A Double-Edged Sword.” *Journal of Molecular Medicine* 85(12): 1351–59.

Lehnardt, Seija. 2010. “Innate Immunity and Neuroinflammation in the CNS: The Role of Microglia in Toll-like Receptor-Mediated Neuronal Injury.” *Glia* 58(3): 253–63.

Li, Ting et al. 2019. “An Update on Reactive Astrocytes in Chronic Pain.” *Journal of neuroinflammation* 16: 1–13.

Liddelow, Shane A., and Ben A. Barres. 2017. “Reactive Astrocytes: Production, Function, and Therapeutic Potential.” *Immunity* 46(6): 957–67.

Louter, Mark A. et al. 2013. “Cutaneous Allodynia as a Predictor of Migraine Chronification.” *Brain* 136(11): 3489–96.

Maniyar, Farooq Husain et al. 2014. “Brain Activations in the Premonitory Phase of Nitroglycerin-Triggered Migraine Attacks.” *Brain* 137(1): 232–41.

Mimura, Junsei, Masatsugu Ema, Kazuhiro Sogawa, and Yoshiaki Fujii-Kuriyama. 1999. “Identification of a Novel Mechanism of Regulation of Ah (Dioxin) Receptor Function.” *Genes & Development* 13(1): 20–25.

Moulton, Eric A. et al. 2014. “Altered Hypothalamic Functional Connectivity with Autonomic Circuits and the Locus Coeruleus in Migraine.” *PLOS ONE* 9(4): e95508.

Natoli, JL et al. 2010. “Global Prevalence of Chronic Migraine: A Systematic Review.” *Cephalalgia* 30(5): 599–609.

Neelamegam, Malinee et al. 2021. “The Effect of Opioids on the Cognitive Function of Older Adults: Results from the Personality and Total Health through Life Study.” *Age and Ageing* 50(5): 1699–1708.

Niedbala, Wanda et al. 2011. “Regulation of Type 17 Helper T-Cell Function by Nitric Oxide during Inflammation.” *Proceedings of the National Academy of Sciences of the United States of America* 108(22): 9220–25.

Ntranos, Achilles et al. 2022. “Bacterial Neurotoxic Metabolites in Multiple Sclerosis Cerebrospinal Fluid and Plasma.” *Brain: A Journal of Neurology* 145(2): 569–83.

Paxinos, George, and Keith BJ Franklin. 2019. *Paxinos and Franklin’s the Mouse Brain in Stereotaxic Coordinates*. Academic press.

Pertovaara, Antti. 2006. “Noradrenergic Pain Modulation.” *Progress in Neurobiology* 80(2): 53–83.

Petersen, Sandra L. et al. 2000. “Distribution of MRNAs Encoding the Arylhydrocarbon Receptor, Arylhydrocarbon Receptor Nuclear Translocator, and Arylhydrocarbon Receptor Nuclear Translocator-2 in the Rat Brain and Brainstem.” *Journal of Comparative Neurology* 427(3): 428–39.

Rothhammer, Veit et al. 2016. “Type I Interferons and Microbial Metabolites of Tryptophan Modulate Astrocyte Activity and Central Nervous System Inflammation via the Aryl Hydrocarbon Receptor.” *Nature Medicine* 22(6): 586–97.

Rothhammer, Veit, and Francisco J. Quintana. 2019. “The Aryl Hydrocarbon Receptor: An Environmental Sensor Integrating Immune Responses in Health and Disease.” *Nature Reviews Immunology* 19(3): 184–97.

Sasa, M., K. Munekiyo, H. Ikeda, and S. Takaori. 1974. “Noradrenaline-Mediated Inhibition by Locus Coeruleus of Spinal Trigeminal Neurons.” *Brain Research* 80(3): 443–60.

Scher, A. I., W. F. Stewart, J. A. Ricci, and R. B. Lipton. 2003. “Factors Associated with the Onset and Remission of Chronic Daily Headache in a Population-Based Study.” *Pain* 106(1–2): 81–89.

Schröcksnadel, Katharina, Barbara Wirleitner, Christiana Winkler, and Dietmar Fuchs. 2006. “Monitoring Tryptophan Metabolism in Chronic Immune Activation.” *Clinica Chimica Acta; International Journal of Clinical Chemistry* 364(1–2): 82–90.

Sheu, Meei-Ling et al. 2022. “Modulation of Aryl Hydrocarbon Receptor Expression Alleviated Neuropathic Pain in a Chronic Constriction Nerve Injury Animal Model.” *International Journal of Molecular Sciences* 23(19): 11255.

Sofroniew, Michael V. 2015. “Astrogliosis.” *Cold Spring Harbor Perspectives in Biology* 7(2): a020420.

Soufli, Imene, Ryma Toumi, Hayet Rafa, and Chafia Touil-Boukoffa. 2016. “Overview of Cytokines and Nitric Oxide Involvement in Immuno-Pathogenesis of Inflammatory Bowel Diseases.” *World Journal of Gastrointestinal Pharmacology and Therapeutics* 7(3): 353–60.

Sun, Daniel, and Tatjana C. Jakobs. 2012. “Structural Remodeling of Astrocytes in the Injured CNS.” *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry* 18(6): 567–88.

Ter Horst, GJ, WJ Meijler, J Korf, and RHA Kemper. 2001. “Trigeminal Nociception-Induced Cerebral Fos Expression in the Conscious Rat.” *Cephalalgia* 21(10): 963–75.

Tuka, Bernadett et al. 2021. “Clinical Relevance of Depressed Kynurenine Pathway in Episodic Migraine Patients: Potential Prognostic Markers in the Peripheral Plasma during the Interictal Period.” *The Journal of Headache and Pain* 22(1): 60.

Unzueta-Larrinaga, Paula et al. 2023. “Isolation and Differentiation of Neurons and Glial Cells from Olfactory Epithelium in Living Subjects.” *Molecular Neurobiology* 60(8): 4472–87.

Vila-Pueyo, Marta et al. 2019. “Divergent Influences of the Locus Coeruleus on Migraine Pathophysiology.” *PAIN* 160(2): 385.

Wang, Yunjie et al. 2018. “A Dual AMPK/Nrf2 Activator Reduces Brain Inflammation After Stroke by Enhancing Microglia M2 Polarization.” *Antioxidants & Redox Signaling* 28(2): 141–63.

Weiller, C. et al. 1995. “Brain Stem Activation in Spontaneous Human Migraine Attacks.” *Nature Medicine* 1(7): 658–60.

van Wissen, Matthijs et al. 2002. “IFN-γ Amplifies IL-6 and IL-8 Responses by Airway Epithelial-Like Cells Via Indoleamine 2,3-Dioxygenase1.” *The Journal of Immunology* 169(12): 7039–44.

Yan, Jiong et al. 2019. “Aryl Hydrocarbon Receptor Signaling Prevents Activation of Hepatic Stellate Cells and Liver Fibrogenesis in Mice.” *Gastroenterology* 157(3): 793-806.e14.

Yeung, Amanda W. S., Andrew C. Terentis, Nicholas J. C. King, and Shane R. Thomas. 2015. “Role of Indoleamine 2,3-Dioxygenase in Health and Disease.” *Clinical Science (London, England: 1979)* 129(7): 601–72.

Zhang, Dan et al. 2010. “Astrogliosis in CNS Pathologies: Is There A Role for Microglia?” *Molecular neurobiology* 41(0): 232–41.

FIGURES

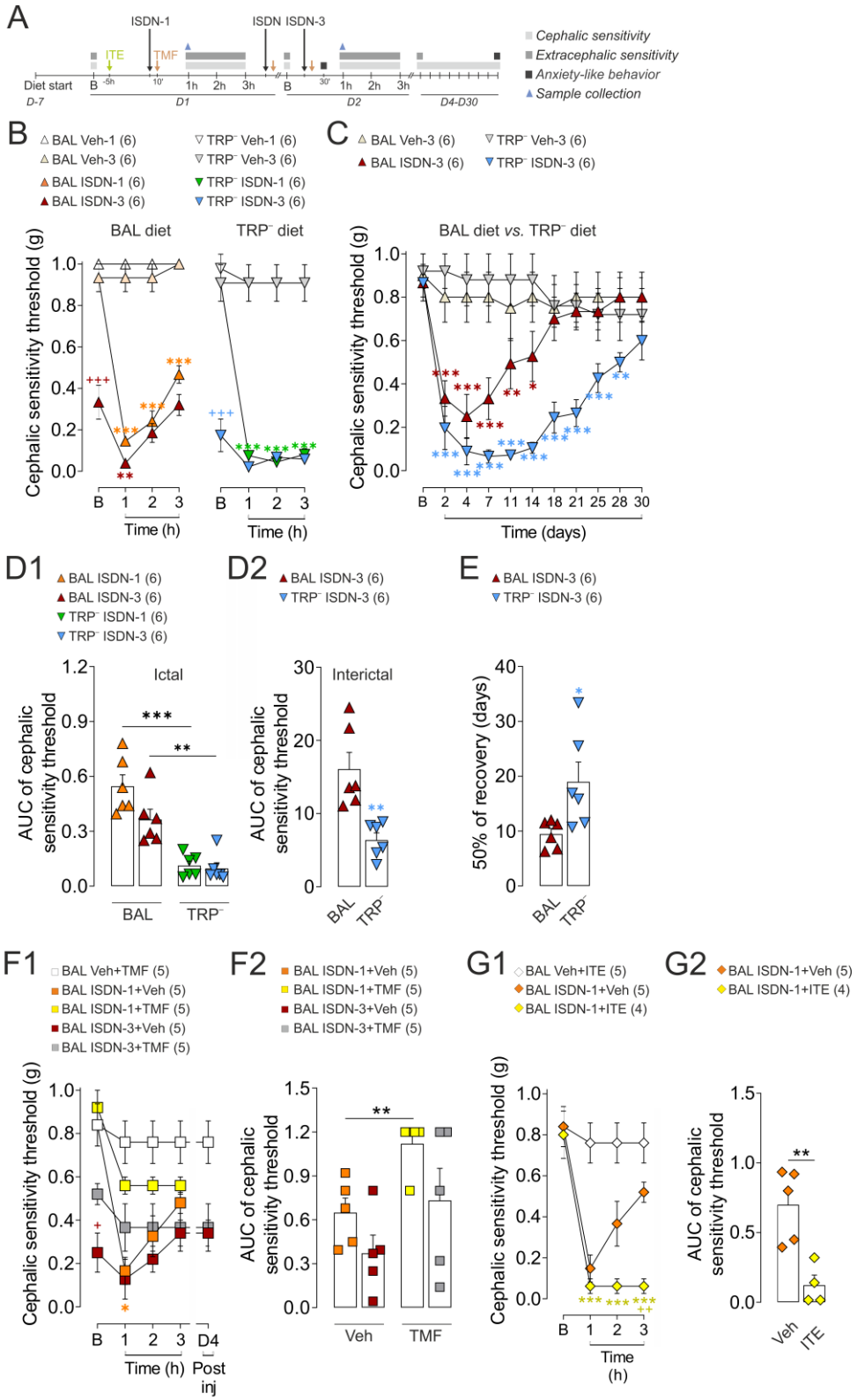

A
ISDN-1
ISDN ISDN-3
ITE
TMF
Diet start
D-7
D1
D2
D4-D30
Cephalic sensitivity
Extracephalic sensitivity
Anxiety-like behavior
Sample collection
B
BAL Veh-1 (6)
BAL Veh-3 (6)
BAL ISDN-1 (6)
BAL ISDN-3 (6)
TRP⁻ Veh-1 (6)
TRP⁻ Veh-3 (6)
TRP⁻ ISDN-1 (6)
TRP⁻ ISDN-3 (6)
C
BAL Veh-3 (6)
BAL ISDN-3 (6)
TRP⁻ Veh-3 (6)
TRP⁻ ISDN-3 (6)
BAL diet
TRP⁻ diet
BAL diet vs. TRP⁻ diet
Cephalic sensitivity threshold (g)
Time (h)
Time (days)
D1
D2
E
Ictal
Interictal
AUC of cephalic sensitivity threshold
50% of recovery (days)
BAL
TRP⁻
F1
F2
G1
G2
BAL Veh+TMF (5)
BAL ISDN-1+Veh (5)
BAL ISDN-1+TMF (5)
BAL ISDN-3+Veh (5)
BAL ISDN-3+TMF (5)
BAL Veh+ITE (5)
BAL ISDN-1+ITE (4)
Post inj
Veh
TMF
ITE
Time (h)

Figure 1. Cephalic Mechanical Sensitivity. A- After one week of a balanced (BAL) or TRP-deficient (TRP^{-}) diet, single/repetitive injections of ISDN (10 mg/kg, i.p.) or vehicle (Veh; 0.9% NaCl) were administered. ITE (10 mg/kg, i.p.) was injected 5 h before ISDN/Veh. TMF (5 mg/kg, i.p.) was injected 10 min after ISDN/veh. Cephalic and extracephalic mechanical sensitivity were measured before (baseline, B), and one, two, and three hours after the Veh/ISDN injection. Measurements were prolonged until 30 days post ISDN-3. Anxiety-like behavior was assessed 30 min and 30 days after ISDN-3. Blood and brain samples were collected at 1 h after ISDN-1 and ISDN-3. **B-** Mechanical sensitivity threshold in response to von Frey stimulation. Data are presented as mean ± S.E.M of (n) mice per group. ***$p < 0.001$ vs. baseline, $^{+++}p < 0.001$ vs. BAL-ISDN group by Tukey HSD posttest following RM 3-way ANOVA. **C-** Persistence of cephalic hypersensitivity. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ vs. baseline by Tukey HSD following RM 3-way ANOVA. **D1-** Area under the curve (AUC) of the acute (ictal) effect of ISDN-1 and ISDN-3 on the cephalic sensitivity. Data represent mean ± S.E.M of (n) mice per group. Symbols represent individual values. **$p < 0.01$ and ***$p < 0.001$ by Tukey HSD following 2-way ANOVA. **D2 -** AUC of the persistent (interictal) effect of ISDN-3 on the cephalic sensitivity. **$p < 0.01$ by Student's t-test. **E-** Fifty percent of recovery of the cephalic sensitivity threshold is represented in days. *$p < 0.05$ by Student's t-test. **F1-** Mechanical sensitivity threshold after a single or repetitive TMF administrations in a BAL diet. *$P < 0.05$ vs. baseline, $^{+}p < 0.05$ vs. BAL-ISDN group by Tukey HSD following RM 3-way ANOVA. **F2-** AUC of the effect of TMF on the cephalic sensitivity. **$p < 0.01$ by Tukey HSD following 2-way ANOVA. **G1-** Mechanical sensitivity threshold after ITE administration at one week of BAL or TRP^{-} diets. ***$p < 0.001$ vs. baseline, $^{++}p < 0.01$ vs. BAL-ISDN group by Tukey HSD following RM 3-way ANOVA. **G2-** AUC of the effect of ITE on the cephalic sensitivity. **$p < 0.01$ by Tukey HSD following 2-way ANOVA.

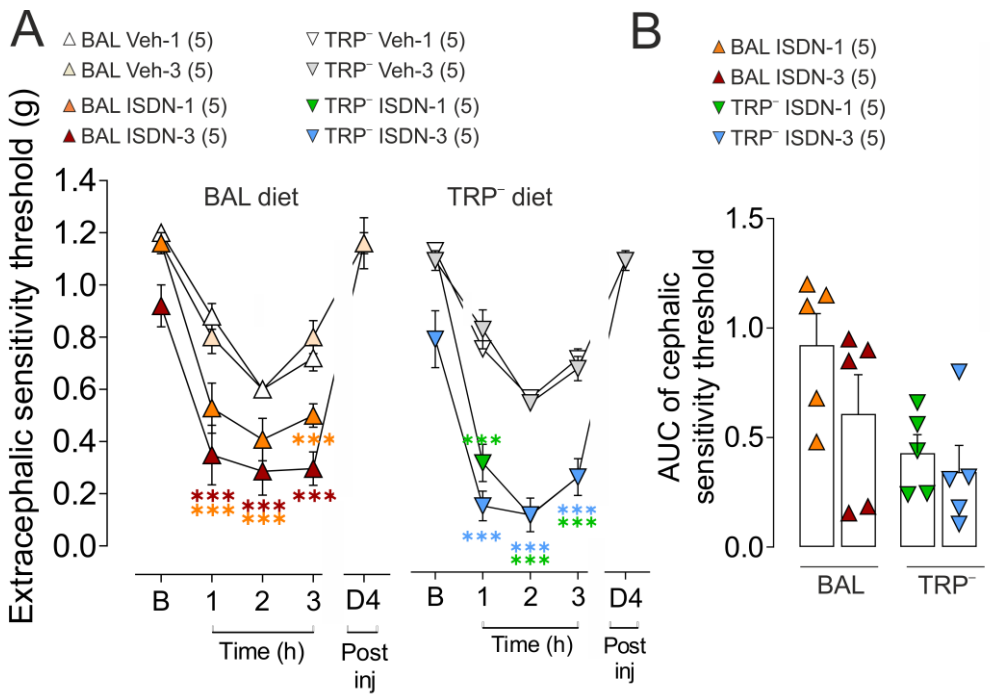


Figure 2. A- Extracephalic Mechanical Sensitivity threshold of mice in response to von Frey stimulation after one (ISDN-1) or repetitive ISDN (ISDN-3) or vehicle (Veh) administration at one week of balanced (BAL) or TRP-deficient (TRP^{-}) diet groups. Data are presented as mean ± S.E.M of (n) mice per group. ***$p < 0.001$ vs. baseline by Tukey HSD posttest following RM 3-way ANOVA. **B-** Area under the curve (AUC) of the effect of ISDN on the extracephalic sensitivity. Data represent mean ± S.E.M of (n) mice per group. Symbols represent individual values. $p > 0.05$ by Tukey HSD following 2-way ANOVA.

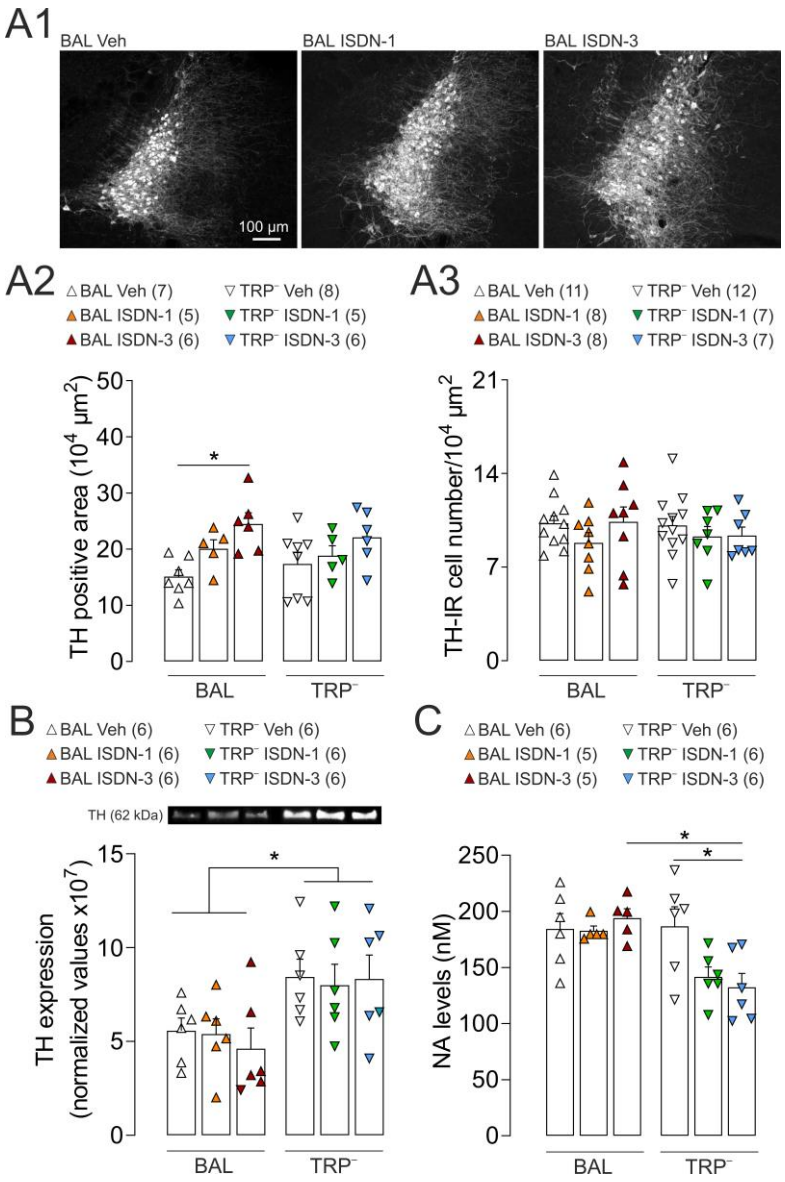


Figure 3. A1- Noradrenergic Functions at the Level of the LC. Representative images of tyrosine hydroxylase (TH)-immunoreactive (IR) stained neurons with their ramifications in the LC under a balanced (BAL) diet coupled with one (ISDN-1) or three (ISDN-3) ISDN administrations or vehicle (Veh). Quantitative assessment of the **A2-** TH positive area and **A3-** TH-IR cell number. Data represent mean ± S.E.M of (n) mice per group. Symbols represent individual values. **p* < 0.05 by Tukey HSD posttest following 2-way ANOVA. **B-** Relative expression of TH protein in the LC. **p* < 0.05 by Tukey HSD following 2-way ANOVA. **C-** Measurements of noradrenaline (NA) levels in the LC. **p* < 0.05 by Tukey HSD following 2-way ANOVA.

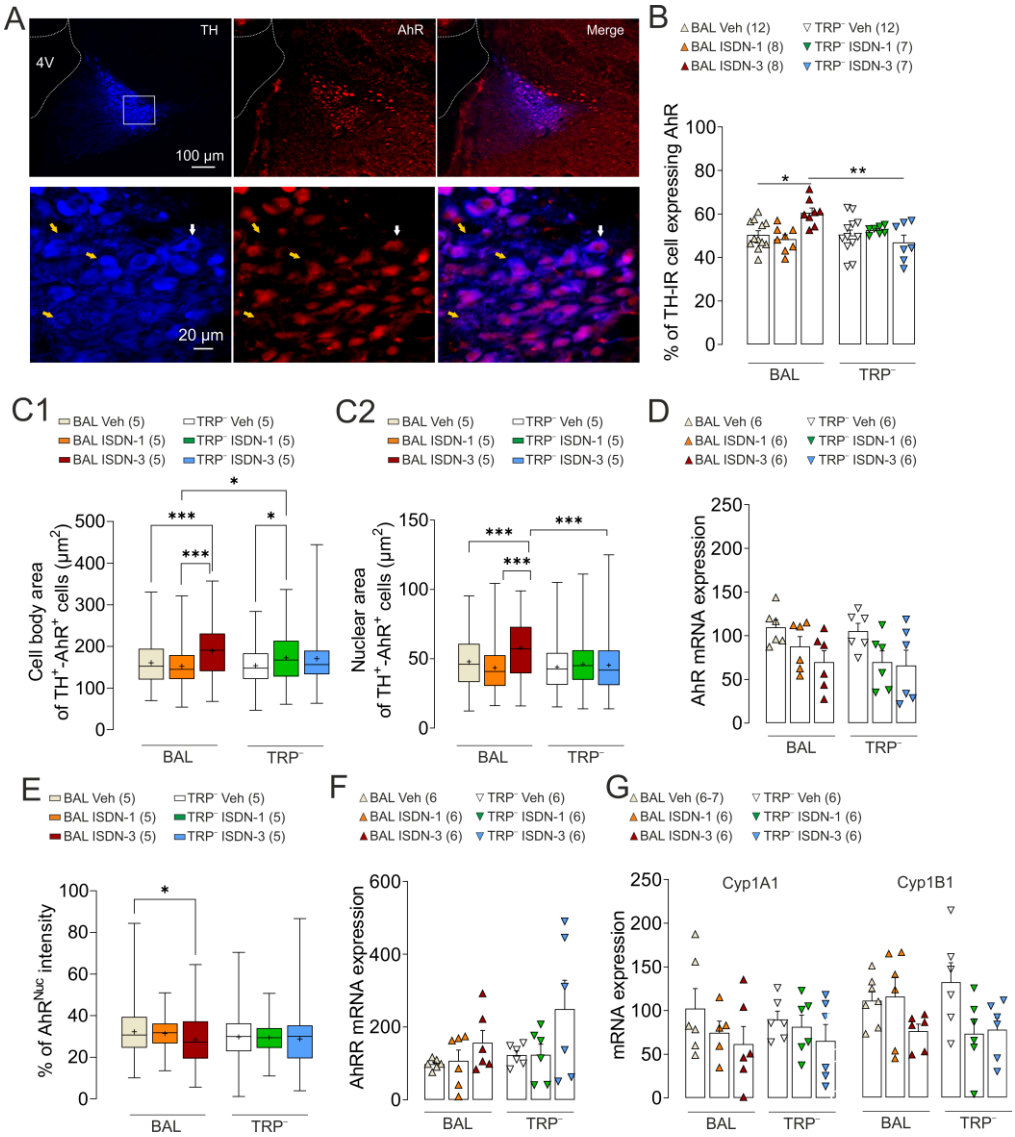

A
TH
AhR
Merge
4V
100 µm
20 µm
B
BAL Veh (12)
TRP⁻ Veh (12)
BAL ISDN-1 (8)
TRP⁻ ISDN-1 (7)
BAL ISDN-3 (8)
TRP⁻ ISDN-3 (7)
% of TH-IR cell expressing AhR
BAL
TRP⁻
C1
BAL Veh (5)
TRP⁻ Veh (5)
BAL ISDN-1 (5)
TRP⁻ ISDN-1 (5)
BAL ISDN-3 (5)
TRP⁻ ISDN-3 (5)
Cell body area of TH⁺-AhR⁺ cells (µm²)
C2
Nuclear area of TH⁺-AhR⁺ cells (µm²)
D
BAL Veh (6
TRP⁻ Veh (6)
BAL ISDN-1 (6)
TRP⁻ ISDN-1 (6)
BAL ISDN-3 (6)
TRP⁻ ISDN-3 (6)
AhR mRNA expression
E
BAL Veh (5)
TRP⁻ Veh (5)
BAL ISDN-1 (5)
TRP⁻ ISDN-1 (5)
BAL ISDN-3 (5)
TRP⁻ ISDN-3 (5)
% of AhR^Nuc intensity
F
BAL Veh (6
TRP⁻ Veh (6)
BAL ISDN-1 (6)
TRP⁻ ISDN-1 (6)
BAL ISDN-3 (6)
TRP⁻ ISDN-3 (6)
AhRR mRNA expression
G
BAL Veh (6-7)
TRP⁻ Veh (6)
BAL ISDN-1 (6)
TRP⁻ ISDN-1 (6)
BAL ISDN-3 (6)
TRP⁻ ISDN-3 (6)
Cyp1A1
Cyp1B1
mRNA expression

Figure 4. AhR Function in the Noradrenergic LC cells. A- Representative images of tyrosine hydroxylase (TH)-immunoreactive (IR) and aryl hydrocarbon receptor (AhR) positive stained cells with their merged image in the LC. Zoomed images show the co-localization of AhR at the level of the TH-positive neurons. Quantitative assessment of the **B-** Percentage (%) of TH-IR cells expressing AhR (AhR^+) was measured under a balanced (BAL) or TRP-deficient (TRP^-) diet coupled with one (ISDN-1) or three (ISDN-3) ISDN administrations. Symbols represent individual values. $*p < 0.05$ and $**p < 0.01$ by Tukey HSD posttest following 2-way ANOVA. Box plots represent the **C1-** cell body and **C2-** nuclear area of TH-IR AhR^+ cells in the total LC. $*p < 0.05$ and $***p < 0.001$ by Tukey HSD post-hoc test following 2-way ANOVA. **D**-Assessment of the activity through measurements of the mRNA expression of the AhR. $p > 0.05$ by Tukey HSD following 2-way ANOVA. Further assessment is done through the quantification of the **E-** Percentage (%) of nuclear AhR intensity. $*p < 0.05$, by Tukey HSD post-hoc test following 2-way ANOVA. Quantifications of the mRNA expression of further downstream AhR genes including the **F-** AhR repressor (AhRR) and **G-** CYP1A1 and CYP1B1. $p > 0.05$ by Tukey HSD following 2-way ANOVA.

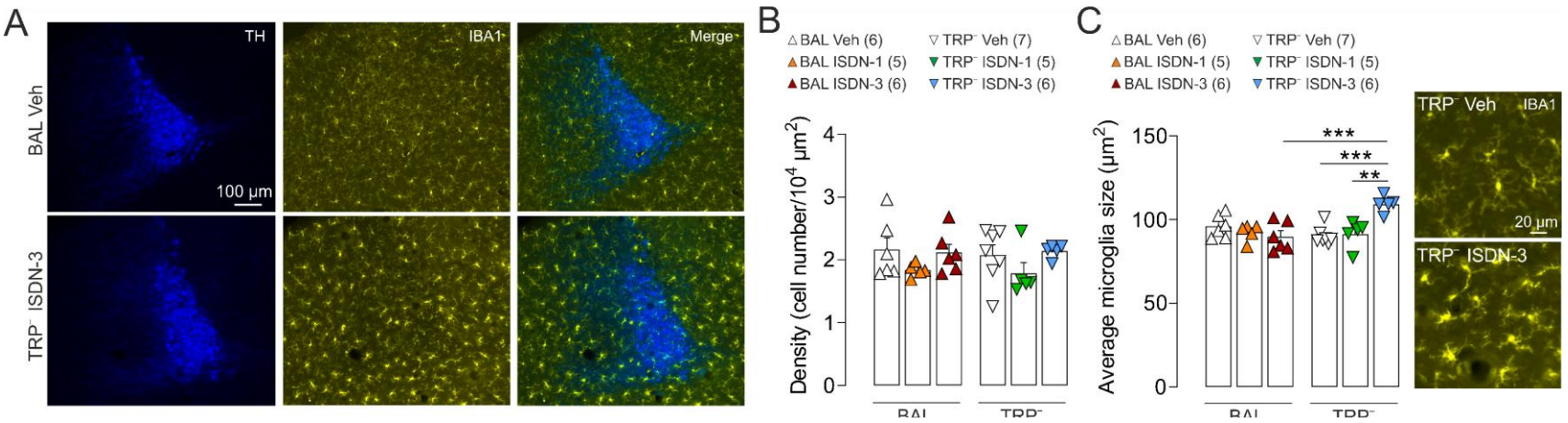


Figure 5. Microglial Reactivity State. A- Representative images of tyrosine hydroxylase (TH) and IBA1-immunoreactive (IR) stained cells with their merged image in the LC under a balanced (BAL) or TRP-free (TRP^{-}) diet coupled with three ISDN administrations or vehicle (Veh). Quantitative assessment of the **B-** Microglia number and **C-** average microglia size. Data represent mean ± S.E.M of (n) mice per group. Symbols represent individual values. ** $p < 0.01$, and *** $p < 0.001$ by Tukey HSD posttest following 2-way ANOVA. The augmentation in the microglial size after ISDN-3 coupled with a TRP-deficient diet is shown in the zoomed images.

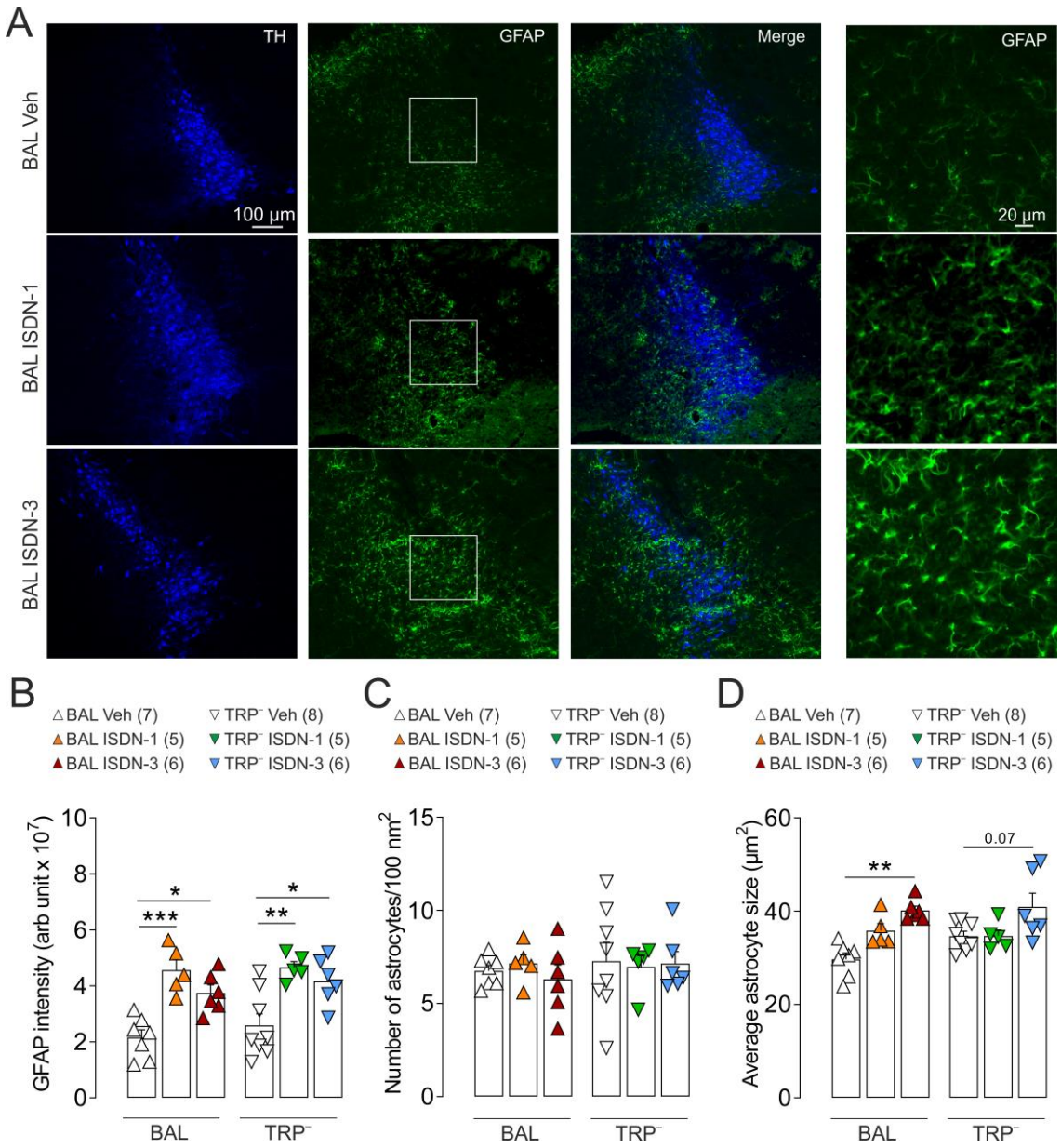


Figure 6. Astrocytic Reactivity State. A- Representative images of tyrosine hydroxylase (TH) and GFAP-immunoreactive (IR) stained cells with their merged image in the LC under a balanced (BAL) diet coupled with one (ISDN-1) or three (ISDN-3) ISDN or vehicle (Veh;) administrations. A zoomed section into the astrocytes stained with GFAP is also shown. Quantitative assessment of **B-** the GFAP Intensity, **C-** the number of astrocytes, and **D-** the astrocyte body size (μm^2) under one week of BAL or TRP-deficient (TRP^{-}) diet. Data represent mean ± S.E.M of (n) mice per group. Symbols represent individual values. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.01$ by Tukey HSD posttest following 2-way ANOVA.

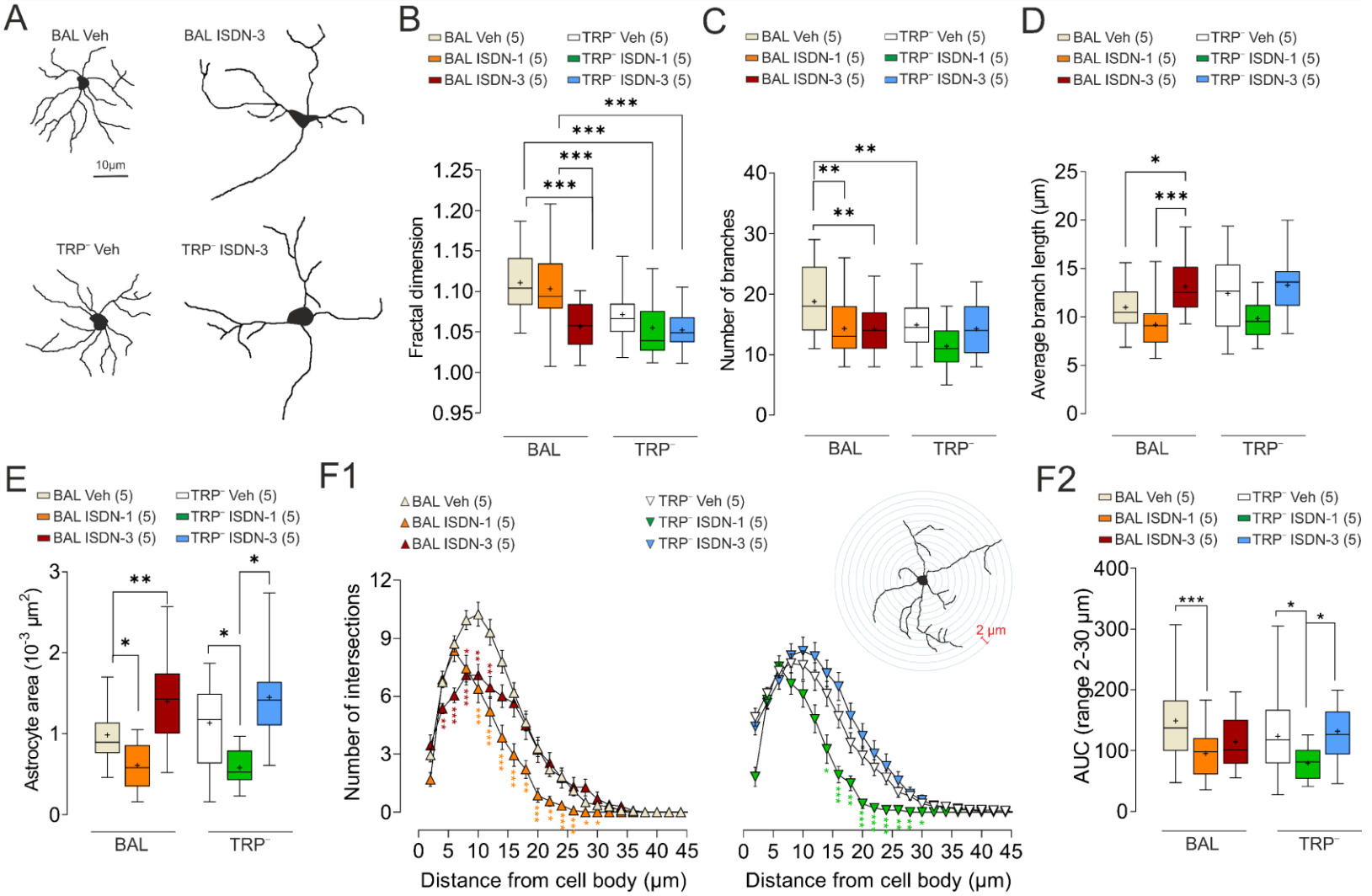

A
BAL Veh
BAL ISDN-3
10µm
TRP⁻ Veh
TRP⁻ ISDN-3
B
BAL Veh (5)
BAL ISDN-1 (5)
BAL ISDN-3 (5)
TRP⁻ Veh (5)
TRP⁻ ISDN-1 (5)
TRP⁻ ISDN-3 (5)
Fractal dimension
BAL
TRP⁻
C
Number of branches
D
Average branch length (µm)
E
Astrocyte area (10⁻³ µm²)
F1
Number of intersections
Distance from cell body (µm)
2 µm
F2
AUC (range 2-30 µm)

Figure 7. Astrocytic Morphological Changes under TRP deficiency and/or ISDN Administration. A- Representative astrocyte 3D reconstructions in the LC under a balanced (BAL) or TRP-deficient (TRP^{-}) diet coupled with one (ISDN-1) or three (ISDN-3) ISDN or vehicle (Veh) administrations. Quantitative assessment of the **B-** Fractal Dimension **C-** number of branches **D-** average branch length and **E-** astrocyte area under ISDN-1 or ISDN-3 administrations at one week of BAL or TRP^{-} diets. Data represent mean ± S.E.M of (n) mice per group. $*p < 0.05$, $**p < 0.01$, and $***p < 0.01$ by Tukey HSD posttest following 2-way ANOVA. **F1-** Sholl analysis of reconstructed astrocytes was performed by counting the number of intersections crossing concentric circles traced around the soma (radii increasing at 2.0 µm steps). $*p < 0.05$, $**p < 0.01$, and $***p < 0.01$ by Tukey HSD following 2-way ANOVA. **F2-** Area under the curve (AUC) of the number of intersections. $*p < 0.05$ and $***p < 0.001$ by Tukey HSD following 2-way ANOVA.

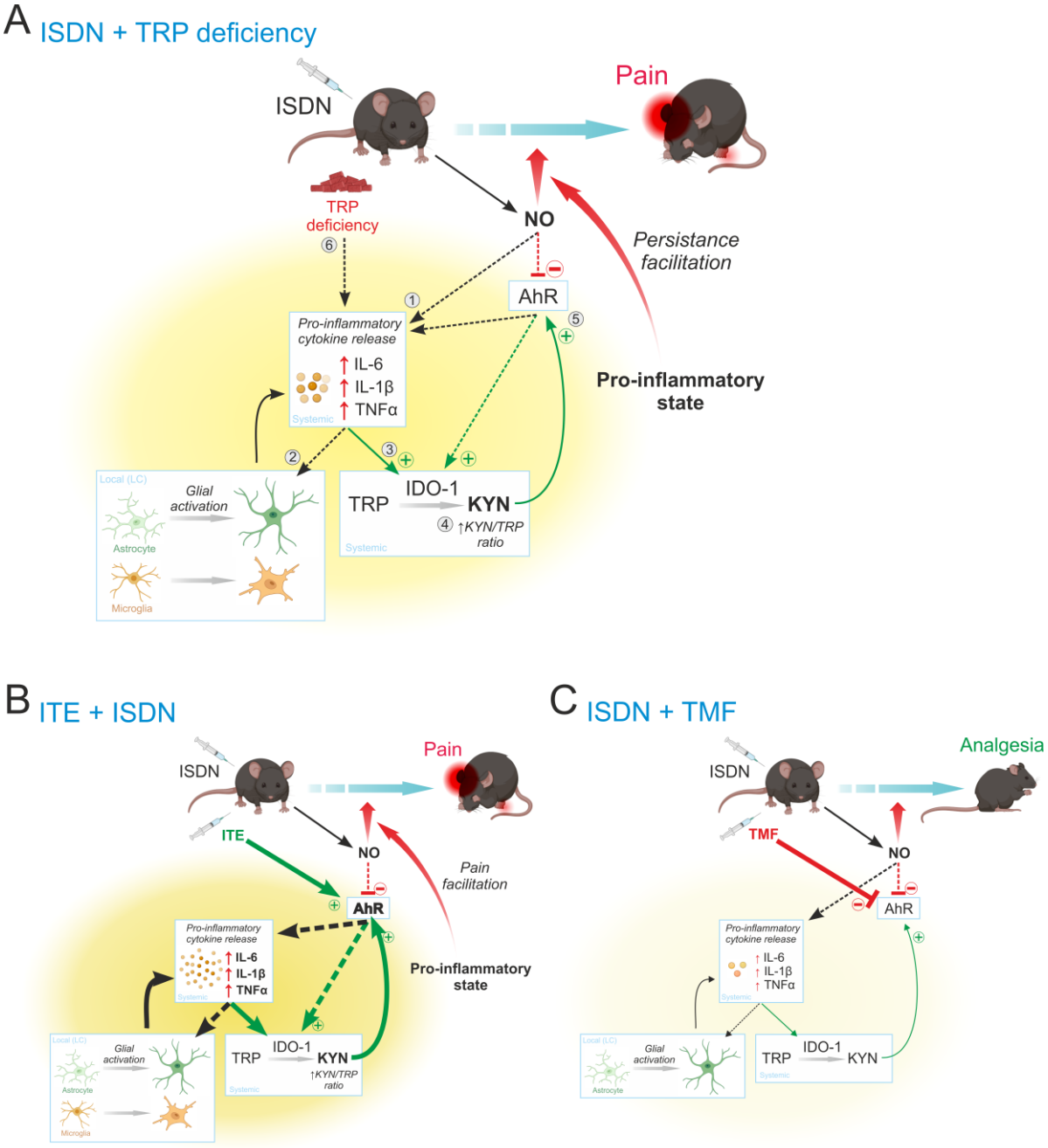

A ISDN + TRP deficiency
ISDN
Pain
TRP deficiency
⑥
NO
Persistance facilitation
AhR
⑤
①
Pro-inflammatory cytokine release
↑ IL-6
↑ IL-1β
↑ TNFα
Systemic
Pro-inflammatory state
②
③
Local (LC)
Glial activation
Astrocyte
Microglia
TRP
IDO-1
KYN
④ ↑KYN/TRP ratio
Systemic
B ITE + ISDN
ISDN
Pain
ITE
NO
Pain facilitation
AhR
Pro-inflammatory cytokine release
↑ IL-6
↑ IL-1β
↑ TNFα
Systemic
Pro-inflammatory state
Local (LC)
Glial activation
Astrocyte
Microglia
TRP
IDO-1
KYN
↑KYN/TRP ratio
Systemic
C ISDN + TMF
ISDN
Analgesia
TMF
NO
AhR
Pro-inflammatory cytokine release
↑ IL-6
↑ IL-1β
↑ TNFα
Systemic
Local (LC)
Glial activation
Astrocyte
TRP
IDO-1
KYN
Systemic

Figure 8. Schematic Summary of the Inflammatory State During Migraine-like Pain and the AhR Involvement. A- Putative Mechanism of ISDN-induced Inflammatory Responses Under TRP deficiency. (1) ISDN-derived nitric oxide (NO) promotes the systemic release (in the blood) of pro-inflammatory cytokines, including interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)α. (2) Pro-inflammatory cytokines lead to local astrogliosis in the locus coeruleus (LC), which releases more pro-inflammatory cytokines in a positive feedback loop. (3) In parallel, TNFα induces the peripheral expression of the enzyme indoleamine 2,3-dioxygenase (IDO; mainly in dendritic cells) that degrades tryptophan (TRP) to kynurenine (KYN). (4) Activation of the KYN pathway leads to a plasmatic TRP reduction and an increase in the KYN/TRP ratio. (5) KYN regulates inflammation through activation of the aryl hydrocarbon receptor (AhR), which in turn, promotes cytokine release and sustains IDO function. Together, these processes generate a pro-inflammatory state that facilitates pain. (6) Prior systemic release of IL-6, IL-1β, and TNFα induced by dietary TRP deficiency for one week establishes an inflammatory pre-state that enhances the ISDN-induced inflammatory responses, as supported by additional microglial activation (2) in the LC. This inflammatory pre-state contributes to pain facilitation and increases CMH persistence. **B and C- Proposed Mechanism for Pro-nociceptive AhR Action in ISDN-induced Migraine-like Pain. B-** Like under TRP deficiency, AhR activation by ITE before ISDN administration could enhance the release of pro-inflammatory cytokines and glial activation, establishing again an inflammatory pre-state that potentiates the ISDN-induced inflammatory response. This process worsens the intensity and prolongs the CMH in mice. **C-** Inhibition of AhR by TMF, administered after ISDN, blocks the AhR activation by KYN. It limits the IDO/AhR- related inflammation, which leads to reduced CMH in mice.

TABLES

Table 1. Summary of Statistical Analyses Corresponding to Figures 1 to 7 and Tables 2 and 3

Fig	Test	$F_{[DFn, DFd]}$ or t_{DF}	*p-value*	$F_{[DFn, DFd]}$	*p-value*	$F_{[DFn, DFd]}$	*p-value*	$F_{interaction\ [DFn, DFd]}$	*p-value*
1B L	RM 3-way	$F_{Time[3,60]}$=29.90	*<0.0001*	$F_{ISDN[1,20]}$=28.60	*<0.0001*	$F_{NB\text{-}INJ[1,20]}$=613.6	*<0.0001*	$F_{[3,60]}$=7.90	*0.0002*
1B R	RM 3-way	$F_{Time[3,60]}$=50.70	*<0.0001*	$F_{ISDN[1,20]}$=3.10	*0.09*	$F_{NB\text{-}INJ[1,20]}$=146	*<0.0001*	$F_{[3,60]}$=20.00	*<0.0001*
1C	RM 3-way	$F_{Time[10,170]}$=14.40	*<0.0001*	$F_{ISDN[1,17]}$=20.40	*0.0003*	$F_{Diet[1,17]}$=3.80	*0.07*	$F_{[10,170]}$=2.20	*0.020*
1D1	2-way	$F_{Diet[1,20]}$=56.30	*<0.0001*	$F_{NB\text{-}INJ[1,20]}$=4.50	*0.05*			$F_{[1,20]}$=3.20	*0.09*
1D2	Student t-Test	$t_{[5,5]}$=5.50	*0.08*						
1E	Welch's Test	$t_{[5,5]}$=12.3	*0.02*						
1F1	RM 3-way	$F_{Time[1.9,30.4]}$=30.60	*<0.0001*	$F_{Treatment[1,16]}$=15.72	*0.001*	$F_{NB\text{-}INJ[1,16]}$=10.30	*0.006*	$F_{[3,48]}$=2.24	*0.10*
1F2	2-way	$F_{Treatment[1,16]}$=0.15	*0.70*	$F_{NB\text{-}INJ[1,16]}$=8.65	*0.001*			$F_{[1,16]}$=5.54	*0.03*
1G1	2-way	$F_{Time[3,24]}$=2.50	*0.09*	$F_{Treatment[3,24]}$=48.2	*<0.0001*			$F_{[8,24]}$=1.10	*0.40*
1G2	Student t-Test	$t_{[4,4]}$=1.60	*0.006*						
2A L	RM 3-way	$F_{Time[2.40,38.46]}$=147.6	*<0.0001*	$F_{ISDN[1,16]}$=33.32	*<0.0001*	$F_{NB\text{-}INJ[1,20]}$=3.84	*0.06*	$F_{[3,48]}$=0.81	*0.49*
2A R	RM 3-way	$F_{Time[2.05,32.82]}$=219.9	*<0.0001*	$F_{ISDN[1,16]}$=61.89	*<0.0001*	$F_{NB\text{-}INJ[1,16]}$=0.08	*0.79*	$F_{[3,48]}$=4.40	*0.008*
2B	2-way	$F_{Diet[1,16]}$=0.09	*0.77*	$F_{NB\text{-}INJ[1,16]}$=7.97	*0.002*			$F_{[1,16]}$=0.94	*0.41*
3A2	2-way	$F_{Diet[1,31]}$=0.09	*0.77*	$F_{ISDN[2,31]}$=7.97	*0.002*			$F_{[2,31]}$=0.94	*0.41*
3A3	2-way	$F_{Diet[1,47]}$= 0.14	*0.71*	$F_{ISDN[2,47]}$=1.21	*0.31*			$F_{[2,47]}$=0.42	*0.66*
3B	2-way	$F_{Diet[1,30]}$= 13.81	*0.0008*	$F_{ISDN[2,30]}$=0.14	*0.87*			$F_{[2,30]}$=0.17	*0.85*
3C	2-way	$F_{Diet[1,28]}$=11.37	*0.002*	$F_{ISDN[2,28]}$=2.42	*0.11*			$F_{[2,28]}$=3.72	*0.04*
4B	2-way	$F_{Diet[1,48]}$=2.91	*0.09*	$F_{ISDN[2,48]}$=1.33	*0.27*			$F_{[2,48]}$=6.96	*0.002*
4C1	2-way	$F_{Diet[1,964]}$=0.15	*0.69*	$F_{ISDN[2,964]}$=12.45	*<0.0001*			$F_{[2,48]}$=9.15	*0.0001*
4C2	2-way	$F_{Diet[1,964]}$=13.72	*0.0002*	$F_{ISDN[2,964]}$=9.50	*<0.0001*			$F_{[2,48]}$=10.65	*0.0001*
4D	2-way	$F_{Diet[1,30]}$=0.75	*0.39*	$F_{ISDN[2,30]}$=5.47	*0.009*			$F_{[2,30]}$=0.21	*0.81*
4E	2-way	$F_{Diet[1,964]}$=3.30	*0.07*	$F_{ISDN[2,964]}$=3.56	*0.03*			$F_{[2,964]}$=1.31	*0.27*
4F	2-way	$F_{Diet[1,30]}$=1.92	*0.18*	$F_{ISDN[2,30]}$=3.52	*0.04*			$F_{[2,30]}$=0.56	*0.57*
4G L	2-way	$F_{Diet[1,29]}$=0.003	*0.96*	$F_{ISDN[2,29]}$=1.83	*0.18*			$F_{[2,29]}$=0.18	*0.83*
4G R	2-way	$F_{Diet[1,31]}$=0.24	*0.63*	$F_{ISDN[2,31]}$=3.92	*0.03*			$F_{[2,31]}$=2.03	*0.15*
5B	2-way	$F_{Diet[1,28]}$=0.09	*0.76*	$F_{ISDN[2,28]}$=2.76	*0.08*			$F_{[2,28]}$=0.08	*0.93*
5C	2-way	$F_{Diet[1,28]}$=3.29	*0.08*	$F_{ISDN[2,28]}$=4.06	*0.03*			$F_{[2,28]}$=11.43	*0.0002*
6B	2-way	$F_{Diet[1,31]}$=1.25	*0.27*	$F_{ISDN[2,31]}$=23.30	*<0.0001*			$F_{[2,31]}$=0.13	*0.88*
6C	2-way	$F_{Diet[1,31]}$=0.42	*0.52*	$F_{ISDN[2,31]}$=0.12	*0.89*			$F_{[2,31]}$=0.22	*0.80*
6D	2-way	$F_{Diet[1,31]}$=0.65	*0.42*	$F_{ISDN[2,31]}$=10.41	*0.0003*			$F_{[2,31]}$=2.09	*0.14*
7B	2-way	$F_{Diet[1,160]}$=29.05	*<0.0001*	$F_{ISDN[2,160]}$=16.83	*<0.0001*			$F_{[2,160]}$=5.56	*0.005*
7C	2-way	$F_{Diet[1,160]}$=9.31	*0.003*	$F_{ISDN[2,160]}$=10.40	*<0.0001*			$F_{[2,160]}$=2.81	*0.06*
7D	2-way	$F_{Diet[1,160]}$=3.03	*0.08*	$F_{ISDN[2,160]}$=21.83	*<0.0001*			$F_{[2,160]}$=0.88	*0.42*
7E	2-way	$F_{Diet[1,160]}$=0.73	*0.39*	$F_{ISDN[2,160]}$=47.60	*<0.0001*			$F_{[2,160]}$=0.62	*0.54*
7G1L	2-way	$F_{Distance[3.5,354.9]}$=178.00	*<0.0001*	$F_{ISDN[2,101]}$=8.91	*0.0003*			$F_{[24,1212]}$=13.2	*<0.0001*
7G1R	2-way	$F_{Distance[3.18,260.5]}$=145.40	*<0.0001*	$F_{ISDN[2,82]}$=5.95	*0.004*			$F_{[24,984]}$=3.03	*<0.0001*
7G2	2-way	$F_{Diet[1,177]}$=0.90	*0.34*	$F_{ISDN[2,177]}$=12.68	*<0.0001*			$F_{[2,177]}$=2.68	*0.07*
Table 2-Plasma									
TRP	2-way	$F_{Diet[1,26]}$=41.04	*<0.0001*	$F_{ISDN[2,26]}$=6.15	*0.007*			$F_{[2,26]}$=1.75	*0.19*
KYN	2-way	$F_{Diet[1,25]}$=11.25	*0.003*	$F_{ISDN[2,25]}$=3.70	*0.04*			$F_{[2,25]}$=0.43	*0.66*
Ratio	2-way	$F_{Diet[1,30]}$=20.94	*<0.0001*	$F_{ISDN[2,30]}$=0.64	*0.53*			$F_{[2,30]}$=1.25	*0.30*
Table 2-LC									
TRP	2-way	$F_{Diet[1,30]}$=59.5	*<0.0001*	$F_{ISDN[2,30]}$=0.64	*0.53*			$F_{[2,30]}$=3.37	*0.05*
KYN	2-way	$F_{Diet[1,28]}$=0.28	*0.60*	$F_{ISDN[2,28]}$=0.04	*0.96*			$F_{[2,28]}$=5.06	*0.01*
Ratio	2-way	$F_{Diet[1,25]}$=1.92	*0.18*	$F_{ISDN[2,25]}$=0.14	*0.87*			$F_{[2,25]}$=0.11	*0.90*
Table 3									
IL-6	2-way	$F_{Diet[1,38]}$=16.30	*0.0003*	$F_{ISDN[2,38]}$=11.22	*0.0001*			$F_{[2,38]}$=17.79	*<0.0001*
IFNg	2-way	$F_{Diet[1,38]}$=3.48	*0.07*	$F_{ISDN[2,38]}$=2.51	*0.09*			$F_{[2,38]}$=0.54	*0.59*
TNFα	2-way	$F_{Diet[1,38]}$=8.45	*0.006*	$F_{ISDN[2,38]}$=17.35	*<0.0001*			$F_{[2,38]}$=5.63	*0.007*
IL1β	2-way	$F_{Diet[1,38]}$=25.69	*<0.0001*	$F_{ISDN[2,38]}$=28.99	*<0.0001*			$F_{[2,38]}$=29.48	*<0.0001*

Data represent *t* and *F* values for Student t-Test, Welch's test, and repeated measure (RM) or not 2 or 3-way ANOVA with corresponding degrees of freedom (DFn, DFd), and *p*-values. ANOVA factors were designed as followed: *Time*, for comparisons between different time points; *Treatment*, for comparisons between TMF or ITE; *Diet*, for comparisons between BAL and TRP^-; *ISDN*, for comparisons between ISDN and Vehicle; *NB-INJ*, number of injections, for comparisons between animals receiving one or three injections; *Distance*, for the distance from the cell body, *Interaction* for effect between two or three factors on the dependent variable. $p < 0.05$ was considered statistically significant. R: Right; L: Left.

Table 2. Levels of Tryptophan (TRP) and Kynurenine (KYN)

	Vehicle	ISDN-1	ISDN-3
$[TRP]_{plasma}$ (nM)			
BAL diet	27.72 ± 1.41	20.52 ± 3.06	17.62 ± 2.5$^{\#}$
TRP^- diet	**5.81 ± 0.73*****	**7.84 ± 2.22*****	**11.01 ± 2.61*****
$[KYN]_{plasma}$ (µM)			
BAL diet	1.63 ± 0.20	0.84 ± 0.14	1.39 ± 0.24
TRP^- diet	1.01 ± 0.11	1.80 ± 0.53	1.37 ± 0.13
$[KYN]/[TRP]\ Ratio_{plasma}$			
BAL diet	85.56 ± 26.09	67.33 ± 15.56	134.85 ± 28.65
TRP^- diet	**209.78 ± 29.72***	**193.01 ± 19.56**$^{+}$	186.27 ± 36.42
$[TRP]_{LC}$ (µM)			
BAL diet	2.55 ± 0.17	3.08 ± 0.07	3.12 ± 0.25
TRP^- diet	**1.69 ± 0.17***	2.42 ± 0.12	**1.81 ± 0.22**$^{+++}$
$[KYN]_{LC}$ (nM)			
BAL diet	119.72 ± 19.47	174.91 ± 8.28	128.82 ± 13.87
TRP^- diet	78.60 ± 16.37	112.44 ± 17.53	96.03 ± 21.44
$[KYN]/[TRP]\ Ratio_{LC}$			
BAL diet	48.45 ± 9.69	56.21 ± 2.91	50.79 ± 9.51
TRP^- diet	42.65 ± 8.32	43.24 ± 7.00	42.84 ± 6.50

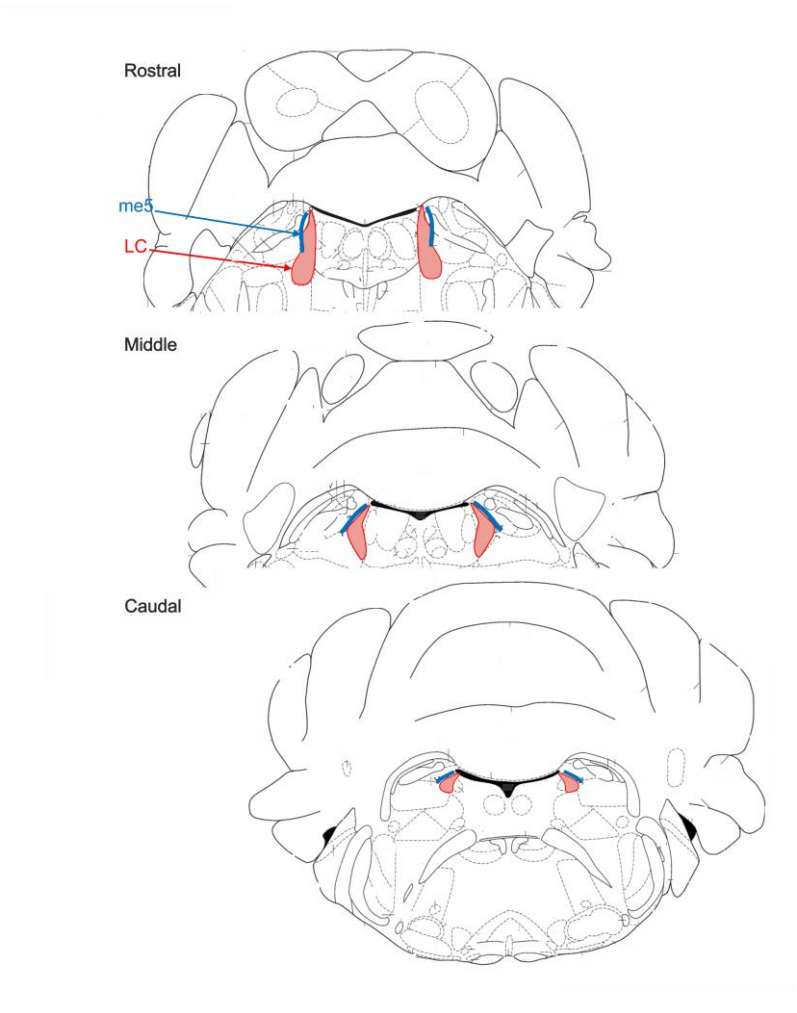
Data represent mean ± SEM of (5-6) mice per group. Levels of TRP and KYN were measured after one week of a balanced (BAL) or Trp-deficient (TRP^-) diet by HPLC. Samples were collected 1 h after ISDN/vehicle administration. $^{*}p$ <0.05 and $^{***}p$ <0.001 *vs.* corresponding BAL group; $^{\#}p$ <0.05 *vs.* BAL-vehicle group;^{+}p <0.05, ^{+++}p <0.001 *vs.* BAL-ISDN group by Tukey's HSD posttest following 2-way ANOVA.

Table 3. Levels of Pro-inflammatory Cytokines In the Plasma

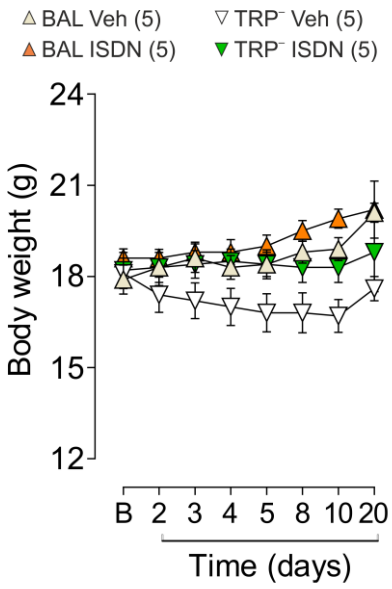
	Vehicle	ISDN-1	ISDN-3
[IL-6] (pg/mL)			
BAL diet	46.76 ± 3.91	**95.40 ± 3.54*****	**89.98 ± 3.08*****
TRP^{-} diet	**96.77 ± 11.17*****	**89.98 ± 90.34*****	**90.34 ± 0.99*****
[TNFα] (pg/mL)			
BAL diet	257.82 ± 15.21	**447.50 ± 11.70*****	**439.45 ± 11.24*****
TRP^{-} diet	**400.33 ± 52.71*****	**456.25 ± 21.16*****	**445.83 ± 8.57*****
[IL-1β] (pg/mL)			
BAL diet	26.75 ± 2.09	**67.28 ± 2.84*****	**76.55 ± 6.24*****
TRP^{-} diet	**70.72 ± 1.75*****	**70.43 ± 1.18*****	**70.56 ± 1.14*****
[IFNγ] (pg/mL)			
BAL diet	208.27 ± 3.39	201.46 ± 1.84	201.45 ± 2.19
TRP^{-} diet	201.91 ± 3.75	199.41 ± 1.34	199.41 ± 1.38

Data represent mean ± SEM of (6-8) mice per group. Levels of cytokines were measured after one week of a balanced (BAL) or Trp-deficient (TRP^{-}) diet by ELISA. Samples were collected 1 h after ISDN/vehicle administration. ***p <0.001 *vs.* corresponding BAL-Vehicle group; by Tukey's HSD posttest following 2-way ANOVA.

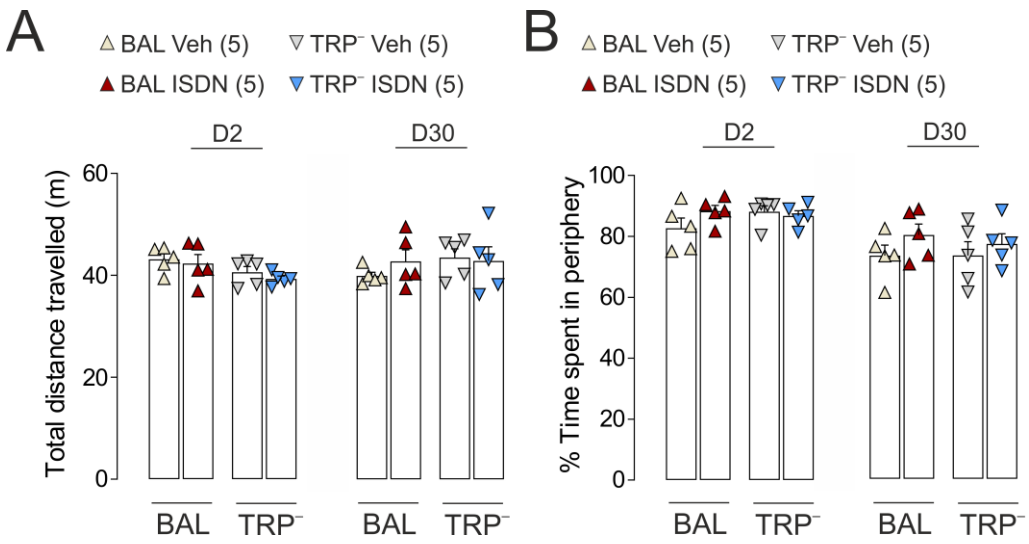
SUPPLEMENTARY FIGURES



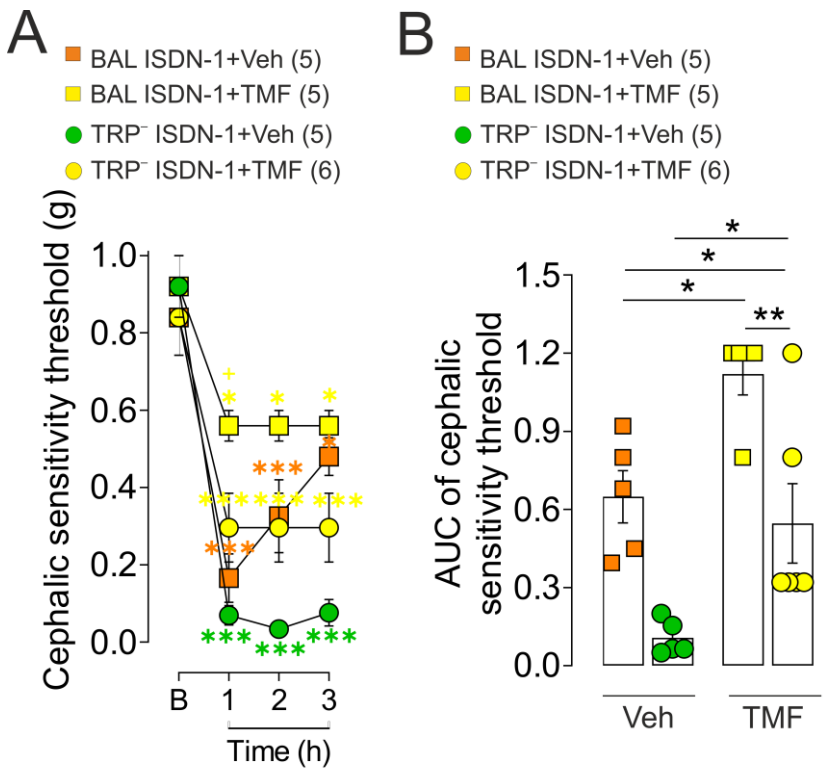
Supplementary Figure S1. Rostro-caudal Sections of the LC. The location and shape of the LC are according to its rostrocaudal orientation. Starting from the most rostral section of the LC (from AP -5.2 to AP -5.34, relative to Bregma), continued through the middle portion (from AP -5.34 to AP -5.52) and finished in the most caudal slide (from AP -5.52 to AP -5.8). me5: mesencephalic trigeminal nucleus. Edited and Adapted from (Paxinos & Franklin, 2019).



Supplementary Figure S2. Body weight of mice after ISDN or vehicle (Veh) administration, in a balanced (BAL) or TRP-deficient (TRP⁻) diet. Data represent mean ± SEM of (n) mice per group. p >0.05 by Tukey HSD posttest following RM 3-way ANOVA.



Supplementary Figure S3. Anxiety-like behavior assessment at 2 and 30 days after ISDN or vehicle (Veh) administration, in a balanced (BAL) or TRP-deficient (TRP⁻) diet through measurement of **A-** total distance traveled and **B-** the percentage of time in the periphery in the open field test. Data represent mean ± SEM of (n) mice per group. p >0.05 by Tukey HSD posttest following 2-way ANOVA.



Supplementary Figure S4. A- Mechanical sensitivity threshold after TMF/Vehicle administration at one week of balanced (BAL) or TRP-deficient (TRP^{-}) diet. Data are presented as mean ± S.E.M of (n) mice per group. *p < 0.05 and ***p < 0.001 vs. baseline, ^{+}p < 0.05 vs. BAL ISDN-1+Veh group by Tukey HSD posttest following RM 3-way ANOVA. **B-** AUC of the cephalic sensitivity after TMF injection in TRP^{-} groups. Symbols represent individual values. *p < 0.05 and **p < 0.01 by Tukey HSD posttest following 2-way ANOVA.

SUPPLEMENTARY TABLES

Table S1. Summary of Statistical Analysis Corresponding to Figures S2 to S4 and Tables S2 and S6

Fig	Test	$F_{[DFn, DFd]}$ or t_{DF}	*p-value*	$F_{[DFn, DFd]}$	*p-value*	$F_{[DFn, DFd]}$	*p-value*	$F_{interaction\ [DFn, DFd]}$	*p-value*
S2	RM 3-way	$F_{Time[7,252]}$=5.82	*<0.0001*	$F_{ISDN[1,36]}$=3.43	*0.07*	$F_{Diet[1,36]}$=5.86	*0.21*	$F_{[7,252]}$=0.93	*0.49*
S3A D2	2-way	$F_{Diet[1,16]}$=5.23	*0.04*	$F_{ISDN[1,16]}$=0.73	*0.41*			$F_{[1,16]}$=0.04	*0.85*
S3A D30	2-way	$F_{Diet[1,16]}$=0.82	*0.38*	$F_{ISDN[1,16]}$=0.30	*0.59*			$F_{[1,16]}$=0.76	*0.40*
S3B D2	2-way	$F_{Diet[1,16]}$=0.71	*0.41*	$F_{ISDN[1,16]}$=0.84	*0.37*			$F_{[1,16]}$=2.31	*0.15*
S3B D30	2-way	$F_{Diet[1,16]}$=0.15	*0.71*	$F_{ISDN[1,16]}$=2.0	*0.18*			$F_{[1,16]}$=0.16	*0.70*
S4A	RM 3-way	$F_{Time[3,48]}$=73.50	*<0.0001*	$F_{ISDN[1,16]}$=26.03	*0.0001*	$F_{Treatment[1,16]}$=20.80	*0.0003*	$F_{[3,48]}$=1.26	*0.30*
S4B	2-way	$F_{Time[1,17]}$=17.63	*0.0006*	$F_{Treatment[1,17]}$=26.44	*<0.0001*			$F_{[1,17]}$=0.02	*0.89*
Table S2									
TH positive area									
R	2-way	$F_{Diet[1,25]}$=2.62	*0.12*	$F_{ISDN[2,25]}$=7.94	*0.002*			$F_{[2,25]}$=0.32	*0.73*
M	2-way	$F_{Diet[1,29]}$=2.49	*0.13*	$F_{ISDN[2,29]}$=3.16	*0.06*			$F_{[2,29]}$=2.00	*0.15*
C	2-way	$F_{Diet[1,29]}$=0.26	*0.61*	$F_{ISDN[2,29]}$=1.76	*0.19*			$F_{[2,29]}$=1.03	*0.37*
TH-IR cell number/104 µm2									
R	2-way	$F_{Diet[1,37]}$=2.40	*0.13*	$F_{ISDN[2,37]}$=2.30	*0.11*			$F_{[2,37]}$=1.33	*0.28*
M	2-way	$F_{Diet[1,38]}$=0.16	*0.69*	$F_{ISDN[2,38]}$=0.52	*0.60*			$F_{[2,38]}$=0.58	*0.56*
C	2-way	$F_{Diet[1,39]}$=0.11	*0.74*	$F_{ISDN[2,39]}$=2.25	*0.12*			$F_{[2,39]}$=1.14	*0.33*
Table S3									
% of TH-IR cells expressing AhR									
R	2-way	$F_{Diet[1,37]}$=0.02	*0.89*	$F_{ISDN[2,37]}$=0.64	*0.53*			$F_{[2,37]}$=4.91	*0.01*
M	2-way	$F_{Diet[1,36]}$=0.92	*0.34*	$F_{ISDN[2,36]}$=0.05	*0.95*			$F_{[2,36]}$=2.46	*0.10*
C	2-way	$F_{Diet[1,38]}$=0.18	*0.67*	$F_{ISDN[2,38]}$=0.74	*0.48*			$F_{[2,38]}$=2.38	*0.11*
Nuclear area (µm2)									
R	2-way	$F_{Diet[1,260]}$=0.02	*0.88*	$F_{ISDN[2,260]}$=2.09	*0.13*			$F_{[2,260]}$=3.35	*0.04*
M	2-way	$F_{Diet[1,371]}$=11.71	*0.0007*	$F_{ISDN[2,371]}$=3.58	*0.03*			$F_{[2,371]}$=9.58	*<0.0001*
C	2-way	$F_{Diet[1,321]}$=10.32	*0.002*	$F_{ISDN[2,321]}$=7.83	*0.0005*			$F_{[2,321]}$=3.37	*0.04*
Cell Body area (µm2)									
R	2-way	$F_{Diet[1,260]}$=0.02	*0.90*	$F_{ISDN[2,260]}$=3.51	*0.03*			$F_{[2,260]}$=1.96	*0.14*
M	2-way	$F_{Diet[1,371]}$=0.18	*0.67*	$F_{ISDN[2,371]}$=2.26	*0.11*			$F_{[2,371]}$=7.18	*0.0009*
C	2-way	$F_{Diet[1,321]}$=1.78	*0.18*	$F_{ISDN[2,321]}$=6.22	*0.0022*			$F_{[2,321]}$=3.37	*0.04*
% of AhRNuc intensity									
R	2-way	$F_{Diet[1,267]}$=0.03	*0.86*	$F_{ISDN[2,267]}$=10.19	*<0.0001*			$F_{[2,267]}$=2.69	*0.07*
M	2-way	$F_{Diet[1,421]}$=2.14	*0.14*	$F_{ISDN[2,421]}$=0.67	*0.51*			$F_{[2,421]}$=1.51	*0.22*
C	2-way	$F_{Diet[1,321]}$=1.75	*0.19*	$F_{ISDN[2,321]}$=7.25	*0.0008*			$F_{[2,321]}$=3.37	*0.04*
Table S4									
Density cell number/104 µm2									
R	2-way	$F_{Diet[1,23]}$=1.69	*0.21*	$F_{ISDN[2,23]}$=2.26	*0.13*			$F_{[2,23]}$=0.20	*0.82*
M	2-way	$F_{Diet[1,26]}$=5.29	*0.03*	$F_{ISDN[2,26]}$=1.08	*0.36*			$F_{[2,26]}$=0.10	*0.90*
C	2-way	$F_{Diet[1,24]}$=0.58	*0.46*	$F_{ISDN[2,24]}$=1.34	*0.28*			$F_{[2,24]}$=1.16	*0.33*
Average microglia size (µm2)									
R	2-way	$F_{Diet[1,23]}$=0.94	*0.34*	$F_{ISDN[2,23]}$=0.92	*0.41*			$F_{[2,23]}$=0.74	*0.49*
M	2-way	$F_{Diet[1,26]}$=0.0007	*0.98*	$F_{ISDN[2,26]}$=5.27	*0.01*			$F_{[2,26]}$=3.35	*0.05*
C	2-way	$F_{Diet[1,24]}$=0.65	*0.43*	$F_{ISDN[2,24]}$=5.20	*0.01*			$F_{[2,24]}$=2.20	*0.13*
Table S5									
GFAP Intensity (arb unit x107)									
R	2-way	$F_{Diet[1,25]}$=0.68	*0.42*	$F_{ISDN[2,25]}$=14.13	*<0.0001*			$F_{[2,25]}$=0.63	*0.54*
M	2-way	$F_{Diet[1,28]}$=0.80	*0.38*	$F_{ISDN[2,28]}$=16.98	*<0.0001*			$F_{[2,28]}$=0.32	*0.73*
C	2-way	$F_{Diet[1,29]}$=1.13	*0.30*	$F_{ISDN[2,29]}$=2.16	*0.13*			$F_{[2,29]}$=0.46	*0.64*
Number of astrocytes/100 nm2									
R	2-way	$F_{Diet[1,25]}$=0.80	*0.38*	$F_{ISDN[2,25]}$=1.51	*0.24*			$F_{[2,25]}$=0.36	*0.70*
M	2-way	$F_{Diet[1,28]}$=0.15	*0.70*	$F_{ISDN[2,28]}$=0.14	*0.87*			$F_{[2,28]}$=0.01	*0.99*
C	2-way	$F_{Diet[1,27]}$=0.39	*0.54*	$F_{ISDN[2,27]}$=0.05	*0.95*			$F_{[2,27]}$=0.86	*0.44*
Average size (µm2)									
R	2-way	$F_{Diet[1,24]}$=2.50	*0.13*	$F_{ISDN[2,24]}$=8.08	*0.002*			$F_{[2,24]}$=0.72	*0.50*
M	2-way	$F_{Diet[1,28]}$=4.78	*0.04*	$F_{ISDN[2,28]}$=6.08	*0.01*			$F_{[2,28]}$=5.99	*0.01*
C	2-way	$F_{Diet[1,27]}$=0.36	*0.55*	$F_{ISDN[2,27]}$=6.52	*0.005*			$F_{[2,27]}$=0.64	*0.54*
Table S6									
Fractal Dimension									
R	2-way	$F_{Diet[1,52]}$=7.77	*0.01*	$F_{ISDN[2,52]}$=19.47	*<0.0001*			$F_{[2,52]}$=1.42	*0.25*
M	2-way	$F_{Diet[1,47]}$=4.74	*0.03*	$F_{ISDN[2,47]}$=2.94	*0.063*			$F_{[2,47]}$=1.58	*0.22*

C	2-way	$F_{Diet[1,49]}$=20.03	*<0.0001*	$F_{ISDN[2,49]}$=5.17	*0.009*	$F_{[2,49]}$=4.75	*0.01*
Average Branch length (μm)							
R	2-way	$F_{Diet[1,52]}$=2.70	*0.11*	$F_{ISDN[2,52]}$=15.71	*<0.0001*	$F_{[2,52]}$=0.37	*0.70*
M	2-way	$F_{Diet[1,47]}$=0.28	*0.60*	$F_{ISDN[2,47]}$=9.45	*0.0004*	$F_{[2,47]}$=2.72	*0.08*
C	2-way	$F_{Diet[1,49]}$=0.94	*0.34*	$F_{ISDN[2,49]}$=3.97	*0.03*	$F_{[2,49]}$=2.15	*0.13*
Number of Branches							
R	2-way	$F_{Diet[1,52]}$=0.34	*0.56*	$F_{ISDN[2,52]}$=7.36	*0.002*	$F_{[2,52]}$=5.73	*0.01*
M	2-way	$F_{Diet[1,47]}$=0.80	*0.37*	$F_{ISDN[2,47]}$=0.65	*0.53*	$F_{[2,47]}$=4.88	*0.01*
C	2-way	$F_{Diet[1,49]}$=18.80	*<0.0001*	$F_{ISDN[2,49]}$=14.56	*<0.0001*	$F_{[2,49]}$=6.05	*0.005*
Astrocyte area (103 μm2)							
R	2-way	$F_{Diet[1,52]}$=1.66	*0.20*	$F_{ISDN[2,52]}$=13.68	*<0.0001*	$F_{[2,52]}$=1.58	*0.22*
M	2-way	$F_{Diet[1,47]}$=1.54	*0.22*	$F_{ISDN[2,47]}$=18.36	*<0.0001*	$F_{[2,47]}$=0.38	*0.68*
C	2-way	$F_{Diet[1,49]}$=0.69	*0.41*	$F_{ISDN[2,49]}$=17.50	*<0.0001*	$F_{[2,49]}$=0.57	*0.57*

Data represent *F* values for repeated measure (RM) or not 2 or 3-way ANOVA with corresponding degrees of freedom (DFn, DFd), and *p*-values. ANOVA factors were designed as followed: *Time*, for comparisons between different time points; *Treatment*, for comparisons between TMF or ITE; *Diet*, for comparisons between BAL and TRP$^-$; *ISDN*, for comparisons between ISDN and Vehicle; *Interaction* for effect between two or three factors on the dependent variable. p <0.05 was considered statistically significant. R: Rostral; M: Middle; C: Caudal; D2: Day 2; D30: Day 30.

Table S2. Effect of ISDN and a TRP-deficient Diet on Noradrenergic LC Cells

	Group	Rostral	Middle	Caudal
TH positive area (10^4 μm^2)	BAL Vehicle	20.05 ± 1.76	17.96 ± 1.84	9.26 ± 1.04
	BAL ISDN-1	28.21 ± 1.33	23.97 ± 1.57	11.12 ± 1.87
	BAL ISDN-3	28.98 ± 3.15	27.67 ± 3.45	14.67 ± 3.16
	TRP$^-$ Vehicle	24.33 ± 3.99	19.82 ± 1.85	12.40 ± 1.39
	TRP$^-$ ISDN-1	29.11 ± 1.72	19.76 ± 3.06	11.77 ± 1.41
	TRP$^-$ ISDN-3	33.44 ± 1.10	21.16 ± 1.56	13.05 ± 1.41
TH-IR cell number/10^4 μm^2	BAL Vehicle	8.33 ± 0.92	13.15 ± 1.04	10.67 ± 1.02
	BAL ISDN-1	11.35 ± 1.31	10.73 ± 1.69	8.29 ± 0.94
	BAL ISDN-3	7.38 ± 0.93	12.81 ± 1.04	12.50 ± 1.33
	TRP$^-$ Vehicle	8.08 ± 1.18	12.17 ± 1.19	10.07 ± 0.70
	TRP$^-$ ISDN-1	7.86 ± 0.77	12.04 ± 0.74	9.89 ± 1.11
	TRP$^-$ ISDN-3	7.01 ± 1.22	11.23 ± 1.29	10.60 ± 1.20

Data represent mean ± SEM of (4-10) animals of the TH positive area and the number of TH-IR (immunoreactive) cells under balanced (BAL) or TRP-deficient (TRP$^-$**)** diets coupled with a vehicle, one (ISDN-1), or three ISDN (ISDN-3) injections. p >0.05 by Tukey's HSD posttest following 2-way ANOVA.

Table S3. Morphological Characteristics of AhR$^+$ TH-IR Cells				
	Group	**Rostral**	**Middle**	**Caudal**
% of TH-IR cell expressing AhR	BAL Vehicle	45.52 ± 3.50	51.69 ± 2.81	51.34 ± 2.51
	BAL ISDN-1	46.73 ± 4.05	51.27 ± 3.04	48.11 ± 2.73
	BAL ISDN-3	59.15 ± 2.85	57.84 ± 2.81	59.97 ± 2.87
	TRP$^-$ Vehicle	52.42 ± 2.39	53.86 ± 2.98	51.11 ± 3.94
	TRP$^-$ ISDN-1	51.77 ± 1.51	52.50 ± 1.65	54.55 ± 1.19
	TRP$^-$ ISDN-3	46.00 ± 4.2	46.08 ± 5.56	50.05 ± 4.38
Nuclear area (μm^2)	BAL Vehicle	46.13 ± 1.73	47.08 ± 2.05	50.40 ± 2.00
	BAL ISDN-1	37.42 ± 2.12	46.97 ± 2.15	42.69 ± 2.16
	BAL ISDN-3	48.65 ± 3.52	**64.18 ± 3.11*****	**61.22 ± 4.03***
	TRP$^-$ Vehicle	40.90 ± 2.83	46.64 ± 2.15	43.12 ± 1.73
	TRP$^-$ ISDN-1	45.93 ± 2.68	47.55 ± 2.01	43.52 ± 2.36
	TRP$^-$ ISDN-3	46.39 ± 3.62	**42.92 ± 3.80**$^{+++}$	**47.49 ± 2.26**$^{+}$
Cell Body (μm^2)	BAL Vehicle	178.53 ± 5.99	147.23 ± 5.74	156.13 ± 5.62
	BAL ISDN-1	172.00 ± 6.11	150.79 ± 5.60	142.64 ± 6.44
	BAL ISDN-3	188.72 ± 13.00	**187.58 ± 9.73****	**192.10 ± 9.35****
	TRP$^-$ Vehicle	155.84 ± 6.44	166.17 ± 5.63	143.16 ± 4.76
	TRP$^-$ ISDN-1	182.94 ± 9.38	170.20 ± 6.76	165.32 ± 9.67
	TRP$^-$ ISDN-3	197.41 ± 16.06	156.34 ± 6.51	158.34 ± 6.75
% of AhR_{Nuc} intensity	BAL Vehicle	27.58 ± 1.11	31.35 ± 1.15	37.80 ± 1.53
	BAL ISDN-1	32.92 ± 1.31	32.24 ± 0.86	**29.59 ± 1.10*****
	BAL ISDN-3	22.91 ± 1.50	29.68 ± 1.47	**30.80 ± 1.88***
	TRP$^-$ Vehicle	29.59 ± 1.64	27.65 ± 1.52	**31.82 ± 1.00****
	TRP$^-$ ISDN-1	28.61 ± 1.03	29.80 ± 0.74	29.86 ± 1.43
	TRP$^-$ ISDN-3	24.57 ± 2.00	30.82 ± 2.60	31.21 ± 2.13

Data represent mean ± SEM of 30-40 cells per animal for 5 animals from the rostrocaudal regions of the LC, under balanced (BAL) or TRP-deficient (TRP$^-$**)** diets coupled with vehicle, one (ISDN-1), or three ISDN (ISDN-3) injections. **p* <0.05, ***p* <0.01, ****p* <0.001 *vs.* vehicle BAL; ^{+}p <0.05, ^{+++}p <0.001 *vs.* opposite group by Tukey's HSD post-hoc test following 2-way ANOVA.

Table S4. Effect of ISDN and TRP-deficient Diet on the Microglia in the LC

	Group	Rostral	Middle	Caudal
Density (cell number/10^4 μm^2)	BAL Vehicle	2.04 ± 0.31	2.30 ± 0.12	1.88 ± 0.22
	BAL ISDN-1	1.68 ± 0.04	1.95 ± 0.04	1.89 ± 0.10
	BAL ISDN-3	1.86 ± 0.29	1.98 ± 0.06	2.33 ± 0.12
	TRP$^-$ Vehicle	1.94 ± 0.31	2.00 ± 0.12	2.42 ± 0.08
	TRP$^-$ ISDN-1	1.62 ± 0.23	1.64 ± 0.02	1.95 ± 0.17
	TRP$^-$ ISDN-3	2.00 ± 0.09	2.12 ± 0.05	2.17 ± 0.11
Average microglia size (μm^2)	BAL Vehicle	97.29 ± 3.56	90.45 ± 1.57	95.58 ± 4.12
	BAL ISDN-1	87.73 ± 2.5	91.24 ± 4.31	96.18 ± 3.17
	BAL ISDN-3	96.19 ± 7.24	93.37 ± 5.14	87.64 ± 2.94
	TRP$^-$ Vehicle	91.90 ± 6.53	88.00 ± 1.87	91.46 ± 2.80
	TRP$^-$ ISDN-1	90.45 ± 4.94	87.68 ± 4.83	90.45 ± 3.35
	TRP$^-$ ISDN-3	114.28 ± 6.16	**110.27 ± 3.00**$^{+\#\#\#}$	**106.87 ± 3.21**$^{++\#\#}$

Data represent mean ± SEM of 3-10 slides per animal from (5-8) mice per group along the rostrocaudal axis of the LC and under balanced (BAL) or TRP-deficient (TRP$^-$**)** diets coupled with vehicle, one (ISDN-1), or three ISDN (ISDN-3) injections. $^{\#}p$ <0.05; $^{\#\#}p$ <0.01 *vs.* TRP$^-$ vehicle group; ^{+}p <0.05; ^{++}p <0.01; ^{+++}p <0.001 *vs.* opposite group by Tukey's HSD post-hoc test following 2-way ANOVA.

Table S5. Effect of ISDN and TRP-deficient diet on Astrocytes in the LC

	Group	Rostral	Middle	Caudal
GFAP Intensity (arb unit x10^7)	BAL Vehicle	2.44 ± 0.39	2.07 ± 0.43	1.84 ± 0.30
	BAL ISDN-1	**5.76 ± 0.37****	**4.70 ± 0.81***	3.01 ± 0.33
	BAL ISDN-3	**5.37 ± 0.39****	**4.38 ± 0.40***	2.84 ± 0.29
	TRP$^-$ Vehicle	3.54 ± 0.65	2.45 ± 0.37	2.69 ± 0.58
	TRP$^-$ ISDN-1	5.96 ± 0.51	**5.54 ± 0.66**$^{\#\#}$	3.4 ± 0.50
	TRP$^-$ ISDN-3	5.25 ± 1.00	4.32 ± 0.52	2.83 ± 0.56
Number of astrocytes/100 nm^2	BAL Vehicle	6.93 ± 0.55	6.85 ± 0.63	7.03 ± 0.41
	BAL ISDN-1	6.76 ± 0.85	7.28 ± 0.94	6.86 ± 0.60
	BAL ISDN-3	5.80 ± 1.08	6.57 ± 1.17	6.45 ± 0.90
	TRP$^-$ Vehicle	8.48 ± 1.01	7.41 ± 1.52	6.64 ± 0.39
	TRP$^-$ ISDN-1	6.71 ± 1.03	7.54 ± 0.41	7.04 ± 0.53
	TRP$^-$ ISDN-3	6.39 ± 1.28	6.89 ± 1.85	7.61 ± 0.74
Average size (μm^2)	BAL Vehicle	30.51 ± 1.93	29.10 ± 1.80	30.19 ± 1.18
	BAL ISDN-1	38.39 ± 1.66	34.60 ± 0.94	35.00 ± 2.33
	BAL ISDN-3	**41.39 ± 0.70****	**39.75 ± 1.40***	**39.56 ± 2.14***
	TRP$^-$ Vehicle	36.47 ± 3.58	**40.56 ± 2.40**$^{+}$	31.85 ± 0.84
	TRP$^-$ ISDN-1	38.37 ± 2.74	32.53 ± 1.51	32.36 ± 1.11
	TRP$^-$ ISDN-3	44.96 ± 3.46	41.64 ± 3.22	37.37 ± 3.56

Data represent mean ± SEM of 3-10 slides per animal from (5-8) mice per group along the rostrocaudal axis of the LC and under balanced (BAL) or TRP-deficient (TRP$^-$**)** diets coupled with vehicle, one (ISDN-1), or three ISDN (ISDN-3) injections. $^{*}p$ <0.05 and $^{**}p$ <0.01 *vs.* BAL vehicle group; $^{\#\#}p$ <0.01 *vs.* TRP$^-$ vehicle group; ^{+}p <0.05 *vs.* opposite group by Tukey's HSD post-hoc test following 2-way ANOVA.

Table S6. Morphometric Analysis of LC Astrocytes

	Group	Rostral	Middle	Caudal
Fractal Dimension	BAL Vehicle	1.11 ± 0.01	1.1 ± 0.01	1.13 ± 0.01
	BAL ISDN-1	1.08 ± 0.01	1.11 ± 0.02	1.13 ± 0.03
	BAL ISDN-3	**1.05 ± 0.01*****	1.06 ± 0.01	**1.06 ± 0.01****
	TRP^{-} Vehicle	1.09 ± 0.01	1.06 ± 0.01	**1.06 ± 0.01****
	TRP^{-} ISDN-1	**1.04 ± 0.01**$^{\#}$	1.07 ± 0.02	**1.06 ± 0.02**$^{++}$
	TRP^{-} ISDN-3	**1.04 ± 0.01**$^{\#\#}$	1.06 ± 0.01	1.06 ± 0.01
Average Branch length (μm)	BAL Vehicle	11.15 ± 0.51	11.61 ± 0.64	10.21 ± 0.73
	BAL ISDN-1	9.04 ± 0.55	10.06 ± 1.16	8.24 ± 0.75
	BAL ISDN-3	13.15 ± 1.04	12.6 ± 0.61	13.66 ± 1.03
	TRP^{-} Vehicle	11.52 ± 0.72	14.1 ± 0.96	12.24 ± 1.31
	TRP^{-} ISDN-1	10.11 ± 0.92	**8.75 ± 0.59**$^{\#\#}$	10.6 ± 0.97
	TRP^{-} ISDN-3	**14.7 ± 0.69**$^{\#}$	12.51 ± 0.91	111.95 ± 1.10
Number of Branches	BAL Vehicle	16.17 ± 1.12	18.33 ± 2.01	21.83 ± 1.38
	BAL ISDN-1	16.67 ± 1.67	14 ± 1.34	**11.17 ± 1.49*****
	BAL ISDN-3	12.86 ± 1.6	14.3 ± 1.39	**15.1 ± 0.97****
	TRP^{-} Vehicle	19.25 ± 1.13	**11.75 ± 0.86***	**12.67 ± 0.76*****
	TRP^{-} ISDN-1	**10.67 ± 0.89**$^{\#\#}$	13.83 ± 1.49	9.67 ± 1.31
	TRP^{-} ISDN-3	**13.83 ± 1.24**$^{\#}$	17.43 ± 1.27	12.33 ± 1.35
Astrocyte area (10^3 μm^2)	BAL Vehicle	0.90 ± 0.10	0.99 ± 0.08	1.06 ± 0.11
	BAL ISDN-1	0.73 ± 0.08	0.62 ± 0.09	0.41 ± 0.12
	BAL ISDN-3	1.42 ± 0.22	1.26 ± 0.14	1.55 ± 0.16
	TRP^{-} Vehicle	1.31 ± 0.10	1.15 ± 0.13	0.94 ± 0.17
	TRP^{-} ISDN-1	0.64 ± 0.13	**0.62 ± 0.10**$^{\#}$	0.49 ± 0.04
	TRP^{-} ISDN-3	1.56 ± 0.18	1.46 ± 0.12	1.28 ± 0.16

Data represent mean ± SEM of 3-10 slides per animal from (3-5) mice per group along the rostrocaudal axis of the LC and under balanced (BAL) or TRP-deficient (TRP^{-}**)** diets coupled with vehicle, one (ISDN-1), or three ISDN (ISDN-3) injections. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 *vs.* BAL vehicle; $^{\#}p$ <0.05 and $^{\#\#}p$ <0.01 *vs.* TRP^{-} vehicle; ^{++}p <0.01 *vs.* opposite group by Tukey's HSD post-hoc test following 2-way ANOVA.

COMPLEMENTARY RESULTS

In this section, additional experiments that were not included in the two primary studies are presented.

I. Study of the neuroinflammatory state in the Sp5C under ISDN-induced migraine-like pain and the contribution of TRP deficiency

Assessment of the glial activation state and the AhR activity under ISDN-induced migraine and TRP-deficient diet conditions were also examined at the level of the spinal trigeminal nucleus caudalis (Sp5c) in female mice. The experimental protocol used was the same as that for the LC in Study 2. Measures were taken 1 h after one (ISDN-1) or three ISDN (ISDN-3) injections, and one week after balanced (BAL) or TRP-deficient (TRP^{-}) diets.

Glial quantification was performed according to the total structure or divided into the three subdivisions of the Sp5c: V1 (innervated by the ophthalmic branch of the trigeminal nerve), V2 (innervated by the maxillary branch), and V3 (innervated by the mandibular branch).

I.1. Astrocytic Activity

ISDN administration did not induce changes in the GFAP intensity in any of the three regions of the Sp5C (Table 1). However, TRP deficiency globally reduced the GFAP labeling in the vehicle and ISDN-injected mice (Table 1). Changes were observed mainly in V1 and V3 regions.

I.2. Microglial Activity

Assessment of the microglial activity showed no change after the administration of ISDN in the microglial density in any of the three regions of the Sp5C (Table 2). Following one week of a TRP-deficient diet, no sign of a change was also recorded.

Table 1. GFAP Intensity in the Sp5c		
	Vehicle	ISDN-3
Total		
BAL diet	2.24 ± 0.22	2.57 ± 0.33
TRP^- diet	1.59 ± 0.16	1.88 ± 0.06
V1		
BAL diet	1.92 ± 0.24	1.91 ± 0.30
TRP^- diet	1.53 ± 0.13	1.38 ± 0.12
V2		
BAL diet	2.15 ± 0.24	2.44 ± 0.32
TRP^- diet	1.37 ± 0.16	1.67 ± 0.12
V3		
BAL diet	2.64 ± 0.28	3.36 ± 0.51
TRP^- diet	1.86 ± 0.33	2.60 ± 0.22

Data represent mean ± SEM of (3-5) Sp5C sections from (5-6) mice per group. p >0.05 by Tukey's HSD post-hoc test following 2-way ANOVA.

Table 2. Microglial Density (cell number/10^4 µm2) in the Sp5c		
	Vehicle	ISDN-3
Total		
BAL diet	2.85 ± 0.20	3.27 ± 0.08
TRP^- diet	2.84 ± 0.27	3.27 ± 0.14
V1		
BAL diet	3.23 ± 0.19	3.20 ± 0.23
TRP^- diet	2.91 ± 0.26	3.36 ± 0.12
V2		
BAL diet	2.85 ± 0.17	3.31 ± 0.14
TRP^- diet	3.13 ± 0.20	3.50 ± 0.18
V3		
BAL diet	3.02 ± 0.12	3.30 ± 0.17
TRP^- diet	2.94 ± 0.12	2.95 ± 0.18

Data represent mean ± SEM of (3-5) Sp5C sections from (5-6) mice per group. p >0.05 by Tukey's HSD post-hoc test following 2-way ANOVA.

I.3. AhR Activity

Assessment of the relative levels of AhR mRNA expression and its respective target genes was done under ISDN-1 and ISDN-3 administrations and a BAL or TRP-deficient diet. The results are presented in the following Table 3, where no changes were observed.

Table 3. Relative mRNA Expression

	Vehicle	ISDN-1	ISDN-3
[AhR]$_{Sp5c}$			
BAL diet	108.3 ± 7.0	103.0 ± 14.5	114.7 ± 45.2
TRP^{-} diet	137.5 ± 11.4	132.0 ± 8.1	74.4 ± 10.1
[CYP1A1]$_{Sp5c}$			
BAL diet	100.0 ± 17.3	78.0 ± 12.9	67.2 ± 16.5
TRP^{-} diet	64.4 ± 13.2	68.1 ± 14.3	58.7 ± 13.9
[CYP1B1]$_{Sp5c}$			
BAL diet	100.0 ± 19.7	55.7 ± 8.6	63.4 ± 20.6
TRP^{-} diet	101.2 ± 25.0	77.7 ± 19.9	68.6 ± 24.0
[AhRR]$_{Sp5c}$			
BAL diet	109.0 ± 12.0	142.6 ± 29.0	151.3 ± 23.4
TRP^{-} diet	149.9 ± 21.6	122.7 ± 10.7	142.5 ± 16.3

Data represent mean ± SEM in AU of (7-9) mice per group. Samples were collected 1 h after ISDN/vehicle administration. p >0.05 by Tukey's HSD post-hoc test following 2-way ANOVA.

Table 4. Summary of Statistical Analyses from 2-way ANOVA Corresponding to Tables 1 to 3

Table	$F_{[DFn, DFd]}$ or t_{DF}	*p-value*	$F_{[DFn, DFd]}$	*p-value*	$F_{interaction\ [DFn, DFd]}$	*p-value*
Table 1						
Total	$F_{Diet[1,18]}=9.90$	*0.006*	$F_{ISDN[1,18]}=2.16$	*0.16*	$F_{[1,18]}=0.008$	*0.93*
V1	$F_{Diet[1,18]}=4.89$	*0.04*	$F_{ISDN[1,18]}=0.15$	*0.70*	$F_{[1,18]}=0.12$	*0.73*
V2	$F_{Diet[1,18]}=12.25$	*0.003*	$F_{ISDN[1,18]}=1.70$	*0.21*	$F_{[1,18]}=0.0003$	*0.98*
V3	$F_{Diet[1,18]}=4.95$	*0.04*	$F_{ISDN[1,18]}=4.35$	*0.05*	$F_{[1,18]}=0.0006$	*0.98*
Table 2						
Total	$F_{Diet[1,17]}=8.56x10^{-5}$	*0.99*	$F_{ISDN[1,17]}=4.16$	0.06	$F_{[1,17]}=0.001$	*0.98*
V1	$F_{Diet[1,17]}=0.14$	*0.72*	$F_{ISDN[1,17]}=0.91$	0.35	$F_{[1,17]}=1.14$	*0.30*
V2	$F_{Diet[1,17]}=1.69$	*0.21*	$F_{ISDN[1,17]}=5.30$	0.03	$F_{[1,17]}=0.05$	*0.83*
V3	$F_{Diet[1,17]}=2.19$	*0.16*	$F_{ISDN[1,17]}=1.00$	0.33	$F_{[1,17]}=0.80$	*0.38*
Table 3						
AhR	$F_{Diet[1,37]}=0.12$	*0.72*	$F_{ISDN[2,37]}=1.07$	*0.35*	$F_{[2,37]}=1.87$	*0.17*
CYP1A1	$F_{Diet[1,35]}=2.10$	*0.16*	$F_{ISDN\ [2,35]}=0.90$	*0.42*	$F_{[2,35]}=0.55$	*0.58*
CYP1B1	$F_{Diet[1,37]}=0.31$	*0.60*	$F_{ISDN\ [2,37]}=1.92$	*0.16*	$F_{[2,37]}=0.14$	*0.87*
AHRR	$F_{Diet[1,34]}=0.06$	*0.81*	$F_{ISDN\ [2,34]}=0.41$	*0.66*	$F_{[2,34]}=1.22$	*0.31*

Data represent *F* values for two-way ANOVA with corresponding degrees of freedom (DFn, DFd), and *p*-values. ANOVA factors were designed as followed: *ISDN*, for comparisons of animals injected with ISDN and those with Vehicle; and *Diet*, for comparisons between BAL and TRP-deficient diets; and *Interaction*, for the effect between two factors on the dependent variable. $p < 0.05$ was considered statistically significant.

II. Study of the effect of TRP supplementation on ISDN-induced cephalic mechanical hypersensitivity

Based on the data obtained throughout this project, we show that the deficiency of TRP is a contributing factor to the augmentation of the cephalic mechanical hypersensitivity (CMH) induced by ISDN. However, the question arises about what would occur if we introduce again TRP in the diet (at the standard dietary level) after ISDN-induced CMH in female mice. Hence, we applied the following protocol: after one week of a TRP-deficient diet, mice were administered with three repetitive ISDN administrations over two days. 72 hours post ISDN-3, the TRP-deficient diet was changed by a BAL diet containing 2.3 g of TRP (Figure 1A). Measurements of the cephalic mechanical sensitivity were done over 30 days to assess the duration of pain persistence. Moreover, we assessed the levels of TRP in the serum 24 h following the TRP supplementation.

Figure 1B showcases the established result in Study 2 that following a TRP deficiency, ISDN-3-injected animals needed 30 days to achieve total recovery of the cephalic mechanical threshold. Re-supplementing the system with TRP, interestingly showed a vast recovery at an approximate time similar to that of mice under a BAL diet (Figure 1B). This was confirmed by the similar AUC of TRP^{Supp} mice that is higher than that of the TRP-deficient counterpart (Figure 1C). Concerning the serum TRP concentration, a significant increase was shown 24 h after the switch from TRP-deficient to the BAL diet, showing similar plasma TRP levels to that of the ISDN-injected mice subjected to the BAL diet and higher than that of the TRP-deficient diet (Figure 1D).

These results have opened up a new door for future experiments in the plan to assess if this fast CMH recovery after TRP diet supplementation is coupled with a reduction of neuroinflammation at the level of different migraine-related regions including the LC and Sp5c, or normalization of inflammatory responses.

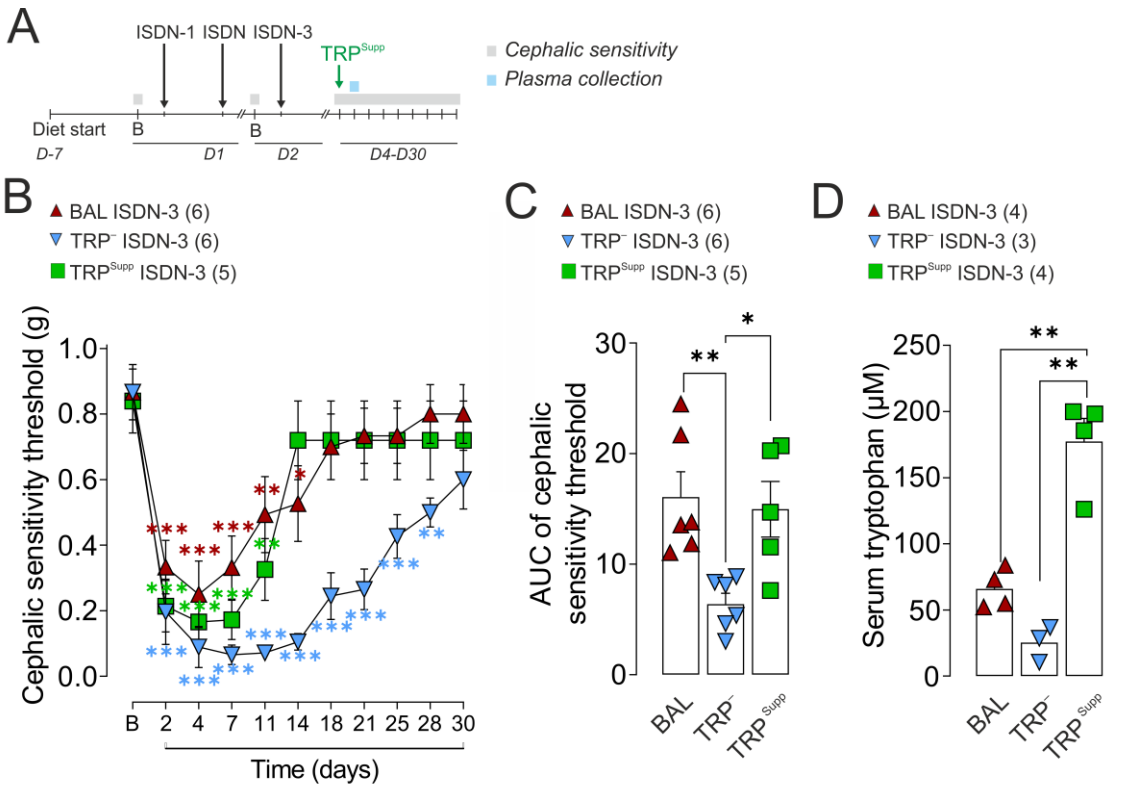


Figure 1. Effect of the Supplementation of TRP on Cephalic Sensitivity. A- After one week of a balanced or TRP-deficient diet, three repetitive injections of ISDN (ISDN-3) were administered. Cephalic mechanical sensitivity was measured before (baseline, B), and 2 to 30 days after ISDN. Change of dietary TRP intake (TRP^{Supp}) was performed 72 hours after the third ISDN administration. **B-** Mechanical sensitivity threshold in response to von Frey stimulation after ISDN. Symbols represent mean ± S.E.M of (n) mice per group. *p <0.05, ** p <0.01, and *** p <0.001 *vs.* baseline by Tukey HSD posttest following 2-way ANOVA. **C-** Area Under the Curve (AUC) of the cephalic sensitivity threshold from 2 to 30 days. * p <0.05 and ** p <0.01 by Tukey HSD following 1-way ANOVA. **C-** Levels of TRP in the serum. Symbols correspond to individual values. ** p <0.01 by Tukey HSD following 1-way ANOVA.

Table 5. Summary of Statistical Analyses from Figure 1

Table	Test	$F_{[DFn, DFd]}$ or t_{DF}	*p-value*	$F_{[DFn, DFd]}$	*p-value*	$F_{interaction\ [DFn, DFd]}$	*p-value*
1B	2- way	$F_{Diet[2,14]}$=6.03	*<0.0001*	$F_{Time[3.5,48.3]}$=	*<0.0001*	$F_{[20,140]}$=3.03	*<0.0001*
1C	1-way	$F_{Diet[2,14]}$=7.39	*0.006*				
1D	1-way	$F_{Diet[2,6]}$=24.10	*0.001*				

Data represent *F* values for one and two-way ANOVA with corresponding degrees of freedom (DFn, DFd), and *p*-values. ANOVA factors were designed as followed: *Time*, for comparisons at different time points; *Diet*, for comparisons between BAL and TRP-deficient diets; and *Interaction*, for the effect between two factors on the dependent variable. p <0.05 was considered statistically significant.

GENERAL DISCUSSION

Within this thesis project, we worked on two central hypotheses, starting with that cephalic mechanical hypersensitivity is coupled with an enhanced inflammatory response that contributes to migraine progression to a chronic state and affects the abortive efficacy of antimigraine drugs. Then we moved toward our second one whereby we hypothesize that TRP-deficient metabolism coupled with an impaired AhR activity could play a role in neuroinflammation during migraine progression. Our work was divided between two primary studies whereby we combined behavioral assessment (pain, light aversion, and anxiety), immunohistochemistry, and biomolecular techniques (RT-PCR, HPLC, western blot, and ELISA) and focused on the level of the LC and Sp5c in an experimental mouse model of migraine-like pain induced by systemic ISDN administrations.

Throughout this journey, we were able to show that:

(1) A single systemic ISDN administration evokes a transitory CMH in both male and female mice, and LH only in females. Both migraine-related symptoms were sensitive to sumatriptan treatment.

(2) Three repeated systemic ISDN administrations induce persistent CMH in female mice, which was concomitant with a systemic increase of plasmatic pro-inflammatory cytokine levels and a local increase of astrocytic reactivity in the LC.

(3) Chronic dietary TRP deficiency:
    a. Reduces systemic TRP and 5-HT levels, but not KYN production.
    b. Blocks the efficacy of sumatriptan to prevent acute ISDN-induced CMH.
    c. Promotes pro-inflammatory cytokine release in the plasma and increases TH expression in the LC.
    d. Worsens acute and persistent ISDN-induced CMH.

(4) TRP-mediated long-lasting ISDN-induced CMH was concomitant with functional impairment of the serotoninergic system in the Sp5C (increased levels of 5-HTR_{1D} after acute ISDN administration), and the noradrenergic activity in the LC (reduced NA release after ISDN-3), and with both astrocytic and microglial enhanced activation.

(5) Finally, AhR plays a pro-nociceptive role in the initiation and progression of ISDN-induced CMH.

ISDN as a Preclinical Model of Migraine

Several preclinical models of migraine-like pain have been developed to study the pathophysiological mechanisms of migraine. We privileged the ISDN model for several reasons. First, ISDN causes headaches in migraineurs but not in healthy subjects (Bellantonio et al. 1997; Castellano et al. 1998), compared to NTG which is less specific (Ramachandran et al. 2012). Second, we previously validated ISDN as a predictive preclinical model of migraine in male rats (Dallel, Descheemaeker, and Luccarini 2018; Flores Ramos et al. 2017). Third, ISDN-induced CMH was correlated with central sensitization in the Sp5C in rats (Flores,2017). In our study, we tested for the first time ISDN in male and female mice. We discovered that, similar to rats, a single ISDN injection elicited reversible CMH and EMH, while repeated ISDN administrations induce persistent CMH. Persistent CMH supports the clinical profile where when the frequency of headaches is more recurrent the higher the chance is to progress from an acute to a chronic state (Ann I. Scher, Midgette, and Lipton 2008). EMH was reversible with no sign of persistence which has been priorly shown in an inflammatory soup model that demonstrated that both hypersensitivities are of a different mechanistic nature (Boyer et al. 2014). These mechanistic differences could be attributed to sensitization (Guy et al. 2010). Furthermore, ISDN only elicited LH in females. This light aversive behavior is similar to that following an acute NTG injection which has previously been described in both male (Markovics et al. 2012) and female mice (Eller et al. 2021). The lack of LH in males could be attributed to the fact that females are more sensitive to NO donors than males, as shown by the higher activity of the NO synthase in females compared to males (Oydanich et al. 2019). Moreover, males showed a higher innate sensibility to light than females, which could support different behavioral and neuronal mechanisms for light adaptation (Chellappa 2021).

ISDN Induces an Abnormal Neuroinflammatory State

Emerging data also suggest that neuroinflammation is a modulator of central sensitization. Increased levels of pro-inflammatory chemokines and cytokines have been detected during ictal and interictal periods in migraine sufferers (Biscetti et al. 2022). In rats, NTG-induced CM was correlated with increased transcriptional activity of IL-6 and TNFα in the trigeminal ganglia and pons (including the LC) (Greco et al.

2017), and with microglial activation in the Sp5C, contributing to central sensitization (He et al. 2019). In our model, a single ISDN administration did not evoke inflammatory responses, while repeated ISDN injections induce an inflammatory reactivity, exhibited by a substantial increase in GFAP reactivity, astrocytic morphological alterations in the LC, and increased levels of pro-inflammatory cytokines at the plasma levels. When compared to sham controls, considerable amounts of cytokine release were observed post-NTG induction in several migraine models, including the robust NTG model (Filippone et al. 2022). Furthermore, we see a considerable morphological change in the astrocytes, with a decrease in complexity and an increase in area and length. Morphological alterations in astrocytes have not been seen in migraine patients. In the presence of damaging insults, however, astrocytes become more reactive and modify their morphology by hypertrophying and process extension towards the location of damage to perform their duty of protection (D. Sun and Jakobs 2012). However, at the level of the Sp5c, no sign of change was seen with respect to both astrocytic and microglial activity. It could be postulated that since the LC has an integral role in the modulation of descending controls of pain, the first step of reactivity is shown at the level of the LC following repeated administrations of the NO donor.

Chronic TRP deficiency is a Contributor to Migraine Pathophysiology and Neuroinflammation

Cephalic, Extracephalic, and Light Hypersensitivities

Tryptophan deficiency has demonstrated clinical evidence as a pivotal player in migraine pathogenesis, primarily in the enhancement of the symptoms of migraine (nausea, headache severity, and photophobia) (P. Drummond 2006). We dysregulated TRP metabolism before inducing migraine, and found a substantial increase in pain persistence, both in acute and interictal periods. This is consistent with clinical models in which dietary acute TRP depletion increases headache severity (P. Drummond 2006), but no research has established its function in pain persistence. Within this project however, we have been able to postulate that TRP deficiency creates an inflammatory state that is further augmented by the action of the NO provided by the administration of ISDN, which I will explain in a further section within the discussion.

TRP deficiency, on the other hand, did not affect ISDN-induced EMH; since the targeted pathway is of a different nature. This disparity in nature might be related to the distinct processes of both hypersensitivities and different manifestations considering in episodic migraine cephalic hypersensitivity is mostly mechanical, whereas extracephalic is mostly thermal (Guy et al. 2010). However, a TRP-deficient diet did not aggravate the ISDN-induced LH in females. This contradicts what the literature has shown as to an increase in LH in migraineurs post-TRP depletion (P. Drummond 2006). This could be due to the pathway targeted by TRP. Since CMH and LH are two distinct pathological pathways, the effect of a TRP-deficient diet could be solely targeting CMH. Moreover, it could be attributed to the timing of the assessment of light hypersensitivity post-ISDN administration, which was one-hour post-ISDN, perhaps assessment at a different time point would showcase an effect on LH.

Neuroinflammation

The metabolism of TRP is linked to aging and generates metabolites that modulate inflammation, maintain energy homeostasis, and influence behavior (Cervenka, Agudelo, and Ruas 2017). TRP metabolites also have a role in the regulation of tissue damage and inflammation. Serotonin is one example, which has been linked to intestinal inflammation (Spohn and Mawe 2017). Modulations in the levels of TRP, such as its deficiency have been shown to compromise immune responses and impair disease resistance in teleost fish (Machado et al. 2019). Furthermore, gut health and intestinal immunity require sufficient dietary TRP for efficient immunological responses and intestinal homeostasis (J. Gao et al. 2018). TRP metabolites are known to support gut microbiota, microbial metabolism, the host's immune system, and host–microbiota synergy (Lamas et al. 2016).

In our results, changes in microglial reactivity required the concomitant impact of TRP dysregulation and repeated ISDN administrations. Understanding the difference between astrocytic and microglial responses requires some understanding of pro-inflammatory cytokine levels. ISDN and TRP both elevated pro-inflammatory mediators such as IL-6, TNF, and IL-1, but not IFNγ. TNF, IL-1, and IFNγ are mostly released by activated astrocytes and microglia, respectively (Liddelow and Barres 2017). As a result, the absence of change in IFNγ expression suggests that the action of ISDN and TRP alone is inadequate to induce activation. Furthermore, the astrocytes

govern the activity of the microglia, meaning that this burst of activity is contingent on an already active astrocytic population.

Noradrenergic Impairment

The LC is an integral noradrenergic center in migraine pathophysiology. Under TRP deficient conditions, we observed that the noradrenergic function of the LC neurons was altered . The TH expression was increased in ISDN-injected mice coupled with a reduced local NA release. TRP depletion has been described to reduce NA levels in rodents (Ardis et al. 2009), although the exact mechanism is not clear. It could involve altered serotoninergic modulation of LC activity (Deen et al. 2018) or enhanced turnover of NA, as described in other chronic pain models (Alba-Delgado et al. 2016). NA has an endogenous analgesic function in the spinal cord (Pertovaara 2006), and low release could contribute to long-lasting CMH induced by TRP deficiency in ISDN-injected mice.

Our results also suggest that morphological changes of LC neurons are necessary to modulate nociceptive responses. We have shown that the effect of TRP metabolism disruption also alters the noradrenergic cell morphology. Repeated ISDN administrations, induce an increase in the cell volume of noradrenergic neurons and more TH-IR projections were observed. However, TRP deficiency inhibited these structural alterations, leading to a concomitant long-lasting CMH. A plethora of studies have described morphological changes in LC neurons in response to stressful stimuli (Borodovitsyna, Joshi, and Chandler 2018). These alterations include an increase in the complexity of the dendritic arborization associated with the expression of genes involved in cell structure and synaptic plasticity with the primary aim of pain amelioration and modulation.

Further insight into the role of the LC will be through local NA inhibition and through electrophysiological analysis in mice under migraine-like conditions.

The TRP-AhR Axis

The action of TRP-derived metabolites might be mediated through AhR since they are endogenous ligands. AhR are important transcription factors with either pro- or anti-inflammatory properties that are found in astrocytes, microglia, and noradrenergic neurons. Hence, coupling the reactivity induced by ISDN and the modulatory role of

AhRs we wanted to assess whether the changes occurring due to TRP depletion occur as a function of mechanistic changes at the level of LC AhRs. ISDN is a potent NO donor, and we know that NO promotes the systemic release of pro-inflammatory cytokines, including IL-6, IL-1β, and TNFα in the blood (Soufli et al. 2016). We observed that TRP deficiency increased IL-6, TNFα, and IL-1β levels in the blood, as described before (van Wissen et al. 2002), maybe by activating AhR. Pro-inflammatory cytokines, mainly TNFα (Gostner et al. 2020), activate the enzyme indoleamine 2,3-dioxygenase (IDO) in peripheral dendritic cells, which transforms TRP to KYN. IDO plays a minor role under physiological circumstances. However, it is strongly activated upon inflammation (Yeung et al. 2015). IDO activity is often measured by the blood KYN/TRP ratio, which is increased in diseases characterized by excessive or chronic inflammation (Schröcksnadel et al. 2006). Our data showed that ISDN also increased peripheral IDO activity thus shifting TRP metabolism toward the production of KYN, leading to low TRP levels, and increased KYN/TRP ratio. This effect could be explained by an indirect action of ISDN on IDO activity: NO produced from ISDN could stimulate TNFα release, which in turn, activates IDO that metabolizes TRP to KYN. Impaired turnover of KYN and TRP has been described in migraineurs, who presented low levels of both metabolites compared to healthy controls (Tuka et al. 2021).

Both levels of TRP and KYN were reduced after ISDN and/or TRP-depleted diet in the LC. IDO expression in the brain is low and restricted to specific brain regions (Kwidzinski and Bechmann 2007). Cerebral TRP metabolism is therefore largely dependent on the transport of TRP and KYN across the blood-brain barrier. Both have access to brain tissue through the large neutral amino acid transporter, hence entering through a competition with other large neutral amino acids leading to their marginally lower levels.

KYN is a ligand of AhR and regulates inflammatory processes through the promotion of cytokine release and hence through the further activation of the IDO creating a cyclic event. This process included the activation of astrocytes and microglia, which progress to a reactive morphological state when both TRP deficiency and repetition of ISDN were combined. NO also controls the activity and expression of AhR by decreasing it (Niedbala et al. 2011). Thus, the final balance between KYN and NO actions on AhR could be a partial reduction of AhR expression after ISDN, leaving

enough active AhR to promote the pronociceptive effect observed after its direct activation by ITE. This enhanced pro-inflammatory state could be a potential inducer of pain facilitation and persistence, as observed by the long-lasting CMH in mice after TRP deficiency. It should be noted that TRP deficiency did not affect ISDN-induced extracephalic hypersensitivity since the targeted pathway is of a different nature. This difference could be due to the different mechanisms of both hypersensitivities considering in episodic migraine cephalic hypersensitivity is mostly mechanical, whereas extracephalic is mostly thermal (Guy et al. 2010).

Our hypothesis is that AhR activation promotes the sensitization of the inflammatory signaling pathways, facilitating nociceptive response and central sensitization of the trigeminal system. Understanding the potential pronociceptive nature of AhR was based on the administration of potent agonists and antagonists. The systemic activation of AhR by the non-toxic agonist ITE, prior to ISDN administration, enhanced the intensity and duration of ictal CMH. On the other hand, AhR inhibition by non-toxic TMF prevents both ictal and interictal ISDN-induced CMH. The blockage of AhR by TMF does not allow KYN to execute its function as a ligand, and hence the IDO/AhR axis is potentially hindered. And when we combined TMF with a TRP-deficient diet, that leads to a lower TMF-induced antalgic effect. Through that, we have been able to show that AhR has a pro-nociceptive action during migraine initiation and progression. For future steps within this part of the project , the AhR knockout model will be used to further understand and identify whether the role of the AhRs is peripheral or central.

Triptan Efficacy

Triptans are highly effective if taken early within the first few minutes of the start of the attack, but when the attack is established, and there is the presence of allodynia (indicative of ongoing central sensitization) patients are no longer responsive (Rami Burstein, Collins, and Jakubowski 2004). Although triptans are recommended as a first-line treatment, approximately 30-40 % of migraineurs suffer from insufficient efficacy or tolerability to triptans (Lipton et al., 2013; Viana et al., 2013). In the case of sumatriptan, for example, non-responders show lower and slower absorption after oral administrations compared to responders (A. Ferrari et al. 2008). Moreover, in women, the efficacy rate of triptans is the same as in men, however, their risk of a headache recurrence is higher (Casteren et al. 2021).

Because sumatriptan works at the level of the serotonergic 5-$HTR_{1B/1D}$ receptors, modulations in serotonergic neurotransmission described in migraineurs may influence the functioning of these receptors and hence be the source of triptan inefficacy. Changes in serotonin levels can be produced by rapid dietary reduction of TRP, its precursor, and a powerful inducer of migraine features such as LH and pain (P. D. Drummond 2005). We demonstrated a lack of efficacy of sumatriptan when it came to both CMH in both sexes and LH in females induced by an acute ISDN administration. Sumatriptan's lack of activity, which is also present in migraineurs, might be attributed to serotoninergic modulations or receptor functioning. Quantification of 5-$HTR_{1B/1D}$ expression reveals that under TRP-deficient circumstances, the receptors' expressions, notably 5-HTR_{1D}, increased significantly, at least at the Sp5C level. This increase in expression, together with low serotonin levels, may lead to migraine episodes (Panconesi 2008). Furthermore, the decrease in sumatriptan effectiveness may be connected to receptor functioning, whereby it is sensitized rather than expressed for $5HTR_{1B}$, for example. This alteration in expression has not been documented in migraine or other pain models. For the next step in this part of the project, we will perform a functionality assessment of 5-$HTR_{1B/1D}$ receptors using patch clamp. This will give insight into the electrophysiological status of these receptors under TRP-deficient conditions coupled with migraine. Moreover, we will assess the efficacy of another triptan within the same model which will allow us to understand if this lack of efficacy is limited to sumatriptan or affects other triptans as well. Since we only assessed the efficacy of sumatriptan at the level of CMH and LH, we will also assess it on EMH under a TRP deficient diet, to see if the effect is also limited to CMH or not.

CONCLUSION

Through our results, we can demonstrate for the first time the pivotal contribution of AhRs as pain mediators through their TRP-derived ligands during migraine progression. Moreover, we have shown the importance of serotoninergic modulation and the contribution of TRP and its metabolites on the effectiveness of sumatriptan. The importance of what we have obtained so far sheds light on the potential clinical importance of this work. To start, what we have obtained is that TRP-deficient diets contribute to the inefficacy of sumatriptan opens a door to further understanding sumatriptan resistance in migraineurs. Potentially altering one's diet and augmenting their daily intake of TRP could ameliorate the efficacy of sumatriptan and hence reduce the number of triptan non-responders. Hence, potentially verifying whether triptan non-responders do have dysregulations in the levels of TRP could be of interest. Further steps could be also to combine a supplementation of TRP coupled with triptan administration among patients resistant to them.

As for the TRP-AhR axis and its nociceptive role, we have been able to showcase a novel mechanism contributing to the augmentation of cephalic hypersensitivity in migraine-like pain. This allows us to further target AhR as a therapeutic means in the field of nociception. However, multiple sides should be taken into account including the physiological and pathological side effects potentially induced through the action on these receptors. Nevertheless, further research is in store to understand this area.

SECONDARY PROJECT

Visualizing Neuronal Dynamics of the Posterior Thalamus in a Mouse Model of Migraine-like Pain

During my thesis, I also implemented a new technique in our lab using a miniscope system for *in vivo* calcium imaging in freely-moving mice (Figure 1). The aim was to investigate the role of the posterior thalamus in the light hypersensitivity (LH) induced by ISDN.

Photophobia or LH is considered the most bothersome symptom reported in 80 % of migraineurs (Choi et al. 2009), during (Lovati et al. 2013) and between headache attacks (Chong, Starling, and Schwedt 2016; Vanagaite et al. 1997). When understating the mechanism by which photophobia arises, four distinct definitions have been accepted: (1) LH is an abnormal sensitivity to light, associated with a reduced photosensitivity threshold, as shown by migraineurs during the interictal period; (2) light can exacerbate or trigger headache; (3) LH is described as a moderate to severe ocular discomfort; and (4) LH is a general aversion to a non-noxious light intensity (Fine and Digre 1995; Lebensohn 1951; Noseda, Kainz, et al. 2010; Noseda, Borsook, and Burstein 2017). Despite the research advances in the last years, the exact mechanisms of different modalities of LH are still unclear.

The thalamus is a central relay for nociceptive information from the lower brain regions to different cortical areas via the trigeminovascular pain pathway (Younis et al. 2019). It is a crucial player in the pathophysiology of migraine. However, it is not only a relay but also a potent integration center of light inputs from the retinal ganglion cells (RGC) and then conveys it to the primary visual cortex (Noseda, Kainz, et al. 2010). Hence, the thalamus could be an integration center for both light and nociceptive information (Rami Burstein, Collins, and Jakubowski 2004). Indeed, Noseda and his team were able to demonstrate the existence of different subpopulations of trigeminovascular neurons at the level of the posterior thalamus (Po) that are either dura sensitive or that respond to both light and dural stimulations (Noseda, Kainz, et al. 2010). However, in Noseda's work, experiments were performed on anesthetized rodents and not under migraine conditions. Stimulation of the Po nuclei was sufficient

to induce light-aversive behavior in a recent mouse model of migraine (Sowers et al. 2020).

Based on their findings, this opened a question of how would Po neurons be affected in terms of functionality during a migraine attack and under light stimulations. Hence we aimed to assess the neuronal activity of these neurons using *in vivo* calcium imaging in free-moving male and female C57BL/6 mice under migraine-like conditions using single or repeated (three, twice per day for two days) intraperitoneal injections of ISDN (Risordan®, Sanofi-Aventis, Paris, France; 10 mg/kg in a 10 ml/kg). Light stimulations were established at 50 or 300 lux. As shown in Study 1 from this thesis, 50 lux illuminance did not induce LH in ISDN-injected mice, while both sexes were photophobic at 300 lux. In the case of males, light aversion to 300 lux was innate. In females, LH was induced by a single ISDN injection. Testing was done in an open field (50x50x30 cm high).

Experiments were done following the ethical guidelines of Directive 2010/63/UE of the European Parliament and the Council and French Decree 2013-118 on the protection of animals used for scientific purposes. Protocols were approved by the French Ministry of Higher Education and Research (Ref. 2018051617409279).

Briefly, mice were anesthetized and fixed on a stereotaxic frame. The scalp was removed. The skull on top of the injection area was carefully drilled and removed using a dental drill. Visualization of the neurons at the level of the Po was done using an injection of a ready-to-image AAV1.Camk2a.GCaMP6f.WPRE.bGHpA virus (Inscopix, CA, USA) which was administered using a glass electrode directly at the level of the Po (AP: -2, MD: 1.2, and DV: -3; Figure 1A) (Paxinos and Franklin 2019). During the same surgical procedure, an integrated micro endoscopic GRIN (Gradient Index) lens (Inscopix, CA, USA) was lowered towards the injection area of the Po. The lens was then secured to the mouse's skull and a cap was placed for protection. A total of 5 males and 3 females were successfully implanted.

Six-week post-surgery, mice were habituated for one week to the testing room with a dummy miniscope placed on their heads. Then, during the testing session, mice were attached to the miniscope (nVoke 2.0, BB-11170203, Inscopix, CA, USA), placed inside the open field, and allowed to move freely for 30 min (Figure 1B). The calcium images were obtained by the nVoke acquisition software (Inscopix, CA, USA; Figure

1C). Baseline measurements of neuronal activity were recorded under the three following conditions: dark (light-OFF), non-aversive light (50 lux), and aversive light (300 lux) each for 40 seconds. ISDN was then administrated, and the same procedure was followed at three-time points post-injection (30, 60, and 180 min). The raw data was then processed with custom-written scripts in Matlab (The MathWorks Inc., 2015), using the subroutine from InscopiX Data Processing software. DF/F and the number of calcium events were used to estimate the cells' synchronicity and activity clusters.

Three main preliminary results were obtained from these experiments:

- One hour after ISDN injection, a significant variation in the activity of Po neurons was observed, concomitant with the peak of cephalic hypersensitivity. Cells that had physiologically increased activity reduced the calcium event, and vice versa, neurons with basal decreased activity increased their responses. However, the global effect could be translated as a reduction of Po neuronal function.

- Light stimulation at 300 lux showed a variation in the activity of the cells, with an overall increase in the calcium response and hence an augmentation of the activity of certain light-sensitive neurons.

- Light stimulation at 300 lux one-hour post-ISDN administration globally increased the calcium activity of Po neurons. Indicating the existence of a subset responding to both pain and light.

- Four different subpopulations of Po neurons were identified, according to their activity after light and/or ISDN stimuli; (1) neurons that- respond to migraine-like pain induced by ISDN, (2) neurons that respond to light (300 lux but not 50 lux), (3) neurons that respond to both nociception and light and (4) non-responder neurons. The proportions of these neurons were different, being neurons that respond to both light and ISDN as the most important subpopulation, followed by ones responding to ISDN alone.

For the moment, the reduced number of recorded neurons from females did not allow us to compare both sexes. For the future steps in this project, more animals are to be added, in addition to a more in-depth analysis of the prior obtained recordings and the ones to come with an assessment of neuronal synchrony and discharge frequency. Moreover, the next experiments will include combined *in vivo* calcium dynamics recording with cephalic and extracephalic von Frey stimulations.

Figure 1. ***In vivo*** **Calcium Imaging in Freely-moving Mice. A-** Area of virus injection and placement of the lens at the level of the posterior thalamus (Po) (marked by the white arrow) on a stereotaxic atlas image (Paxinos & Franklin, 2019). **B-** A mouse connected to the miniscope. **C-** A recording of active posterior thalamic neurons (marked by the white arrow). Created with Biorender.com.

REFERENCES

Burstein, Rami, Beth Collins, and Moshe Jakubowski. 2004. "Defeating Migraine Pain with Triptans: A Race against the Development of Cutaneous Allodynia." *Annals of Neurology* 55(1): 19–26.

Choi, J. Y. et al. 2009. "Usefulness of a Photophobia Questionnaire in Patients with Migraine." *Cephalalgia* 29(9): 953–59.

Chong, Catherine D, Amaal J Starling, and Todd J Schwedt. 2016. "Interictal Photosensitivity Associates with Altered Brain Structure in Patients with Episodic Migraine." *Cephalalgia* 36(6): 526–33.

Fine, P G, and K B Digre. 1995. "A Controlled Trial of Regional Sympatholysis in the Treatment of Photo-Oculodynia Syndrome." *Journal of neuro-ophthalmology* 15(2): 90–94.

Lebensohn, James E. 1951. "Photophobia: Mechanism and Implications*." *American Journal of Ophthalmology* 34(9): 1294–1300.

Lovati, Carlo et al. 2013. "Central Sensitization in Photophobic and Non-Photophobic Migraineurs: Possible Role of Retino Nuclear Way in the Central Sensitization Process." *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* 34 Suppl 1: S133-135.

Noseda, Rodrigo et al. 2010. "A Neural Mechanism for Exacerbation of Headache by Light." *Nature Neuroscience* 13(2): 239–45.

Noseda, Rodrigo, David Borsook, and Rami Burstein. 2017. "Neuropeptides and Neurotransmitters That Modulate Thalamo-Cortical Pathways Relevant to Migraine Headache." *Headache: The Journal of Head and Face Pain* 57(S2): 97–111.

Paxinos, George, and Keith BJ Franklin. 2019. *Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates*. Academic press.

Sowers, Levi P. et al. 2020. "Stimulation of Posterior Thalamic Nuclei Induces Photophobic Behavior in Mice." *Headache: The Journal of Head and Face Pain* 60(9): 1961–81.

Vanagaite, J et al. 1997. "Light-Induced Discomfort and Pain in Migraine." *Cephalalgia* 17(7): 733–41.

Younis, Samaira, Anders Hougaard, Rodrigo Noseda, and Messoud Ashina. 2019. "Current Understanding of Thalamic Structure and Function in Migraine." *Cephalalgia* 39(13): 1675–82.

REFERENCES

A

Abbott, B. D. et al. 1999. "RT-PCR Quantification of AHR, ARNT, GR, and CYP1A1 MRNA in Craniofacial Tissues of Embryonic Mice Exposed to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin and Hydrocortisone." *Toxicological Sciences: An Official Journal of the Society of Toxicology* 47(1): 76–85.

Abbott, S. B. et al. 2012. "C1 Neurons Excite Locus Coeruleus and A5 Noradrenergic Neurons along with Sympathetic Outflow in Rats." *The Journal of Physiology* 590(12): 2897–2915.

Abraira, Victoria E., and David D. Ginty. 2013. "The Sensory Neurons of Touch." *Neuron* 79(4): 618–39.

Afridi, S. K. et al. 2005. "A PET Study Exploring the Laterality of Brainstem Activation in Migraine Using Glyceryl Trinitrate." *Brain* 128(4): 932–39.

Aggarwal, Milan, Veena Puri, and Sanjeev Puri. 2012. "Serotonin and CGRP in Migraine." *Annals of Neurosciences* 19(2): 88–94.

Agudelo, Leandro Z. et al. 2018. "Kynurenic Acid and Gpr35 Regulate Adipose Tissue Energy Homeostasis and Inflammation." *Cell Metabolism* 27(2): 378-392.e5.

Agus, Allison, Julien Planchais, and Harry Sokol. 2018. "Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease." *Cell Host & Microbe* 23(6): 716–24.

Ahn, Andrew H. 2010. "On the Temporal Relationship Between Throbbing Migraine Pain and Arterial Pulse." *Headache: The Journal of Head and Face Pain* 50(9): 1507–10.

Akahoshi, Eiichi, Seiko Yoshimura, Saeko Uruno, and Mitsuko Ishihara-Sugano. 2009. "Effect of Dioxins on Regulation of Tyrosine Hydroxylase Gene Expression by Aryl Hydrocarbon Receptor: A Neurotoxicology Study." *Environmental Health* 8(1): 24.

Akerman, Simon, Philip R. Holland, and Peter J. Goadsby. 2011. "Diencephalic and Brainstem Mechanisms in Migraine." *Nature Reviews. Neuroscience* 12(10): 570–84.

Al Bander, Zahraa, Marloes Dekker Nitert, Aya Mousa, and Negar Naderpoor. 2020. "The Gut Microbiota and Inflammation: An Overview." *International Journal of Environmental Research and Public Health* 17(20): 7618.

Alba-Delgado, Cristina et al. 2015. "Subpopulations of PKCγ Interneurons within the Medullary Dorsal Horn Revealed by Electrophysiologic and Morphologic Approach." *PAIN* 156(9): 1714.

Alba-Delgado, Cristina et al. 2018. "5-HT2A Receptor-Induced Morphological Reorganization of PKCγ-Expressing Interneurons Gates Inflammatory Mechanical Allodynia in Rat." *Journal of Neuroscience* 38(49): 10489–504.

Alba-Delgado, Cristina, Alberto Cebada-Aleu, Juan Antonio Mico, and Esther Berrocoso. 2016. "Comorbid Anxiety-like Behavior and Locus Coeruleus Impairment in Diabetic Peripheral Neuropathy: A Comparative Study with the Chronic Constriction Injury Model." *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 71: 45–56.

Allen, Nicola J., and Ben A. Barres. 2009. "Glia — More than Just Brain Glue." *Nature* 457(7230): 675–77.

Amakura, Yoshiaki et al. 2003. "Screening of the Inhibitory Effect of Vegetable Constituents on the Aryl Hydrocarbon Receptor-Mediated Activity Induced by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin." *Biological & Pharmaceutical Bulletin* 26(12): 1754–60.

Amano, N., J. W. Hu, and B. J. Sessle. 1986. "Responses of Neurons in Feline Trigeminal Subnucleus Caudalis (Medullary Dorsal Horn) to Cutaneous, Intraoral, and Muscle Afferent Stimuli." *Journal of Neurophysiology* 55(2): 227–43.

Amaral, David G., and Harry M. Sinnamon. 1977. "The Locus Coeruleus: Neurobiology of a Central Noradrenergic Nucleus." *Progress in Neurobiology* 9(3): 147–96.

Amin, Faisal Mohammad et al. 2013. "Magnetic Resonance Angiography of Intracranial and Extracranial Arteries in Patients with Spontaneous Migraine without Aura: A Cross-Sectional Study." *The Lancet Neurology* 12(5): 454–61.

Amor, Sandra, Fabiola Puentes, David Baker, and Paul Van Der Valk. 2010. "Inflammation in Neurodegenerative Diseases." *Immunology* 129(2): 154–69.

Andreatta, Marta et al. 2012. "Altered Processing of Emotional Stimuli in Migraine: An Event-Related Potential Study." *Cephalalgia* 32(15): 1101–8.

Andreou, Anna P. et al. 2010. "Animal Models of Headache: From Bedside to Bench and Back to Bedside." *Expert Review of Neurotherapeutics* 10(3): 389–411.

Anthony, M., H. Hinterberger, and J. W. Lance. 1967. "Plasma Serotonin in Migraine and Stress." *Archives of Neurology* 16(5): 544–52.

Ardis, T. C. et al. 2009. "Effect of Acute Tryptophan Depletion on Noradrenaline and Dopamine in the Rat Brain." *Journal of Psychopharmacology (Oxford, England)* 23(1): 51–55.

Arnsten, A. F. T., and P. S. Goldman-Rakic. 1984. "Selective Prefrontal Cortical Projections to the Region of the Locus Coeruleus and Raphe Nuclei in the Rhesus Monkey." *Brain Research* 306(1): 9–18.

Arnsten, Amy F. T. 2009. "Stress Signalling Pathways That Impair Prefrontal Cortex Structure and Function." *Nature Reviews Neuroscience* 10(6): 410–22.

Arzani, Mahsa et al. 2020. "Gut-Brain Axis and Migraine Headache: A Comprehensive Review." *The Journal of Headache and Pain* 21(1): 15.

Asghar, M. S. et al. 2010. “Dilation by CGRP of Middle Meningeal Artery and Reversal by Sumatriptan in Normal Volunteers.” *Neurology* 75(17): 1520–26.

Aston-Jones, G., H. Akaoka, P. Charléty, and G. Chouvet. 1991. “Serotonin Selectively Attenuates Glutamate-Evoked Activation of Noradrenergic Locus Coeruleus Neurons.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 11(3): 760–69.

Aston-Jones, Gary et al. 1986. “The Brain Nucleus Locus Coeruleus: Restricted Afferent Control of a Broad Efferent Network.” *Science* 234(4777): 734–37.

Aston-Jones, Gary, and Jonathan D. Cohen. 2005. “Adaptive Gain and the Role of the Locus Coeruleus–Norepinephrine System in Optimal Performance.” *Journal of Comparative Neurology* 493(1): 99–110.

Aurora, S. K., Y. Cao, S. M. Bowyer, and K. M. A. Welch. 1999. “The Occipital Cortex Is Hyperexcitable in Migraine; Evidence from TMS, FMRI and MEG Studies (Wolff Award 1999).” *Headache* 39: 469–76.

Aurora, Sheena K., and Mitchell F. Brin. 2017. “Chronic Migraine: An Update on Physiology, Imaging, and the Mechanism of Action of Two Available Pharmacologic Therapies.” *Headache: The Journal of Head and Face Pain* 57(1): 109–25.

Avona, Amanda et al. 2019. “Dural Calcitonin Gene-Related Peptide Produces Female-Specific Responses in Rodent Migraine Models.” *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 39(22): 4323–31.

Aydın, Meliha et al. 2015. “Plasma Cytokine Levels in Migraineurs During and Outside of Attacks.” *Electronic Journal of General Medicine* 12(4): 307–12.

B

Baba, Hiroshi, Koki Shimoji, and Megumu Yoshimura. 2000. “Norepinephrine Facilitates Inhibitory Transmission in Substantia Gelatinosa of Adult Rat Spinal Cord (Part 1) Effects on Axon Terminals of GABAergic and Glycinergic Neurons.” *The Journal of the American Society of Anesthesiologists* 92(2): 473–473.

Bachiller, Sara et al. 2018. “Microglia in Neurological Diseases: A Road Map to Brain-Disease Dependent-Inflammatory Response.” *Frontiers in Cellular Neuroscience* 12. https://www.frontiersin.org/articles/10.3389/fncel.2018.00488 (May 13, 2023).

Badawy, Abdulla A-B. 2017. “Kynurenine Pathway of Tryptophan Metabolism: Regulatory and Functional Aspects.” *International Journal of Tryptophan Research* 10: 1178646917691938.

Bahra, A. et al. 2001. “Brainstem Activation Specific to Migraine Headache.” *Lancet (London, England)* 357(9261): 1016–17.

Bakırhan, Hande, Hilal Yıldıran, and Tugba Uyar Cankay. 2022. "Associations between Diet Quality, DASH and Mediterranean Dietary Patterns and Migraine Characteristics." *Nutritional Neuroscience* 25(11): 2324–34.

Baldacci, F. et al. 2013. "Photophobia Is Associated with Allodynia during Migraine Attack." *Journal of the Neurological Sciences* 333: e487.

Baldassarri, Stephen R. et al. 2020. "Inverse Changes in Raphe and Cortical 5-HT1B Receptor Availability after Acute Tryptophan Depletion in Healthy Human Subjects." *Synapse* 74(10): e22159.

Bansal, Tarun, Robert C. Alaniz, Thomas K. Wood, and Arul Jayaraman. 2010. "The Bacterial Signal Indole Increases Epithelial-Cell Tight-Junction Resistance and Attenuates Indicators of Inflammation." *Proceedings of the National Academy of Sciences of the United States of America* 107(1): 228–33.

Barnes, Nicholas M., and Trevor Sharp. 1999. "A Review of Central 5-HT Receptors and Their Function." *Neuropharmacology* 38(8): 1083–1152.

Barroso, Andreia, João Vitor Mahler, Pedro Henrique Fonseca-Castro, and Francisco J. Quintana. 2021. "The Aryl Hydrocarbon Receptor and the Gut–Brain Axis." *Cellular & Molecular Immunology* 18(2): 259–68.

Bartsch, T., and P. J. Goadsby. 2003. "Increased Responses in Trigeminocervical Nociceptive Neurons to Cervical Input after Stimulation of the Dura Mater." *Brain* 126(8): 1801–13.

Bartsch, T., Y. E. Knight, and P. J. Goadsby. 2004. "Activation of 5-HT1B/1D Receptor in the Periaqueductal Gray Inhibits Nociception." *Annals of Neurology* 56(3): 371–81.

Bartsch, Thorsten, and Peter J. Goadsby. 2002. "Stimulation of the Greater Occipital Nerve Induces Increased Central Excitability of Dural Afferent Input." *Brain* 125(7): 1496–1509.

Basbaum, Allan I., Diana M. Bautista, Grégory Scherrer, and David Julius. 2009. "Cellular and Molecular Mechanisms of Pain." *Cell* 139(2): 267–84.

Bates, E. A. et al. 2010. "Sumatriptan Alleviates Nitroglycerin-Induced Mechanical and Thermal Allodynia in Mice." *Cephalalgia: An International Journal of Headache* 30(2): 170–78.

Bellantonio, P et al. 1997. "Haemodynamic Correlates of Early and Delayed Responses to Sublingual Administration of Isosorbide Dinitrate in Migraine Patients: A Transcranial Doppler Study." *Cephalalgia* 17(3): 183–87.

Benjamin, Laura et al. 2004. "Hypothalamic Activation after Stimulation of the Superior Sagittal Sinus in the Cat: A Fos Study." *Neurobiology of Disease* 16(3): 500–505.

Berman, Nancy E.J. et al. 2006. “Serotonin in Trigeminal Ganglia of Female Rodents: Relevance to Menstrual Migraine.” *Headache: The Journal of Head and Face Pain* 46(8): 1230–45.

Berridge, Craig W, and Barry D Waterhouse. 2003. “The Locus Coeruleus–Noradrenergic System: Modulation of Behavioral State and State-Dependent Cognitive Processes.” *Brain Research Reviews* 42(1): 33–84.

Bigal, Marcelo E. et al. 2003. “Assessment of Migraine Disability Using the Migraine Disability Assessment (MIDAS) Questionnaire: A Comparison of Chronic Migraine With Episodic Migraine.” *Headache: The Journal of Head and Face Pain* 43(4): 336–42.

Alba-Delgado, Cristina et al. 2008. “Concepts and Mechanisms of Migraine Chronification.” *Headache: The Journal of Head and Face Pain* 48(1): 7–15.

Bilgiç, Başar et al. 2016. “Volumetric Differences Suggest Involvement of Cerebellum and Brainstem in Chronic Migraine.” *Cephalalgia: An International Journal of Headache* 36(4): 301–8.

Biringer, Roger Gregory. 2023. “Migraine Signaling Pathways: Purine Metabolites That Regulate Migraine and Predispose Migraineurs to Headache.” *Molecular and Cellular Biochemistry*. https://doi.org/10.1007/s11010-023-04701-7 (August 2, 2023).

Biscetti, Leonardo et al. 2022. “Immunological Findings in Patients with Migraine and Other Primary Headaches: A Narrative Review.” *Clinical and Experimental Immunology* 207(1): 11–26.

Biskup, Caroline Sarah et al. 2012. “Effects of Acute Tryptophan Depletion on Brain Serotonin Function and Concentrations of Dopamine and Norepinephrine in C57BL/6J and BALB/CJ Mice.” *PLoS ONE* 7(5): e35916.

Bloudek, L. M. et al. 2012. “Cost of Healthcare for Patients with Migraine in Five European Countries: Results from the International Burden of Migraine Study (IBMS).” *The Journal of Headache and Pain* 13(5): 361–78.

Blumenfeld, Andrew M. et al. 2021. “Efficacy of Ubrogepant Based on Prior Exposure and Response to Triptans: A Post Hoc Analysis.” *Headache: The Journal of Head and Face Pain* 61(3): 422–29.

Boadle-Biber, Margaret C. 1993. “Regulation of Serotonin Synthesis.” *Progress in Biophysics and Molecular Biology* 60(1): 1–15.

Boardman, HF, E Thomas, PR Croft, and DS Millson. 2003. “Epidemiology of Headache in an English District.” *Cephalalgia* 23(2): 129–37.

Bobker, D. H., and J. T. Williams. 1989. “Serotonin Agonists Inhibit Synaptic Potentials in the Rat Locus Ceruleus in Vitro via 5-Hydroxytryptamine1A and 5-Hydroxytryptamine1B Receptors.” *The Journal of Pharmacology and Experimental Therapeutics* 250(1): 37–43.

Bogdanov, Volodymyr Borysovych et al. 2011. "Migraine Preventive Drugs Differentially Affect Cortical Spreading Depression in Rat." *Neurobiology of Disease* 41(2): 430–35.

Bolay, Hayrunnisa et al. 2015. "Gender Influences Headache Characteristics with Increasing Age in Migraine Patients." *Cephalalgia* 35(9): 792–800.

Bonaventure, P., A. Schotte, P. Cras, and J. E. Leysen. 1997. "Autoradiographic Mapping of 5-HT1B-and 5-HT1D Receptors in Human Brain Using [3H] Alniditan, a New Radioligand." *Receptors & channels* 5(3–4): 225–30.

Borgdorff, Piet. 2018. "Arguments against the Role of Cortical Spreading Depression in Migraine." *Neurological Research* 40(3): 173–81.

Borodovitsyna, Olga, Neal Joshi, and Daniel Chandler. 2018. "Persistent Stress-Induced Neuroplastic Changes in the Locus Coeruleus/Norepinephrine System." *Neural Plasticity* 2018: 1892570.

Borsook, D. et al. 2014. "Sex and the Migraine Brain." *Neurobiology of Disease* 68: 200–214.

Bosi, Annalisa et al. 2020. "Tryptophan Metabolites Along the Microbiota-Gut-Brain Axis: An Interkingdom Communication System Influencing the Gut in Health and Disease." *International Journal of Tryptophan Research : IJTR* 13: 1178646920928984.

Boyer, Nelly et al. 2017. "Propranolol Treatment Prevents Chronic Central Sensitization Induced by Repeated Dural Stimulation." *PAIN* 158(10): 2025.

Boyer, Nelly, Radhouane Dallel, Alain Artola, and Lénaïc Monconduit. 2014. "General Trigeminospinal Central Sensitization and Impaired Descending Pain Inhibitory Controls Contribute to Migraine Progression." *Pain* 155(7): 1196–1205.

Bradnam, Lynley, and Christine Barry. 2013. "The Role of the Trigeminal Sensory Nuclear Complex in the Pathophysiology of Craniocervical Dystonia." *Journal of Neuroscience* 33(47): 18358–67.

Bravo-Ferrer, Isabel et al. 2019. "Lack of the Aryl Hydrocarbon Receptor Accelerates Aging in Mice." *FASEB journal: official publication of the Federation of American Societies for Experimental Biology* 33(11): 12644–54.

Brennan, K. C. et al. 2013. "Casein Kinase Iδ Mutations in Familial Migraine and Advanced Sleep Phase." *Science Translational Medicine* 5(183): 183ra56, 1–11.

Brennan, Kevin C. et al. 2007. "Distinct Vascular Conduction With Cortical Spreading Depression." *Journal of Neurophysiology* 97(6): 4143–51.

Breslau, N. et al. 2000. "Headache and Major Depression: Is the Association Specific to Migraine?" *Neurology* 54(2): 308–13.

Breton-Provencher, Vincent, and Mriganka Sur. 2019. "Active Control of Arousal by a Locus Coeruleus GABAergic Circuit." *Nature Neuroscience* 22(2): 218–28.

Brighina, Filippo et al. 2011. “Abnormal Facilitatory Mechanisms in Motor Cortex of Migraine with Aura.” *European Journal of Pain* 15(9): 928–35.

Brightwell, J. J., and B. K. Taylor. 2009. “Noradrenergic Neurons in the Locus Coeruleus Contribute to Neuropathic Pain.” *Neuroscience* 160(1): 174–85.

Browne, Caroline A., Gerard Clarke, Timothy G. Dinan, and John F. Cryan. 2012. “An Effective Dietary Method for Chronic Tryptophan Depletion in Two Mouse Strains Illuminates a Role for 5-HT in Nesting Behaviour.” *Neuropharmacology* 62(5–6): 1903–15.

Brozzi, Flora, Cataldo Arcuri, Ileana Giambanco, and Rosario Donato. 2009. “S100B Protein Regulates Astrocyte Shape and Migration via Interaction with Src Kinase *.” *Journal of Biological Chemistry* 284(13): 8797–8811.

Bruinstroop, Eveline et al. 2012. “Hypothalamic Neuropeptide Y (NPY) Controls Hepatic VLDL-Triglyceride Secretion in Rats via the Sympathetic Nervous System.” *Diabetes* 61(5): 1043–50.

Bruinvels, Anne T., Jose M. Palacios, and Daniel Hoyer. 1993. “Autoradiographic Characterisation and Localisation of 5-HT 1D Compared to 5-HT 1B Binding Sites in Rat Brain.” *Naunyn-Schmiedeberg’s archives of pharmacology* 347: 569–82.

Bruno, P. P., F. Carpino, G. Carpino, and A. Zicari. 2007. “An Overview on Immune System and Migraine.” *European Review for Medical and Pharmacological Sciences* 11(4): 245–48.

Bücheler, M. M, K Hadamek, and L Hein. 2002. “Two A2-Adrenergic Receptor Subtypes, A2A and A2C, Inhibit Transmitter Release in the Brain of Gene-Targeted Mice.” *Neuroscience* 109(4): 819–26.

Bukharaeva, Ellya, Venera Khuzakhmetova, Svetlana Dmitrieva, and Andrei Tsentsevitsky. 2021. “Adrenoceptors Modulate Cholinergic Synaptic Transmission at the Neuromuscular Junction.” *International Journal of Molecular Sciences* 22(9): 4611.

Burstein, R., M. F. Cutrer, and D. Yarnitsky. 2000. “The Development of Cutaneous Allodynia during a Migraine Attack Clinical Evidence for the Sequential Recruitment of Spinal and Supraspinal Nociceptive Neurons in Migraine.” *Brain: A Journal of Neurology* 123 (Pt 8): 1703–9.

Burstein, Rami et al. 2010. “Thalamic Sensitization Transforms Localized Pain into Widespread Allodynia.” *Annals of Neurology* 68(1): 81–91.

Burstein, Rami, Beth Collins, and Moshe Jakubowski. 2004. “Defeating Migraine Pain with Triptans: A Race against the Development of Cutaneous Allodynia.” *Annals of Neurology* 55(1): 19–26.

Burstein, Rami, and Moshe Jakubowski. 2005. “Unitary Hypothesis for Multiple Triggers of the Pain and Strain of Migraine.” *Journal of Comparative Neurology* 493(1): 9–14.

Burstein, Rami, Hiroyoshi Yamamura, Amy Malick, and Andrew M. Strassman. 1998. "Chemical Stimulation of the Intracranial Dura Induces Enhanced Responses to Facial Stimulation in Brain Stem Trigeminal Neurons." *Journal of Neurophysiology* 79(2): 964–82.

Buse, Dawn C. et al. 2013. "Psychiatric Comorbidities of Episodic and Chronic Migraine." *Journal of Neurology* 260(8): 1960–69.

Buse, Dawn C. et al. 2019. "Life With Migraine: Effects on Relationships, Career, and Finances From the Chronic Migraine Epidemiology and Outcomes (CaMEO) Study." *Headache* 59(8): 1286–99.

Bushman, Elisa T., Michael W. Varner, and Kathleen B. Digre. 2018. "Headaches Through a Woman's Life." *Obstetrical & Gynecological Survey* 73(3): 161–73.

Bushong, Eric A., Maryann E. Martone, Ying Z. Jones, and Mark H. Ellisman. 2002. "Protoplasmic Astrocytes in CA1 Stratum Radiatum Occupy Separate Anatomical Domains." *Journal of Neuroscience* 22(1): 183–92.

Buzzi, M. G., and M. A. Moskowitz. 1990. "The Antimigraine Drug, Sumatriptan (GR43175), Selectively Blocks Neurogenic Plasma Extravasation from Blood Vessels in Dura Mater." *British Journal of Pharmacology* 99(1): 202–6.

Bylund, D. B. et al. 1994. "International Union of Pharmacology Nomenclature of Adrenoceptors." *Pharmacological Reviews* 46(2): 121–36.

C

Cady, Roger K. et al. 2009. "Elevated Saliva Calcitonin Gene-Related Peptide Levels during Acute Migraine Predict Therapeutic Response to Rizatriptan." *Headache: The Journal of Head and Face Pain* 49(9): 1258–66.

Cahir, Marie, Tara Ardis, Gavin P. Reynolds, and Stephen J. Cooper. 2007. "Acute and Chronic Tryptophan Depletion Differentially Regulate Central 5-HT1A and 5-HT2A Receptor Binding in the Rat." *Psychopharmacology* 190(4): 497–506.

Calhoun, Anne, and Sutapa Ford. 2008. "Elimination of Menstrual-Related Migraine Beneficially Impacts Chronification and Medication Overuse." *Headache* 48(8): 1186–93.

Cameron, Chris et al. 2015. "Triptans in the Acute Treatment of Migraine: A Systematic Review and Network Meta-Analysis." *Headache: The Journal of Head and Face Pain* 55(S4): 221–35.

Cao, Hong, and Yu-Qiu Zhang. 2008. "Spinal Glial Activation Contributes to Pathological Pain States." *Neuroscience and Biobehavioral Reviews* 32(5): 972–83.

Capuani, Clizia et al. 2016. "Defective Glutamate and K+ Clearance by Cortical Astrocytes in Familial Hemiplegic Migraine Type 2." *EMBO Molecular Medicine* 8(8): 967–86.

Caronna, Edoardo et al. 2021. “Anti-CGRP Monoclonal Antibodies in Chronic Migraine with Medication Overuse: Real-Life Effectiveness and Predictors of Response at 6 Months.” *The Journal of Headache and Pain* 22(1): 120.

Carpenter, Linda L. et al. 1998. “Tryptophan Depletion During Continuous CSF Sampling in Healthy Human Subjects.” *Neuropsychopharmacology* 19(1): 26–35.

Casili, Giovanna et al. 2020. “Dimethyl Fumarate Alleviates the Nitroglycerin (NTG)-Induced Migraine in Mice.” *Journal of Neuroinflammation* 17(1): 59.

Cassone, Vincent M. 1990. “Effects of Melatonin on Vertebrate Circadian Systems.” *Trends in Neurosciences* 13(11): 457–64.

Castellano, A. E. et al. 1998. “Indomethacin Increases the Effect of Isosorbide Dinitrate on Cerebral Hemodynamic in Migraine Patients: Pathogenetic and Therapeutic Implications.” *Cephalalgia* 18(9): 622–30.

Casteren, Daphne S. van et al. 2021. “Sex Differences in Response to Triptans: A Systematic Review and Meta-Analysis.” *Neurology* 96(4): 162–70.

Cedarbaum, Jesse M., and George K. Aghajanian. 1978. “Afferent Projections to the Rat Locus Coeruleus as Determined by a Retrograde Tracing Technique.” *Journal of Comparative Neurology* 178(1): 1–15.

Cervenka, Igor, Leandro Z. Agudelo, and Jorge L. Ruas. 2017. “Kynurenines: Tryptophan’s Metabolites in Exercise, Inflammation, and Mental Health.” *Science* 357(6349): eaaf9794.

Chamba, G. et al. 1991. “Distribution of Alpha-1 and Alpha-2 Binding Sites in the Rat Locus Coeruleus.” *Brain Research Bulletin* 26(2): 185–93.

Chanda, Mona Lisa et al. 2013. “Behavioral Evidence for Photophobia and Stress-Related Ipsilateral Head Pain in Transgenic Cacna1a Mutant Mice.” *PAIN*® 154(8): 1254–62.

Chandler, Daniel J., Wen-Jun Gao, and Barry D. Waterhouse. 2014. “Heterogeneous Organization of the Locus Coeruleus Projections to Prefrontal and Motor Cortices.” *Proceedings of the National Academy of Sciences* 111(18): 6816–21.

Chandler, Daniel, and Barry Waterhouse. 2012. “Evidence for Broad Versus Segregated Projections from Cholinergic and Noradrenergic Nuclei to Functionally and Anatomically Discrete Subregions of Prefrontal Cortex.” *Frontiers in Behavioral Neuroscience* 6. https://www.frontiersin.org/articles/10.3389/fnbeh.2012.00020 (May 9, 2023).

Chandley, Michelle J. et al. 2014. “Elevated Gene Expression of Glutamate Receptors in Noradrenergic Neurons from the Locus Coeruleus in Major Depression.” *International Journal of Neuropsychopharmacology* 17(10): 1569–78.

Chang, Jeff et al. 2017. “Impaired Intestinal Permeability Contributes to Ongoing Bowel Symptoms in Patients With Inflammatory Bowel Disease and Mucosal Healing.” *Gastroenterology* 153(3): 723-731.e1.

Charbit, Annabelle R., Simon Akerman, and Peter J. Goadsby. 2011. “Trigeminocervical Complex Responses after Lesioning Dopaminergic A11 Nucleus Are Modified by Dopamine and Serotonin Mechanisms.” *Pain* 152(10): 2365–76.

Charles, Andrew C., and Serapio M. Baca. 2013. “Cortical Spreading Depression and Migraine.” *Nature Reviews Neurology* 9(11): 637–44.

Charles, Anthony, and K. C. Brennan. 2009. “Cortical Spreading Depression—New Insights and Persistent Questions.” *Cephalalgia* 29(10): 1115–24.

Chauvel, Virginie et al. 2012. “Effect of Systemic Kynurenine on Cortical Spreading Depression and Its Modulation by Sex Hormones in Rat.” *Experimental Neurology* 236(2): 207–14.

Chellappa, Sarah L. 2021. “Individual Differences in Light Sensitivity Affect Sleep and Circadian Rhythms.” *Sleep* 44(2): zsaa214.

Chen, Shih-Pin et al. 2017. “Inhibition of the P2X7–PANX1 Complex Suppresses Spreading Depolarization and Neuroinflammation.” *Brain* 140(6): 1643–56.

Chen, Zhiye et al. 2017. “Altered Functional Connectivity of Amygdala Underlying the Neuromechanism of Migraine Pathogenesis.” *The Journal of Headache and Pain* 18(1): 7.

Chimerel, Catalin et al. 2014. “Bacterial Metabolite Indole Modulates Incretin Secretion from Intestinal Enteroendocrine L Cells.” *Cell Reports* 9(4): 1202–8.

Choi, J. Y. et al. 2009. “Usefulness of a Photophobia Questionnaire in Patients with Migraine.” *Cephalalgia* 29(9): 953–59.

Chong, Catherine D, Amaal J Starling, and Todd J Schwedt. 2016. “Interictal Photosensitivity Associates with Altered Brain Structure in Patients with Episodic Migraine.” *Cephalalgia* 36(6): 526–33.

Christie, MacDonald J., J. T. Williams, P. B. Osborne, and C. E. Bellchambers. 1997. “Where Is the Locus in Opioid Withdrawal?” *Trends in Pharmacological Sciences* 18(4): 134–40.

Chung, Sena et al. 2022. “Common Bacterial Metabolite Indole Directly Activates Nociceptive Neuron through Transient Receptor Potential Ankyrin 1 Channel.” *Pain* 163(8): 1530–41.

Clark, Anna K., Elizabeth A. Old, and Marzia Malcangio. 2013. “Neuropathic Pain and Cytokines: Current Perspectives.” *Journal of Pain Research* 6: 803–14.

Clayton, Edwin C., Janusz Rajkowski, Jonathan D. Cohen, and Gary Aston-Jones. 2004. "Phasic Activation of Monkey Locus Ceruleus Neurons by Simple Decisions in a Forced-Choice Task." *Journal of Neuroscience* 24(44): 9914–20.

Comai, Stefano, Antonella Bertazzo, Martina Brughera, and Sara Crotti. 2020. "Chapter Five - Tryptophan in Health and Disease." In *Advances in Clinical Chemistry*, ed. Gregory S. Makowski. Elsevier, 165–218. https://www.sciencedirect.com/science/article/pii/S0065242319300745 (May 20, 2023).

Coppola, G., F. Pierelli, and J. Schoenen. 2007. "Is the Cerebral Cortex Hyperexcitable or Hyperresponsive in Migraine?" *Cephalalgia: An International Journal of Headache* 27(12): 1427–39.

Coppola, Gianluca et al. 2017. "Cerebral Gray Matter Volume in Patients with Chronic Migraine: Correlations with Clinical Features." *The Journal of Headache and Pain* 18(1): 115.

Croop, Robert et al. 2021. "Oral Rimegepant for Preventive Treatment of Migraine: A Phase 2/3, Randomised, Double-Blind, Placebo-Controlled Trial." *The Lancet* 397(10268): 51–60.

Csati, A. et al. 2012. "Distribution of Vasoactive Intestinal Peptide, Pituitary Adenylate Cyclase-Activating Peptide, Nitric Oxide Synthase, and Their Receptors in Human and Rat Sphenopalatine Ganglion." *Neuroscience* 202: 158–68.

Cuartero, María I. et al. 2014. "L-Kynurenine/Aryl Hydrocarbon Receptor Pathway Mediates Brain Damage After Experimental Stroke." *Circulation* 130(23): 2040–51.

Curran, D. A., H. Hinterberger, and J. W. Lance. 1965. "Total Plasma Serotonin, 5-Hydroxyindoleacetic Acid and p-Hydroxy-m-Methoxymandelic Acid Excretion in Normal and Migrainous Subjects." *Brain: A Journal of Neurology* 88(5): 997–1010.

Curto, Martina et al. 2015. "Altered Kynurenine Pathway Metabolites in Serum of Chronic Migraine Patients." *The Journal of Headache and Pain* 17: 47.

D

Dahlstroem, A., and K. Fuxe. 1964. "Evidence for the Existence of Monoamine Neurons in the Central Nervous System. I. Demonstration of Monoarnines in Cell Badies of Brainstem Neurons." *Acta Physiologica Scandinavica. Supplementum*: SUPPL 232:1-55.

Dahlström, A., and K. Fuxe. 1964. "Localization of Monoamines in the Lower Brain Stem." *Experientia* 20(7): 398–99.

Dallel, Radhouane, Amélie Descheemaeker, and Philippe Luccarini. 2018. "Recurrent Administration of the Nitric Oxide Donor, Isosorbide Dinitrate, Induces a Persistent Cephalic Cutaneous Hypersensitivity: A Model for Migraine

Progression." *Cephalalgia: An International Journal of Headache* 38(4): 776–85.

Dallel, Radhouane, Luis Villanueva, Alain Woda, and Daniel Voisin. 2003. "[Neurobiology of trigeminal pain]." *Medecine Sciences: M/S* 19(5): 567–74.

D'Andrea, Giovanni et al. 2014. "Tryptamine Levels Are Low in Plasma of Chronic Migraine and Chronic Tension-Type Headache." *Neurological Sciences* 35(12): 1941–45.

De Fusco, Maurizio et al. 2003. "Haploinsufficiency of ATP1A2 Encoding the Na+/K+ Pump Alpha2 Subunit Associated with Familial Hemiplegic Migraine Type 2." *Nature Genetics* 33(2): 192–96.

De Leo, Joyce A., Vivianne L. Tawfik, and Michael L. LaCroix-Fralish. 2006. "The Tetrapartite Synapse: Path to CNS Sensitization and Chronic Pain." *Pain* 122(1–2): 17–21.

De Logu, Francesco et al. 2019. "Migraine-Provoking Substances Evoke Periorbital Allodynia in Mice." *The Journal of Headache and Pain* 20(1): 18.

Deen, Marie et al. 2017. "Serotonergic Mechanisms in the Migraine Brain - a Systematic Review." *Cephalalgia: An International Journal of Headache* 37(3): 251–64.

Deen, Marie et al. 2018. "High Brain Serotonin Levels in Migraine between Attacks: A 5-HT4 Receptor Binding PET Study." *NeuroImage : Clinical* 18: 97–102.

Deen, Marie et al. 2019. "Migraine Is Associated with High Brain 5-HT Levels as Indexed by 5-HT4 Receptor Binding." *Cephalalgia* 39(4): 526–32.

Denison, Michael S., and Scott R. Nagy. 2003. "Activation of the Aryl Hydrocarbon Receptor by Structurally Diverse Exogenous and Endogenous Chemicals." *Annual Review of Pharmacology and Toxicology* 43: 309–34.

Denuelle, M. et al. 2011. "A PET Study of Photophobia during Spontaneous Migraine Attacks." *Neurology* 76(3): 213–18.

Denuelle, Marie et al. 2007. "Hypothalamic Activation in Spontaneous Migraine Attacks." *Headache: The Journal of Head and Face Pain* 47(10): 1418–26.

Derry, Christopher J., Sheena Derry, and R. Andrew Moore. 2014. "Sumatriptan (All Routes of Administration) for Acute Migraine Attacks in Adults-Overview of Cochrane Reviews." *Cochrane Database of Systematic Reviews* (5).

Deutch, Ariel Y., Menek Goldstein, and Robert H. Roth. 1986. "Activation of the Locus Coeruleus Induced by Selective Stimulation of the Ventral Tegmental Area." *Brain Research* 363(2): 307–14.

Dever, Daniel P. et al. 2016. "Aryl Hydrocarbon Receptor (AhR) Deletion in Cerebellar Granule Neuron Precursors Impairs Neurogenesis." *Developmental neurobiology* 76(5): 533–50.

Dichgans, Martin et al. 2005. “Mutation in the Neuronal Voltage-Gated Sodium Channel SCN1A in Familial Hemiplegic Migraine.” *Lancet (London, England)* 366(9483): 371–77.

Diener, Hans-Christoph et al. 2004. “Topiramate in Migraine Prophylaxis--Results from a Placebo-Controlled Trial with Propranolol as an Active Control.” *Journal of Neurology* 251(8): 943–50.

Dimitrova, Alexandra K. et al. 2013. “Prevalence of Migraine in Patients With Celiac Disease and Inflammatory Bowel Disease.” *Headache: The Journal of Head and Face Pain* 53(2): 344–55.

Dodds, K. N. et al. 2016. “Glial Contributions to Visceral Pain: Implications for Disease Etiology and the Female Predominance of Persistent Pain.” *Translational Psychiatry* 6(9): e888–e888.

Dodick, David W. et al. 2020. “Ubrogepant, an Acute Treatment for Migraine, Improved Patient-Reported Functional Disability and Satisfaction in 2 Single-Attack Phase 3 Randomized Trials, ACHIEVE I and II.” *Headache* 60(4): 686–700.

Dolwick, Kristine M. et al. 1993. “Cloning and Expression of a Human Ah Receptor CDNA.” *Molecular pharmacology* 44(5): 911–17.

Dreier, Jens P. et al. 2009. “Cortical Spreading Ischaemia Is a Novel Process Involved in Ischaemic Damage in Patients with Aneurysmal Subarachnoid Haemorrhage.” *Brain* 132(7): 1866–81.

Drummond, PD. 2006. “Tryptophan Depletion Increases Nausea, Headache and Photophobia in Migraine Sufferers.” *Cephalalgia* 26(10): 1225–33.

Drummond, Peter D. 2005. “Effect of Tryptophan Depletion on Symptoms of Motion Sickness in Migraineurs.” *Neurology* 65(4): 620–22.

Ducros, A. et al. 2021. “Revised Guidelines of the French Headache Society for the Diagnosis and Management of Migraine in Adults. Part 2: Pharmacological Treatment.” *Revue Neurologique* 177(7): 734–52.

von Düring, M. et al. 1990. “Neuropeptide Y- and Substance P-like Immunoreactive Nerve Fibers in the Rat Dura Mater Encephali.” *Anatomy and Embryology* 182(4): 363–73.

E

Ebersberger, A., M. Ringkamp, P. W. Reeh, and H. O. Handwerker. 1997. “Recordings From Brain Stem Neurons Responding to Chemical Stimulation of the Subarachnoid Space.” *Journal of Neurophysiology* 77(6): 3122–33.

Edelmayer, Rebecca M. et al. 2009. “Medullary Pain Facilitating Neurons Mediate Allodynia in Headache-Related Pain.” *Annals of Neurology* 65(2): 184–93.

Edmeads, John, and Joan A. Mackell. 2002. “The Economic Impact of Migraine: An Analysis of Direct and Indirect Costs.” *Headache: The Journal of Head and Face Pain* 42(6): 501–9.

Edvinsson, Jacob C. A. et al. 2022. “Lasmiditan and 5-Hydroxytryptamine in the Rat Trigeminal System; Expression, Release and Interactions with 5-HT1 Receptors.” *The Journal of Headache and Pain* 23(1): 26.

Edvinsson, L., S. Rosendal-Helgesen, and R. Uddman. 1983. “Substance P: Localization, Concentration and Release in Cerebral Arteries, Choroid Plexus and Dura Mater.” *Cell and Tissue Research* 234(1): 1–7.

Edvinsson, Lars, Kristian Agmund Haanes, and Karin Warfvinge. 2019. “Does Inflammation Have a Role in Migraine?” *Nature Reviews. Neurology* 15(8): 483–90.

Ehrlich, Amy M. et al. 2020. “Indole-3-Lactic Acid Associated with Bifidobacterium-Dominated Microbiota Significantly Decreases Inflammation in Intestinal Epithelial Cells.” *BMC Microbiology* 20(1): 357.

Eigenbrodt, Anna K. et al. 2022. “Premonitory Symptoms in Migraine: A Systematic Review and Meta-Analysis of Observational Studies Reporting Prevalence or Relative Frequency.” *The Journal of Headache and Pain* 23(1): 140.

Eikermann-Haerter, Katharina, and Michael A. Moskowitz. 2008. “Animal Models of Migraine Headache and Aura.” *Current Opinion in Neurology* 21(3): 294–300.

Eller, Olivia C. et al. 2021. “Voluntary Wheel Running Partially Attenuates Early Life Stress-Induced Neuroimmune Measures in the Dura and Evoked Migraine-Like Behaviors in Female Mice.” *Frontiers in Physiology* 12. https://www.frontiersin.org/articles/10.3389/fphys.2021.665732 (July 28, 2023).

Erzurumlu, Reha S., Yasunori Murakami, and Filippo M. Rijli. 2010. “Mapping the Face in the Somatosensory Brainstem.” *Nature Reviews Neuroscience* 11(4): 252–63.

Escher, Claus M, Lejla Paracka, Dirk Dressler, and Katja Kollewe. 2017. “Botulinum Toxin in the Management of Chronic Migraine: Clinical Evidence and Experience.” *Therapeutic Advances in Neurological Disorders* 10(2): 127–35.

Estave, Paige M. et al. 2021. “Learning the Full Impact of Migraine through Patient Voices: A Qualitative Study.” *Headache: The Journal of Head and Face Pain* 61(7): 1004–20.

F

Farajdokht, Fereshteh et al. 2017. “Chronic Ghrelin Treatment Reduced Photophobia and Anxiety-like Behaviors in Nitroglycerin- Induced Migraine: Role of Pituitary Adenylate Cyclase-Activating Polypeptide.” *The European Journal of Neuroscience* 45(6): 763–72.

Fazio, Francesco et al. 2014. “Cinnabarinic Acid, an Endogenous Agonist of Type-4 Metabotropic Glutamate Receptor, Suppresses Experimental Autoimmune Encephalomyelitis in Mice.” *Neuropharmacology* 81: 237–43.

Felten, David L., and John R. Sladek. 1983. “Monoamine Distribution in Primate Brain V. Monoaminergic Nuclei: Anatomy, Pathways and Local Organization.” *Brain Research Bulletin* 10(2): 171–284.

Fernstrom, John D. et al. 2013. “The Ingestion of Different Dietary Proteins by Humans Induces Large Changes in the Plasma Tryptophan Ratio, a Predictor of Brain Tryptophan Uptake and Serotonin Synthesis.” *Clinical Nutrition* 32(6): 1073–76.

Ferrari, Anna et al. 2008. “Interindividual Variability of Oral Sumatriptan Pharmacokinetics and of Clinical Response in Migraine Patients.” *European Journal of Clinical Pharmacology* 64(5): 489–95.

Ferrari, M. D. et al. 2022. “Migraine.” *Nature Reviews Disease Primers* 8(1). https://hdl.handle.net/1887/3502231 (May 1, 2023).

Ferrari, Michel D. et al. 2015. “Migraine Pathophysiology: Lessons from Mouse Models and Human Genetics.” *The Lancet. Neurology* 14(1): 65–80.

Ferrari, Michel D., P. J. Goadsby, K. I. Roon, and Richard B. Lipton. 2002. “Triptans (Serotonin, 5-HT1B/1D Agonists) in Migraine: Detailed Results and Methods of a Meta-Analysis of 53 Trials.” *Cephalalgia* 22(8): 633–58.

Fila, Michal et al. 2023. “Nutrition and Calcitonin Gene Related Peptide (CGRP) in Migraine.” *Nutrients* 15(2): 289.

Filippone, Alessia et al. 2022. “BAY-117082-Driven NLRP3 Inflammasome Inhibition Resolves Nitro-Glycerine (NTG) Neuronal Damage in in Vivo Model of Migraine.” *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie* 156: 113851.

Fine, P G, and K B Digre. 1995. “A Controlled Trial of Regional Sympatholysis in the Treatment of Photo-Oculodynia Syndrome.” *Journal of neuro-ophthalmology* 15(2): 90–94.

Flores Ramos, José María et al. 2017. “The Nitric Oxide Donor, Isosorbide Dinitrate, Induces a Cephalic Cutaneous Hypersensitivity, Associated with Sensitization of the Medullary Dorsal Horn.” *Neuroscience* 344: 157–66.

Fontaine, Denys et al. 2018. “Dural and Pial Pain-Sensitive Structures in Humans: New Inputs from Awake Craniotomies.” *Brain: A Journal of Neurology* 141(4): 1040–48.

Franklin, M., P.J. Cowen, and R.D. Craven. 1995. “The Effects of a Low Tryptophan Diet on Brain 5 -HT Metabolism and 5-HT-Mediated Neuroendocrine Responses in the Male Rat.” *Journal of Psychopharmacology* 9(4): 336–41.

Fu, Jiaqi et al. 2018. “Shared Epitope-Aryl Hydrocarbon Receptor Crosstalk Underlies the Mechanism of Gene-Environment Interaction in Autoimmune Arthritis.” *Proceedings of the National Academy of Sciences of the United States of America* 115(18): 4755–60.

Fukuda, Itsuko et al. 2015. “Catechins in Tea Suppress the Activity of Cytochrome P450 1A1 through the Aryl Hydrocarbon Receptor Activation Pathway in Rat Livers.” *International Journal of Food Sciences and Nutrition* 66(3): 300–307.

G

Ganapathy, Vadivel, Naren Gupta, and Robert G. Martindale. 2006. “Protein Digestion and Absorption.” In *Physiology of the Gastrointestinal Tract*, Elsevier Inc., 1667–92. http://www.scopus.com/inward/record.url?scp=84884017598&partnerID=8YFLogxK (July 2, 2023).

Gao, Jing et al. 2018. “Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism.” *Frontiers in Cellular and Infection Microbiology* 8. https://www.frontiersin.org/articles/10.3389/fcimb.2018.00013 (June 4, 2023).

Gao, Kan, Chun-long Mu, Aitak Farzi, and Wei-yun Zhu. 2020. “Tryptophan Metabolism: A Link Between the Gut Microbiota and Brain.” *Advances in Nutrition* 11(3): 709–23.

Garland, Scott G., Steven M. Smith, and John G. Gums. 2019. “Erenumab: A First-in-Class Monoclonal Antibody for Migraine Prevention.” *Annals of Pharmacotherapy* 53(9): 933–39.

Georgescu, Doina et al. 2019. “Migraine without Aura and Subclinical Atherosclerosis in Young Females: Is Gut Microbiota to Blame?” *Medicina* 55(12): 786.

Gesi, M. et al. 2000. “The Role of the Locus Coeruleus in the Development of Parkinson’s Disease.” *Neuroscience & Biobehavioral Reviews* 24(6): 655–68.

Ghaemi, Amir et al. 2016. “Immunomodulatory Effect of Toll-Like Receptor-3 Ligand Poly I:C on Cortical Spreading Depression.” *Molecular Neurobiology* 53(1): 143–54.

Ghaemi, Amir et al. 2018. “Astrocyte-Mediated Inflammation in Cortical Spreading Depression.” *Cephalalgia* 38(4): 626–38.

Giffin, N. J. et al. 2003. “Premonitory Symptoms in Migraine: An Electronic Diary Study.” *Neurology* 60(6): 935–40.

Giffin, Nicola J. et al. 2016. “The Migraine Postdrome: An Electronic Diary Study.” *Neurology* 87(3): 309–13.

Gil-Gouveia, Raquel, António G. Oliveira, and Isabel Pavão Martins. 2016. “The Impact of Cognitive Symptoms on Migraine Attack-Related Disability.” *Cephalalgia: An International Journal of Headache* 36(5): 422–30.

Gilhus, Nils Erik, and Günther Deuschl. 2019. "Neuroinflammation — a Common Thread in Neurological Disorders." *Nature Reviews Neurology* 15(8): 429–30.

Gilsbach, Ralf, and Lutz Hein. 2008. "Presynaptic Metabotropic Receptors for Acetylcholine and Adrenaline/Noradrenaline." In *Pharmacology of Neurotransmitter Release*, Handbook of Experimental Pharmacology, eds. Thomas C. Südhof and Klaus Starke. Berlin, Heidelberg: Springer, 261–88. https://doi.org/10.1007/978-3-540-74805-2_9 (June 25, 2023).

Giorgi, Filippo Sean et al. 2021. "The Connections of Locus Coeruleus with Hypothalamus: Potential Involvement in Alzheimer's Disease." *Journal of Neural Transmission* 128(5): 589–613.

Goadsby, Peter J. et al. 2017. "Pathophysiology of Migraine: A Disorder of Sensory Processing." *Physiological Reviews* 97(2): 553–622.

Goadsby, Peter J., and Karen L. Hoskin. 1999. "Differential Effects of Low Dose CP122,288 and Eletriptan on Fos Expression Due to Stimulation of the Superior Sagittal Sinus in Cat☆." *PAIN* 82(1): 15.

Goadsby, Peter J., and Yolande Knight. 1997. "Inhibition of Trigeminal Neurones after Intravenous Administration of Naratriptan through an Action at 5-Hydroxy-Tryptamine (5-HT1B/1D) Receptors." *British Journal of Pharmacology* 122(5): 918–22.

Goadsby, Peter J., and Richard B. Lipton. 1997. "A Review of Paroxysmal Hemicranias, SUNCT Syndrome and Other Short-Lasting Headaches with Autonomic Feature, Including New Cases." *Brain: a journal of neurology* 120(1): 193–209.

Gomez Perdiguero, Elisa et al. 2015. "Tissue-Resident Macrophages Originate from Yolk-Sac-Derived Erythro-Myeloid Progenitors." *Nature* 518(7540): 547–51.

Gormley, Padhraig et al. 2016. "Meta-Analysis of 375,000 Individuals Identifies 38 Susceptibility Loci for Migraine." *Nature Genetics* 48(8): 856–66.

Gostner, Johanna M. et al. 2020. "Tryptophan Metabolism and Related Pathways in Psychoneuroimmunology: The Impact of Nutrition and Lifestyle." *Neuropsychobiology* 79(1): 89–99.

Goya-Jorge, Elizabeth, María Elisa Jorge Rodríguez, Maité Sylla-Iyarreta Veitía, and Rosa M. Giner. 2021. "Plant Occurring Flavonoids as Modulators of the Aryl Hydrocarbon Receptor." *Molecules* 26(8): 2315.

Greco, Rosaria et al. 2017. "Effects of Kynurenic Acid Analogue 1 (KYNA-A1) in Nitroglycerin-Induced Hyperalgesia: Targets and Anti-Migraine Mechanisms." *Cephalalgia* 37(13): 1272–84.

Grzanna, R., and M. E. Molliver. 1980. "The Locus Coeruleus in the Rat: An Immunohistochemical Delineation." *Neuroscience* 5(1): 21–40.

Guillemin, Gilles J., George Smythe, Osamu Takikawa, and Bruce J. Brew. 2005. "Expression of Indoleamine 2,3-Dioxygenase and Production of Quinolinic Acid by Human Microglia, Astrocytes, and Neurons." *Glia* 49(1): 15–23.

Gupta, Saurabh, Stephanie J. Nahas, and B. Lee Peterlin. 2011. "Chemical Mediators of Migraine: Preclinical and Clinical Observations." *Headache* 51(6): 1029–45.

Gursoy-Ozdemir, Yasemin et al. 2004. "Cortical Spreading Depression Activates and Upregulates MMP-9." *Journal of Clinical Investigation* 113(10): 1447–55.

Guy, N. et al. 2010. "Are There Differences between Cephalic and Extracephalic Cutaneous Allodynia in Migraine Patients?" *Cephalalgia: An International Journal of Headache* 30(7): 881–86.

Gyires, Klára, Zoltán S. Zádori, Tamás Török, and Péter Mátyus. 2009. "A2-Adrenoceptor Subtypes-Mediated Physiological, Pharmacological Actions." *Neurochemistry International* 55(7): 447–53.

H

Haddjeri, Nasser, Claude De Montigny, and Pierre Blier. 1997. "Modulation of the Firing Activity of Noradrenergic Neurones in the Rat Locus Coeruleus by the 5-Hydroxtryptamine System." *British journal of pharmacology* 120(5): 865–75.

Hahn, Mark E. et al. 2006. "Unexpected Diversity of Aryl Hydrocarbon Receptors in Non-Mammalian Vertebrates: Insights from Comparative Genomics." *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 305A(9): 693–706.

Haines, D. E. 1991. "On the Question of a Subdural Space." *The Anatomical Record* 230(1): 3–21.

Han, Sung Gu, Seong-Su Han, Michal Toborek, and Bernhard Hennig. 2012. *Toxicology and Applied Pharmacology* 261(2): 181–88.

Hanieh, Hamza. 2014. "Toward Understanding the Role of Aryl Hydrocarbon Receptor in the Immune System: Current Progress and Future Trends." *BioMed Research International* 2014: 520763.

Hanneman, W. H. et al. 1996. "Stimulation of Calcium Uptake in Cultured Rat Hippocampal Neurons by 2,3,7,8-Tetrachlorodibenzo-p-Dioxin." *Toxicology* 112(1): 19–28.

Hara, Masamitsu et al. 2017. "Interaction of Reactive Astrocytes with Type I Collagen Induces Astrocytic Scar Formation through the Integrin–N-Cadherin Pathway after Spinal Cord Injury." *Nature medicine* 23(7): 818–28.

Harriott, Andrea M., Lauren C. Strother, Marta Vila-Pueyo, and Philip R. Holland. 2019. "Animal Models of Migraine and Experimental Techniques Used to Examine Trigeminal Sensory Processing." *The Journal of Headache and Pain* 20(1): 91.

Hayashida, Ken-ichiro, Hideaki Obata, Kunie Nakajima, and James C. Eisenach. 2008. "Gabapentin Acts within the Locus Coeruleus to Alleviate Neuropathic Pain." *Anesthesiology* 109(6): 1077–84.

He, Wei et al. 2019. "Microglial NLRP3 Inflammasome Activation Mediates IL-1β Release and Contributes to Central Sensitization in a Recurrent Nitroglycerin-Induced Migraine Model." *Journal of Neuroinflammation* 16(1): 78.

Hentall, Ian D., Riza Mesigil, Alberto Pinzon, and Brian R. Noga. 2003. "Temporal and Spatial Profiles of Pontine-Evoked Monoamine Release in the Rat's Spinal Cord." *Journal of Neurophysiology* 89(6): 2943–51.

Herd, Clare P. et al. 2018. "Botulinum Toxins for the Prevention of Migraine in Adults." *Cochrane Database of Systematic Reviews* (6). https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD011616.pub2/full (June 25, 2023).

Ho, Tony W. et al. 2014. "Randomized Controlled Trial of the CGRP Receptor Antagonist Telcagepant for Migraine Prevention." *Neurology* 83(11): 958–66.

Hoffmann, Jan et al. 2015. "Evidence for Orexinergic Mechanisms in Migraine." *Neurobiology of Disease* 74: 137–43.

Hoffmann, Jan, Serapio M Baca, and Simon Akerman. 2019. "Neurovascular Mechanisms of Migraine and Cluster Headache." *Journal of Cerebral Blood Flow & Metabolism* 39(4): 573–94.

Hoffmann, Jan, and Andrew Charles. 2018. "Glutamate and Its Receptors as Therapeutic Targets for Migraine." *Neurotherapeutics: The Journal of the American Society for Experimental NeuroTherapeutics* 15(2): 361–70.

Höglund, Erik, Øyvind Øverli, and Svante Winberg. 2019. "Tryptophan Metabolic Pathways and Brain Serotonergic Activity: A Comparative Review." *Frontiers in Endocrinology* 10. https://www.frontiersin.org/articles/10.3389/fendo.2019.00158 (August 12, 2023).

Holets, V. R. et al. 1988. "Locus Coeruleus Neurons in the Rat Containing Neuropeptide Y, Tyrosine Hydroxylase or Galanin and Their Efferent Projections to the Spinal Cord, Cerebral Cortex and Hypothalamus." *Neuroscience* 24(3): 893–906.

Holstege, Gert. 1987. "Some Anatomical Observations on the Projections from the Hypothalamus to Brainstem and Spinal Cord: An HRP and Autoradiographic Tracing Study in the Cat." *Journal of Comparative Neurology* 260(1): 98–126.

Horvath, Gabriella A et al. 2011. "Hemiplegic Migraine, Seizures, Progressive Spastic Paraparesis, Mood Disorder, and Coma in Siblings with Low Systemic Serotonin." *Cephalalgia* 31(15): 1580–86.

Hou, Mingyan et al. 2001. "5-HT1B and 5-HT1D Receptors in the Human Trigeminal Ganglion: Co-Localization with Calcitonin Gene-Related Peptide, Substance P and Nitric Oxide Synthase." *Brain Research* 909(1): 112–20.

Hougaard, Anders et al. 2013. "Provocation of Migraine with Aura Using Natural Trigger Factors." *Neurology* 80(5): 428–31.

Hougaard, Anders et al. 2017. "Increased Brainstem Perfusion, but No Blood-Brain Barrier Disruption, during Attacks of Migraine with Aura." *Brain* 140(6): 1633–42.

Houle, Timothy T., Dana P. Turner, and Donald B. Penzien. 2012. "How Does the Migraine Attack Stop? It's NOT the Trigger: Common Headache Triggers Do Not Predict Cessation of Pain." *Headache* 52(1): 189–90.

Howorth, Patrick W, Anja G Teschemacher, and Anthony E Pickering. 2009. "Retrograde Adenoviral Vector Targeting of Nociresponsive Pontospinal Noradrenergic Neurons in the Rat in Vivo." *The Journal of Comparative Neurology* 512(2): 141–57.

Hu, James W., and Alain Woda. 2013. "Trigeminal Brainstem Nuclear Complex, Physiology." In *Encyclopedia of Pain*, eds. Gerald F. Gebhart and Robert F. Schmidt. Berlin, Heidelberg: Springer, 4060–65. https://doi.org/10.1007/978-3-642-28753-4_4604 (July 12, 2023).

Hubbard, Troy D. et al. 2015. "Adaptation of the Human Aryl Hydrocarbon Receptor to Sense Microbiota-Derived Indoles." *Scientific Reports* 5: 12689.

Humphrey, PPA, and PJ Goadsby. 1994. "The Mode of Action of Sumatriptan Is Vascular? A Debate." *Cephalalgia* 14(6): 401–10.

I

IHS. 2018. "Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd Edition." *Cephalalgia: An International Journal of Headache* 38(1): 1–211.

Isler, H. 1992. "The Galenic Tradition and Migraine." *Journal of the History of the Neurosciences* 1(3): 227–33.

Itoi, K., and N. Sugimoto. 2010. "The Brainstem Noradrenergic Systems in Stress, Anxiety and Depression." *Journal of Neuroendocrinology* 22(5): 355–61.

J

Jackson, Daniel P. et al. 2014. "Ah Receptor–Mediated Suppression of Liver Regeneration through NC-XRE–Driven P21Cip1 Expression." *Molecular Pharmacology* 85(4): 533–41.

Jackson, Jeffrey L. et al. 2019. "Beta-Blockers for the Prevention of Headache in Adults, a Systematic Review and Meta-Analysis." *PloS One* 14(3): e0212785.

Jakate, Abhijeet et al. 2020. “Evaluation of the Pharmacokinetic Interaction of Ubrogepant Coadministered With Sumatriptan and of the Safety of Ubrogepant With Triptans.” *Headache: The Journal of Head and Face Pain* 60(7): 1340–50.

Jalgaonkar, Sharmila et al. 2023. “Analgesic, Anti-Inflammatory, and Antioxidant Potential of S-Adenosyl L-Methionine on Nitroglycerine Induced Migraine in Mice Models.” *Journal of Pharmacy and Pharmacology Research* 07(01). https://www.fortunejournals.com/articles/analgesic-antiinflammatory-and-antioxidant-potential-of-sadenosyl-lmethionine-on-nitroglycerine-induced-migraine-in-mice-models.html (July 18, 2023).

James, Tony et al. 2021. “Locus Coeruleus in Memory Formation and Alzheimer’s Disease.” *European Journal of Neuroscience* 54(8): 6948–59.

Jans, L. A. W., C. K. J. Lieben, and A. Blokland. 2007. “Influence of Sex and Estrous Cycle on the Effects of Acute Tryptophan Depletion Induced by a Gelatin-Based Mixture in Adult Wistar Rats.” *Neuroscience* 147(2): 304–17.

Jensen, Rigmor, and Lars J. Stovner. 2008. “Epidemiology and Comorbidity of Headache.” *The Lancet. Neurology* 7(4): 354–61.

Jha, Mithilesh Kumar, Myungjin Jo, Jae-Hong Kim, and Kyoungho Suk. 2019. “Microglia-Astrocyte Crosstalk: An Intimate Molecular Conversation.” *The Neuroscientist* 25(3): 227–40.

Ji, Ru-Rong et al. 2018. “Neuroinflammation and Central Sensitization in Chronic and Widespread Pain.” *Anesthesiology* 129(2): 343–66.

Ji, Ru-Rong, Temugin Berta, and Maiken Nedergaard. 2013. “Glia and Pain: Is Chronic Pain a Gliopathy?” *Pain* 154(0 1): S10–28.

Jin, Un-Ho et al. 2014. “Microbiome-Derived Tryptophan Metabolites and Their Aryl Hydrocarbon Receptor-Dependent Agonist and Antagonist Activities.” *Molecular Pharmacology* 85(5): 777–88.

Jin, Xin et al. 2016. “Identification of a Group of GABAergic Neurons in the Dorsomedial Area of the Locus Coeruleus.” *PLOS ONE* 11(1): e0146470.

John, Ann-Marie, and J. Milton Bell. 1976. “Amino Acid Requirements of the Growing Mouse.” *The Journal of Nutrition* 106(9): 1361–67.

Jones, Lauren A., Emily W. Sun, Alyce M. Martin, and Damien J. Keating. 2020. “The Ever-Changing Roles of Serotonin.” *The international journal of biochemistry & cell biology* 125: 105776.

Jones, S. L., and G. F. Gebhart. 1986. “Quantitative Characterization of Ceruleospinal Inhibition of Nociceptive Transmission in the Rat.” *Journal of Neurophysiology* 56(5): 1397–1410.

Julius, David, and Allan I. Basbaum. 2001. “Molecular Mechanisms of Nociception.” *Nature* 413(6852): 203–10.

Juricek, Ludmila, and Xavier Coumoul. 2018. “The Aryl Hydrocarbon Receptor and the Nervous System.” *International Journal of Molecular Sciences* 19(9): 2504.

K

Karatas, Hulya et al. 2013. “Spreading Depression Triggers Headache by Activating Neuronal Panx1 Channels.” *Science* 339(6123): 1092–95.

Karolewicz, Beata, Craig A Stockmeier, and Gregory A Ordway. 2005. “Elevated Levels of the NR2C Subunit of the NMDA Receptor in the Locus Coeruleus in Depression.” *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 30(8): 1557–67.

Kashem, Sakeen W. et al. 2015. “Nociceptive Sensory Fibers Drive Interleukin-23 Production from CD301b+ Dermal Dendritic Cells and Drive Protective Cutaneous Immunity.” *Immunity* 43(3): 515–26.

Kaur, Harrisham, Chandrani Bose, and Sharmila S. Mande. 2019. “Tryptophan Metabolism by Gut Microbiome and Gut-Brain-Axis: An in Silico Analysis.” *Frontiers in Neuroscience* 13: 1365.

Kawasaki, Yasuhiko, Eiichi Kumamoto, Hidemasa Furue, and Megumu Yoshimura. 2003. “A2Adrenoceptor–Mediated Presynaptic Inhibition of Primary Afferent Glutamatergic Transmission in Rat Substantia Gelatinosa Neurons.” *Anesthesiology* 98(3): 682–89.

Kelman, L. 2007. “The Triggers or Precipitants of the Acute Migraine Attack.” *Cephalalgia* 27(5): 394–402.

Kessler, M., T. Terramani, G. Lynch, and M. Baudry. 1989. “A Glycine Site Associated with N-Methyl-d-Aspartic Acid Receptors: Characterization and Identification of a New Class of Antagonists.” *Journal of Neurochemistry* 52(4): 1319–28.

Keszthelyi, D., F. J. Troost, and A. a. M. Masclee. 2009. “Understanding the Role of Tryptophan and Serotonin Metabolism in Gastrointestinal Function.” *Neurogastroenterology & Motility* 21(12): 1239–49.

Khan, Johra et al. 2021. “Genetics, Pathophysiology, Diagnosis, Treatment, Management, and Prevention of Migraine.” *Biomedicine & Pharmacotherapy* 139: 111557.

Kiguchi, Norikazu, Yuka Kobayashi, and Shiroh Kishioka. 2012. “Chemokines and Cytokines in Neuroinflammation Leading to Neuropathic Pain.” *Current Opinion in Pharmacology* 12(1): 55–61.

Kim, Dong W. et al. 2000. “The RelA NF-KB Subunit and the Aryl Hydrocarbon Receptor (AhR) Cooperate to Transactivate the c-Myc Promoter in Mammary Cells.” *Oncogene* 19(48): 5498–5506.

Kimball, R. W., A. P. Friedman, and E. Vallejo. 1960. “Effect of Serotonin in Migraine Patients.” *Neurology* 10: 107–11.

Kimura, Akihiro et al. 2008. “Aryl Hydrocarbon Receptor Regulates Stat1 Activation and Participates in the Development of Th17 Cells.” *Proceedings of the National Academy of Sciences of the United States of America* 105(28): 9721–26.

Kimura, Eiki et al. 2021. “Neurons Expressing the Aryl Hydrocarbon Receptor in the Locus Coeruleus and Island of Calleja Major Are Novel Targets of Dioxin in the Mouse Brain.” *Histochemistry and Cell Biology* 156(2): 147–63.

Kimura, Eiki, and Chiharu Tohyama. 2017. “Embryonic and Postnatal Expression of Aryl Hydrocarbon Receptor MRNA in Mouse Brain.” *Frontiers in Neuroanatomy* 11: 4.

Kirthi, Varo, Sheena Derry, and R. Andrew Moore. 2013. “Aspirin with or without an Antiemetic for Acute Migraine Headaches in Adults.” *The Cochrane Database of Systematic Reviews* 2013(4). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6483629/ (July 18, 2023).

Knight, Y. E, and P. J Goadsby. 2001. “The Periaqueductal Grey Matter Modulates Trigeminovascular Input: A Role in Migraine?11Presented in Part at the Xth International Headache Congress, Barcelona, Spain, 24–26 June 1999 [Knight and Goadsby, Cephalalgia 19 (1999) 315].” *Neuroscience* 106(4): 793–800.

Koga, Hitoshi et al. 2005. “Activation of Presynaptic GABAA Receptors Increases Spontaneous Glutamate Release onto Noradrenergic Neurons of the Rat Locus Coeruleus.” *Brain Research* 1046(1): 24–31.

Kogelman, Lisette J. A. et al. 2019. “Migraine Polygenic Risk Score Associates with Efficacy of Migraine-Specific Drugs.” *Neurology Genetics* 5(6). https://ng.neurology.org/content/5/6/e364 (August 2, 2023).

Konopelski, Piotr et al. 2019. “Indole-3-Propionic Acid, a Tryptophan-Derived Bacterial Metabolite, Reduces Weight Gain in Rats.” *Nutrients* 11(3): 591.

Körtési, Tamás, Eleonóra Spekker, and László Vécsei. 2022. “Exploring the Tryptophan Metabolic Pathways in Migraine-Related Mechanisms.” *Cells* 11(23): 3795.

Kraft, Andrew D., and G. Jean Harry. 2011. “Features of Microglia and Neuroinflammation Relevant to Environmental Exposure and Neurotoxicity.” *International Journal of Environmental Research and Public Health* 8(7): 2980–3018.

Kuburas, Adisa, and Andrew F. Russo. 2023. “Shared and Independent Roles of CGRP and PACAP in Migraine Pathophysiology.” *The Journal of Headache and Pain* 24(1): 34.

Kuypers, H. G. 1958. “Corticobular Connexions to the Pons and Lower Brain-Stem in Man: An Anatomical Study.” *Brain: A Journal of Neurology* 81(3): 364–88.

Kwiat, G. C., and A. I. Basbaum. 1992. “The Origin of Brainstem Noradrenergic and Serotonergic Projections to the Spinal Cord Dorsal Horn in the Rat.” *Somatosensory & Motor Research* 9(2): 157–73.

Kwidzinski, Erik, and Ingo Bechmann. 2007. “IDO Expression in the Brain: A Double-Edged Sword.” *Journal of Molecular Medicine* 85(12): 1351–59.

L

Lahvis, G. P. et al. 2000. “Portosystemic Shunting and Persistent Fetal Vascular Structures in Aryl Hydrocarbon Receptor-Deficient Mice.” *Proceedings of the National Academy of Sciences of the United States of America* 97(19): 10442–47.

Lahvis, Garet P. et al. 2005. “The Aryl Hydrocarbon Receptor Is Required for Developmental Closure of the Ductus Venosus in the Neonatal Mouse.” *Molecular Pharmacology* 67(3): 714–20.

Lai, T.-H., J.-L. Fuh, and S.-J. Wang. 2009. “Cranial Autonomic Symptoms in Migraine: Characteristics and Comparison with Cluster Headache.” *Journal of Neurology, Neurosurgery & Psychiatry* 80(10): 1116–19.

Lamas, Bruno et al. 2016. “CARD9 Impacts Colitis by Altering Gut Microbiota Metabolism of Tryptophan into Aryl Hydrocarbon Receptor Ligands.” *Nature Medicine* 22(6): 598–605.

Lamb, Yvette N. 2019. “Fremanezumab in the Prevention of Migraine: A Profile of Its Use.” *Drugs & Therapy Perspectives* 35(12): 592–600.

Lambert, Geoffrey A. 2010. “The Lack of Peripheral Pathology in Migraine Headache.” *Headache: The Journal of Head and Face Pain* 50(5): 895–908.

Lang, Roland et al. 2015. “Physiology, Signaling, and Pharmacology of Galanin Peptides and Receptors: Three Decades of Emerging Diversity” ed. Arthur Christopoulos. *Pharmacological Reviews* 67(1): 118–75.

Larigot, Lucie et al. 2022. “Aryl Hydrocarbon Receptor and Its Diverse Ligands and Functions: An Exposome Receptor.” *Annual Review of Pharmacology and Toxicology* 62(1): 383–404.

Lassen, L. H. et al. 2002. “CGRP May Play a Causative Role in Migraine.” *Cephalalgia: An International Journal of Headache* 22(1): 54–61.

Laurell, Katarina et al. 2016. “Premonitory Symptoms in Migraine: A Cross-Sectional Study in 2714 Persons.” *Cephalalgia* 36(10): 951–59.

Lauritzen, Martin et al. 2011. “Clinical Relevance of Cortical Spreading Depression in Neurological Disorders: Migraine, Malignant Stroke, Subarachnoid and Intracranial Hemorrhage, and Traumatic Brain Injury.” *Journal of Cerebral Blood Flow & Metabolism* 31(1): 17–35.

Lebensohn, James E. 1951. “Photophobia: Mechanism and Implications*.” *American Journal of Ophthalmology* 34(9): 1294–1300.

Lee, Sun Hwa, Yeonwook Kang, and Soo-Jin Cho. 2017. "Subjective Cognitive Decline in Patients with Migraine and Its Relationship with Depression, Anxiety, and Sleep Quality." *The Journal of Headache and Pain* 18(1): 77.

Lehnardt, Seija. 2010. "Innate Immunity and Neuroinflammation in the CNS: The Role of Microglia in Toll-like Receptor-Mediated Neuronal Injury." *Glia* 58(3): 253–63.

Léna, C. et al. 1999. "Diversity and Distribution of Nicotinic Acetylcholine Receptors in the Locus Ceruleus Neurons." *Proceedings of the National Academy of Sciences of the United States of America* 96(21): 12126–31.

Lesch, K. Peter et al. 1993. "Regional Brain Expression of Serotonin Transporter MRNA and Its Regulation by Reuptake Inhibiting Antidepressants." *Molecular Brain Research* 17(1): 31–35.

Levy, Dan, Rami Burstein, and Andrew M. Strassman. 2005. "Calcitonin Gene–Related Peptide Does Not Excite or Sensitize Meningeal Nociceptors: Implications for the Pathophysiology of Migraine." *Annals of Neurology* 58(5): 698–705.

Levy, Dan, Alejandro Labastida-Ramirez, and Antoinette MaassenVanDenBrink. 2019. "Current Understanding of Meningeal and Cerebral Vascular Function Underlying Migraine Headache." *Cephalalgia: An International Journal of Headache* 39(13): 1606–22.

Li, Lishi et al. 2011. "The Functional Organization of Cutaneous Low-Threshold Mechanosensory Neurons." *Cell* 147(7): 1615–27.

Li, Ting et al. 2019. "An Update on Reactive Astrocytes in Chronic Pain." *Journal of neuroinflammation* 16: 1–13.

Liddelow, Shane A. et al. 2017. "Neurotoxic Reactive Astrocytes Are Induced by Activated Microglia." *Nature* 541(7638): 481–87.

Liddelow, Shane A., and Ben A. Barres. 2017. "Reactive Astrocytes: Production, Function, and Therapeutic Potential." *Immunity* 46(6): 957–67.

Lin, Li, Yue Dai, and Yufeng Xia. 2022. "An Overview of Aryl Hydrocarbon Receptor Ligands in the Last Two Decades (2002–2022): A Medicinal Chemistry Perspective." *European Journal of Medicinal Chemistry* 244: 114845.

Linde, M. et al. 2012. "The Cost of Headache Disorders in Europe: The Eurolight Project." *European Journal of Neurology* 19(5): 703–11.

Linde, Mattias, Wim M. Mulleners, Edward P. Chronicle, and Douglas C. McCrory. 2013. "Topiramate for the Prophylaxis of Episodic Migraine in Adults." *Cochrane Database of Systematic Reviews* (6). https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD010610/full (May 1, 2023).

Lindén, Jere, Sanna Lensu, and Raimo Pohjanvirta. 2014. "Effect of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) on Hormones of Energy Balance in a

TCDD-Sensitive and a TCDD-Resistant Rat Strain." *International Journal of Molecular Sciences* 15(8): 13938–66.

Lipton, R. B. et al. 2000. "Migraine, Quality of Life, and Depression: A Population-Based Case-Control Study." *Neurology* 55(5): 629–35.

Lipton, Richard B. et al. 2000. "Efficacy and Safety of Acetaminophen in the Treatment of Migraine: Results of a Randomized, Double-Blind, Placebo-Controlled, Population-Based Study." *Archives of internal medicine* 160(22): 3486–92.

Lipton, Richard B. et al. 2013. "Impact of NSAID and Triptan Use on Developing Chronic Migraine: Results From the American Migraine Prevalence and Prevention (AMPP) Study." *Headache: The Journal of Head and Face Pain* 53(10): 1548–63.

Long, Ting et al. 2020. "Microglia P2X4R-BDNF Signalling Contributes to Central Sensitization in a Recurrent Nitroglycerin-Induced Chronic Migraine Model." *The Journal of Headache and Pain* 21(1): 4.

Louter, Mark A. et al. 2013. "Cutaneous Allodynia as a Predictor of Migraine Chronification." *Brain* 136(11): 3489–96.

Lovati, Carlo et al. 2013. "Central Sensitization in Photophobic and Non-Photophobic Migraineurs: Possible Role of Retino Nuclear Way in the Central Sensitization Process." *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* 34 Suppl 1: S133-135.

Luppi, P. -H. et al. 1995. "Afferent Projections to the Rat Locus Coeruleus Demonstrated by Retrograde and Anterograde Tracing with Cholera-Toxin B Subunit and Phaseolus Vulgaris Leucoagglutinin." *Neuroscience* 65(1): 119–60.

Luque, J. M., P. Malherbe, and J. G. Richards. 1994. "Localization of GABAA Receptor Subunit MRNAs in the Rat Locus Coeruleus." *Molecular Brain Research* 24(1): 219–26.

Lv, Qi et al. 2018. "Norisoboldine, a Natural Aryl Hydrocarbon Receptor Agonist, Alleviates TNBS-Induced Colitis in Mice, by Inhibiting the Activation of NLRP3 Inflammasome." *Chinese Journal of Natural Medicines* 16(3): 161–74.

M

Ma, Qing-Ping. 2001. "Co-Localization of 5-HT1B/1D/1F Receptors and Glutamate in Trigeminal Ganglia in Rats." *Neuroreport* 12(8): 1589–91.

MaassenVanDenBrink, A. et al. 1998. "5-HT1B Receptor Polymorphism and Clinical Response to Sumatriptan." *Headache* 38(4): 288–91.

MacGregor, E. Anne. 2016. "Diagnosing Migraine." *The Journal of Family Planning and Reproductive Health Care* 42(4): 280–86.

Machado, M. et al. 2019. “Dietary Tryptophan Deficiency and Its Supplementation Compromises Inflammatory Mechanisms and Disease Resistance in a Teleost Fish.” *Scientific Reports* 9(1): 7689.

Mainero, Caterina, Jasmine Boshyan, and Nouchine Hadjikhani. 2011. “Altered Functional Magnetic Resonance Imaging Resting-State Connectivity in Periaqueductal Gray Networks in Migraine.” *Annals of Neurology* 70(5): 838–45.

Malick, Amy et al. 2001. “A Neurohistochemical Blueprint for Pain-Induced Loss of Appetite.” *Proceedings of the National Academy of Sciences* 98(17): 9930–35.

Malick, Amy, Rew M. Strassman, and Rami Burstein. 2000. “Trigeminohypothalamic and Reticulohypothalamic Tract Neurons in the Upper Cervical Spinal Cord and Caudal Medulla of the Rat.” *Journal of Neurophysiology* 84(4): 2078–2112.

Manabe, T., A. Yamamoto, K. Satoh, and K. Ichihara. 2001. “Tolerance to Nitroglycerin Induced by Isosorbide-5-Mononitrate Infusion in Vivo.” *Biological & Pharmaceutical Bulletin* 24(12): 1370–72.

Maness, Eden B. et al. 2022. “Role of the Locus Coeruleus and Basal Forebrain in Arousal and Attention.” *Brain Research Bulletin* 188: 47–58.

Maniyar, Farooq Husain et al. 2014. “Brain Activations in the Premonitory Phase of Nitroglycerin-Triggered Migraine Attacks.” *Brain* 137(1): 232–41.

Mansour, Alfred et al. 1994. “μ-Opioid Receptor MRNA Expression in the Rat CNS: Comparison to μ-Receptor Binding.” *Brain Research* 643(1): 245–65.

Marcus, Jacob N. et al. 2001. “Differential Expression of Orexin Receptors 1 and 2 in the Rat Brain.” *Journal of Comparative Neurology* 435(1): 6–25.

Markovics, Adrienn et al. 2012. “Pituitary Adenylate Cyclase-Activating Polypeptide Plays a Key Role in Nitroglycerol-Induced Trigeminovascular Activation in Mice.” *Neurobiology of Disease* 45(1): 633–44.

Martin, Vincent T., and Brinder Vij. 2016. “Diet and Headache: Part 2.” *Headache: The Journal of Head and Face Pain* 56(9): 1553–62.

Martin, William J et al. 1999. “Differential Effects of Neurotoxic Destruction of Descending Noradrenergic Pathways on Acute and Persistent Nociceptive Processing.” *Pain* 80(1): 57–65.

Mason, Bianca N. et al. 2017. “Induction of Migraine-Like Photophobic Behavior in Mice by Both Peripheral and Central CGRP Mechanisms.” *The Journal of Neuroscience* 37(1): 204–16.

Matejuk, Agata, and Richard M. Ransohoff. 2020. “Crosstalk Between Astrocytes and Microglia: An Overview.” *Frontiers in Immunology* 11. https://www.frontiersin.org/articles/10.3389/fimmu.2020.01416 (May 13, 2023).

Mateo, Yolanda, Joseba Pineda, and J. Javier Meana. 1998. “Somatodendritic A2-Adrenoceptors in the Locus Coeruleus Are Involved in the In Vivo Modulation of Cortical Noradrenaline Release by the Antidepressant Desipramine.” *Journal of Neurochemistry* 71(2): 790–98.

Mathew, Ninan T. 2011. “Pathophysiology of Chronic Migraine and Mode of Action of Preventive Medications.” *Headache* 51 Suppl 2: 84–92.

Matsumura, Fumio. 2009. “The Significance of the Nongenomic Pathway in Mediating Inflammatory Signaling of the Dioxin-Activated Ah Receptor to Cause Toxic Effects.” *Biochemical Pharmacology* 77(4): 608–26.

Matynia, Anna et al. 2012. “Intrinsically Photosensitive Retinal Ganglion Cells Are the Primary but Not Exclusive Circuit for Light Aversion.” *Experimental Eye Research* 105: 60–69.

May, Arne. 2011. “Experience-Dependent Structural Plasticity in the Adult Human Brain.” *Trends in Cognitive Sciences* 15(10): 475–82.

McMenamin, Paul G. et al. 2003. “Macrophages and Dendritic Cells in the Rat Meninges and Choroid Plexus: Three-Dimensional Localisation by Environmental Scanning Electron Microscopy and Confocal Microscopy.” *Cell and tissue research* 313: 259–69.

McNaughton, F. L., and W. H. Feindel. 1977. “Innervation of Intracranial Structures: A Reappraisal.” *Physiological aspects of clinical neurology. Oxford, England: Blackwell Scientific*: 279–93.

Melone, Marcello, Chiara Ciriachi, Daniela Pietrobon, and Fiorenzo Conti. 2019. “Heterogeneity of Astrocytic and Neuronal GLT-1 at Cortical Excitatory Synapses, as Revealed by Its Colocalization With Na+/K+-ATPase α Isoforms.” *Cerebral Cortex* 29(8): 3331–50.

Melzack, R., and P. D. Wall. 1965. “Pain Mechanisms: A New Theory.” *Science (New York, N.Y.)* 150(3699): 971–79.

Mendell, Lorne M. 2014. “Constructing and Deconstructing the Gate Theory of Pain.” *Pain* 155(2): 210–16.

Mermet-Joret, Noëmie et al. 2022. “Postnatal Development of Inner Lamina II Interneurons of the Rat Medullary Dorsal Horn.” *PAIN* 163(5): 984.

Meßlinger, Karl et al. 1993. “Innervation of the Dura Mater Encephali of Cat and Rat: Ultrastructure and Calcitonin Gene-Related Peptide-like and Substance P-like Immunoreactivity.” *Anatomy and Embryology* 188(3): 219–37.

Mimura, Junsei, Masatsugu Ema, Kazuhiro Sogawa, and Yoshiaki Fujii-Kuriyama. 1999. “Identification of a Novel Mechanism of Regulation of Ah (Dioxin) Receptor Function.” *Genes & Development* 13(1): 20–25.

Minen, Mia Tova et al. 2016. “Migraine and Its Psychiatric Comorbidities.” *Journal of Neurology, Neurosurgery, and Psychiatry* 87(7): 741–49.

Moisset, X. et al. 2017. “Migraine Prevalence in Inflammatory Bowel Disease Patients: A Tertiary-Care Centre Cross-Sectional Study.” *European Journal of Pain (London, England)* 21(9): 1550–60.

Moisset, Xavier et al. 2013. “Migraine Headaches and Pain with Neuropathic Characteristics: Comorbid Conditions in Patients with Multiple Sclerosis.” *Pain* 154(12): 2691–99.

Moja, Egidio A. et al. 1988. “Decrease in Plasma Tryptophan after Tryptophan-Free Amino Acid Mixtures in Man.” *Life Sciences* 42(16): 1551–56.

Møllgård, Kjeld et al. 2023. “A Mesothelium Divides the Subarachnoid Space into Functional Compartments.” *Science* 379(6627): 84–88.

Moreno-Ajona, David, María Dolores Villar-Martínez, and Peter J. Goadsby. 2022. “New Generation Gepants: Migraine Acute and Preventive Medications.” *Journal of Clinical Medicine* 11(6): 1656.

Moskowitz, Michael A. 1984. “The Neurobiology of Vascular Head Pain.” *Annals of Neurology* 16(2): 157–68.

Moskowitz, Michael A., and Christian Waeber. 1996. “Migraine Enters the Molecular Era.” *The Neuroscientist* 2(3): 191–200.

Moulton, Eric A. et al. 2008. “Interictal Dysfunction of a Brainstem Descending Modulatory Center in Migraine Patients.” *PLOS ONE* 3(11): e3799.

Moulton, Eric A. et al. 2014. “Altered Hypothalamic Functional Connectivity with Autonomic Circuits and the Locus Coeruleus in Migraine.” *PLOS ONE* 9(4): e95508.

Muneer, Ather. 2020. “Kynurenine Pathway of Tryptophan Metabolism in Neuropsychiatric Disorders: Pathophysiologic and Therapeutic Considerations.” *Clinical Psychopharmacology and Neuroscience* 18(4): 507–26.

Munro, Gordon, Inger Jansen-Olesen, and Jes Olesen. 2017. “Animal Models of Pain and Migraine in Drug Discovery.” *Drug Discovery Today* 22(7): 1103–11.

Murphy, D. L. et al. 1989. “Serotoninergic Function in Neuropsychiatric Disorders.” In *Serotonin: Actions, Receptors, Pathophysiology*, Satellite Symposia of the IUPHAR 10th International Congress of Pharmacology, eds. Ewan J. Mylecharane, James A. Angus, Ivan S. de la Lande, and Patrick P. A. Humphrey. London: Palgrave Macmillan UK, 257–64. https://doi.org/10.1007/978-1-349-10114-6_31 (May 20, 2023).

Murray, Iain A. et al. 2010. “Antagonism of Aryl Hydrocarbon Receptor Signaling by 6,2’,4’-Trimethoxyflavone.” *The Journal of Pharmacology and Experimental Therapeutics* 332(1): 135–44.

N

Nakahama, Taisuke et al. 2011. “Aryl Hydrocarbon Receptor Deficiency in T Cells Suppresses the Development of Collagen-Induced Arthritis.” *Proceedings of the National Academy of Sciences* 108(34): 14222–27.

Natoli, JL et al. 2010. “Global Prevalence of Chronic Migraine: A Systematic Review.” *Cephalalgia* 30(5): 599–609.

Nazari, Fatemeh, and Maryam Eghbali. 2012. “Migraine and Its Relationship with Dietary Habits in Women.” *Iranian Journal of Nursing and Midwifery Research* 17(2 Suppl1): S65–71.

Neeb, Lars et al. 2017. “Structural Gray Matter Alterations in Chronic Migraine: Implications for a Progressive Disease?” *Headache* 57(3): 400–416.

Neelamegam, Malinee et al. 2021. “The Effect of Opioids on the Cognitive Function of Older Adults: Results from the Personality and Total Health through Life Study.” *Age and Ageing* 50(5): 1699–1708.

Nichols, David E., and Charles D. Nichols. 2008. “Serotonin Receptors.” *Chemical Reviews* 108(5): 1614–41.

Niedbala, Wanda et al. 2011. “Regulation of Type 17 Helper T-Cell Function by Nitric Oxide during Inflammation.” *Proceedings of the National Academy of Sciences of the United States of America* 108(22): 9220–25.

Nierenburg, Hida del C. et al. 2015. “Systematic Review of Preventive and Acute Treatment of Menstrual Migraine.” *Headache: The Journal of Head and Face Pain* 55(8): 1052–71.

Noseda, Rodrigo, Vanessa Kainz, et al. 2010. “A Neural Mechanism for Exacerbation of Headache by Light.” *Nature Neuroscience* 13(2): 239–45.

Noseda, Rodrigo, Luis Constandil, et al. 2010. “Changes of Meningeal Excitability Mediated by Corticotrigeminal Networks: A Link for the Endogenous Modulation of Migraine Pain.” *Journal of Neuroscience* 30(43): 14420–29.

Noseda, Rodrigo et al. 2011. “Cortical Projections of Functionally Identified Thalamic Trigeminovascular Neurons: Implications for Migraine Headache and Its Associated Symptoms.” *Journal of Neuroscience* 31(40): 14204–17.

Noseda, Rodrigo, David Borsook, and Rami Burstein. 2017. “Neuropeptides and Neurotransmitters That Modulate Thalamo-Cortical Pathways Relevant to Migraine Headache.” *Headache: The Journal of Head and Face Pain* 57(S2): 97–111.

Noseda, Rodrigo, and Rami Burstein. 2013. “Migraine Pathophysiology: Anatomy of the Trigeminovascular Pathway and Associated Neurological Symptoms, Cortical Spreading Depression, Sensitization, and Modulation of Pain.” *Pain* 154 Suppl 1: S44-53.

Noseda, Rodrigo, David Copenhagen, and Rami Burstein. 2019. "Current Understanding of Photophobia, Visual Networks and Headaches." *Cephalalgia: An International Journal of Headache* 39(13): 1623–34.

Ntranos, Achilles et al. 2022. "Bacterial Neurotoxic Metabolites in Multiple Sclerosis Cerebrospinal Fluid and Plasma." *Brain: A Journal of Neurology* 145(2): 569–83.

Nyholt, Dale R et al. 2015. "Concordance of Genetic Risk across Migraine Subgroups: Impact on Current and Future Genetic Association Studies." *Cephalalgia* 35(6): 489–99.

O

Oh, Kyungmi et al. 2014. "Combination of Anxiety and Depression Is Associated with an Increased Headache Frequency in Migraineurs: A Population-Based Study." *BMC Neurology* 14(1): 238.

Ohtake, Fumiaki et al. 2003. "Modulation of Oestrogen Receptor Signalling by Association with the Activated Dioxin Receptor." *Nature* 423(6939): 545–50.

Olesen, Jes, Rami Burstein, Messoud Ashina, and Peer Tfelt-Hansen. 2009. "Origin of Pain in Migraine: Evidence for Peripheral Sensitisation." *The Lancet. Neurology* 8(7): 679–90.

Olesen, Jes, Bo Larsen, and Martin Lauritzen. 1981. "Focal Hyperemia Followed by Spreading Oligemia and Impaired Activation of Rcbf in Classic Migraine." *Annals of Neurology* 9(4): 344–52.

Olszewski, Jerzy. 1950. "On the Anatomical and Functional Organization of the Spinal Trigeminal Nucleus." *Journal of Comparative Neurology* 92(3): 401–13.

O'Mahony, S. M. et al. 2015. "Serotonin, Tryptophan Metabolism and the Brain-Gut-Microbiome Axis." *Behavioural Brain Research* 277: 32–48.

Ornstein, K. et al. 1987. "Biochemical and Radioautographic Evidence for Dopaminergic Afferents of the Locus Coeruleus Originating in the Ventral Tegmental Area." *Journal of Neural Transmission* 70(3): 183–91.

Ossipov, Michael H., Gregory O. Dussor, and Frank Porreca. 2010. "Central Modulation of Pain." *The Journal of Clinical Investigation* 120(11): 3779–87.

Oydanich, Marko et al. 2019. "Mechanisms of Sex Differences in Exercise Capacity." *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 316(6): R832–38.

P

Palego, Lionella, Laura Betti, Alessandra Rossi, and Gino Giannaccini. 2016. "Tryptophan Biochemistry: Structural, Nutritional, Metabolic, and Medical Aspects in Humans." *Journal of amino acids* 2016.

Palkovits, M. et al. 1977. “Serotonergic Innervation of the Forebrain: Effect of Lesions on Serotonin and Tryptophan Hydroxylase Levels.” *Brain Research* 130(1): 121–34.

Panconesi, Alessandro. 2008. “Serotonin and Migraine: A Reconsideration of the Central Theory.” *The Journal of Headache and Pain* 9(5): 267–76.

Pardridge, William M., and Gary Fierer. 1990. “Transport of Tryptophan into Brain from the Circulating, Albumin-Bound Pool in Rats and in Rabbits.” *Journal of Neurochemistry* 54(3): 971–76.

Park, JungWook et al. 2014. “Differential Trigeminovascular Nociceptive Responses in the Thalamus in the Familial Hemiplegic Migraine 1 Knock-in Mouse: A Fos Protein Study.” *Neurobiology of Disease* 64: 1–7.

Parpura, Vladimir, and Alexei Verkhratsky. 2012. “Homeostatic Function of Astrocytes: Ca2+ and Na+ Signalling.” *Translational Neuroscience* 3(4): 334–44.

Pavlovic, Jelena M. et al. 2017. “Sex-Related Influences in Migraine.” *Journal of Neuroscience Research* 95(1–2): 587–93.

Paxinos, George, and Keith BJ Franklin. 2019. *Paxinos and Franklin’s the Mouse Brain in Stereotaxic Coordinates*. Academic press.

Pelclová, Daniela et al. 2006. “Adverse Health Effects in Humans Exposed to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD).” *Reviews on Environmental Health* 21(2): 119–38.

Pellegrino Baena, Cristina et al. 2018. “Migraine and Cognitive Function: Baseline Findings from the Brazilian Longitudinal Study of Adult Health: ELSA-Brasil.” *Cephalalgia* 38(9): 1525–34.

Penfield, Wilder, and FRANCIS McNaughton. 1940. “Dural Headache and Innervation of the Dura Mater.” *Archives of Neurology & Psychiatry* 44(1): 43–75.

Pennisi, E., G. Cruccu, M. Manfredi, and G. Palladini. 1991. “Histometric Study of Myelinated Fibers in the Human Trigeminal Nerve.” *Journal of the Neurological Sciences* 105(1): 22–28.

Penzien, Donald B. et al. 2015. “Well-Established and Empirically Supported Behavioral Treatments for Migraine.” *Current Pain and Headache Reports* 19(7): 34.

Peres, Mario F. P. et al. 2006. “Potential Therapeutic Use of Melatonin in Migraine and Other Headache Disorders.” *Expert Opinion on Investigational Drugs* 15(4): 367–75.

Peres, MFP et al. 2004. “Cerebrospinal Fluid Glutamate Levels in Chronic Migraine.” *Cephalalgia* 24(9): 735–39.

Perini, Francesco et al. 2005. “Plasma Cytokine Levels in Migraineurs and Controls.” *Headache* 45(7): 926–31.

Peris, Francesc et al. 2017. “Towards Improved Migraine Management: Determining Potential Trigger Factors in Individual Patients.” *Cephalalgia* 37(5): 452–63.

Pertovaara, Antti. 2006. “Noradrenergic Pain Modulation.” *Progress in Neurobiology* 80(2): 53–83.

Petersen, Sandra L. et al. 2000. “Distribution of MRNAs Encoding the Arylhydrocarbon Receptor, Arylhydrocarbon Receptor Nuclear Translocator, and Arylhydrocarbon Receptor Nuclear Translocator-2 in the Rat Brain and Brainstem.” *Journal of Comparative Neurology* 427(3): 428–39.

Ping, H. X., H. Q. Wu, and G. Q. Liu. 1990. “Modulation of Neuronal Activity of Locus Coeruleus in Rats Induced by Excitatory Amino Acids.” *Zhongguo Yao Li Xue Bao = Acta Pharmacologica Sinica* 11(3): 193–95.

Pinheiro, Carina F. et al. 2021. “Interictal Photophobia and Phonophobia Are Related to the Presence of Aura and High Frequency of Attacks in Patients with Migraine.” *Applied Sciences* 11(6): 2474.

Poland, A, E Glover, and A S Kende. 1976. “Stereospecific, High Affinity Binding of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin by Hepatic Cytosol. Evidence That the Binding Species Is Receptor for Induction of Aryl Hydrocarbon Hydroxylase.” *Journal of Biological Chemistry* 251(16): 4936–46.

Polderman, Tinca J. C. et al. 2015. “Meta-Analysis of the Heritability of Human Traits Based on Fifty Years of Twin Studies.” *Nature Genetics* 47(7): 702–9.

Porreca, Frank, Michael H. Ossipov, and G. F. Gebhart. 2002. “Chronic Pain and Medullary Descending Facilitation.” *Trends in Neurosciences* 25(6): 319–25.

Powell, Domonica N. et al. 2020. “Indoles from the Commensal Microbiota Act via the AHR and IL-10 to Tune the Cellular Composition of the Colonic Epithelium during Aging.” *Proceedings of the National Academy of Sciences* 117(35): 21519–26.

Poyan Mehr, Ali et al. 2018. “De Novo NAD+ Biosynthetic Impairment in Acute Kidney Injury in Humans.” *Nature Medicine* 24(9): 1351–59.

Pradhan, Amynah A. et al. 2014. “Characterization of a Novel Model of Chronic Migraine.” *PAIN*® 155(2): 269–74.

Pradhan, Amynah A., Zachariah Bertels, and Simon Akerman. 2018. “Targeted Nitric Oxide Synthase Inhibitors for Migraine.” *Neurotherapeutics* 15(2): 391–401.

Price, Theodore J., and Christopher M. Flores. 2007. “Critical Evaluation of the Colocalization Between Calcitonin Gene-Related Peptide, Substance P, Transient Receptor Potential Vanilloid Subfamily Type 1 Immunoreactivities, and Isolectin B4 Binding in Primary Afferent Neurons of the Rat and Mouse.” *The Journal of Pain* 8(3): 263–72.

Procházková, Jirina et al. 2011. “The Interplay of the Aryl Hydrocarbon Receptor and β-Catenin Alters Both AhR-Dependent Transcription and Wnt/β-Catenin Signaling in Liver Progenitors.” *Toxicological Sciences: An Official Journal of the Society of Toxicology* 122(2): 349–60.

Proietti-Cecchini, A., J. Afra, and J. Schoenen. 1997. “Intensity Dependence of the Cortical Auditory Evoked Potentials as a Surrogate Marker of Central Nervous System Serotonin Transmission in Man: Demonstration of a Central Effect for the 5HT1B/1D Agonist Zolmitriptan (311C90, Zomig).” *Cephalalgia: An International Journal of Headache* 17(8): 849–54; discussion 799.

Pudovkina, Olga L., Thomas I. F. H. Cremers, and Ben H. C. Westerink. 2002. “The Interaction between the Locus Coeruleus and Dorsal Raphe Nucleus Studied with Dual-Probe Microdialysis.” *European Journal of Pharmacology* 445(1–2): 37–42.

Pudovkina, Olga L., Yukie Kawahara, Jan de Vries, and Ben HC Westerink. 2001. “The Release of Noradrenaline in the Locus Coeruleus and Prefrontal Cortex Studied with Dual-Probe Microdialysis.” *Brain research* 906(1–2): 38–45.

Puledda, Francesca, and Kevin Shields. 2018. “Non-Pharmacological Approaches for Migraine.” *Neurotherapeutics* 15(2): 336–45.

Q

Qiu, Ju, and Liang Zhou. 2013. “Aryl Hydrocarbon Receptor Promotes RORγt^{+} Group 3 ILCs and Controls Intestinal Immunity and Inflammation.” *Seminars in Immunopathology* 35(6): 657–70.

Quintana, Francisco J. et al. 2010. “An Endogenous Aryl Hydrocarbon Receptor Ligand Acts on Dendritic Cells and T Cells to Suppress Experimental Autoimmune Encephalomyelitis.” *Proceedings of the National Academy of Sciences of the United States of America* 107(48): 20768–73.

R

Raghavendra, Vasudeva, Flobert Tanga, and Joyce A. DeLeo. 2003. “Inhibition of Microglial Activation Attenuates the Development but Not Existing Hypersensitivity in a Rat Model of Neuropathy.” *Journal of Pharmacology and Experimental Therapeutics* 306(2): 624–30.

Rahmann, A. et al. 2008. “Vasoactive Intestinal Peptide Causes Marked Cephalic Vasodilation, but Does Not Induce Migraine.” *Cephalalgia: An International Journal of Headache* 28(3): 226–36.

Raja, Srinivasa N. et al. 2020. “The Revised International Association for the Study of Pain Definition of Pain: Concepts, Challenges, and Compromises.” *Pain* 161(9): 1976–82.

Ramachandran, Roshni et al. 2012. “A Naturalistic Glyceryl Trinitrate Infusion Migraine Model in the Rat.” *Cephalalgia* 32(1): 73–84.

Ransohoff, Richard M., and V. Hugh Perry. 2009. “Microglial Physiology: Unique Stimuli, Specialized Responses.” *Annual Review of Immunology* 27: 119–45.

Rattanawong, Wanakorn, Alan Rapoport, and Anan Srikiatkhachorn. 2022. “Neurobiology of Migraine Progression.” *Neurobiology of Pain (Cambridge, Mass.)* 12: 100094.

Ray, Bronson S., and Harold G. Wolff. 1940. “Experimental Studies on Headache: Pain-Sensitive Structures of the Head and Their Significance in Headache.” *Archives of surgery* 41(4): 813–56.

Razeghi Jahromi, Soodeh, Zeinab Ghorbani, et al. 2019. “Association of Diet and Headache.” *The Journal of Headache and Pain* 20(1): 106.

Razeghi Jahromi, Soodeh, Mansoureh Togha, et al. 2019. “The Association between Dietary Tryptophan Intake and Migraine.” *Neurological Sciences* 40(11): 2349–55.

Recober, Ana, and Andrew Russo. 2007. “Olcegepant, a Non-Peptide CGRP1 Antagonist for Migraine Treatment.” *IDrugs : the investigational drugs journal* 10: 566–74.

Reddy, Nihaal et al. 2021. “The Complex Relationship between Estrogen and Migraines: A Scoping Review.” *Systematic Reviews* 10(1): 72.

Ren, Caixia et al. 2018. “Low Levels of Serum Serotonin and Amino Acids Identified in Migraine Patients.” *Biochemical and Biophysical Research Communications* 496(2): 267–73.

Renehan, W. E., M. F. Jacquin, R. D. Mooney, and R. W. Rhoades. 1986. “Structure-Function Relationships in Rat Medullary and Cervical Dorsal Horns. II. Medullary Dorsal Horn Cells.” *Journal of Neurophysiology* 55(6): 1187–1201.

Renthal, William. 2018. “Localization of Migraine Susceptibility Genes in Human Brain by Single-Cell RNA Sequencing.” *Cephalalgia: An International Journal of Headache* 38(13): 1976–83.

Ressler, Kerry J., and Charles B. Nemeroff. 2000. “Role of Serotonergic and Noradrenergic Systems in the Pathophysiology of Depression and Anxiety Disorders.” *Depression and Anxiety* 12(S1): 2–19.

Rexed, Bror. 1952. “The Cytoarchitectonic Organization of the Spinal Cord in the Cat.” *Journal of Comparative Neurology* 96(3): 415–95.

Reyes, Beverly A. S., Rita J. Valentino, Guangping Xu, and Elisabeth J. Van Bockstaele. 2005. “Hypothalamic Projections to Locus Coeruleus Neurons in Rat Brain.” *European Journal of Neuroscience* 22(1): 93–106.

Richard, Dawn M. et al. 2009. “L-Tryptophan: Basic Metabolic Functions, Behavioral Research and Therapeutic Indications.” *International Journal of Tryptophan Research* 2: IJTR-S2129.

Roager, Henrik M., and Tine R. Licht. 2018. “Microbial Tryptophan Catabolites in Health and Disease.” *Nature Communications* 9(1): 3294.

Robert, Claude et al. 2013. “Paraventricular Hypothalamic Regulation of Trigeminovascular Mechanisms Involved in Headaches.” *Journal of Neuroscience* 33(20): 8827–40.

Romani, Luigina et al. 2014. “Microbiota Control of a Tryptophan–AhR Pathway in Disease Tolerance to Fungi.” *European Journal of Immunology* 44(11): 3192–3200.

Romanos, Jennifer et al. 2020. “Astrocyte Dysfunction Increases Cortical Dendritic Excitability and Promotes Cranial Pain in Familial Migraine.” *Science Advances* 6(23): eaaz1584.

Roth, William et al. 2021. “Tryptophan Metabolism and Gut-Brain Homeostasis.” *International Journal of Molecular Sciences* 22(6): 2973.

Rothhammer, Veit et al. 2016. “Type I Interferons and Microbial Metabolites of Tryptophan Modulate Astrocyte Activity and Central Nervous System Inflammation via the Aryl Hydrocarbon Receptor.” *Nature Medicine* 22(6): 586–97.

Rothhammer, Veit, and Francisco J. Quintana. 2019. “The Aryl Hydrocarbon Receptor: An Environmental Sensor Integrating Immune Responses in Health and Disease.” *Nature Reviews Immunology* 19(3): 184–97.

S

Sacco, Simona, Christian Lampl, et al. 2022. “European Headache Federation (EHF) Consensus on the Definition of Effective Treatment of a Migraine Attack and of Triptan Failure.” *The journal of headache and pain* 23(1): 1–10.

Sacco, Simona, Faisal Mohammad Amin, et al. 2022. “European Headache Federation Guideline on the Use of Monoclonal Antibodies Targeting the Calcitonin Gene Related Peptide Pathway for Migraine Prevention – 2022 Update.” *The Journal of Headache and Pain* 23(1): 67.

Saito, Hiroto et al. 2017. “Ascending Projections of Nociceptive Neurons from Trigeminal Subnucleus Caudalis: A Population Approach.” *Experimental Neurology* 293: 124–36.

Sakai, Y. et al. 2008. “Sumatriptan Normalizes the Migraine Attack-Related Increase in Brain Serotonin Synthesis.” *Neurology* 70(6): 431–39.

Sakurai, Shunya, Toshiyuki Shimizu, and Umeharu Ohto. 2017. “The Crystal Structure of the AhRR-ARNT Heterodimer Reveals the Structural Basis of the Repression of AhR-Mediated Transcription.” *The Journal of Biological Chemistry* 292(43): 17609–16.

Salisbury, Richard L., and Courtney E. W. Sulentic. 2015. “The AhR and NF-KB/Rel Proteins Mediate the Inhibitory Effect of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin

on the 3' Immunoglobulin Heavy Chain Regulatory Region." *Toxicological Sciences: An Official Journal of the Society of Toxicology* 148(2): 443–59.

Salminen, Antero. 2022. "Role of Indoleamine 2,3-Dioxygenase 1 (IDO1) and Kynurenine Pathway in the Regulation of the Aging Process." *Ageing Research Reviews* 75: 101573.

Sampaolo, Simone et al. 2017. "First Study on the Peptidergic Innervation of the Brain Superior Sagittal Sinus in Humans." *Neuropeptides* 65: 45–55.

Sara, Susan J., and Sebastien Bouret. 2012. "Orienting and Reorienting: The Locus Coeruleus Mediates Cognition through Arousal." *Neuron* 76(1): 130–41.

Sasa, M., K. Munekiyo, H. Ikeda, and S. Takaori. 1974. "Noradrenaline-Mediated Inhibition by Locus Coeruleus of Spinal Trigeminal Neurons." *Brain Research* 80(3): 443–60.

Schain, Aaron J. et al. 2018. "Activation of Pial and Dural Macrophages and Dendritic Cells by Cortical Spreading Depression." *Annals of Neurology* 83(3): 508–21.

Schain, Aaron J., Agustin Melo-Carrillo, Andrew M. Strassman, and Rami Burstein. 2017. "Cortical Spreading Depression Closes Paravascular Space and Impairs Glymphatic Flow: Implications for Migraine Headache." *Journal of Neuroscience* 37(11): 2904–15.

Schepelmann, K. et al. 1997. "Activation of Trigeminal Brain Stem Neurons by Chemical Stimulation of the Dura Mater Encephali–Preparation for Studying Meningeal Nociception in the Rat: Eine Präparation Zum Studium Der Meningealen Nozizeption Bei Der Ratte." *Der Schmerz* 11: 322–27.

Scher, A. I., W. F. Stewart, J. A. Ricci, and R. B. Lipton. 2003. "Factors Associated with the Onset and Remission of Chronic Daily Headache in a Population-Based Study." *Pain* 106(1–2): 81–89.

Scher, Ann I., Lynn A. Midgette, and Richard B. Lipton. 2008. "Risk Factors for Headache Chronification." *Headache: The Journal of Head and Face Pain* 48(1): 16–25.

Schmidt, Katharina et al. 2019. "Localization of the Locus Coeruleus in the Mouse Brain." *Journal of Visualized Experiments: JoVE* (145).

Schneggenburger, Ralf, and Erwin Neher. 2005. "Presynaptic Calcium and Control of Vesicle Fusion." *Current Opinion in Neurobiology* 15(3): 266–74.

Schoenen, Jean et al. 2013. "Migraine Prevention with a Supraorbital Transcutaneous Stimulator: A Randomized Controlled Trial." *Neurology* 80(8): 697–704.

Schröcksnadel, Katharina, Barbara Wirleitner, Christiana Winkler, and Dietmar Fuchs. 2006. "Monitoring Tryptophan Metabolism in Chronic Immune Activation." *Clinica Chimica Acta; International Journal of Clinical Chemistry* 364(1–2): 82–90.

Schueler, Markus, Winfried L. Neuhuber, Roberto De Col, and Karl Messlinger. 2014. "Innervation of Rat and Human Dura Mater and Pericranial Tissues in the Parieto-Temporal Region by Meningeal Afferents." *Headache: The Journal of Head and Face Pain* 54(6): 996–1009.

Schuh-Hofer, Sigrid et al. 2007. "Increased Serotonin Transporter Availability in the Brainstem of Migraineurs." *Journal of Neurology* 254(6): 789–96.

Schwarz, Lindsay A., and Liqun Luo. 2015. "Organization of the Locus Coeruleus-Norepinephrine System." *Current Biology* 25(21): R1051–56.

Schwedt, Todd J. et al. 2014. "Allodynia and Descending Pain Modulation in Migraine: A Resting State Functional Connectivity Analysis." *Pain Medicine* 15(1): 154–65.

Schwedt, Todd J. et al. 2014. "Chronic Migraine." *Bmj* 348.

Scott, Lesley J. 2020. "Ubrogepant: First Approval." *Drugs* 80(3): 323–28.

Segal, M. 1979. "Serotonergic Innervation of the Locus Coeruleus from the Dorsal Raphe and Its Action on Responses to Noxious Stimuli." *The Journal of Physiology* 286: 401–15.

Seki, Kenjiro, Satomi Yoshida, and Manoj Kumar Jaiswal. 2018. "Molecular Mechanism of Noradrenaline during the Stress-Induced Major Depressive Disorder." *Neural Regeneration Research* 13(7): 1159–69.

Seng, Elizabeth K., Paul R. Martin, and Timothy T. Houle. 2022. "Lifestyle Factors and Migraine." *The Lancet Neurology* 21(10): 911–21.

Seok, Seung-Hyeon et al. 2018. "Trace Derivatives of Kynurenine Potently Activate the Aryl Hydrocarbon Receptor (AHR)." *The Journal of Biological Chemistry* 293(6): 1994–2005.

Sessle, B J, J W Hu, R Dubner, and G E Lucier. 1981. "Functional Properties of Neurons in Cat Trigeminal Subnucleus Caudalis (Medullary Dorsal Horn). II. Modulation of Responses to Noxious and Nonnoxious Stimuli by Periaqueductal Gray, Nucleus Raphe Magnus, Cerebral Cortex, and Afferent Influences, and Effect of Naloxone." *Journal of Neurophysiology* 45(2): 193–207.

Sessle, Barry J. 2000. "Acute and Chronic Craniofacial Pain: Brainstem Mechanisms of Nociceptive Transmission and Neuroplasticity, and Their Clinical Correlates." *Critical Reviews in Oral Biology & Medicine* 11(1): 57–91.

Shabbir, Faisal et al. 2013. "Effect of Diet on Serotonergic Neurotransmission in Depression." *Neurochemistry International* 62(3): 324–29.

Sheu, Meei-Ling et al. 2022. "Modulation of Aryl Hydrocarbon Receptor Expression Alleviated Neuropathic Pain in a Chronic Constriction Nerve Injury Animal Model." *International Journal of Molecular Sciences* 23(19): 11255.

Shibata, Mamoru, and Norihiro Suzuki. 2017. “Exploring the Role of Microglia in Cortical Spreading Depression in Neurological Disease.” *Journal of Cerebral Blood Flow & Metabolism* 37(4): 1182–91.

Sicuteri, Federigo, A. Testi, and B. Anselmi. 1961. “Biochemical Investigations in Headache: Increase in the Hydroxyindoleacetic Acid Excretion during Migraine Attacks.” *International Archives of Allergy and Applied Immunology* 19(1): 55–58.

Silberstein, S. D. et al. 2012. “Evidence-Based Guideline Update: Pharmacologic Treatment for Episodic Migraine Prevention in Adults: Report of the Quality Standards Subcommittee of the American Academy of Neurology and the American Headache Society.” *Neurology* 78(17): 1337–45.

Silberstein, Stephen D. 2015. “Preventive Migraine Treatment.” *Continuum : Lifelong Learning in Neurology* 21(4 Headache): 973–89.

Simpson, Kimberly L. et al. 1997. “Lateralization and Functional Organization of the Locus Coeruleus Projection to the Trigeminal Somatosensory Pathway in Rat.” *Journal of Comparative Neurology* 385(1): 135–47.

Socała, Katarzyna et al. 2021. “The Role of Microbiota-Gut-Brain Axis in Neuropsychiatric and Neurological Disorders.” *Pharmacological Research* 172: 105840.

Sofroniew, Michael V. 2015. “Astrogliosis.” *Cold Spring Harbor Perspectives in Biology* 7(2): a020420.

Solvay, Marie et al. 2023. “Tryptophan Depletion Sensitizes the AHR Pathway by Increasing AHR Expression and GCN2/LAT1-Mediated Kynurenine Uptake, and Potentiates Induction of Regulatory T Lymphocytes.” *bioRxiv*: 2023–01.

Somjen, George G. 2001. “Mechanisms of Spreading Depression and Hypoxic Spreading Depression-Like Depolarization.” *Physiological Reviews* 81(3): 1065–96.

Song, Gyun Jee, and Kyoungho Suk. 2017. “Pharmacological Modulation of Functional Phenotypes of Microglia in Neurodegenerative Diseases.” *Frontiers in Aging Neuroscience* 9. https://www.frontiersin.org/articles/10.3389/fnagi.2017.00139 (July 23, 2023).

Song, Jiasheng et al. 2002. “A Ligand for the Aryl Hydrocarbon Receptor Isolated from Lung.” *Proceedings of the National Academy of Sciences of the United States of America* 99(23): 14694–99.

Song, Ping, Tharmarajan Ramprasath, Huan Wang, and Ming-Hui Zou. 2017. “Abnormal Kynurenine Pathway of Tryptophan Catabolism in Cardiovascular Diseases.” *Cellular and Molecular Life Sciences* 74(16): 2899–2916.

Soufli, Imene, Ryma Toumi, Hayet Rafa, and Chafia Touil-Boukoffa. 2016. “Overview of Cytokines and Nitric Oxide Involvement in Immuno-Pathogenesis of

Inflammatory Bowel Diseases." *World Journal of Gastrointestinal Pharmacology and Therapeutics* 7(3): 353–60.

Sowers, Levi P. et al. 2020. "Stimulation of Posterior Thalamic Nuclei Induces Photophobic Behavior in Mice." *Headache: The Journal of Head and Face Pain* 60(9): 1961–81.

Spohn, Stephanie N., and Gary M. Mawe. 2017. "Non-Conventional Features of Peripheral Serotonin Signalling — the Gut and Beyond." *Nature Reviews Gastroenterology & Hepatology* 14(7): 412–20.

Starke, Klaus. 2001. "Presynaptic Autoreceptors in the Third Decade: Focus on A2-Adrenoceptors." *Journal of Neurochemistry*.

Steiner, Timothy J., and Lars Jacob Stovner. 2023. "Global Epidemiology of Migraine and Its Implications for Public Health and Health Policy." *Nature Reviews Neurology* 19(2): 109–17.

van der Stelt, Hiske M., Laus M. Broersen, Berend Olivier, and Herman G. M. Westenberg. 2004. "Effects of Dietary Tryptophan Variations on Extracellular Serotonin in the Dorsal Hippocampus of Rats." *Psychopharmacology* 172(2): 137–44.

Stewart, W. F. et al. 2008. "Cumulative Lifetime Migraine Incidence in Women and Men." *Cephalalgia: An International Journal of Headache* 28(11): 1170–78.

Strasser, Barbara, Johanna M. Gostner, and Dietmar Fuchs. 2016. "Mood, Food, and Cognition: Role of Tryptophan and Serotonin." *Current Opinion in Clinical Nutrition and Metabolic Care* 19(1): 55–61.

Strassman, A. M., S. A. Raymond, and R. Burstein. 1996. "Sensitization of Meningeal Sensory Neurons and the Origin of Headaches." *Nature* 384(6609): 560–64.

Strassman, Andrew M., and Dan Levy. 2004. "The Anti-Migraine Agent Sumatriptan Induces a Calcium-Dependent Discharge in Meningeal Sensory Neurons." *NeuroReport* 15(9): 1409.

Sun, Daniel, and Tatjana C. Jakobs. 2012. "Structural Remodeling of Astrocytes in the Injured CNS." *The Neuroscientist: A Review Journal Bringing Neurobiology, Neurology and Psychiatry* 18(6): 567–88.

Sun, Lijun. 2021. "Recent Advances in the Development of AHR Antagonists in Immuno-Oncology." *RSC Medicinal Chemistry* 12(6): 902–14.

Sutin, E. L., and D. M. Jacobowitz. 1991. "Chapter 1 - Neurochemicals in the Dorsal Pontine Tegmentum." In *Progress in Brain Research*, Neurobiology of the Locus Coeruleus, eds. C. D. Barnes and O. Pompeiano. Elsevier, 3–14. https://www.sciencedirect.com/science/article/pii/S0079612308637966 (May 9, 2023).

Swanson, L. W. 1976. "The Locus Coeruleus: A Cytoarchitectonic, Golgi and Immunohistochemical Study in the Albino Rat." *Brain Research* 110(1): 39–56.

Swanson, L. W. 1982. “The Projections of the Ventral Tegmental Area and Adjacent Regions: A Combined Fluorescent Retrograde Tracer and Immunofluorescence Study in the Rat.” *Brain Research Bulletin* 9(1): 321–53.

T

Taheri, Parichehr et al. 2020. “Nitric Oxide Role in Anxiety-like Behavior, Memory and Cognitive Impairments in Animal Model of Chronic Migraine.” *Heliyon* 6(12): e05654.

Taipa, R. et al. 2018. “Inflammatory Pathology Markers (Activated Microglia and Reactive Astrocytes) in Early and Late Onset Alzheimer Disease: A Post Mortem Study.” *Neuropathology and Applied Neurobiology* 44(3): 298–313.

Tajti, J, R Uddman, and L Edvinsson. 2001. “Neuropeptide Localization in the ‘Migraine Generator’ Region of the Human Brainstem.” *Cephalalgia* 21(2): 96–101.

Takizawa, Tsubasa et al. 2016. “Temporal Profiles of High-Mobility Group Box 1 Expression Levels after Cortical Spreading Depression in Mice.” *Cephalalgia* 36(1): 44–52.

Takizawa, Tsubasa et al. 2020. “Non-Invasively Triggered Spreading Depolarizations Induce a Rapid pro-Inflammatory Response in Cerebral Cortex.” *Journal of Cerebral Blood Flow & Metabolism* 40(5): 1117–31.

Tanaka, Masatoshi et al. 1989. “Met-Enkephalin, Injected during the Early Phase of Stress, Attenuates Stress-Induced Increases in Noradrenaline Release in Rat Brain Regions.” *Pharmacology Biochemistry and Behavior* 32(3): 791–95.

Tang, Yu, and Weidong Le. 2016. “Differential Roles of M1 and M2 Microglia in Neurodegenerative Diseases.” *Molecular Neurobiology* 53(2): 1181–94.

Tang, Yuanyuan et al. 2020. “Gut Microbiota Dysbiosis Enhances Migraine-Like Pain Via TNFα Upregulation.” *Molecular Neurobiology* 57(1): 461–68.

Targowska-Duda, Katarzyna M. et al. 2020. “NOP Receptor Agonist Attenuates Nitroglycerin-Induced Migraine-like Symptoms in Mice.” *Neuropharmacology* 170: 108029.

Tassorelli, Cristina, and Shirley A. Joseph. 1995. “Systemic Nitroglycerin Induces Fos Immunoreactivity in Brainstem and Forebrain Structures of the Rat.” *Brain Research* 682(1): 167–81.

Taylor, Bradley K., and Allan I. Basbaum. 1995. “Neurochemical Characterization of Extracellular Serotonin in the Rostral Ventromedial Medulla and Its Modulation by Noxious Stimuli.” *Journal of Neurochemistry* 65(2): 578–89.

Taylor, Bradley K., and Karin N. Westlund. 2017. “The Noradrenergic Locus Coeruleus as a Chronic Pain Generator.” *Journal of Neuroscience Research* 95(6): 1336–46.

Ter Horst, GJ, WJ Meijler, J Korf, and RHA Kemper. 2001. "Trigeminal Nociception-Induced Cerebral Fos Expression in the Conscious Rat." *Cephalalgia* 21(10): 963–75.

Terlizzi, Rossana et al. 2018. "Epigenetic DNA Methylation Changes in Episodic and Chronic Migraine." *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* 39(Suppl 1): 67–68.

Tfelt-Hansen, P., P. De Vries, and P. R. Saxena. 2000. "Triptans in Migraine: A Comparative Review of Pharmacology, Pharmacokinetics and Efficacy." *Drugs* 60(6): 1259–87.

Tfelt-Hansen, Peer. 2007. "Parenteral vs. Oral Sumatriptan and Naratriptan: Plasma Levels and Efficacy in Migraine. A Comment." *The Journal of Headache and Pain* 8(5): 273–76.

Thomsen, L.l., C. Kruuse, H.k. Iversen, and J. Olesen. 1994. "A Nitric Oxide Donor (Nitroglycerin) Triggers Genuine Migraine Attacks." *European Journal of Neurology* 1(1): 73–80.

Thorlund, Kristian et al. 2014. "Comparative Efficacy of Triptans for the Abortive Treatment of Migraine: A Multiple Treatment Comparison Meta-Analysis." *Cephalalgia: An International Journal of Headache* 34(4): 258–67.

Tomkiewicz, C. et al. 2013. "The Aryl Hydrocarbon Receptor Regulates Focal Adhesion Sites through a Non-Genomic FAK/Src Pathway." *Oncogene* 32(14): 1811–20.

Totah, Nelson K. B., Nikos K. Logothetis, and Oxana Eschenko. 2019. "Noradrenergic Ensemble-Based Modulation of Cognition over Multiple Timescales." *Brain Research* 1709: 50–66.

Tricklebank, Mark, and Eileen Daly. 2019. *The Serotonin System: History, Neuropharmacology, and Pathology*. Academic Press.

Tsuda, Makoto et al. 2003. "P2X4 Receptors Induced in Spinal Microglia Gate Tactile Allodynia after Nerve Injury." *Nature* 424(6950): 778–83.

Tsuda, Makoto, Simon Beggs, Michael W. Salter, and Kazuhide Inoue. 2013. "Microglia and Intractable Chronic Pain." *Glia* 61(1): 55–61.

Tsuji, Ai et al. 2023. "The Tryptophan and Kynurenine Pathway Involved in the Development of Immune-Related Diseases." *International Journal of Molecular Sciences* 24(6): 5742.

Tsuruoka, Masayoshi et al. 2003. "Unilateral Hindpaw Inflammation Induces Bilateral Activation of the Locus Coeruleus and the Nucleus Subcoeruleus in the Rat." *Brain Research Bulletin* 61(2): 117–23.

Tuka, Bernadett et al. 2021. "Clinical Relevance of Depressed Kynurenine Pathway in Episodic Migraine Patients: Potential Prognostic Markers in the Peripheral

Plasma during the Interictal Period." *The Journal of Headache and Pain* 22(1): 60.

U

Uematsu, Akira et al. 2017. "Modular Organization of the Brainstem Noradrenaline System Coordinates Opposing Learning States." *Nature Neuroscience* 20(11): 1602–11.

Uematsu, Akira, Bao Zhen Tan, and Joshua P. Johansen. 2015. "Projection Specificity in Heterogeneous Locus Coeruleus Cell Populations: Implications for Learning and Memory." *Learning & Memory* 22(9): 444–51.

Unzueta-Larrinaga, Paula et al. 2023. "Isolation and Differentiation of Neurons and Glial Cells from Olfactory Epithelium in Living Subjects." *Molecular Neurobiology* 60(8): 4472–87.

Uthman, Olalekan A. 2016. "Global, Regional, and National Disability-adjusted Life Years (DALYs) for 315 Diseases and Injuries and Healthy Life Expectancy (HALE) for 195 Countries and Territories, 1990-2015 : A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors (GBD) 2015 Study." *Lancet* 388(10053): 1603–58.

V

Valentino, Rita J., and Elisabeth Van Bockstaele. 2014. "Endogenous Opioids: The Downside of Opposing Stress." *Neurobiology of Stress* 1: 23–32.

Van Bockstaele, Elisabeth J, Eric E. O Colago, and Sue Aicher. 1998. "Light and Electron Microscopic Evidence for Topographic and Monosynaptic Projections from Neurons in the Ventral Medulla to Noradrenergic Dendrites in the Rat Locus Coeruleus." *Brain Research* 784(1): 123–38.

Van Den Maagdenberg, Arn MJM, Dale R. Nyholt, and Verneri Anttila. 2019. "Novel Hypotheses Emerging from GWAS in Migraine?" *The journal of headache and pain* 20(1): 1–7.

Vanagaite, J et al. 1997. "Light-Induced Discomfort and Pain in Migraine." *Cephalalgia* 17(7): 733–41.

Varnäs, Katarina, Håkan Hall, Pascal Bonaventure, and Göran Sedvall. 2001. "Autoradiographic Mapping of 5-HT1B and 5-HT1D Receptors in the Post Mortem Human Brain Using [3H] GR 125743." *Brain research* 915(1): 47–57.

Varnäs, Katarina, Christer Halldin, and Håkan Hall. 2004. "Autoradiographic Distribution of Serotonin Transporters and Receptor Subtypes in Human Brain." *Human Brain Mapping* 22(3): 246–60.

Vasile, Flora, Elena Dossi, and Nathalie Rouach. 2017. "Human Astrocytes: Structure and Functions in the Healthy Brain." *Brain Structure and Function* 222(5): 2017–29.

Veloso, Felix, Krishna Kumar, and Cory Toth. 1998. “Headache Secondary to Deep Brain Implantation.” *Headache: The Journal of Head and Face Pain* 38(7): 507–15.

Verkhratsky, Alexei, Nancy Ann Oberheim Bush, Maiken Nedergaard, and Arthur Butt. 2018. “The Special Case of Human Astrocytes.” *Neuroglia* 1(1): 21–29.

Viana, Michele et al. 2013. “Triptan Nonresponders: Do They Exist and Who Are They?” *Cephalalgia: An International Journal of Headache* 33(11): 891–96.

Vila-Pueyo, Marta. 2018. “Targeted 5-HT1F Therapies for Migraine.” *Neurotherapeutics* 15(2): 291–303.

Vila-Pueyo, Marta. 2019. “Divergent Influences of the Locus Coeruleus on Migraine Pathophysiology.” *PAIN* 160(2): 385.

Vogel, Christoph F. A. et al. 2014. “Cross-Talk between Aryl Hydrocarbon Receptor and the Inflammatory Response: A Role for Nuclear Factor-KB.” *The Journal of Biological Chemistry* 289(3): 1866–75.

Vos, Theo et al. 2016. “Global, Regional, and National Incidence, Prevalence, and Years Lived with Disability for 310 Diseases and Injuries, 1990–2015: A Systematic Analysis for the Global Burden of Disease Study 2015.” *The Lancet* 388(10053): 1545–1602.

W

Walsh, A. E. S. et al. 1995. “Dieting Decreases Plasma Tryptophan and Increases the Prolactin Response to D-Fenfluramine in Women but Not Men.” *Journal of Affective Disorders* 33(2): 89–97.

Wang, Dan et al. 2000. “γ-Aminobutyric Acid- and Glycine-Immunoreactive Neurons Postsynaptic to Substance P-Immunoreactive Axon Terminals in the Superficial Layers of the Rat Medullary Dorsal Horn.” *Neuroscience Letters* 288(3): 187–90.

Wang, Fengzhi, Jiaoqi Wang, Yumeng Cao, and Zhongxin Xu. 2020. “Serotonin–Norepinephrine Reuptake Inhibitors for the Prevention of Migraine and Vestibular Migraine: A Systematic Review and Meta-Analysis.” *Regional Anesthesia & Pain Medicine* 45(5): 323–30.

Wang, Qimeng et al. 2018. “Aryl Hydrocarbon Receptor Inhibits Inflammation in DSS-Induced Colitis via the MK2/p-MK2/TTP Pathway.” *International Journal of Molecular Medicine* 41(2): 868–76.

Wang, Qiong, Yingjuan Liu, Jianxu Zhang, and Weiwen Wang. 2020. “Corticotropin-Releasing Factor Receptors in the Locus Coeruleus Modulate the Enhancement of Active Coping Behaviors Induced by Chronic Predator Odor Inoculation in Mice.” *Frontiers in Psychology* 10. https://www.frontiersin.org/articles/10.3389/fpsyg.2019.03028 (June 25, 2023).

Wang, Xing, Yuqi Chen, Jinlei Song, and Chao You. 2021. "Efficacy and Safety of Monoclonal Antibody Against Calcitonin Gene-Related Peptide or Its Receptor for Migraine: A Systematic Review and Network Meta-Analysis." *Frontiers in Pharmacology* 12. https://www.frontiersin.org/articles/10.3389/fphar.2021.649143 (May 1, 2023).

Wang, Yunjie et al. 2018. "A Dual AMPK/Nrf2 Activator Reduces Brain Inflammation After Stroke by Enhancing Microglia M2 Polarization." *Antioxidants & Redox Signaling* 28(2): 141–63.

Warnock, Julia K., Lawrence J. Cohen, Harvey Blumenthal, and Jordan E. Hammond. 2017. "Hormone-Related Migraine Headaches and Mood Disorders: Treatment with Estrogen Stabilization." *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy* 37(1): 120–28.

Weiller, C. et al. 1995. "Brain Stem Activation in Spontaneous Human Migraine Attacks." *Nature Medicine* 1(7): 658–60.

Welch, K. Michael A., Vijaya Nagesh, Sheena K. Aurora, and Neil Gelman. 2001. "Periaqueductal Gray Matter Dysfunction in Migraine and Chronic Daily Headache May Be Due to Free Radical Damage." *The Journal of Headache and Pain* 2: s33–41.

Wells, Rebecca Erwin et al. 2021. "Effectiveness of Mindfulness Meditation vs Headache Education for Adults With Migraine." *JAMA Internal Medicine* 181(3): 317–28.

Wendimu, Menbere Y., and Shelley B. Hooks. 2022. "Microglia Phenotypes in Aging and Neurodegenerative Diseases." *Cells* 11(13): 2091.

Westlund, K. N., and J. D. Coulter. 1980. "Descending Projections of the Locus Coeruleus and Subcoeruleus/Medial Parabrachial Nuclei in Monkey: Axonal Transport Studies and Dopamine-Beta-Hydroxylase Immunocytochemistry." *Brain Research* 2(3): 235–64.

Williams, W. A. et al. 1999. "Effects of Acute Tryptophan Depletion on Plasma and Cerebrospinal Fluid Tryptophan and 5-Hydroxyindoleacetic Acid in Normal Volunteers." *Journal of Neurochemistry* 72(4): 1641–47.

Willis, W. D., and K. N. Westlund. 1997. "Neuroanatomy of the Pain System and of the Pathways That Modulate Pain." *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society* 14(1): 2–31.

van Wissen, Matthijs et al. 2002. "IFN-γ Amplifies IL-6 and IL-8 Responses by Airway Epithelial-Like Cells Via Indoleamine 2,3-Dioxygenase1." *The Journal of Immunology* 169(12): 7039–44.

Wolf, Susanne A., HWGM Boddeke, and Helmut Kettenmann. 2017. "Microglia in Physiology and Disease." *Annual review of physiology* 79: 619–43.

Wong, Janice W. Y. et al. 2018. “Effects of Dietary Acute Tryptophan Depletion (ATD) on NPY Serum Levels in Healthy Adult Humans Whilst Controlling for Methionine Supply—A Pilot Study.” *Nutrients* 10(5): 594.

Wu, Jr-Wei et al. 2022. “The Use of Neuroimaging for Predicting Sumatriptan Treatment Response in Patients With Migraine.” *Frontiers in Neurology* 13. https://www.frontiersin.org/articles/10.3389/fneur.2022.798695 (August 2, 2023).

Wu, Shouyi et al. 2022. “A C-Fos Activation Map in Nitroglycerin/Levcromakalim-Induced Models of Migraine.” *The Journal of Headache and Pain* 23(1): 128.

X

Xie, Hong-Ying et al. 2015. “Increases in PKC Gamma Expression in Trigeminal Spinal Nucleus Is Associated with Orofacial Thermal Hyperalgesia in Streptozotocin-Induced Diabetic Mice.” *Journal of Chemical Neuroanatomy* 63: 13–19.

Xue, Zhaohui et al. 2017. “Mechanisms and Therapeutic Prospects of Polyphenols as Modulators of the Aryl Hydrocarbon Receptor.” *Food & Function* 8(4): 1414–37.

Y

Yan, Jiong et al. 2019. “Aryl Hydrocarbon Receptor Signaling Prevents Activation of Hepatic Stellate Cells and Liver Fibrogenesis in Mice.” *Gastroenterology* 157(3): 793-806.e14.

Yao, Song T, and Andrew J Lawrence. 2005. “Purinergic Modulation of Cardiovascular Function in the Rat Locus Coeruleus.” *British Journal of Pharmacology* 145(3): 342–52.

Yeung, Amanda W. S., Andrew C. Terentis, Nicholas J. C. King, and Shane R. Thomas. 2015. “Role of Indoleamine 2,3-Dioxygenase in Health and Disease.” *Clinical Science (London, England: 1979)* 129(7): 601–72.

Young, Simon N. 2013. “Acute Tryptophan Depletion in Humans: A Review of Theoretical, Practical and Ethical Aspects.” *Journal of Psychiatry & Neuroscience : JPN* 38(5): 294–305.

Young, W. S., and M. J. Kuhar. 1980. “Noradrenergic Alpha 1 and Alpha 2 Receptors: Light Microscopic Autoradiographic Localization.” *Proceedings of the National Academy of Sciences of the United States of America* 77(3): 1696–1700.

Younis, Samaira, Anders Hougaard, Rodrigo Noseda, and Messoud Ashina. 2019. “Current Understanding of Thalamic Structure and Function in Migraine.” *Cephalalgia* 39(13): 1675–82.

Yu, Edward et al. 2017. “Increases in Plasma Tryptophan Are Inversely Associated with Incident Cardiovascular Disease in the Prevención Con Dieta Mediterránea (PREDIMED) Study.” *The Journal of Nutrition* 147(3): 314–22.

Yücel, Yavuz et al. 2016. "Association of Polymorphisms within the Serotonin Receptor Genes 5-HTR1A, 5-HTR1B, 5-HTR2A and 5-HTR2C and Migraine Susceptibility in a Turkish Population." *Clinical Psychopharmacology and Neuroscience* 14(3): 250–55.

Z

Zelante, Teresa et al. 2013. "Tryptophan Catabolites from Microbiota Engage Aryl Hydrocarbon Receptor and Balance Mucosal Reactivity via Interleukin-22." *Immunity* 39(2): 372–85.

Zhang, Dan et al. 2010. "Astrogliosis in CNS Pathologies: Is There A Role for Microglia?" *Molecular neurobiology* 41(0): 232–41.

Zhang, Leyi et al. 2022. "Temporal Characteristics of Astrocytic Activation in the TNC in a Mice Model of Pain Induced by Recurrent Dural Infusion of Inflammatory Soup." *The Journal of Headache and Pain* 23(1): 8.

Zhang, XiChun et al. 2011. "Activation of Central Trigeminovascular Neurons by Cortical Spreading Depression." *Annals of Neurology* 69(5): 855–65.

Zhang, Yue et al. 2019. "Transcutaneous Auricular Vagus Nerve Stimulation at 1 Hz Modulates Locus Coeruleus Activity and Resting State Functional Connectivity in Patients with Migraine: An FMRI Study." *NeuroImage : Clinical* 24: 101971.

Zhao, Huiying et al. 2016. "Gene-Based Pleiotropy across Migraine with Aura and Migraine without Aura Patient Groups." *Cephalalgia* 36(7): 648–57.

Zhao, Shou-cai et al. 2017. "Regulation of Microglial Activation in Stroke." *Acta Pharmacologica Sinica* 38(4): 445–58.

Zhao, Ze-Hua et al. 2019. "Indole-3-Propionic Acid Inhibits Gut Dysbiosis and Endotoxin Leakage to Attenuate Steatohepatitis in Rats." *Experimental & Molecular Medicine* 51(9): 1–14.

Ziegler, Dewey K. et al. 1987. "Migraine Prophylaxis: A Comparison of Propranolol and Amitriptyline." *Archives of Neurology* 44(5): 486–89.

Zis, Panagiotis, Thomas Julian, and Marios Hadjivassiliou. 2018. "Headache Associated with Coeliac Disease: A Systematic Review and Meta-Analysis." *Nutrients* 10(10): 1445.

Zwart, J.-A. et al. 2003. "Depression and Anxiety Disorders Associated with Headache Frequency. The Nord-Trøndelag Health Study." *European Journal of Neurology* 10(2): 147–52.

RÉSUMÉ EN FRANÇAIS

INTRODUCTION ET OBJECTIFS

La migraine est un trouble neurobiologique caractérisé par des céphalées sévères, unilatérales et pulsatiles, souvent accompagnées de nausées et/ou de vomissements ainsi que de troubles sensoriels et moteurs. Chez environ 3% des patients, ces manifestations épisodiques prennent une forme chronique, avec une augmentation des crises, ainsi que des troubles sensoriels associés. Les mécanismes subjacents cette progression ne sont pas totalement clairs. L'un de plus fréquents symptômes de la migraine est l'hypersensibilité cutanée céphalique, qui a été corrélée avec une sensibilisation des régions centrales du système trigéminal chez les patients, mais également chez les modèles précliniques. Cette sensibilisation central est modulé par les voies descendantes de la douleur au niveau du tronc cérébral, qui elles aussi sont altérées chez le migraineux. Des études plus récentes ont suggéré la réponse neuroinflammatoire comme un modulateur de la sensibilisation centrale ainsi que de la fonction des noyaux supra-spinaux régulateurs de la douleur. Cette neuroinflammation pourrait contribuer à la persistance de la douleur et au passage à l'état chronique. En effet, pendant la céphalée il y a lieu un processus d'inflammation neurogène qui active les voies nociceptives trigéminées et déclenche la douleur. De plus, les migraineurs présentent des niveaux anormalement élevés de cytokines pro-inflammatoires pendant et entre les crises. Finalement, la migraine est comorbide avec d'autres troubles inflammatoires. Nous émettons donc l'hypothèse que la l'hypersensibilité cutanée céphalique est associée à un état pro-inflammatoire généralisé qui contribue à la progression de la migraine d'épisodique à chronique, et qui pourrai en plus affecter l'activité thérapeutique des antimigraineurs. Le processus par lequel la signalisation inflammatoire est initiée et son effet sur les systèmes de modulation de la douleur sont inconnus. C'est là qu'intervient le TRP et ses métabolites, qui ont été largement impliqués dans la pathophysiologie de la migraine. Le déficit aigu du TRP dans le régime aggrave les crises et l'hypersensibilité sensorielle. Ses dérivés modulent en plus la réponse inflammatoire par plusieurs mécanismes, dont l'activation des AhR. Le rôle des AhR n'a jamais été étudié dans le contexte de la migraine, mais son implication a été démontrée dans d'autres maladies inflammatoires chroniques comorbides avec la migraine. Notre deuxième hypothèse est donc que le métabolisme du TRP, par le biais des AhR, pourraient jouer un rôle clé dans la neuroinflammation au cours de la chronification de la migraine.

Nous avons focalisé notre étude sur deux structures cérébrales impliqués dans la migraine : le LC et le Sp5C, chez un modèle murin expérimental de douleur migraineuse induite par l'administration systémique d'ISDN. Nous avons choisi ces deux régions pour plusieurs raisons : (1) le Sp5C est un acteur clé de l'intégration nociceptive au niveau du système trigémino-vasculaire, et son activation contribue à la sensibilisation centrale, et donc à l'hypersensibilité cutanée ; (2) le LC est la principale source de noradrénaline du système nerveux central, et son rôle comme modulateur de la progression de la douleur a été confirmé dans d'autres modèles de douleur chronique. Il est aussi impliqué dans la migraine, et module le système trigéminal par ses projections noradrénergiques descendantes. Notre approche expérimentale a donc combiné l'évaluation comportementale (de la douleur, de l'aversion à la lumière et de l'anxiété), l'immunohistochimie et les techniques biomoléculaires (RT-PCR, HPLC, western blot et ELISA).

Les objectifs spécifiques de chaque étude ont été les suivants :

. Étude I :

Évaluer les conséquences d'une déficience alimentaire en TRP sur :

- l'apparition de la douleur de type migraineuse suite à l'administration aigüe de l'ISDN chez les souris males and femelles. Nous avons évalué deux des symptômes sensoriels les plus répandus : l'hypersensibilité mécanique céphalique et l'hypersensibilité à la lumière.

- l'efficacité du sumatriptan (un antimigraineux préconisé pour traiter la crise de céphalée) pour bloquer les hypersensibilités sensorielles induites par l'ISDN.

. Étude II :

- Évaluer la contribution du métabolisme du TRP dans la chronification de la migraine induite par l'administration répétée d'ISDN chez des souris femelles, à travers de l'évaluation comportementale de la sensibilité céphalique, et de la fonction noradrénergique du LC.

- Examiner l'effet de l'administration répétée d'ISDN, seule ou combinée à une déficience en TRP, sur la réponse inflammatoire systémique et locale au niveau du LC.

- Caractériser la contribution des AhR dans la progression de la migraine par l'évaluation comportementale de la sensibilité céphalique après son activation/inhibition pharmacologique, et par l'analyse d'immunofluorescence et biomoléculaire au niveau du LC.

RÉSULTATS

. Étude I :

La présente étude a démontré que le déficit chronique du TRP et donc la dérégulation de son métabolisme (1) prolonge l'hypersensibilités céphalique ictale et (2) réduit l'efficacité du sumatriptan sur l'hypersensibilité céphalique et à la lumière induites par l'administration aigue de l'ISDN chez les deux sexes. Ces changements sont associés à une augmentation de l'expression des récepteurs sérotoninergique du type 1D dans le Sp5C et à une réduction des taux sériques de sérotonine.

. Étude II :

Dans cette étude, nous avons pu montrer que la persistance de l'hypersensibilité céphalique induite par l'administration répétée de l'ISDN est concomitante à un état inflammatoire généralisé, qui se manifeste par une augmentation de la libération de cytokines pro-inflammatoires dans le plasma, une réactivité astrocytaire et une modification de l'expression des AhR dans le LC. La deficiency en TRP favorise cette état pro-inflammatoire, altère la fonction noradrénergique du LC et stimule l'activation des cellules microgliales, processus qui contribue à la persistance de l'hypersensibilité céphalique induite par l'ISDN. Nous démontrons aussi le rôle pro-nociceptif des AhR dans l'état inflammatoire provoqué par l'ISDN. Ces résultats appuient le rôle du LC dans la chronicisation de la migraine et proportionnent un nouvel mécanisme, basé sur l'axe TRP-AhR, jamais ne décrit auparavant dans sa physiopathologie.

DISCUSSION

Evidences cliniques et précliniques montrent que la carence en TRP joue un rôle central dans la pathogenèse de la migraine, notamment en renforçant les signes de la migraine (nausées, sévérité des maux de tête et photophobie). La dérégulation du métabolisme du TRP, a travers d'une déplétion nutritionnel, augment substantielle la persistance de la douleur induite par l'ISDN, à la fois dans les périodes aiguës et intericales. Ceci est cohérent avec les modèles cliniques dans lesquels le déficit aigu du TRP augmente la sévérité des maux de tête. Cependant, aucune recherche n'a établi sa fonction dans la persistance de la douleur migraineuse. Dans le cadre de ce projet, nous avons cependant pu postuler que la carence en TRP crée un état inflammatoire qui est encore renforcé par l'action du NO fourni par l'administration d'ISDN, ce que j'expliquerai dans une autre section de la discussion.

Cette disparité pourrait être liée aux processus distincts des deux hypersensibilités et aux différentes manifestations, étant donné que dans la migraine épisodique, l'hypersensibilité céphalique est principalement mécanique, tandis que l'hypersensibilité extra céphalique est principalement thermique. Cependant, un régime sans TRP n'a pas aggravé l'hypersensibilité à la lumière induite par l'ISDN chez les femelles. Ceci est en contradiction avec les résultats de la littérature concernant l'augmentation de l'hypersensibilité à la lumière chez les migraineux après une déplétion en TRP cela pourrait être dû à la voie ciblée par la TRP. Étant donné que la CMH et l'hypersensibilité à la lumière impliquent deux voies pathologiques distinctes, l'effet d'un régime sans TRP pourrait cibler uniquement la CMH. En outre, cela pourrait être attribué au moment de l'évaluation de l'hypersensibilité à la lumière après l'administration de l'ISDN, qui a eu lieu une heure après l'administration de l'ISDN, peut-être qu'une évaluation à un moment différent mettrait en évidence un effet sur l'hypersensibilité à la lumière.

De manière surprenante, les changements dans la réactivité microgliale ont nécessité l'impact combiné de TRP et de doses répétées de RNIS. Pour comprendre la différence entre les réponses astrocytaires et microgliales, il faut comprendre les niveaux de cytokines pro-inflammatoires. L'ISDN et la TRP ont tous deux augmenté les médiateurs pro-inflammatoires tels que l'IL-6, le TNFα et l'IL-1, mais pas l'IFNγ. Le TNF, l'IL-1 et l'IFN sont principalement libérés par les astrocytes et la microglie

activés, respectivement. Par conséquent, l'absence de changement dans l'expression de l'IFN suggère que l'action de l'ISDN et de la TRP seule est inadéquate pour induire l'activation. De plus, les astrocytes régissent l'activité de la microglie, ce qui signifie que ce sursaut d'activité dépend d'une population astrocytaire déjà active.

L'action des métabolites dérivés du TRP pourrait être médiée par les AhR puisqu'il s'agit de ligands endogènes. Les AhR sont d'importants facteurs de transcription aux propriétés pro- ou anti-inflammatoires que l'on trouve dans les astrocytes, la microglie et les neurones noradrénergiques. Par conséquent, en associant la réactivité induite par l'ISDN et le rôle modulateur des AhR, nous avons voulu évaluer si les changements dus à la déplétion des TRP se produisent en fonction de changements mécanistiques au niveau des AhR de la LC. L'ISDN est un puissant donneur de NO, et nous savons que le NO favorise la libération systémique de cytokines pro-inflammatoires, notamment l'IL-6, l'IL-1β et le TNFα dans le sang. Nous avons observé que la carence en TRP augmentait les niveaux d'IL-6, de TNFα et d'IL-1β dans le sang, comme décrit précédemment, peut-être en activant AhR. Les cytokines pro-inflammatoires, principalement le TNFα, activent l'enzyme indoleamine 2,3-dioxygénase (IDO) dans les cellules dendritiques périphériques, qui transforme le TRP en KYN. L'IDO joue un rôle mineur dans des circonstances physiologiques. Cependant, elle est fortement activée en cas d'inflammation. L'activité de l'IDO est souvent mesurée par le rapport KYN/TRP dans le sang, qui augmente dans les maladies caractérisées par une inflammation excessive ou chronique. Nos données ont montré que l'ISDN augmentait également l'activité périphérique de l'IDO, déplaçant ainsi le métabolisme de la TRP vers la production de KYN, conduisant à de faibles niveaux de TRP et à une augmentation du rapport KYN/TRP. Cet effet pourrait s'expliquer par une action indirecte de l'ISDN sur l'activité de l'IDO : Le NO produit par l'ISDN pourrait stimuler la libération de TNFα qui, à son tour, active l'IDO qui métabolise la TRP en KYN (Figure 8A). Une altération du renouvellement du KYN et de la TRP a été décrite chez les migraineux, qui présentaient de faibles niveaux de ces deux métabolites par rapport aux témoins sains.

Les niveaux de TRP et de KYN ont tous deux été réduits après un régime alimentaire appauvri en ISDN et/ou en TRP dans le LC. L'expression de l'IDO dans le cerveau est faible et limitée à des régions cérébrales spécifiques. Le métabolisme cérébral de la TRP dépend donc largement du transport de la TRP et du KYN à travers

la barrière hémato-encéphalique. Tous deux accèdent au tissu cérébral par l'intermédiaire du transporteur des grands acides aminés neutres, entrant ainsi en compétition avec d'autres grands acides aminés neutres, ce qui se traduit par des niveaux marginalement plus faibles.

Le KYN est un ligand de l'AhR et régule les processus inflammatoires en favorisant la libération de cytokines et donc en activant l'IDO, ce qui crée un événement cyclique. Ce processus comprend l'activation des astrocytes et de la microglie, qui évoluent vers un état morphologique réactif lorsque la déficience en TRP et la répétition de l'ISDN sont combinées. Le NO contrôle également l'activité et l'expression de l'AhR en la diminuant. Ainsi, l'équilibre final entre les actions de KYN et de NO sur AhR pourrait être une réduction partielle de l'expression d'AhR après ISDN, laissant suffisamment d'AhR actif pour promouvoir l'effet pro-nociceptif observé après son activation directe par ITE. Cet état pro-inflammatoire renforcé pourrait être un inducteur potentiel de la facilitation et de la persistance de la douleur, comme observé par la CMH de longue durée chez les souris après une déficience en TRP. Il convient de noter que la déficience en TRP n'a pas affecté l'hypersensibilité extra-céphalique induite par l'ISDN, car la voie ciblée est d'une nature différente. Cette différence pourrait être due aux mécanismes différents des deux hypersensibilités, étant donné que dans la migraine épisodique, l'hypersensibilité céphalique est principalement mécanique, alors que l'hypersensibilité extracéphalique est principalement thermique.

Notre hypothèse est que l'activation des AhR favorise la sensibilisation des voies de signalisation inflammatoires, qui réagissent de manière démesurée après l'administration d'ISDN, facilitant la réponse nociceptive et la sensibilisation centrale du système trigéminal. La compréhension de la nature pronociceptive potentielle du AhR a été basée sur l'administration d'agonistes et d'antagonistes puissants. L'activation systémique du AhR par l'agoniste non toxique ITE, avant l'administration de l'ISDN, a renforcé l'intensité et la durée du CMH ictal. D'autre part, l'inhibition des AhR par le TMF non toxique prévient à la fois les CMH ictales et interictales induites par l'ISDN. Le blocage de l'AhR par le TMF ne permet pas à la KYN d'exécuter sa fonction de ligand, et donc l'axe IDO/AhR est potentiellement entravé. Et lorsque nous avons combiné le TMF avec un régime déficient en TRP, l'effet antalgique induit par le TMF est moindre. Grâce à cela, nous avons pu montrer que les AhR ont une action pro-nociceptive pendant l'initiation et la progression de la migraine.

Les triptans sont très efficaces s'ils sont pris tôt, dans les premières minutes suivant le début de la crise, mais lorsque la crise est établie et qu'il y a présence d'allodynie (indiquant une sensibilisation centrale continue), les patients ne répondent plus. Bien que les triptans soient recommandés comme traitement de première intention, environ 30 à 40 % des migraineux souffrent d'une efficacité ou d'une tolérance insuffisante aux triptans. Dans le cas du sumatriptan, par exemple, les non-répondeurs présentent une absorption plus faible et plus lente après les administrations orales que les répondeurs. De plus, chez les femmes, le taux d'efficacité des triptans est le même que chez les hommes, mais le risque de récidive des maux de tête est plus élevé. Il est également important d'ajouter qu'une efficacité et/ou une tolérance insuffisante avec un triptan ne permet pas toujours de prédire les résultats avec un autre triptan et que le passage à un autre triptan peut être bénéfique pour les patients.

Le sumatriptan agissant au niveau des récepteurs sérotoninergiques 5-$HTR_{1B/1D}$, les modulations de la neurotransmission sérotoninergique décrites chez les migraineux peuvent influencer le fonctionnement de ces récepteurs et donc être à l'origine de l'inefficacité des triptans. Des changements dans les niveaux de sérotonine peuvent être produits par une réduction alimentaire rapide de TRP, son précurseur, et un puissant inducteur des caractéristiques de la migraine telles que l'hypersensibilité à la lumière et la douleur. Nous avons démontré le manque d'efficacité du sumatriptan en ce qui concerne la CMH chez les deux sexes et l'hypersensibilité à la lumière chez les femmes, induites par l'administration aiguë d'ISDN. Le manque d'activité du Sumatriptan, également présent chez les migraineux, pourrait être attribué à des modulations sérotoninergiques ou au fonctionnement des récepteurs. La quantification de l'expression des récepteurs 5-$HTR_{1B/1D}$ révèle que dans des conditions d'appauvrissement en TRP, l'expression des récepteurs, notamment du 5-HTR_{1D}, a augmenté de manière significative, au moins au niveau du Sp5C. Cette augmentation de l'expression, associée à de faibles niveaux de sérotonine, peut entraîner des épisodes migraineux. En outre, la diminution de l'efficacité du sumatriptan peut être liée au fonctionnement du récepteur, qui est sensibilisé plutôt qu'exprimé pour le 5-HTR_{1B}, par exemple. Cette altération de l'expression n'a pas été documentée dans la migraine ou d'autres modèles de douleur. Pour la prochaine étape de cette partie du projet, nous effectuerons une évaluation de la fonctionnalité des récepteurs 5-$HTR_{1B/1D}$ par électrophysiologie in vivo et in vitro, and par essaies d'extravasation plasmatique des

molécules à travers des vaisseaux sanguins. Cela permettra de mieux comprendre l'état électrophysiologique de ces récepteurs dans des conditions de déficience en TRP associées à la migraine. En outre, nous évaluerons l'efficacité d'un autre triptan dans le même modèle, ce qui nous permettra de comprendre si ce manque d'efficacité est limité au sumatriptan ou s'il affecte également d'autres triptans.

CONCLUSION

Grâce à nos résultats, nous pouvons démontrer pour la première fois la contribution essentielle des AhR comme pronociceptifs par le biais de leurs ligands dérivés des TRP dans l'état inflammatoire de la migraine induite par l'ISDN. En outre, nous avons montré l'importance de la modulation sérotoninergique et la contribution d'un apport alimentaire quotidien de TRP sur l'efficacité du sumatriptan. L'importance de ce que nous avons obtenu jusqu'à présent met en lumière une importance clinique potentielle. Pour commencer, ce que nous avons obtenu, à savoir qu'une alimentation déficiente en TRP contribue à l'inefficacité du sumatriptan, ouvre la voie à une meilleure compréhension de l'inefficacité du sumatriptan chez les migraineux. La modification du régime alimentaire et l'augmentation de l'apport quotidien en TRP pourraient améliorer l'efficacité du sumatriptan et donc réduire le nombre de non-répondeurs au triptan.

En ce qui concerne l'axe TRP-AhR et son rôle nociceptif, nous avons pu mettre en évidence un nouveau mécanisme contribuant à l'augmentation de l'hypersensibilité céphalique dans la douleur migraineuse. Cela nous permet de cibler davantage les AhR comme moyen thérapeutique dans le domaine de la nociception. Toutefois, il convient de prendre en compte de multiples aspects, notamment les effets secondaires physiologiques et pathologiques potentiellement induits par l'action sur ces récepteurs. Quoi qu'il en soit, des recherches plus approfondies sont nécessaires pour comprendre ce domaine.