The Delta Opioid Receptor: A Therapeutic Target For Headache Disorders

BY

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THESIS

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Dedicated to my wife, Shenetta Moye

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Authorship Statement

This thesis includes concepts, figures, and methods that have been previously published. Specifically, Chapter 1 draws on concepts that have been previously published as reviews. Chapters 2 and 3 have been peer-reviewed and published in *Cephalalgia* and *Neuropharmacology*, respectively. Briefly, the title, authors, and contribution of each author is as follows:

Chapter 1
Vicente-Sanchez, A., Segura, L. and Pradhan, A.A. (2016). "The delta opioid receptor tool box." Neuroscience 338: 145-159.

Section 1.9.2. in the introduction draws on concepts from this review. A. Vicente-Sanchez was the primary author, I (Segura, L.) wrote the section which reviewed mutant animal models of the delta opioid receptor, and A.A. Pradhan edited the review.

Moye, L. S., Pradhan, A. A. (2017). “From blast to bench: A translational mini-review of posttraumatic headache.” J Neurosci Res;95(6): 1347-1354.

Section 1.3. in the introduction draws on concepts from this review. I (Moye, L.S.) was the primary author, and A.A. Pradhan edited this mini-review.

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LIST OF ABBREVIATIONS

Ca^{2+}	Calcium
CGRP	Calcitonin gene-related peptide
CRLR	Calcitonin receptor-like receptor
CREB	cAMP response element binding
DHE	Dihydroergotamine
DOR	Delta opioid receptor
eNOS	Endothelial nitric oxide synthase
iNOS	Inducible nitric oxide synthase
KOR	Kappa opioid receptor
MOR	Mu opioid receptor
NMDA	N-methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
NOS	Nitric oxide synthase
NSAID	Non-steroidal anti-inflammatory drug
NTG	Nitroglycerin
PACAP-38	Pituitary adenylate cyclase-activating polypeptide-38
RAMP1	Receptor activity-modifying protein 1
RCP	Receptor component protein
sGC	Soluble guanylyl cyclase
SSRI	Selective serotonin reuptake inhibitors
TG	Trigeminal ganglia
TNC	Trigeminal nucleus caudalis

SUMMARY

Headache disorders are incredibly common, and those with a migraine-like phenotype are one of the most disabling neurological disorders. Migraine affects up to 12% of the general population, and the incidence of this disorder increases every year. In the United States, there are over 30 million migraine patients, of which 3 million experience chronic migraine. Approximately 3% of patients with episodic migraines will progress into a chronic condition, and the rate of this transformation is increasing, as well. Despite the very high prevalence of headache disorders, therapeutic strategies are limited. There is a small pool of therapies currently used to treat migraine, and most patients either do not find these medications effective or they find the side effects too averse. The development of effective anti-migraine therapeutics is dependent on a thorough characterization of potential targets in preclinical animal models. In this thesis, I behaviorally and molecularly characterize the delta opioid receptor (DOR) as a promising novel target for the treatment of headache disorders. Under the scope of this characterization, I developed and thoroughly characterized a mouse model of post-traumatic headache. I pharmacologically validated this mouse model of post-traumatic headache and used it to screen the DOR as a promising therapeutic. Additionally, I characterized the effect of DOR activation in multiple models of headache, and demonstrate the unique interplay between the DOR, the pro-migraine peptide calcitonin gene-related peptide (CGRP), and the CGRP receptor, in the trigeminovascular system.

DORs have been shown to be highly effective in chronic pain states, have a low abuse liability, and have been shown to positively modulate emotional state. They are anti-hyperalgesic, anti-allodynic, anxiolytic, antidepressant, and have limited addictive properties. Clearly, the DOR has multiple characteristics that would suggest that it could be a promising new treatment for headache disorders. Here, I show the anti-allodynic effect of DOR activation in multiple models of headache and demonstrate the protective effect of chronic DOR activation in nitroglycerin (NTG) models of headache. Specifically, SNC80, a hallmark DOR agonist, reversed peripheral and cephalic hypersensitivity in the NTG mouse model of chronic migraine, post-traumatic headache, medication overuse headache (medication overuse headache), and opioid-induced hyperalgesia (opioid-induced hyperalgesia). Furthermore, chronic DOR activation prevented the development of basal hypersensitivity in the NTG model of chronic migraine and post-traumatic headache. The mechanism driving migraine-associated pain may lie within the trigeminovascular system, specifically the trigeminal ganglia (TG) and its main output center the trigeminal nucleus caudalis (TNC). To better understand how DOR activation regulates migraine-associated pain, I characterized the expression of the pro-migraine neuropeptide CGRP, components of the CGRP receptor activity modifying protein 1 (RAMP1) and calcitonin receptor-like receptor (CRLR), and the DOR within the TG and TNC. In the NTG model of chronic migraine, I found increased expression of CGRP and DOR within the TG and TNC. Interestingly, chronic DOR activation normalized increased CGRP expression, which proposes that both CGRP and DOR regulate aspects of migraine-associated pain. Additionally, I found a high co-expression of RAMP1, CRLR, and DOR within the TNC, which suggests that the DOR may directly affect the CGRP

receptor within the same cell, and that the downstream effects may normalize CGRP tone within the trigeminovascular system.

Taken together, the results from this thesis demonstrate the powerful potential of DOR agonists for the treatment of headache disorders. Additionally, these data suggest that DOR activation may blunt CGRP from propagating its pro-pain effects within the trigeminovascular system.

1. INTRODUCTION

1.1. PREFACE

Migraines have had an infamous prominence throughout history. One of the earliest written descriptions of migraine-associated symptoms appears around 400 BC from Hippocrates. Considered the father of medicine, he describes a patient's painful migraine and visual disturbances which strongly resemble aura:

> *As for Phoenix, this is more or less what he felt in his right eye:*
> *Most of the time he seemed to see something shining before him like a light, usually in part of the right eye. At the end of a moment, a violent pain supervened in the right temple, then in all the head and neck, and where the head is attached to the spine. Vomiting, when it became possible, was able to divert the pain and render it more moderate.* (Leroux 2016)

Throughout history, many other migraineurs and physicians would describe headache pain in poems, memoirs, and other works of literature. However, a clinical description of migraine did not appear in medical texts until the 18th century. In 1712, physicians first described the five major types of headache, including megrim which is now referred to as migraine with aura *(*1712*)*. Over 200 years after this description, scientists published the first treatment for migraine, ergotamine tartrate (Graham and Wolff 1938). Interestingly, ergotamine tartrate tablets are still used today to treat migraine-like headaches. They are now one of a variety of currently available abortive and prophylactic therapies. All these agents have had overall life-changing effects in some migraine patients but unfortunately do not yield similar positive results in other migraine patients. Up to 48% of migraineurs find their treatments to be ineffective and up to 53.2% of migraineurs are dissatisfied with treatment side effects (Blumenfeld, Bloudek et al. 2013). This dissatisfaction contributes to other risk factors, such as the development and/or exacerbation of mood disorders, that may worsen migraine and complicate treatment (May and Schulte 2016). To date,

there is no cure for migraine and no treatment is 100% effective in all migraine patients. Additionally, there are minimal options for migraineurs that do not respond to traditional migraine therapies. The development of more effective therapies for headache disorders, including migraine, is dependent on a thorough characterization of novel compounds in preclinical animal models of headache. The detailed characterization of a promising therapeutic target, the delta opioid receptor (DOR), in multiple models of headache is the primary goal of this thesis. Following the introduction, I present data supporting this proposal in manuscript form, including 2 peer-reviewed publications, and 1 that is in preparation for publication. These data include the detailed characterization and pharmacological validation of a novel mouse model of post-traumatic headache, the behavioral effect of acute DOR activation in the nitroglycerin (NTG) model of chronic migraine, a novel model of post-traumatic headache, and established models of medication overuse headache and opioid-induced hyperalgesia; and chronic DOR activation in the NTG model of chronic migraine and post-traumatic headache. The final component of this thesis focuses on the molecular characterization of DOR within the NTG model of chronic migraine where I explore the interplay between DOR and migraine-associated molecules in the trigeminovascular pathway. Altogether, the culmination of these data demonstrates the powerful potential of DOR activation as a possible therapy for headache disorders.

In this introduction, I will provide general background on migraines and DORs, and each chapter will also have a short introduction that is specific to that project. First, I will start by highlighting the current state of migraine, focusing on the symptoms and anatomical

regions implicated in the different phases of a migraine attack. Next, I will discuss secondary headache disorders, with a strong focus on post-traumatic headache, medication overuse headache, and opioid-induced hyperalgesia. Then, I will discuss current treatments for migraine, specifically abortive and prophylactic drugs. Next, I will discuss the NTG model of chronic migraine, which is heavily used in this thesis, and the molecular interactions between nitric oxide and calcitonin gene-related peptide (CGRP). I will then review the DOR, including a brief introduction to the other members of the opioid receptor family: the mu opioid receptor (MOR), the kappa opioid receptor (KOR), and the nociception/orphanin FQ peptide receptor (NOP). Next, I will discuss characteristics of the DOR, and highlight the various mutant mouse models currently available to study DOR trafficking and function. Finally, I will conclude by clearly stating the aims and organization of this thesis.

1.2. MIGRAINE

Migraine is a common and incredibly incapacitating neurological disorder affecting up to 12% of the worldwide population (WHO 2011, Woldeamanuel and Cowan 2017). This disorder has grave socio-economic and personal consequences, and is ranked as the third most prevalent disorder in the world (2017). The International Classification of Headache Disorders (ICHD) classifies migraine as a primary headache, a disorder which is due to the headache condition itself and not a symptom of another underlying disease (ICHD3b 2013). Within the broad definition of migraine, the ICHD further classifies this condition into migraine without aura, migraine with aura, and chronic migraine (ICHD3b 2013). Migraine without aura is defined by a migraine-like headache, and migraine with

aura includes neurological symptoms such as nausea, photophobia, and visual or sensory disturbances that usually precede the migraine-like headache (ICHD3b 2013). Up to one-third of migraine patients present with aura, and this migraine subtype occurs more commonly in women than men (Russell, Rasmussen et al. 1995, ICHD3b 2013). Clinically, migraines are characterized by recurrent headache attacks which can last approximately 4-72 hours (ICHD3b 2013). These headache attacks are unilateral, have a pulsating quality, can be aggravated by physical activity, and are strongly associated with nausea, photophobia, and phonophobia (ICHD3b 2013). In migraine with aura, fully reversible neurological symptoms can precede the headache and duration may range from 30 minutes to 72 hours. These reversible neurological symptoms disrupt the visual, sensory, and motor systems, and can cause aberrant changes in speech or language. Although migraine starts out as an episodic disorder, it commonly progresses to a chronic disorder (Bigal, Serrano et al. 2008). Chronic migraine is characterized by recurrent headache attacks resulting in 15 or more headache days per month for over 3 months, and this chronic disorder is incredibly difficult to treat. Episodic migraine transforms to chronic migraine at a rate of 2.5% per year, and certain risks, like stress, anxiety, and head/neck trauma, can increase the rate of transformation (Stovner, Hagen et al. 2007, Bigal, Serrano et al. 2008, Stewart, Wood et al. 2008, Victor, Hu et al. 2010, ICHD3b 2013). The mechanisms underlying the progression from episodic to chronic migraine remain unclear, and many factors can contribute to the chronification of migraine in different patients.

While both episodic and chronic migraine are incapacitating, chronic migraine is by far more disabling and is associated with multiple comorbid disorders. There is no cure for chronic migraine, but medications may help reduce headache days which would provide a relatively higher quality of life. Several factors play a role in the progression to chronic migraine, including pre-existing comorbid disorders, genetic predispositions, and lifestyle choices that increase stress and anxiety (Bigal, Serrano et al. 2008, Cevoli, Sancisi et al. 2009, Bigal and Lipton 2011). Migraine chronification can happen at different rates for patients, and medication overuse may contribute to maintaining this chronic disorder (Bigal and Lipton 2011). Despite well-managed medication therapy and nonpharmacological interventions, chronic migraine may continue to be refractory, and even those who successfully reduce their headache days may relapse.

Effectively diagnosing patients with the correct migraine type and closely monitoring their headache days may improve health outcomes. The diagnostic criteria for migraine (Table 1) guides clinicians in categorizing these different headache subtypes and provides a template for treating migraine patients with the most effective therapeutics. Additionally, the breakdown of symptoms associated with each headache type promotes the development of preclinical animal models that encompass the broad symptomology associated with migraine.

Table 1: Diagnostic criteria for migraine

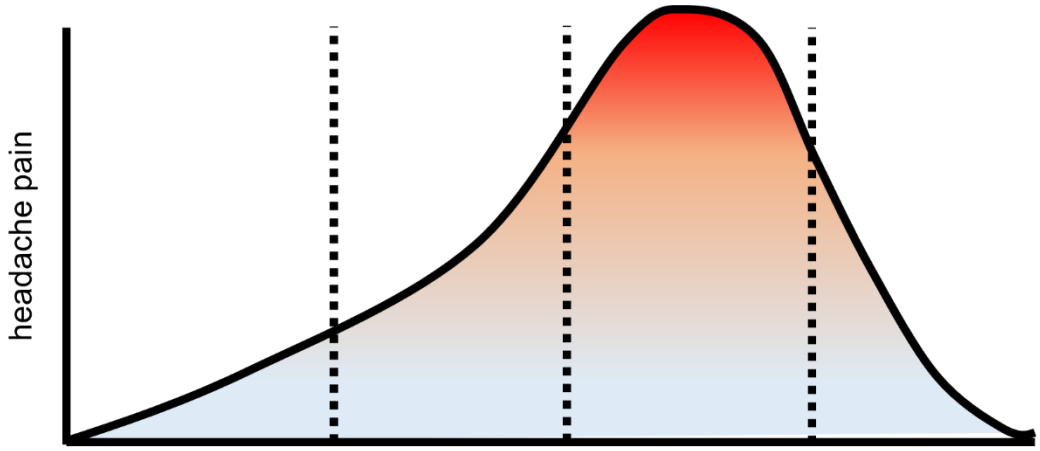
Migraine without aura	Migraine with aura

At least five occurrences of the following:
• Headache attacks lasting 4-72 hours, with at least two of the following four characteristics:
  ○ Unilateral location
  ○ Pulsating quality
  ○ Moderate or severe pain intensity
  ○ Aggravation by or causing avoidance of routine physical activity
• During headache attack, at least one of the following:
  ○ Nausea and/or vomiting
  ○ Photophobia or phonophobia
	Headache attacks described under "Migraine without aura", accompanied with 1 or more of the following fully reversible aura symptoms:
	○ Visual
	○ Sensory
	○ Speech and/or language
	○ Motor
	○ Brainstem
	○ Retinal
	At least three of the following six characteristics:
	○ At least one aura symptom spreads gradually over 5 minutes
	○ Two or more aura symptoms occur in succession
	○ Each individual aura symptom lasts 5-60 minutes, except for motor symptoms which may last up to 72 hours
	○ At least one aura symptom is unilateral
	○ At least one aura symptom is positive, such as feelings of scintillations, pens, and needles
	○ The aura is accompanied, or followed within 60 minutes, by a headache attack

Chronic migraine	
• Headache attacks (with or without aura) on 15 or more days per month for over 3 months • Headache attacks must be due to migraine, and not a secondary headache, on 8 or more days per month for over 3 months	

**Adapted from ICHD, 3rd beta edition.*

There are multiple theories underlying migraine, and undoubtedly there are environmental and genetic influences that contribute to the development of this disorder. Interestingly, migraines are cyclical. Not only can recurrent migraine attacks repetitively emerge on a daily, weekly, or monthly basis, but migraine attacks also cycle through different phases (Goadsby, Holland et al. 2017). The distinct phases of a migraine attack encompass their own distinct symptomology, and it is possible for some symptoms to worsen as migraine progresses from an episodic to a chronic disorder. The phases of a migraine attack are as follows: prodrome or the premonitory phase, aura (if applicable), headache, and postdrome (Figure 1, (The American Migraine Foundation)). The headache attack phase is the most disabling phase of a migraine. However, it is also important to note the distressing symtpoms that occur during the interictal periods, or the periods in between migraine attacks. The stress of not knowing when another migraine attack will occur or how long the headache attack will last can contribute to the exacerbation of migraine (The American Migraine Foundation). Thus, it is reasonable to acknowledge that migraine affects the patient constantly, and results in disabling symptoms both during the headache phase and the interictal periods.



Phase	prodrome	aura	headache	postdrome
Duration	hours to days	5-60 minutes	4-72 hours	24-48 hours
Symptoms	• Fatigue • Mood changes • Food cravings • Yawning • Muscle tenderness • Photophobia	• Visual disturbances • Sensory changes • Motor changes • Speech and/or language disturbances	• Unilateral headache • Nausea • Photophobia • Phonophobia	• Fatigue • Stiff neck • Difficulty concentrating
Brain regions implicated	• Hypothalamus • Brainstem • Limbic system • Cortical areas	• Cortical areas, specifically visual cortex	• Meninges, dura • Trigeminal ganglion • Trigeminal cervical complex • Brain stem • Hypothalamus • Thalamus • Basal ganglia nuclei • Cortical areas	• Hypothalamus • Brainstem • Limbic system • Cortical areas

Figure 1: Phases of a migraine attack, adapted from The American Migraine Foundation (The American Migraine Foundation). Phase, duration, symptoms, brain regions and headache pain are charted in the above schematic. Headache pain progressively increases throughout the phases of migraine, hitting peak pain levels during the headache phase. The duration of each phase varies, and multiple brain regions may be implicated depending on the symptoms of each phase.

Below, I will discuss the different phases of a migraine attack, and focus on the anatomical brain regions implicated based on the symptomology of each phase. The prodrome or premonitory phase marks the beginning of the migraine attack. Most people will experience this phase but not necessarily before every migraine attack. Next, aura may occur and those who experience it may endure periods of blurry vision, vision loss, or blind spots in their visual field. Then, the headache phase includes a severe unilateral pulsating headache, and can include nausea, photophobia, and phonophobia. Finally, postdrome concludes the migraine attack, and the length of this phase can vary. The interictal state, or the time period between headache attacks, also varies between patients and migraineurs may experience anxiety due to the uncertainty of when the next migraine attack will occur (Lipton, Hamelsky et al. 2000). Understanding the symptoms associated with each phase, and the brain regions implicated during each phase, may provide insight into the underlying pathophysiology of migraine attacks

1.2.1. PRODROME, THE PREMONITORY PHASE

The premonitory phase allows some patients to predict migraine headache up to 12 hours of its onset (Giffin, Ruggiero et al. 2003). Common symptoms include fatigue, mood changes, food cravings, yawning, muscle tenderness, and photophobia. These symptoms suggest that the following are involved: hypothalamus, brainstem, limbic system, and certain cortical areas (Maniyar, Sprenger et al. 2014). Technological advancements, such as the use of live imaging, allows researchers to visualize migraines as they occur. With functional neuroimaging, positron emission tomography scans with H2(15)O were used to measure cerebral blood flow as a marker for neuronal activity in

migraineurs without pain and then during a migraine (Maniyar, Sprenger et al. 2014). In this study, nitroglycerin (NTG), a known human migraine trigger, was used to trigger premonitory symptoms. NTG triggered neural activity in the posterolateral hypothalamus, midbrain tegmental area, periaqueductal gray, dorsal pons and various cortical areas such as the occipital, temporal, and prefrontal cortex (Maniyar, Sprenger et al. 2014). These data suggest that these regions are crucial to the premonitory phase, and that overactivation of these brain regions may trigger the onset of a headache attack. In addition to being cyclical, migraine is also a multifactorial disease. The multitude of symptoms, and the variety of brain regions activated in these imaging studies, suggests that there may be a malfunctioning neural circuitry in migraineurs. Because of this neurological disorder, or of the genetics underlying this disease, the brain of a migraineur could be altered structurally and functionally. It is also possible that repeated attacks alter the structural and functional neural circuitry, which further complicates the pathophysiology of this disorder. This dysfunction can lead to additional molecular, anatomical, and functional abnormalities that sensitize the brain and worsen headache (Burstein, Noseda et al. 2015). Recently, researchers imaged the brain of a migraineur every day for one month, which included untreated migraine attacks as well as the interictal phases (Schulte and May 2016). In this landmark study, a migraine patient had magnetic resonance imaging done every day for 30 days, which encompassed three complete, untreated migraine attacks (Schulte and May 2016). In this study, hypothalamic activity as a response to trigeminal nociceptive stimulation was altered during the 24 hours prior to headache onset (Schulte and May 2016). The hypothalamus showed altered functional coupling with the spinal trigeminal nuclei (Schulte and May 2016).

These functional imaging studies implicate the hypothalamus as a “migraine generator”, a region that plays a key role in facilitating or amplifying pain transmission during a migraine attack. These data also demonstrate the critical role of trigeminal nuclei, and the connection between the trigeminal nuclei to other nodes in the brain, in generating migraine attacks.

There are various theories explaining how migraine triggers can contribute to a migraine attack. One theory is that different migraine triggers can activate a wide variety of brain areas, which ultimately change the parasympathetic innervations of the meninges. In this theory, migraine triggers can activate multiple hypothalamic, limbic, and cortical areas, all of which contain projections to the preganglionic parasympathetic neurons in the superior salivatory nucleus (Burstein and Jakubowski 2005). The superior salivatory nucleus activates postganglionic parasympathetic neurons in the sphenopalatine ganglion, which results in vasodilation and local release of inflammatory molecules that can activate meningeal nociceptors. Bidirectional trafficking of the trigeminovascular system forms a feedback loop, meaning that the trigeminovascular system activates the same brain areas that triggered its own activity. This perpetual feedback loop could theoretically drive a migraine attack for hours/days, and may, in part, explain the cyclical nature of this disease (Burstein and Jakubowski 2005). Within this loop, regions can become sensitized to each subsequent activation and may result in heightened migraine pain. In addition to the hypothalamus, it is also possible that the thalamus transmits nociceptive signals to the cortex, which can contribute to this pro-migraine signaling (Noseda and Burstein 2013). The abnormal functioning of these pathways in response to everyday triggers leads to the

manifestation of symptoms in the premonitory phase and can also trigger a cascade of events that ultimately manifests in a headache attack. Prodrome may last a few hours or a few days, and many migraineurs are able to use these symptoms to predict the onset of a migraine attack. Following prodrome, a migraineur may experience aura or progress directly to the headache phase.

1.2.2. AURA

Approximately one-third of migraine attacks are preceded by aura, and the most prevalent aura symptoms include visual disturbances, sensory, speech/language, and motor disturbances, and consequently a disruption of higher order function (Eriksen, Thomsen et al. 2004, ICHD3b 2013). Visual aura has been the best characterized of the aforementioned symptoms. Visual aura is thought to be very similar to cortical spreading depression that has been seen in animals. Thus, cortical spreading depression is thought to be the neurophysiological correlate of migraine (Leo 1944, Pietrobon and Moskowitz 2013). Cortical spreading depression is characterized by a slowly propagating wave of depolarization, followed by inhibition of cortical activity for up to 30 minutes (Lauritzen 1994, Somjen 2001, Smith, Bradley et al. 2006, Pietrobon and Moskowitz 2013). Cortical spreading depression is initiated by local elevations in extracellular potassium (K+) that depolarizes neurons for approximately 30-50 seconds (Smith, Bradley et al. 2006). It is possible that the accumulation of extracellular K+ occurs as a result of repeated depolarization and that this pool can further depolarize the cells from which it was originally released (Grafstein 1956, Smith, Bradley et al. 2006, Pietrobon and Moskowitz 2014). The efflux of K+ disrupts cell membrane ionic gradients, leading to an influx of

sodium (Na+) and calcium (Ca^{2+}), and release of glutamate (Charles and Brennan 2009). Furthermore, cortical spreading depression propagation may be mediated by gap junctions between neurons and glial cells (Somjen 2001). By impacting cortical areas that may relay down to deeper brain regions, cortical spreading depression may also activate trigeminal nociception and trigger headache (Zhang, Levy et al. 2010, Karatas, Erdener et al. 2013, Pietrobon and Moskowitz 2013). Although cortical spreading depression has not yet been seen in human migraine patients, electrophysiological events similar to cortical spreading depression have been observed in patients during the aura phase of migraine as well as after severe traumatic brain injuries (Lauritzen, Dreier et al. 2011). Using high-field functional magnetic resonance imaging, visual aura was recorded in three subjects (Hadjikhani, Sanchez Del Rio et al. 2001). During the onset of an aura, blood oxygenation level-dependent signals increased, then decreased, within the occipital cortex (Hadjikhani, Sanchez Del Rio et al. 2001). Imaging studies such as these suggest that cortical spreading depression-like electrophysiological events in the visual cortex may generate visual aura. These disturbances can last 5-60 minutes, and immediately precede the headache attack.

1.2.3. HEADACHE

The headache phase of a migraine attack results in a throbbing, pulsating unilateral headache. This headache can be accompanied with nausea, photophobia, and phonophobia. The migraine attack can last anywhere from 4 to 72 hours and is incredibly disabling. The trigeminovascular pathway is well characterized, and activation of this pathway may explain migraine-associated pain (Figure 2) (Burstein, Noseda et al. 2015).

The trigeminovascular pathway conveys nociceptive information from the meninges to the central areas of the brain, and subsequently to the cortex. Fibers originating from the trigeminal ganglion innervate the meninges and large cerebral arteries (Burstein, Noseda et al. 2015, Goadsby, Holland et al. 2017). Nociceptive innervation occurs mainly through the ophthalmic branch of the trigeminal nerve (Goadsby, Holland et al. 2017). Afferents from the trigeminal ganglion converge with other inputs from adjacent skin, pericranial, and paraspinal muscle, and other C1-C2 innervated tissues before synapsing on second-order neurons in the trigeminal cervical complex. The trigeminal cervical complex encompasses the trigeminal nucleus caudalis (TNC) and the dorsal horn of the upper cervical spinal cord (C1-C2) (Burstein, Yamamura et al. 1998, Bartsch and Goadsby 2003, Noseda and Burstein 2013, Goadsby, Holland et al. 2017). The trigeminal cervical complex transmits signals to the brain stem, thalamic, hypothalamic, and basal ganglia nuclei (Malick, Strassman et al. 2000). These nuclei then project to multiple cortical areas that are involved in processing the cognitive, emotional, and sensory-discriminative aspects of migraine-associated pain (Noseda, Jakubowski et al. 2011). The characterization of the trigeminovascular pathway has been integral to understanding the pathogenesis of migraine, and activating this pathway is crucial to the generation of migraine-like pain.

Activation of the trigeminovascular pathway explains the distribution of migraine-associated pain seen during a headache attack. Whether altered parasympathetic tone activates this pathway, or whether there are multiple factors contributing to this activation needs further elucidation. If the activation begins in the periphery, nociceptive neurons

that innervate the dura mater are stimulated and then they release various inflammatory peptides. These peptides can include neurokinin A, nitric oxide, and vasoactive neuropeptides like vasoactive intestinal polypeptide, calcitonin gene-related peptide (CGRP), substance P, and pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38). These neurotransmitters bind to their respective receptors, causing signaling along the trigeminovascular pathway. This pathway ultimately projects to the TNC in the brain stem, the cervical spinal cord area, and subsequently projecting those pain signals to higher areas like the thalamus and cortex. The activation of this pathway leads to arterial vasodilation and mast cell degranulation (Messlinger, Hanesch et al. 1993, Messlinger, Fischer et al. 2011, Amin, Hougaard et al. 2014). Once activated by these endogenous migraine mediators, both peripheral and central sensitization can occur.

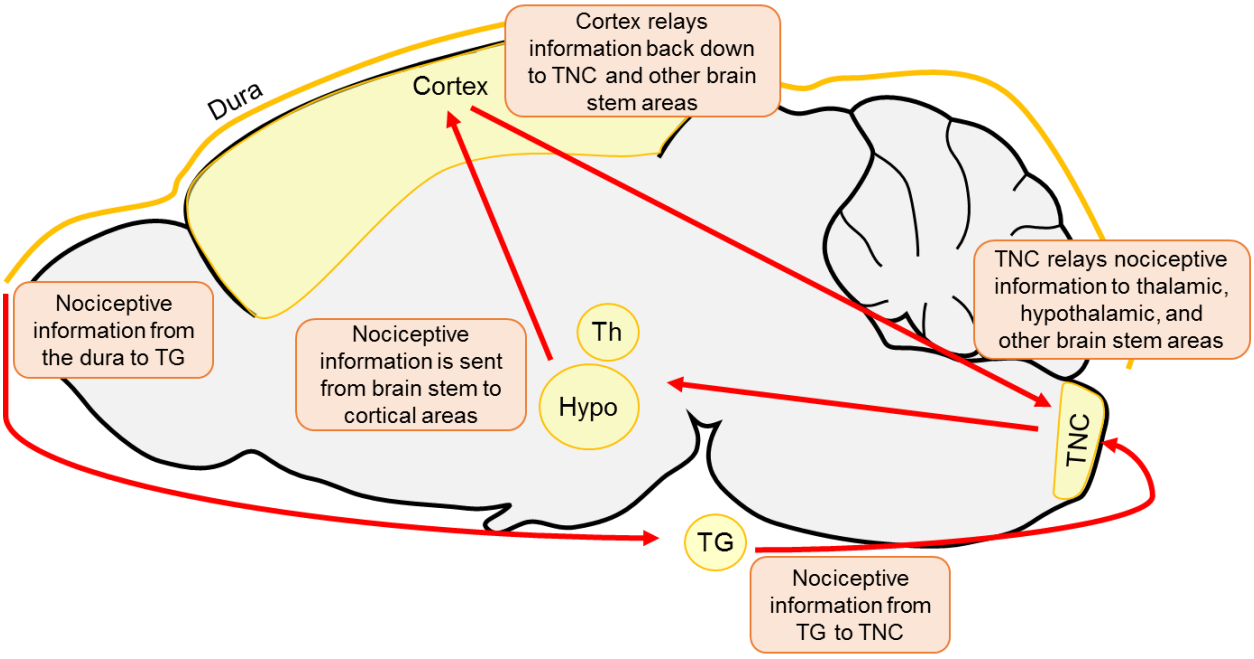


Figure 2: Trigeminovascular theory of migraine. Schematic of the proposed trigeminovascular theory of migraine. In the schematic, nociceptive information is thought to be projected towards the trigeminal ganglia (TG), and then further propagated towards the trigeminal nucleus caudalis (TNC). Once in the TNC, it is thought that nociceptive information is sent into subcortical regions like the thalamus, hypothalamus, which sends information directly to the cortex. Once in the cortex, it is possible that the cortex relays nociceptive information back to the TNC.

Peripheral sensitization occurs when peripheral trigeminovascular neurons become sensitized to dural stimuli. Specifically, the threshold of these neurons in response to stimuli decreases while the magnitude of their response increases (Burstein, Noseda et al. 2015). This phenomenon is partially responsible for the characteristic throbbing pain of migraine, and the exacerbation of migraine pain by bending over, coughing, or other stimuli. The sensitization, or increased sensitivity to sensory stimulation, could be caused by the hyper-responsiveness of primary afferent fibers (De Felice, Ossipov et al. 2010, De Felice, Ossipov et al. 2010). While the exact mechanism underlying peripheral sensitization is not yet fully understood, certain animal studies have shown the importance of the trigeminovascular pathway in sensitization. Activating the

trigeminovascular pathway can result in a feedback loop, and a one-time activation of this pathway can result in molecular changes that ultimately re-activate the same pathway. For example, rat studies have shown that dural mast cell degranulation can lead to prolonged activation of trigeminal pain pathway (Levy, Kainz et al. 2012). Activation of this pathway also leads to the release of neurotransmitters, including the pro-migraine peptide CGRP.

CGRP regulates migraine attacks in multiple ways and exploring the role of CGRP is of immense interest to this thesis work. Animal studies have shown that CGRP release is critical to the initiation and maintenance of peripheral sensitization (Iyengar, Ossipov et al. 2017). While CGRP release is maintained and peripheral sensitization occurs, this could result in a lower threshold to stimuli. In fact, repeated CGRP injection into rat paws decreased the threshold to noxious mechanical stimuli, suggesting that chronic CGRP injections altered the threshold level and less stimuli was required to result in mechanical pain (Nakamura-Craig and Gill 1991). In addition to sensitization in the periphery, central sensitization can also occur. Central sensitization contributes to the development and maintenance of chronic headache pain. Specifically, these changes in the central nervous system further maintain pain. Sensitization of trigeminovascular neurons in the trigeminovascular complex, and subsequently those in the mid brain, may also be responsible for cephalic and extracephalic cutaneous allodynia, respectively (Noseda and Burstein 2013). Over 63% of migraine patients experience cutaneous allodynia, and this phenomenon is associated with increased migraine frequency and severity (Burstein, Collins et al. 2004, Bigal, Ashina et al. 2008, Charles and Brennan 2008, Lipton, Bigal et

al. 2008, Burstein, Jakubowski et al. 2010). Cutaneous allodynia, or pain resulting from an innocuous stimuli, is more common and more severe in chronic migraineurs than in other headache patients (Bigal, Ashina et al. 2008). Under the scope of central sensitization, it is possible that migraine-associated hyperalgesia is also mediated by thalamic neurons (Burstein, Jakubowski et al. 2010). In rats, chemically stimulating, and subsequently sensitizing, the cranial dura resulted in long-lasting hyperexcitability to innocuous and noxious paw stimulation, as recorded from sensory neurons in the posterior thalamus (Burstein, Jakubowski et al. 2010). In these rats where sensitization had already occurred, innocuous mechanical stimuli such as lightly brushing the paw resulted in large bouts of neuronal firing, and did not produce any neuronal firing in control rats (Burstein, Jakubowski et al. 2010). In migraine patients that were having a migraine attack, stimulating hand skin resulted in larger blood oxygenation level-dependent signals in the posterior thalamus, as compared to when the migraine patients were free of the migraine (Burstein, Jakubowski et al. 2010). These results suggest that the extracephalic allodynia associated with migraine may be mediated, in part, by sensitized thalamic neurons. These results also suggest that sensitizing the cranial dura can lead to sensitizing thalamic neurons, and that this nociceptive information can evoke and spread allodynia beyond the cephalic region. A migraine attack may last 4 to 72 hours and is incredibly debilitating. At the end of a migraine attack, migraineurs will enter the postdrome phase which encompasses symptoms that are very similar to the prodrome.

1.2.4. POSTDROME

Postdrome is the final stage of a migraine attack, and postdrome symptoms mimic those during the premonitory phase (Blau 1991, Giffin, Lipton et al. 2016). Postdrome symptoms include feeling tired/weary, having difficulty concentrating, and a stiff neck (Giffin, Lipton et al. 2016). There is a return to a pre-headache state within 24 hours after migraine pain has resolved, and the severity of the migraine is not associated with the duration of the postdrome (Kelman 2006, Giffin, Lipton et al. 2016). As symptoms are similar to the prodrome, similar brain regions involved in the prodrome may also be involved in the postdrome. Constant activation of these brain regions may sensitize the circuitry to subsequent triggers, which feeds into the cyclical nature of migraine (The American Migraine Foundation).

Overall, migraine is a multifaceted disease, and there are multiple symptoms associated with all phases of migraine. Additionally, migraine is an ongoing disorder, and the migraine patient may experience some symptoms at all times (Stovner, Hagen et al. 2007). For example, sensitivity to light may occur at all times, but be most intense during the headache phase of migraine. The Global Burden of Headache study found that 46% of the adult population worldwide has an active headache disorder, 11% of which is for migraine (Stovner, Hagen et al. 2007). These headache disorders, including migraine, are the 10 most disabling conditions for both sexes, and the 5 most disabling for women (Stovner, Hagen et al. 2007, Victor, Hu et al. 2010). The median age of migraine onset occurs at 25 years for women and 24 years for men, with 75% of the migraine population developing migraine before the age of 35 (Stewart, Wood et al. 2008). Migraine develops

during peak productivity years for both men and women. Direct costs due to migraine are $1 billion annually, and $5.6-$17.2 billion in indirect costs (lost time at work, school, home) (Lipton, Stewart et al. 2001, Adelman, Adelman et al. 2004, Goldberg 2005). Unfortunately, this ranking is increasing with time, and further research is required to better treat patients with these disorders (Murray, Vos et al. 2012, Vos, Flaxman et al. 2012).

1.3. SECONDARY HEADACHE DISORDERS WITH A MIGRAINE-LIKE PHENOTYPE

Secondary headache disorders are due to an underlying disease, and not the headache condition itself (ICHD3b 2013). Some secondary headache disorders have a migraine-like phenotype and can be difficult to treat. These headaches can be attributed to a trauma or injury to the head or neck, a cranial or intracranial disorder, substance use or withdrawal, infection, or a psychiatric disorder (ICHD3b 2013). Here, I will briefly cover post-traumatic headache which has a migraine-like phenotype and develops after mild traumatic brain injuries, and medication overuse headache which is observed following chronic use of therapeutics.

Post-traumatic headache is one of the most frequent and disabling disorders following mild traumatic brain injury (Couch and Bearss 2001). Consisting of blows, blasts, and jolts, traumatic brain injuries are penetrating injuries to the head that disrupt normal functioning of the brain for any period of time (National Center for Injury Prevention and Control 2003). More than 1.5 million Americans experience a traumatic brain injury annually, and at least 75 percent of these injuries are mild traumatic brain injury (National

Center for Injury Prevention and Control 2003, Faul M 2010). Currently, mild traumatic brain injuries cost the nation $17 billion per year, including direct and indirect healthcare expenses (National Center for Injury Prevention and Control 2003). Defined as a secondary headache, post-traumatic headache develops within 7 days of traumatic brain injury, or within 7 days of regaining consciousness post-traumatic brain injury (ICHD3b 2013). While acute post-traumatic headache is resolved within 3 months of onset after injury, chronic post-traumatic headache can persist beyond 3 months (ICHD3b 2013). Up to 90% of mild traumatic brain injury patients develop a migraine-like post-traumatic headache, which can persist up to a year post-injury (Couch and Bearss 2001). Post-traumatic headache leads to decreased quality of life and chronic disability, affecting persons of all ages, races/ethnicities, and income levels (Coronado, Xu et al. 2011). Some experts argue that post-traumatic headache may develop outside the 7-day window and further research is needed to investigate the progression from mild traumatic brain injury to post-traumatic headache.

Post-traumatic headache has been recognized as a public health concern for decades (Ross 1945, ICHD3b 2013). Considered a “silent epidemic”, mild traumatic brain injury-related symptoms are often not immediately visible, and thus not instantaneously treated (National Center for Injury Prevention and Control 2003). This delay in treatment has been a concern among physicians treating mild traumatic brain injury patients (Rosenthal 1992). The delay or lack of post-traumatic headache treatment carries the potential of exacerbating post-traumatic symptoms, ultimately resulting in both economic and social costs. Although few studies have speculated on the genesis of post-traumatic headache,

meta-analyses of epidemiological data have suggested that mild traumatic brain injury lays the foundation for downstream progression to post-traumatic headache (Couch and Bearss 2001, National Center for Injury Prevention and Control 2003, Coronado, Xu et al. 2011). Recent epidemiological data has provided insight on the relationship between mild traumatic brain injury and post-traumatic headache (Ross 1945, National Center for Injury Prevention and Control 2003, Coronado, Xu et al. 2011). Post-traumatic headache patients were more likely to have suffered from a mild, not moderate/severe, TBI (National Center for Injury Prevention and Control 2003, Faul M 2010). To date, the only factors that predict the development of post-traumatic headache following mild traumatic brain injury is sex (females develop post-traumatic headache more often than males), prior headache disorder (e.g. migraine), and a family history of headache disorders (Mihalik, Register-Mihalik et al. 2013, Walker, Marwitz et al. 2013). However, this data is limited to post-traumatic headache patients who visited an emergency room, their primary care doctor, or a headache center for their headache pain, and there may be post-traumatic headache patients who do not realize that they have this secondary headache. Moreover, these data are reflective of civilian populations. In military populations, the incidence of TBI is much more pronounced (Theeler, Lucas et al. 2013). Due to advancements in protective gear, military personnel are withstanding blast-related injuries that were once fatal, and experiencing unprecedented side effects (Warden 2006, T Tanielian 2008). Veterans from Iraq and Afghanistan (Operation Iraqi Freedom and Operation Enduring Freedom, respectively) returned to the United States with headache post-traumatic brain injury (Warden 2006, Theeler, Lucas et al. 2013). Another study found that 77% of soldiers with chronic post-traumatic headache experienced blast-induced traumatic brain

injuries (Erickson 2011). Retroactive studies show that up to 78% of soldiers returning from combat with deployment-related concussion suffered from episodic headache, and 20% from chronic daily headache (Theeler, Lucas et al. 2013). Post-traumatic headache is a residual consequence of a majority of blast traumatic brain injury cases (Cernak and Noble-Haeusslein 2010, D'Onofrio, Russo et al. 2014). To date, the relatively limited number of animal models of post-traumatic headache has severely impeded further elucidation of the mechanisms underlying this disorder. A majority of the mouse models of post-traumatic headache are based on moderate to severe traumatic brain injuries, although a majority of post-traumatic headache patients suffer from mild traumatic brain injuries. These mild traumatic brain injury-induced migraines are incredibly difficult to treat, and Chapter 2 of this thesis is dedicated to developing and characterizing a mouse model of mild traumatic brain injury-induced migraine.

In addition to post-traumatic headache, medication overuse headache is also a secondary headache disorder that is difficult to treat. In this thesis, I focus on medication overuse headache by sumatriptan, and I also explore cephalic hyperalgesia in a model of opioid-induced hyperalgesia. Medication overuse headache and opioid-induced hyperalgesia has been previously seen after recurrent use of triptans and opioids, respectively.

Medication overuse headache and opioid-induced hyperalgesia are paradoxical phenomena where frequent use of an anti-nociceptive drug results in hyperalgesia. In this section, I will first describe medication overuse headache to triptans, and then describe opioid-induced hyperalgesia to morphine. Triptans are serotonin receptor agonists with

selectivity for the 5-HT_{1B}, 5-HT_{1D}, and 5-HT_{1F} receptors (Ahn and Basbaum 2005). Triptans are commonly prescribed as abortive migraine therapeutics, and the exact mechanism of action by which triptans regulate migraine is currently unclear (Ahn and Basbaum 2005). Chronic use of triptans can lead to medication overuse headache, and medication overuse headache has a worldwide prevalence of 1-2% (Kristoffersen and Lundqvist 2014). Medication overuse headache complicates the treatment of migraine and is also a complex disorder. To date, the first line treatment for medication overuse headache is withdrawal from the drug, which further complicates the treatment of both the primary migraine and medication overuse headache. More detailed information regarding medication overuse headache will be discussed in Chapter 3 of this thesis.

In addition to medication overuse headache by triptans, overuse of opioids can result in opioid-induced hyperalgesia. Opioids are typically used in U.S. emergency room settings to treat headaches that have a migraine-like phenotype, and opioid-induced hyperalgesia in headache could possibly be another form of medication overuse headache (Bigal and Lipton 2009). Although the true prevalence of opioid-induced hyperalgesia is unknown, chronic exposure to opioids is a public health concern and may result in addiction as well as opioid-induced hyperalgesia (Lee, Silverman et al. 2011). While opioids may provide acute relief, regular use of these compounds contributes to the progression of cephalic pain from an episodic to a chronic pain state (Bigal and Lipton 2009). Patients are dependent on these abortive treatments, although they do not provide sufficient pain relief in all patients (Visser, de Vriend et al. 1996, Bigal, Serrano et al. 2008, Lipton, Buse et al. 2013). Interestingly, opioids like hydrocodone, oxycodone, and meperidine have a poor

efficacy for treating headaches. A major concern with using these opioids are that they are MOR-based agonists and they have an extremely high addiction potential. The rewarding properties of opioids could also interfere with the painful characteristics of opioid-induced hyperalgesia (Bigal and Lipton 2008, Bigal and Lipton 2009). The first line treatment for opioid-induced hyperalgesia is withdrawal from the overused drug, which complicates the treatment for primary headaches. Additionally, patients that withdraw from their overused medications have high headache relapse rates after initial successful withdrawal (Katsarava, Limmroth et al. 2003). Novel therapeutics that have fewer adverse effects are needed to bypass the chronification of headache pain and to avoid the addictive potential of opioids. Both medication overuse headache and opioid-induced hyperalgesia have been modeled in rodents, and Chapter 3 of this thesis focuses on exploring DOR agonists as a potential therapy for medication overuse headache and opioid-induced hyperalgesia, in addition to post-traumatic headache and chronic migraine.

Trauma and medication overuse can increase the susceptibility to developing chronic migraine, and the management of migraine and secondary headache disorders can be difficult (Weatherall 2015). The lack of treatments for cephalic pain makes this patient population more vulnerable to drug abuse. While triptan abuse is concerning, opioid abuse in pain patients has led to a public health crisis, specifically by contributing to the national opioid epidemic. A recent study found that over 50% of patients had been prescribed opioids for their migraine, and 20% still use these opioids regularly (Minen, Lindberg et al. 2015). Interestingly, another study found that opioids were administered

in over half of all emergency room visits for migraine, and that there was a significant association between repeat visits to the emergency room and opioid prescription (Friedman, West et al. 2015). The over-prescription of opioids to treat headache can lead to dependence on the prescribed drug, and the addictive properties of these compounds is concerning. In the U.S., the number of opioid-related overdose deaths has quadrupled in the past 20 years (CDC 2016). The sale of these MOR agonists has more than quadrupled since 1999 (Justic 2011, Paulozzi LJ 2011), and this increase has had detrimental consequences. We now know that prescription opioids have not meaningfully impacted the amount of pain that Americans report (Daubresse, Chang et al. 2013, Chang, Daubresse et al. 2014), but they have contributed significantly to the 18-year increase in overdose deaths (CDC 2016). To date, nearly half of all U.S. opioid-related overdose deaths involve a MOR-based prescription opioid (CDC 2016). The dependence on MOR-based therapies to treat pain has cultivated this public health issue, and the lack of alternatives to these prescription opioids could further foster the opioid epidemic. To combat this issue, the mechanisms underlying pain disorders must be elucidated and novel therapeutic targets like DOR agonists must be explored.

1.4. CURRENT TREATMENTS FOR MIGRAINE

Despite significant advancements in understanding the complex etiology of migraine, there are still limited effective treatments for this disorder. Here, the current state of migraine treatments will be discussed. Migraine medications can generally be divided into abortive and prophylactic treatments. Abortive treatments stop established headache pain during an attack. Prophylactic treatments prevent a migraine attack and are

chronically taken by the patient. In addition to these therapeutics, migraineurs may also benefit from nonpharmacological treatments such as behavioral changes to diet, physical exercise, and avoiding stressful situations. To date, no therapeutic is 100% effective in all patients, and no migraine therapeutic is completely devoid of side effects. While approximately half of migraine patients respond to currently available therapeutics, a majority remain with refractory headaches which are unresponsive to migraine treatments and incredibly difficult to treat. Here, I will discuss the benefits and setbacks of these currently available treatments.

1.5. NONPHARMACOLOGICAL TREATMENTS FOR MIGRAINE

Nonpharmacological treatments include biofeedback, behavioral modifications, psychosocial interventions like relaxation and stress management, acupuncture, impulse magnetic-field therapy, and exercise. These interventions may help control migraine attacks and better predict the onset of a migraine attack (Wells and Loder 2012). Nondrug interventions have few side effects, and often result in an overall positive impact on the migraine patient. While beneficial on their own, these interventions are most beneficial in combination with pharmacological therapy. In a meta-analysis of acupuncture studies, needling acupuncture, in conjunction with medication therapy, was found to improve headache intensity, frequency, and response rate (Sun and Gan 2008). Neurostimulation has also recently been employed to help migraineurs manage their pain. High-cervical spinal cord stimulation in patients with chronic migraine reduced pain and significantly improved quality of life (De Agostino, Federspiel et al. 2015). In a randomized controlled clinical trial, a one-hour treatment with external trigeminal nerve stimulation also

significantly relieved headache pain relief (Chou, Shnayderman Yugrakh et al. 2018). By incorporating nonpharmacological interventions into a patient's treatment plan, migraineurs may feel more in control of their disorder and may learn to understand how lifestyle can exacerbate or alleviate a migraine attack. Despite the positive impact of nonpharmacological treatments, they are underused. It is possible that psychological factors such as lack of motivation, poor awareness of triggers, and maladaptive coping styles can hinder patient compliance (Matsuzawa, Lee et al. 2018). Ultimately, effective communication between the physician and migraine patient is needed to treat the migraine with the most effective treatments possible.

1.6. PHARMACOLOGICAL TREATMENTS OF MIGRAINE

Pharmacological treatments for migraine have been used since the late 1930s. With the breakthrough of ergotamine tartrate as the first documented medication therapy, the field of medicine has grown to incorporate many other abortive and prophylactic therapeutics. In addition to prescribed abortive or prophylactic therapeutics, migraine patients also use over-the-counter drugs to alleviate their migraine pain. The Migraine in America Symptoms and Treatment study is a longitudinal, internet-based panel study of symptoms, approaches to management, and unmet treatment needs among U.S. adult migraineurs (Lipton, Munjal et al. 2018). The Migraine in America Symptoms and Treatment study found that 95.1% of migraineurs currently use acute treatment, with 58.9% of migraineurs using over-the-counter drugs, 11.3% using exclusively prescription drugs, and 20.5% using a combination of both (Lipton, Munjal et al. 2018). The overuse of medication has led to the development of secondary headache disorders and has

complicated the treatment of migraine. Here, I will discuss abortive therapeutics that are used to treat established pain, and prophylactic therapeutics which are used to prevent the development of headache pain (Figure 3).

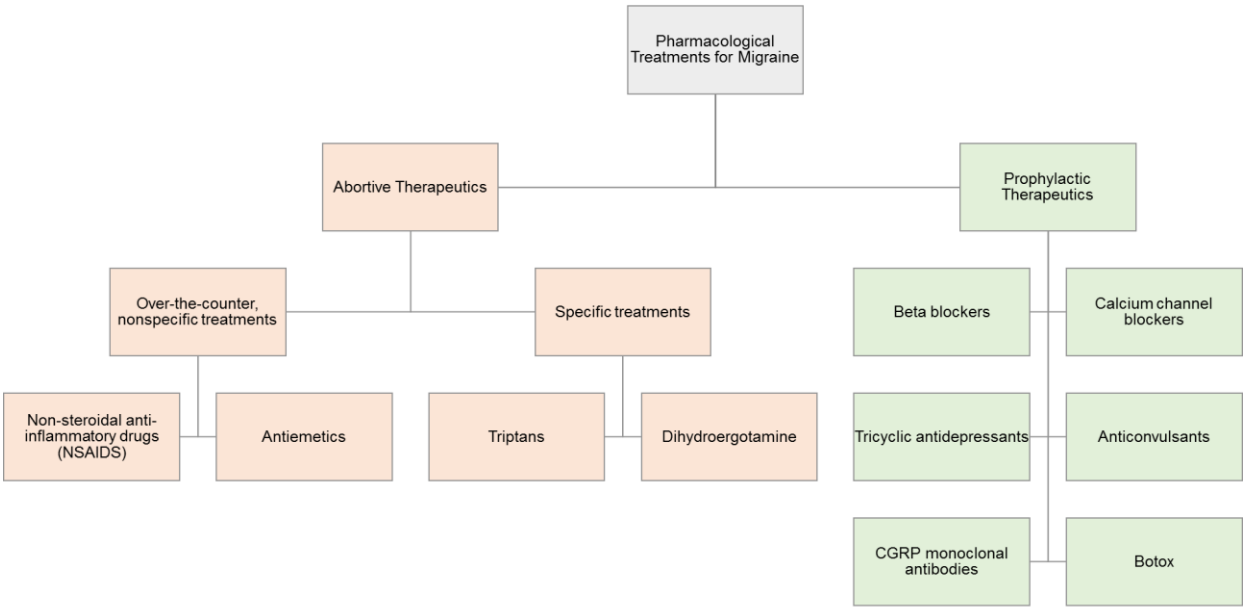


Figure 3: Schematic of pharmacological treatments for migraine. Pharmacological treatments for migraine can be divided into abortive and prophylactic therapies. Under the umbrella term “abortive therapeutics”, there exist over the counter treatments that may not be specific for migraine, but are considered pain relieving, as well as specific treatments for migraine that are often prescribed. Under the umbrella term “prophylactic treatments”, there exist a handful of treatments that are intended to be taken to prevent the onset of a migraine attack. The treatments for migraine continue to grow, and with time the schematic could also grow to encompass novel therapeutics approved for migraine.

1.6.1. ABORTIVE THERAPEUTICS FOR MIGRAINE

Abortive therapeutics can be classified as nonspecific treatments like analgesics and non-steroidal anti-inflammatory drugs (NSAIDS), or specific treatments like ergot derivatives and triptans (Antonaci, Ghiotto et al. 2016). Abortive therapeutics are not meant to be used chronically and are typically used in conjunction with preventive therapies. While

preventive therapies are meant to reduce attack frequency and severity over the life of the disorder, abortive therapeutics are acute treatments to stop a headache attack. Mentioned previously, the Migraine in America Symptoms and Treatment study found that 95.1% of migraineurs use acute treatments for their headache, with a majority using over-the-counter medications (Lipton, Munjal et al. 2018). The over-the-counter medications included NSAIDs like aspirin, acetaminophen, and paracetamol with caffeine (Diamond, Bigal et al. 2007, Lipton, Bigal et al. 2007). One reason for a high use of over-the-counter drugs opposed to specific treatments is accessibility. Patients may not be enrolled in health plans that give them access to these treatments. Also, many patients may not be aware that they have migraines. In The American Migraine Prevalence and Prevention (AMPP) study, researchers evaluated the epidemiology, burden, and patterns of healthcare utilization for migraine by mailing surveys to 120,000 US households. Household members answered survey questions based on the criteria outlined in the Second Edition of The International Classification of Headache Disorders (ICHD-2), and only 56.2% of those household members that met the criteria for migraine had ever received a medical diagnosis of their headache (Patel, Bigal et al. 2004). This leaves approximately half of migraineurs self-reporting and self-managing their headache disorder. In this study, approximately half (49%) treated their headaches with only over-the-counter medications, 20.1% exclusively used prescription medications, and 28.8% interchanged using both nonspecific and specific treatments (Patel, Bigal et al. 2004). This study shows that migraine may be underdiagnosed and thus undertreated, and that migraineurs that self-medicate may not be effectively doing so with only over-the-counter medications.

Nonspecific treatments are sometimes used in combination with specific treatments. Mentioned previously, the first documented abortive therapeutic was ergotamine tartrate, and has been used since the early 20th century. The ergot derivatives, ergotamine tartrate and dihydroergotamine, are serotonin receptor agonists and they also interact with other receptors (5-HT1$_A$, 5-HT$_5$, 5-HT$_2$, 5-HT$_7$, α-adrenoreceptors, D2 receptors). Ergot derivatives are vasoconstrictors, have a long duration of action, and they also result in adverse side effects such as nausea, vomiting, cramps, and sleepiness (Antonaci, Ghiotto et al. 2016). Within the currently available ergot derivatives, dihydroergotamine is better tolerated but also less effective. The route of administration, as well as whether ergot derivatives are used in combination with another acute medication, also affects the efficacy of ergot derivatives. A meta-analysis of 11 studies found that dihydroergotamine alone was less effective than dihydroergotamine administered with an antiemetic which are drugs that are effective against vomiting and nausea. However, frequent use of ergot derivatives results in ergot-induced headaches, which is a secondary medication overuse headache that can further complicate migraine. One way to circumvent ergot-induced medication overuse headache is by pairing the drug with a preventive therapy. However, there are now other abortive therapeutics, like triptans, that migraine patients can use.

Similar to the ergot derivatives, triptans are also 5-HT agonists and have vasoconstrictive properties. Triptans are the first specific therapy for migraine and are highly selective for the 5-HT$_{1B/1D}$ receptors. These vasoconstrictors do not penetrate the blood brain barrier, which suggests that they may exert their anti-hyperalgesic effects on peripheral targets

(Ferrari and Saxena 1992). In an immunohistochemical study in rats, 5-HT_{1B} and 5-HT_{1D} receptors were found on trigeminal ganglia (TG) and on myelinated A-fibers in the TG, a peripheral region implicated in migraine-associated pain (Ma, Hill et al. 2001). Triptans have also been shown to inhibit the release of vasoactive neuropeptides within the trigeminovascular system (Ferrari and Saxena 1992). The pro-migraine neuropeptide, CGRP is highly implicated in the pathophysiology of migraine. In the rat TG, approximately 50% of CGRP+ neurons also expressed 5-HT_{1B} and 5-HT_{1D} receptors (Ma, Hill et al. 2001). These results demonstrate the colocalization of the migraine generator CGRP with 5-HT_{1B} and 5-HT_{1D} receptors, and this suggests that sumatriptan may regulate migraine-associated pain by modulating CGRP through activation of these serotonergic receptors. In cultured trigeminal neurons, sumatriptan inhibits the secretion of CGRP from sensory neurons (Durham and Russo 1999). In another study, applying sumatriptan to individual mouse dural CGRP positive nociceptive fiber terminations caused an inhibition in the amplitude of action potentials (Baillie, Ahn et al. 2012). The mechanism by which triptans modulate CGRP secretion is well characterized, and these results provide insight on the importance of regulating the net CGRP tone within the trigeminovascular system. Sumatriptan is the oldest drug in this class, and there are six second-generation triptans (zolmitriptan, naratriptan, rizatriptan, almotriptan, eletriptan and frovatriptan). While triptans are effective in stopping a headache attack, they also result in adverse side effects like flushing, tingling, neck pain and chest pressure. Considering the vasoconstrictive properties of these 5-HT agonists, migraine patients with cardiovascular issues should avoid these treatments. Additionally, frequent use of triptans can lead to medication overuse headache which can become refractory. Interestingly,

women were more likely to take triptans (17.7% women vs. 14.3% men, p<0.001) (Lipton, Munjal et al. 2018). However, this may be due to the higher prevalence of migraine in women. Like the ergot derivatives, triptans are most effective when taken in combination with a preventive therapy.

1.6.2. PROPHYLACTIC THERAPEUTICS FOR MIGRAINE

Prophylactic therapeutics are administered to prevent the frequency and intensity of a headache attack. In general, preventive treatment is recommended when migraine attack affects the patient's daily functioning 2-3 migraine days per month (Lipton, Bigal et al. 2007). Only a subset of migraineurs take preventive therapies, although approximately half would benefit from chronic treatment (Lipton, Bigal et al. 2007). In the AMPP study, only 38.7% of migraineurs used preventive treatments, and only 12.4% of migraineurs currently used these treatments (Patel, Bigal et al. 2004). Interestingly, men were also more likely than women to take preventive medication (14.5% men vs. 10.4% women, p<0.001) (Lipton, Munjal et al. 2018). To date, the variety of drugs that can be used to prevent a migraine attack include angiotensin receptor blockers and angiotensin-converting-enzyme inhibitors, antiepileptic drugs, antidepressants, and β-blockers (Silberstein, Holland et al. 2012). Overall, these agents were not originally formulated for the prophylaxis of migraine but have shown antinociceptive and preventive effects for migraine. Here, I will briefly give a background on these preventive therapies.

Angiotensin receptor blockers and angiotensin-converting-enzyme inhibitors have been found to be possibly effective for migraine prevention. angiotensin receptor blockers and

angiotensin-converting-enzyme inhibitors are antihypertensive agents and are used to treat high blood pressure and heart failure. Briefly, they help relax blood vessels and have been found to also prevent migraine. In a crossover study, chronic administration of the angiotensin receptor blocker candesartan over 12 weeks reduced the mean number of headache days (Tronvik, Stovner et al. 2003). In migraineurs aged 18 to 65, the placebo group had an average 18.5 headache days per month while the candesartan group had 13.6 headache days per month (Tronvik, Stovner et al. 2003). Similarly, chronic treatment with the angiotensin-converting-enzyme inhibitor lisinopril over 12 weeks reduced headache severity and days with headache (Schrader, Stovner et al. 2001). The exact mechanism by which these antihypertensive agents prevent migraine is unclear. However, animal studies have shown that reducing activity of the angiotensin receptors may regulate hyperalgesia (Halker, Starling et al. 2016). Specifically, administering the neuropeptide angiotensin II in the caudal ventrolateral medulla resulted in hyperalgesia, which was attenuated by the angiotensin type 1 antagonist losartan (Marques-Lopes, Pinto et al. 2009). There are a few ways in which the renin angiotensin system may regulate migraine-associated pain. First, there may be a genetic predisposition towards angiotensin-converting-enzyme polymorphisms in migraineurs (Kowa, Fusayasu et al. 2005, Horasanli, Atac et al. 2013). Specifically, an angiotensin-converting-enzyme polymorphism was present in 81.1% of migraineurs, while it was only present in 59.1% of non-migraine patients (Horasanli, Atac et al. 2013). This polymorphism was strongly associated with migraineurs with aura (Kowa, Fusayasu et al. 2005). The renin angiotensin system may also regulate hyperalgesia via nitric oxide. Specifically, angiotensin activates nuclear factor kappa B, which is subsequently associated with the

expression of inducible nitric oxide synthase (Reuter, Chiarugi et al. 2002). Nitric oxide, which is in part produced from inducible nitric oxide synthase, has been shown to induce migraine-associated pain, specifically with the nitric oxide donor NTG (Iversen, Olesen et al. 1989). These results suggest that angiotensin receptor blockers and angiotensin-converting-enzyme inhibitors may regulate migraine-associated pain by ultimately affecting downstream targets like nuclear factor kappa B and nitric oxide.

Another class of drugs commonly used for migraine prophylaxis is antiepileptic drugs. There have been promising results supporting the use of antiepileptic drugs like gabapentin and topiramate. A study found that chronic gabapentin over 12 weeks reduced the monthly migraine rate by at least 50%, and that the average number of migraine days during a headache attack was also reduced (Mathew, Rapoport et al. 2001). Additionally, topiramate has also been well characterized in the prevention of migraine (Storey, Calder et al. 2001, Brandes, Saper et al. 2004, Gupta, Singh et al. 2007, Ashtari, Shaygannejad et al. 2008, Millan-Guerrero, Isais-Millan et al. 2008). Within the first month of using topiramate, migraine patients had significantly less migraine days per month (Storey, Calder et al. 2001, Brandes, Saper et al. 2004). While migraine prevention has been well studied with topiramate, the mechanism by which topiramate prevents migraine-associated pain is unclear. However, it is possible that topiramate regulates migraine by having multiple effects on different targets, and the net effect leads to a reduction in migraine-associated pain. Topiramate blocks voltage-dependent sodium channels in rat cerebellar granule cells, calcium channels in the dentate gyrus of the rat hippocampus (Zona, Ciotti et al. 1997, Zhang, Velumian et al. 2000), and enhances the

inhibitory effect of GABA by reducing postsynaptic calcium buildup (Qian and Noebels 2003). The enhancement of GABA-medicated inhibition by topiramate has multiple downstream effects on a variety of pathways and affecting the net GABAergic tone may have a neuroprotective effect in the context of migraine.

Epilepsy and migraine may share similar pathophysiological mechanisms, and both disorders have a paroxysmal nature. In addition to a malfunctioning GABAergic, and possibly glutamatergic, system, it is possible that epilepsy has a closer connection to migraine than previously thought. Epilepsy occurs more commonly in migraineurs, and the prevalence of migraine is higher in patients with epilepsy (Haut, Bigal et al. 2006). Valproate, an antiepileptic drug, has also been found to be effective in preventing migraines. Valproate increases GABA levels and inhibits voltage-sensitive calcium channels, which ultimately reduce activation of the trigeminal nerve (Shahien and Beiruti 2012). Although the mechanisms by which valproate exerts its antimigraine effects are thought to be GABA-dependent, further research is needed to confirm the underlying mechanisms involved.

In addition to antihypertensive and anticonvulsant agents, antidepressants have also been found to prevent the chronification of migraine. Tricyclic antidepressants (TCAs) like amitriptyline and selective serotonin reuptake inhibitors (SSRIs) like fluoxetine show promising results (Adly, Straumanis et al. 1992, Saper, Silberstein et al. 1994). TCAs have been broadly used for migraine prophylaxis, and TCAs mainly exert their antidepressant effects by modulating the reuptake of norepinephrine and serotonin

(Silberstein 2006). A proposed mechanism by which TCAs modulate migraine-associated pain include modulating serotonergic tone. Specifically, TCAs can inhibit norepinephrine and serotonin reuptake which can tap into the body's natural pain modulation system, and TCAs may also enhance endogenous opioids and subsequently affect nociceptive pathways (Sawynok, Esser et al. 2001, Colombo, Annovazzi et al. 2004, Ramadan 2004, Garza and Swanson 2006). When compared to the anticonvulsant topiramate, the TCA amitriptyline was just as effective in reducing monthly migraines (Dodick, Freitag et al. 2009). In combination, amitriptyline and topiramate may be beneficial for migraine patients who also suffer from comorbid depression (Keskinbora and Aydinli 2008).

Similar to TCAs, SSRIs are also used to prevent migraine. After three months of fluoxetine treatment, migraine patients had less headache frequency, but unchanged headache severity (Saper, Silberstein et al. 1994). In another study, fluoxetine also reduced headache frequency after the first month of treatment (Adly, Straumanis et al. 1992). These antidepressants are high-affinity selective serotonin reuptake inhibitors (SSRIs) and thus inhibit 5-HT and norepinephrine uptake, resulting in more 5-HT and norepinephrine in the synaptic cleft. The modulation of serotoninergic tone could result in a protective effect, and partially explain how antidepressants prevent migraine chronification (Punay and Couch 2003, Silberstein 2006). These agents maintain the downregulated state of β-adrenergic receptors, increase the number and function of $GABA_B$ receptors, and overall contribute to a decreased excitatory tone (Pilc and Lloyd 1984, Lloyd, Thuret et al. 1985, Suzdak and Gianutsos 1986, Asakura, Tsukamoto et al. 1987, Gray and Green 1987, Ferrari, Odink et al. 1989, Martin, Pichat et al. 1989, Pratt

and Bowery 1993, Berman, Puri et al. 2006, Cornelisse, Van der Harst et al. 2007). Interestingly, this same mechanism may also give insight into why SSRIs may not be optimally suited as long-term anti-migraine agents. Enhanced 5-HT in the synaptic cleft may act on various 5-HT receptors, and continual activation of these 5-HT receptors may result in headache pain that is similar to the medication overuse headache seen after chronic triptan use. A common side effect of SSRIs like fluoxetine is headache, suggesting that while enhanced monoaminergic activity may be effective for treating depressive episodes and provide temporary headache relief, chronic 5-HT stimulation may lead to adverse effects like headache (Ferguson 2001).

Additional drugs that are used for migraine prophylaxis include β-blockers. β-blockers are β adrenergic receptor antagonists, and they block the receptor from adrenaline and norepinephrine. β-blockers like metoprolol and propranolol have been found to be effective in reducing migraine frequency, duration, and attack severity (Rao, Das et al. 2000, Diener, Hartung et al. 2001, Schellenberg, Lichtenthal et al. 2008). Although the exact mechanism underlying how β-blockers prevent migraine chronification are unknown, it is possible that the vasoconstricting effects of these drugs plays a role in the physiological maintenance of the vasculature (Boyer, Signoret-Genest et al. 2017).

Another interesting drug that has recently gained popularity for the treatment of migraine is onabotulinum toxin A (Escher, Paracka et al. 2017). Although onabotulinum toxin A is now approved by the US Food and Drug Administration for the prevention of chronic migraine, it was initially used to treat strabismus, and then later used for cosmetic

procedures (Manni, Bagolini et al. 1989). When onabotulinum toxin A is injected intramuscularly or subcutaneously, it is internalized by motor neurons and translocated to the cytosol (Escher, Paracka et al. 2017). Once inside the cytosol, onabotulinum toxin A cleaves SNAP-25, a protein that mediates the fusion of a vesicle with the cell membrane (Escher, Paracka et al. 2017). By cleaving SNAP-25, onabotulinum toxin A eliminates the protein that is critical for vesicular fusion, which will ultimately inhibit the release of neurotransmitters from the presynaptic vesicles (Escher, Paracka et al. 2017). The inhibition of presynaptic neurotransmitter release may, in part, underlie the mechanism by which onabotulinum toxin A regulates migraine-associated pain. The ability of onabotulinum toxin A to prevent neurotransmitter release via SNAP-25 is powerful. However, there are a substantial number of migraineurs that do not get relief from this procedure or the relief is not immediate (Aurora, Dodick et al. 2010, Dodick, Turkel et al. 2010). A possible explanation for why onabotulinum toxin A does not provide immediate relief in all migraine patients is that onabotulinum toxin A does not block calcium and soluble NSF attachment protein receptor (SNARE)-independent neurotransmitter release, which has been shown to occur in sensory neurons (Purkiss, Welch et al. 2000, Demarque, Represa et al. 2002). Interestingly, CGRP secretion can occur via a calcium and SNAP-25 dependent mechanism, which would be blocked with onabotulinum toxin A treatment (Durham and Masterson 2013). In the same study, CGRP secretion that occurs through a calcium and SNAP-25 independent mechanism is not altered in response to onabotulinum toxin A treatment (Durham and Masterson 2013). These two mechanisms by which CGRP can be secreted from trigeminal neurons suggest that there are at least 2 pools that release CGRP from the trigeminal system. The amount of CGRP released

from these 2 pools may be dependent on severity of the migraine, and may also suggest that there are multiple ways to induce a migraine attack. While results with onabotulinum toxin A treatment are promising, further research should explore the variety of ways that CGRP secretion occurs over the course of a migraine attack.

One of the key neuropeptides that may be involved in the pathophysiology of migraine is CGRP. Next, I will discuss the success of monoclonal antibodies targeted against CGRP and the CGRP receptor for the acute and preventive treatment of migraine. Small molecule antibodies targeted against the CGRP receptor have gone through phase 2 and 3 clinical trials, and have been found to be effective in treating acute migraine (Olesen, Diener et al. 2004, Ho, Ferrari et al. 2008, Diener, Barbanti et al. 2011, Hewitt, Aurora et al. 2011, Marcus, Goadsby et al. 2014, Voss, Lipton et al. 2016). There have been mild to moderate adverse effects, and no liver toxicity associated with these monoclonal antibodies (Diener, Barbanti et al. 2011, Marcus, Goadsby et al. 2014, Voss, Lipton et al. 2016). There has been success with monoclonal antibodies targeted against the CGRP receptor, and the U.S. Food and Drug Administration (FDA) recently approved Aimovig™ (erenumab), a CGRP receptor antagonist, and Emgality™ (galcanezumab), a CGRP blocker, for the treatment of migraine (U.S. Food & Drug Administration 2018). In addition to antibodies targeted against the CGRP receptor, monoclonal antibodies targeted against the peptide CGRP have also yielded positive results. In particular, eptinezumab (ALD403), galcanezumab (LY2951742), and fremanezumab (TEV-48215) have significantly reduced migraine days per month. Antagonizing the CGRP pathway by directly modulating CGRP or the CGRP receptor is a compelling approach and has thus

far not raised any safety issues. However, the long-term safety profile of these compounds has not yet been seen, and it is important to determine the long-term effects of antagonizing the CGRP pathway in migraine patients (Tso and Goadsby 2017).

1.7. MODELING MIGRAINE

Preclinical animal models of chronic migraine and migraine-like headaches can be used to explore the underlying pathophysiology of this disease state and can also be used to screen novel therapeutics. There are multiple models of migraine available, such as the dural inflammation model of migraine pain and a model focused on electrostimulating the trigeminovascular pathway (Phebus and Johnson 2001, Akerman, Holland et al. 2013, Strassman and Burstein 2013, Burgos-Vega, Quigley et al. 2018). Here, I will briefly cover the NTG model of chronic migraine that is primarily used in this thesis, a model which has been argued to be the best validated and most studied human model of migraine, as well as detailed methods of additional animal models are included within each chapter (Olesen and Jansen-Olesen 2012).

One approach to modeling migraine is the quantification of increased sensory sensitivity in response to known migraine triggers like NTG. NTG is a vasodilator, and is commonly prescribed to treat chest pain in coronary artery disease (Boden, Padala et al. 2015). In addition to being used in the treatment of cardiovascular disorders, a notable side effect of associated with NTG is migraine-like headache (Bagdy, Riba et al. 2010). NTG reliably triggers headache in normal subjects, and migraine without aura in migraine-susceptible patients (Iversen, Olesen et al. 1989, Christiansen, Thomsen et al. 1999, Afridi, Matharu

et al. 2005, Olesen 2008). NTG is commonly used to induced migraine in humans, and the use of this migraine trigger has been established in humans (Olesen 2008, Olesen 2010). In rodents, NTG has been used to induce migraine-associated pain, and thus we can quantify the sensory hypersensitivity associated with migraine (Bates, Nikai et al. 2010, Markovics, Kormos et al. 2012). Acute NTG was previously shown to produce thermal and mechanical allodynia in mice, which was reversed by the abortive migraine therapy sumatriptan (Bates, Nikai et al. 2010) and a CGRP receptor antagonist (Capuano, Greco et al. 2014). These results pharmacologically validated the use of NTG in models of migraine and shed light on the role of CGRP within migraine. NTG has also been used to determine whether certain genes are important for migraine susceptibility. In a transgenic mouse model of familial migraine, mice expressing a human migraine gene (casein kinase 1 delta) showed a greater sensitivity to NTG-induced hyperalgesia compared to wild-type controls (Brennan, Bates et al. 2013). Furthermore, NTG has also been shown to produce light-aversive behavior which similar to photophobia in migraineurs (Markovics, Kormos et al. 2012), and increased meningeal blood flow in mice (Greco, Meazza et al. 2011, Markovics, Kormos et al. 2012).

While NTG was first used as a trigger to induce a single episode of migraine, our lab has adapted this model to study chronic migraine. Using chronic intermittent injections of NTG, we can study the progression of migraine from an acute to chronic state (Pradhan, Smith et al. 2014). The NTG model of chronic migraine has been well characterized in humans and rodents. In rodents, each NTG treatment evokes hypersensitivity which peaks at 2 hours and lasts for several hours after each injection (Pradhan, Smith et al.

2014, Pradhan, Smith et al. 2014, Moye and Pradhan 2017). Chronic treatment with NTG also results in the development of a progressive and sustained basal hypersensitivity, in which mice remain hypersensitive to mechanical stimulation days after NTG administration (Pradhan, Smith et al. 2014, Tipton, Tarash et al. 2015). These results parallel the allodynia that may occur both between and during migraine attacks in chronic migraine patients. Furthermore, migraine preventatives like topiramate and propranolol can block NTG-induced basal hypersensitivity (Pradhan, Smith et al. 2014, Tipton, Tarash et al. 2015). Taken together, these results indicate that NTG effectively models migraine-like symptoms in rodents (Erdener and Dalkara 2014). I use the NTG model of chronic migraine in Chapters 2-4, and Chapter 4 focuses on the effect of chronic NTG on the pro-migraine peptide CGRP, the CGRP receptor, and DOR. Chapter 4 also explores the interplay between CGRP, the CGRP receptor, and DOR, providing insight into the molecular underpinnings of NTG-induced hypersensitivity.

1.8. MOLECULAR INTERACTIONS INVOLVED IN MIGRAINE

1.8.1. NITRIC OXIDE DYSREGULATION

Nitric oxide is a gaseous chemical messenger that is involved in a variety of physiological processes. Nearly all tissues produce nitric oxide, although concentration of nitric oxide is highest in the brain (Cherian, Hlatky et al. 2004). nitric oxide is synthesized from the amino acid L-arginine to L-citrulline by the enzyme nitric oxide synthase (NOS) (Cherian, Hlatky et al. 2004). Three isozymes of NOS exist: endothelial nitric oxide synthase (eNOS, NOS3), neuronal nitric oxide synthase (nNOS, NOS1), and inducible nitric oxide synthase (iNOS, NOS2) (Cherian, Hlatky et al. 2004). Both eNOS and nNOS are

constitutively expressed, and regulated by Ca^{2+}/calmodulin; nNOS is activated by an influx of Ca^{2+} via N-methyl-D-aspartate (NMDA) receptors, and is kept in close proximity to NMDA receptors via the scaffolding protein PSD-95 (Feil and Kleppisch 2008). In contrast to the constitutive forms of NOS, iNOS is upregulated following toxic or inflammatory stimuli. Many triggers can lead in inflammation, and traumas like traumatic brain injuries have been shown to increase nitric oxide production (Villalba, Sonkusare et al. 2014). Specifically, there are two peaks in nitric oxide production after traumatic brain injury, immediately after injury and a few hours-days later (Cherian, Hlatky et al. 2004). The initial immediate peak in nitric oxide is probably due to eNOS, and nNOS (Cherian, Hlatky et al. 2004). The second, late peak of nitric oxide may be due to iNOS (Cherian, Hlatky et al. 2004). Animal studies of post-traumatic headache have demonstrated that nitric oxide is a key player in the pathogenesis of post-traumatic headache, and its upregulation may contribute to migraine-like pain (Daiutolo, Tyburski et al. 2016). In addition to traumatic brain injuries, nitric oxide is fundamental to the pathophysiology of migraine. Multiple studies have demonstrated the role of nitric oxide in migraine, and NOS inhibitors, which subsequently limit availability of nitric oxide, can block headache (Ashina, Bendtsen et al. 1999). NTG, a nitric oxide donor, is a human migraine trigger and these studies suggest that nitric oxide may be involved in all phases of a migraine attack.

The primary receptor for nitric oxide is soluble guanylyl cyclase (sGC), a heterodimeric enzyme that converts guanosine triphosphate (GTP) to cyclic guanosine-3'-5'-monophosphate (cGMP) (Figure 4) (Denninger and Marletta 1999). There are several

different effectors for the intracellular second messenger, cGMP. Classically, the main receptor for cGMP includes cGMP-dependent protein kinases (cGKs) (Feil and Kleppisch 2008, Russwurm, Russwurm et al. 2013, Pradhan, Bertels et al. 2018). cGK belongs to the serine/threonine kinase family. cGK is known to phosphorylate a myriad of proteins, such as IP_3 receptor-associated cGMP kinase substrate (IRAG), which inhibits the IP_3 receptor and leads to reduced Ca^{2+} release from inner stores. The decrease in intracellular Ca^{2+} leads to a decrease in the Ca^{2+}/calmodulin complex, which binds to the myosin light chain kinase (MLCK). With a decrease in MLCK, relaxation of the vessel occurs. The vasodilation theory of migraine says that headache pain may be due to the dilation of cranial vessels, and now migraine theories have grown to take into account genetic predispositions and environmental factors.

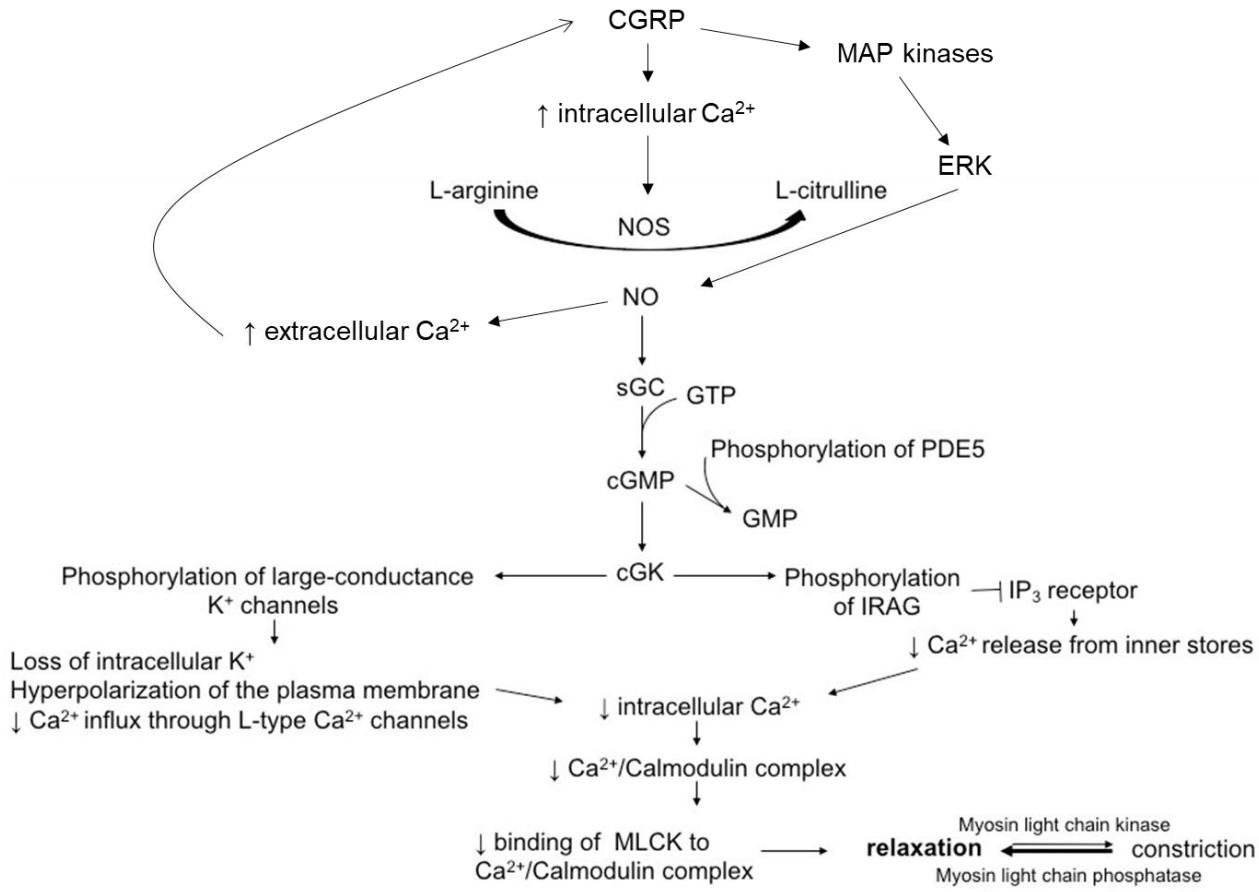


Figure 4: Schematic of the vasodilation theory of migraine. One theory of migraine touches on the physiological relationship between vasodilation and migraine-like pain. Within this theory, it is thought that certain molecules like nitric oxide and CGRP are intertwined, and that dysregulation at the level of CGRP and nitric oxide may contribute to the migraine-like phenotype we see in migraineurs. In a healthy trigeminovascular system, CGRP would contribute to nitric oxide production, and nitric oxide would exert its effects on downstream targets such as sGC, and it would also utilize extracellular Ca^{2+} to contribute to the production of CGRP. In a dysregulated environment, it is possible that enhanced CGRP release contributes to enhanced nitric oxide production, which may alter Ca^{2+} levels that further promote increased CGRP release. In this scenario, it would be ideal to blunt the effects of CGRP on the production of nitric oxide by limiting the ability of CGRP to continue contributing to its own enhanced secretion.

In addition to cGK, cGMP also acts on phosphodiesterase (PDE) 5, which accelerates termination of cGMP signal. cGMP is degraded by a few cGMP-specific PDEs, and by dual-substrate PDEs that hydrolyze both cAMP and cGMP. Through PDEs, cGMP can modulate itself or cAMP. For example, cGMP increases levels of cAMP by binding to

PDE3A, specifically by inhibiting the ability of PDE3A to hydrolyze cAMP. cGMP can also lower cyclic nucleotide levels by binding to PDE2A, or PDE5A. Furthermore, the PDE1 family is activated by the binding of Ca^{2+}/calmodulin, which provides an avenue for cross talk between Ca^{2+} and cyclic nucleotide signaling pathways. PDEs offer a platform for cross talk between cAMP and cGMP (Omori and Kotera 2007), which can form a feedback loop that may be involved in the chronification of migraine (Lamping 2001).

Interestingly, nitric oxide can also act via cGMP-independent pathways. For example, nitric oxide can modulate cellular functions by S-nitrosylation of nuclear proteins associated with cAMP response element binding (CREB) proteins. CREB proteins are involved in the regulation of DNA binding, and thus CRE-mediated gene expression. The relationship between nitric oxide and CREB could be key to the transcription of certain pro-migraine neuropeptides, such as CGRP (Freeland, Liu et al. 2000). nitric oxide has been speculated to regulate synthesis and release of CGRP, which is of importance to migraine and migraine-like headaches (Bellamy, Bowen et al. 2006). The role of nitric oxide in migraine is pertinent, as nitric oxide may act via sGC and also via cGMP-independent mechanisms to cause a cascade of signaling effects in nociceptive pathways. Sildenafil, a phosphodiesterase type 5 (PDE-5) inhibitor, results in headaches. These effects are well described, and it is possible the sildenafil exerts its pro-headache effects by increasing cGMP, which can have effects on downstream signaling pathways that may contribute to increased vasodilation (Olesen 2008, Pradhan, Bertels et al. 2018). Pro-migraine effects of nitric oxide appear to be critically mediated by sGC activation (Ben Aissa, Tipton et al. 2017).

Given the prominent role of CGRP in migraine, recent studies have focused on elucidated the relationship between CGRP and nitric oxide. An *in vitro* study investigated the effects of CGRP on nitric oxide using primary human mandibular osteoblasts (Yan, Yinghui et al. 2011). The results from this study suggest that CGRP quickly induces nitric oxide production by elevating intracellular calcium levels, which would stimulate eNOS activity, not necessarily nNOS and iNOS (Yan, Yinghui et al. 2011). Interestingly, NTG-induced CGRP release is absent in eNOS knockout mice, suggesting that eNOS may be the link between CGRP and nitric oxide (Lee, Xu et al. 2003). These results suggest that CGRP may act upstream of nitric oxide. However, it is also possible that there is a feedback loop and that while CGRP contributes to the production of nitric oxide, downstream effects of NO production can propagate effects that will ultimately stimulate CGRP.

1.8.2. CALCITONIN-GENE RELATED PEPTIDE AS A PRO-MIGRAINE PEPTIDE

Calcitonin gene-related peptide (CGRP) is now considered a pro-migraine neuropeptide, and it is now accepted that the CGRP pathway is critical to the development and maintenance of migraine (Edvinsson 2015). Since the discovery of CGRP in the 1980s, this neuropeptide has been studied as a vasodilator and pro-pain peptide within the pain pathway (Edvinsson 2015). CGRP was first localized to thin, non-myelinated nerve fibers in the cerebral vasculature and the trigeminal ganglion (Uddman, Edvinsson et al. 1985, Edvinsson, Ekman et al. 1987, Edvinsson 2017). CGRP was found to be abundant within the trigeminovascular system, a circuitry which plays a major role in the regulation of

headache (Lassen, Haderslev et al. 2002, Bigal, Walter et al. 2013). The initial experiments focused on examining CGRP in the trigeminal ganglion, and Edvinsson's group showed the vasodilatory effects of CGRP (Edvinsson 2015). Based on this finding, Edvinsson pioneered the study of CGRP in patients, with a specific focus in measuring CGRP from the jugular vein of patients with trigeminal neuralgia (Goadsby, Edvinsson et al. 1988). Using this method, increased levels of CGRP in plasma were seen in migraine patients for the first time in the clinic in the 1990s (Goadsby, Edvinsson et al. 1990). In retrospect, this finding would be the first time that CGRP was implicated in migraine, and now there are FDA-approved migraine therapeutics based on CGRP (U.S. Food & Drug Administration 2018). Here, I will talk about the role of CGRP in migraine and speculate on ways that CGRP regulates migraine-associated pain.

One way that migraine could progress is through the disruption of the trigeminovascular system, which has afferents projecting from the dura to the trigeminal ganglia (TG) (Goadsby and Edvinsson 1993). Discovered 30 years ago, CGRP could possibly have been a consequence of alternative RNA processing of the calcitonin gene (Amara, Jonas et al. 1982). CGRP has two major forms: α and β (Brain and Grant 2004). CGRPα and CGRPβ are synthesized from two distinct genes at different sites on human chromosome 11 (Wimalawansa, Morris et al. 1990). While CALC I can undergo alternative splicing to produce either calcitonin or CGRPα, CALC II only produces CGRPβ (Alevizaki, Shiraishi et al. 1986, Steenbergh, Hoppener et al. 1986). Both forms have over 90% homology and both share similar biological activities (Steenbergh, Hoppener et al. 1986). However, CGRPα is the principal form found in the central and peripheral nervous system, while

CGRPβ is found mainly in the enteric nervous system (Morris, Panico et al. 1984, Amara, Arriza et al. 1985, Mulderry, Ghatei et al. 1985). CGRP is a highly potent vasodilator and possesses protective mechanisms that play a role in the cardiovascular system and wound healing. This migraine generator is a 37-amino acid peptide, and is primarily localized to C and Aδ sensory fibers, and released from these sensory nerves in the pain pathways (Zaidi, Breimer et al. 1987). It is a well-characterized biomarker of migraine and is increased in blood plasma and saliva during migraine attacks (Cernuda-Morollon, Larrosa et al. 2013).

Once synthesized, CGRP is stored in large, dense-core vesicles within the nerve terminal. After depolarization, CGRP is released from the terminal via Ca^{2+}-dependent exocytosis, which is mediated by classical exocytotic pathways that involve soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins (Russell, King et al. 2014). Synthesis of CGRP is upregulated in tissues undergoing an inflammatory response and may be linked to the release of nerve growth factor (NGF) from macrophages. Interestingly, traumas like traumatic brain injuries can induce macrophage subsets in the brain, which may further implicate CGRP in migraine-like headaches like post-traumatic headache (Hsieh, Kim et al. 2013).

CGRP is expressed in a major portion of primary afferent neurons, and acts on the CGRP receptor (Breimer, MacIntyre et al. 1988). The functional CGRP receptor consists of the calcitonin receptor-like receptor (CRLR), complemented by the receptor activity-modifying protein 1 (RAMP1), and by an intracellular component, the receptor

component protein (RCP) (Seiler, Nusser et al. 2013). RAMP1 is responsible for the specificity of CGRP, while the association of CRLR with other RAMPs changes the specificity of the receptor to other proteins (Seiler, Nusser et al. 2013). RCP couples the receptor to the intracellular signaling pathway through $G\alpha_s$ proteins and adenylate cyclase (Evans, Rosenblatt et al. 2000). Following $G\alpha_s$-dependent stimulation of adenylate cyclase, cAMP is increased (Russell, King et al. 2014). The increase of cAMP leads to activation of protein kinase A (PKA), and in some cases the opening of ATP-sensitive K^+ channels (Russell, King et al. 2014). Increased cAMP does lead to phosphorylation of the CREB protein via a PKA-dependent pathway. The effect of CGRP on CRE-mediated gene expression could be key to understanding the molecular underpinnings of migraine. Furthermore, CGRP activates mitogen-activated protein kinases (MAPKs) (Russell, King et al. 2014). CGRP has also been found to have a neuroprotective effect against oxidative stress-induced apoptosis by activating extracellular signal-regulated kinase 1/2 (ERK1/2) and p38 MAPKs (Schaeffer, Vandroux et al. 2003).

Furthermore, recent work has speculated that CGRP and inducible nitric oxide synthase may be interacting in mediating allodynia, a phenomenon where innocuous stimuli are perceived as painful (Daiutolo, Tyburski et al. 2016). It is possible that nitric oxide regulates CGRP expression by triggering signaling mechanisms that stimulate CGRP synthesis and release (Bellamy, Bowen et al. 2006). The mechanisms of nitric oxide stimulation of CGRP secretion required extracellular Ca^{2+} (Bellamy, Bowen et al. 2006). Interestingly, activation of CGRP receptors has been demonstrated to regulate nitric

oxide release, as seen with CGRP treatment increasing inducible nitric oxide synthase expression and nitric oxide release in primary cultures of rat trigeminal ganglia (Li, Vause et al. 2008). In the NTG model of migraine, MK-8825, a CGRP antagonist, reversed NTG-induced hyperalgesia when administered with NTG and also prevented NTG-induced hyperalgesia when administered 30-60 minutes before administering NTG (Greco, Mangione et al. 2014). In a double-blind-cross-over study, 13 migraine patients were administered NTG and then were subsequently administered either BIBN4096BS, a CGRP antagonist, or a placebo (Tvedskov, Tfelt-Hansen et al. 2010). Interestingly, there was no difference between the groups and thus blocking CGRP did not reverse NTG-induced migraine in migraineurs (Tvedskov, Tfelt-Hansen et al. 2010). Altogether, these results suggest that CGRP may be upstream of nitric oxide, and this would explain why blocking CGRP after NTG had been administered did not block the onset of migraine. The relationship between CGRP and nitric oxide is intertwined, and key to elucidating the molecular underpinnings of migraine.

1.9. OPIOID RECEPTORS

The opioid receptor family includes the mu opioid receptor (MOR), the kappa opioid receptor (KOR), the nociception/orphanin FQ (NOP) receptor, and the delta opioid receptor (DOR). These opioid receptors are G-protein coupled receptors, and they primarily act through the $G\alpha_{i/o}$ protein subunits ((Dhawan, Cesselin et al. 1996)). Endogenous opioid peptides are molecules that bind to opioid receptors. Leu-enkephalin and met-enkephalin are the most selective for the DOR (Hughes, Smith et al. 1975, Hughes, Kosterlitz et al. 1997), β-endorphin is equally selective for MOR and DOR (Loh,

Tseng et al. 1976, Waterfield, Leslie et al. 1979), dynorphins bind to KOR (Goldstein, Tachibana et al. 1979), and nociception/orphanin FQ is the endogenous ligand that binds to NOP (Meunier, Mollereau et al. 1995, Reinscheid, Nothacker et al. 1995).

Although these opioid receptors are found throughout the body, the main effects most relevant to analgesia are mediated by ORs found in the brain and spinal cord. Here, I will briefly cover MOR, KOR, and NOP, and dedicate a majority of this section to discussing DOR.

1.9.1. MU OPIOID RECEPTOR, KAPPA OPIOID RECEPTOR, AND NOCICEPTIN/ORPHANIN FQ RECEPTOR

MOR is best known for its analgesic properties, but also is incredibly important in regulating reward and MOR-based agonists have high addictive potential. The MOR has been extensively studied for its antinociceptive and rewarding properties, and it has also been studied in relation to the DOR (Kieffer and Evans 2009, Gaveriaux-Ruff and Kieffer 2011, Lutz and Kieffer 2012). MOR-based agonists have been at the forefront of the national opioid epidemic. MOR has a broad neuroanatomical distribution (Mansour, Khachaturian et al. 1988, Mansour, Fox et al. 1995), and is expressed in pain-related areas like the periaqueductal gray and rostroventral medulla (Goodman, Snyder et al. 1980, Arvidsson, Riedl et al. 1995, Mansour, Fox et al. 1995). MOR agonists are anti-nociceptive, and their anti-nociceptive properties can be blocked by the MOR antagonist naloxone (Tseng and Fujimoto 1985). Additionally, intrathecal administrations of MOR agonists like morphine, codeine, meperidine, methadone, fentanyl, and DAMGO result in

increased latencies in the hotplate and tail flick tests, which can be blocked by administering MOR antagonists (Yaksh and Rudy 1976, Yaksh, Kohl et al. 1977, Pick, Paul et al. 1991). Chronic use of MOR agonists can result in opioid-induced hyperalgesia, as discussed previously.

The KOR is mainly known for its dysphoric properties (Wee and Koob 2010). Specifically, KOR activation has been shown to decrease reward, and plays a role in stress-mediated behaviors (Knoll and Carlezon 2010). KORs are distributed in patches in the striatum, and are incredibly dense in the nucleus accumbens, the pyramidal and molecular layers of the hippocampus, the granular layer of the dentate gyrus, within the thalamus, and in the hindbrain (Tempel and Zukin 1987). Overall, the KOR is expressed in most major brain areas, suggesting that the KOR may play a role in multiple behaviors (Tempel and Zukin 1987). Due to the anti-reward properties of the KOR, this receptor has been studied in regards to addiction. Specifically, the anti-reward properties of KOR activation could be particularly helpful in mediating the rewarding properties of MOR-based agonists. Additionally, the KOR has also recently been implicated in depression, and there are a few clinical trials that have used KOR antagonists for the treatment of depression (Harrison 2013). KOR agonists have also been shown to be pain relieving, but this effect is variable and may be dependent on stress-induced anti-nociception (Taylor, Roberts et al. 2015). Additionally, the interplay between KOR and DOR may provide interesting data regarding the treatment of pain. This interplay will be discussed further in this section.

NOP is the least characterized opioid receptor and is also the most recently discovered (Toll, Bruchas et al. 2016). NOP is expressed in multiple brain regions and is thought to be involved in a variety of central processes. In regard to pain, NOP has been found in the periaqueductal gray, thalamic nuclei, somatosensory cortex, rostral ventral medulla, lateral parabrachial nucleus, spinal cord, and dorsal root ganglia (Neal, Mansour et al. 1999, Florin, Meunier et al. 2000). With the advent of the NOP-eGFP knock in mouse, researchers can now explore the expression of NOP within the central nervous system (Ozawa, Brunori et al. 2015). Using this knock in mouse, NOP was found to be expressed in the spinal cord, specifically in the most superficial lamina of the spinal cord, which is a region implicated in pain (Neumann, Braz et al. 2008, Basbaum, Bautista et al. 2009). It will be interesting to see ongoing research involving NOP in pain, and possibly the interplay between NOP and DOR.

Another interesting and emerging field involves heterodimerization, and the functional consequence of heterodimerization among the 4 different opioid receptors may yield interesting results. MOR and DOR are both expressed in the caudate putamen and nucleus accumbens, which is also rich in dopamine (Narita, Funada et al. 2001, Wang, Tawfik et al. 2018). MOR and DOR are both located on primary afferents that terminate in the spinal cord, and there is evidence that a combination of both MOR and DOR are present on C, Aβ and Aδ fibers (Dado, Law et al. 1993, Arvidsson, Dado et al. 1995, Mansour, Fox et al. 1995). In contrast to the intracellular profile of the DOR, MOR has been found to be primarily expressed on the cell surface (Garzon and Pickel 2001). MOR-DOR heterodimers have been proposed, although there seems to be low expression of

these heterodimers within the central nervous system (Wang, Tawfik et al. 2018). MOR-DOR heterodimers are of particular interest because of the unique analgesic potential that they could offer. DOR-selective drugs have been found to enhance the potency of MOR-selective drugs, and these results promote the idea that MOR-DOR heterodimers exist and may be a potential therapeutic target (Gomes, Jordan et al. 2000). While it is possible that MOR-DOR heterodimers exist and play a role in analgesia, it may be more plausible that MOR and DOR work in concert and that either a MOR/DOR agonist could result in a synergistic effect of both opioid receptors. Specifically, MOR ligands have been found to allosterically enhance DOR radioligand binding, and DOR ligands also enhanced MOR radioligand binding in an established model of MOR-DOR heteromers (Gomes, Ijzerman et al. 2011). These results suggest that MOR ligands could also allosterically modulate the DOR, and vice versa (Gomes, Ijzerman et al. 2011).

KOR-DOR heterodimers have also been proposed, and it has been shown that KOR antagonism may regulate DOR agonist potency and efficacy (Jacobs, Pando et al. 2018). While activation of KOR results in dysphoria and hallucinations (Martin and Eisenach 2001), and upregulation of this receptor is associated with hyperalgesia (Wang, Gardell et al. 2001), the synergistic effect of KOR antagonism on DOR agonism may yield promising treatments for pain. The NOP receptor has been the least studied of the ORs, and there are limited antibodies targeted against the NOP receptor. The NOP receptor heterodimerizes with DOR in the periaqueductal gray and the medial vestibular nucleus, which are regions that modulate nociceptive processing and vestibular reflex (Sulaiman, Niklasson et al. 1999, Vaughan, Bagley et al. 2003, Evans, You et al. 2010).

1.9.2. DELTA OPIOID RECEPTOR

DOR agonists are a promising alternative to current therapeutics for chronic migraine and other headache disorders. Here, I will first discuss the anatomy and regulation of the DOR. Then, I will focus on behavioral data related to the DOR. Finally, I will introduce the DOReGFP knock in mouse model, which is an integral tool to the last chapter of this thesis.

1.9.2.1. REGULATION OF DELTA OPIOID RECEPTORS

DORs are $G\alpha_{i/o}$-protein coupled receptors (Gendron, Cahill et al. 2016). Following activation by an agonist (e.g. SNC80) or endogenous ligand (e.g. enkephalin), the Gα and Gβγ subunits dissociate from one another and act on various intracellular effector pathways, causing inhibition of cAMP formation (Al-Hasani and Bruchas 2011). Additionally, activation of DORs modulates Ca^{2+} and K^+ ion channels (Al-Hasani and Bruchas 2011, Pradhan, Befort et al. 2011, Gendron, Cahill et al. 2016, Vicente-Sanchez, Segura et al. 2016). After Gα dissociates from Gβγ, the Gα protein subunit directly interacts with the G-protein gated inward rectifying K^+ channel ($K_{ir}3$). Furthermore, activation of the DOR causes a reduction in Ca^{2+} currents, which is mediated by binding of the dissociated Gβγ protein subunit directly interacting with the Ca^{2+} channel. Since activation of DORs inhibits adenylate cyclase activity and subsequent cAMP formation, cAMP-dependent Ca^{2+} influx is also reduced. Activation of the DOR leads to hyperpolarization and inhibition of the neural activity of the cell. By activating DOR, it may be possible to inhibit the neural activity of the cell and thus be used as a pharmacotherapy.

DOR expression is widely distributed within the central nervous system, and has been well characterized using radioligand autoradiography and immunohistochemistry (Gouarderes, Tellez et al. 1993, Arvidsson, Dado et al. 1995, Mansour, Fox et al. 1995, Cahill, McClellan et al. 2001, Pradhan and Clarke 2005, Erbs, Faget et al. 2015). DORs are expressed in layers I, II, and VIa of the neocortex, diffusely in the striatum, moderately in the pars reticulata of the substantia nigra and in the interpeduncular nucleus (Tempel and Zukin 1987). While MORs and KORs are widely distributed in most major brain regions, DORs were only present in the forebrain and two midbrain structures (Tempel and Zukin 1987). Human autoradiography studies showed high DOR expression in the caudate, putamen, temporal cortex, and amygdala (Blackburn, Cross et al. 1988). Interestingly, there may be phylogenetic differences in the expression of DOR. Specifically, there is a high DOR mRNA expression in large dorsal root ganglion cells in the rat, but in the human dorsal root ganglion DOR mRNA was detected over small and medium-sized cells (Mennicken, Zhang et al. 2003). These results highlight the importance of translational impact, and that the exact processes in a mouse model may not be exactly replicated in a migraine patient.

The differential staining of the DOR depending on whether the antibody was tagged to the C or N terminus of the DOR also shed light on the functionality of the DOR. Most DOR expression was found to be cytosolic, and researchers began to wonder whether only plasma membrane bound DORs were functional. However, research now shows that trafficking of the DOR plays a major role in its functionality (Pradhan and Clarke 2005, Cahill, Holdridge et al. 2007). Despite the exhaustive effort that went in to characterizing

the DOR, it was later determined that antibodies targeted against the DOR resulted in varying data, and that they were differentially labeling cellular and subcellular domains (Cahill, McClellan et al. 2001).

DORs undergo a process of maturation in which the GPCRs are exocytosed from the endoplasmic reticulum (ER) to the Golgi complex, and then trafficked to the plasma membrane (Gendron, Cahill et al. 2016). While in the ER, up to 50% of the DORs may be degraded. Remaining receptors form ternary complexes with calnexin and Ca^{2+}-sensing ATPases to regulate maturation of the receptor in a Ca^{2+}-dependent manner (Gendron, Cahill et al. 2016). Once successfully folded proteins have been exported from the ER to the Golgi complex, they undergo post-translational modifications such as glycosylation (Gendron, Cahill et al. 2016). Within the Golgi complex, chaperone proteins escort the receptors to the plasma membrane. Receptors may be sorted to the constitutive or the regulated vesicular pathway. In the regulated vesicular pathway, specialized secretory vesicles are exported to the plasma membrane in response to a signal. Interestingly, ligands can stabilize different active states of the receptor, and thus produce different receptor-effector complexes. Specifically, a ligand can promote the pathway involving G-protein signaling, or arrestin-mediated signaling (Violin and Lefkowitz 2007). The varying receptor conformations initiate differing signaling and receptor trafficking events. This concept is referred to as ligand-directed signaling and adds a level of complexity to the relationship between a ligand and its receptor (Violin and Lefkowitz 2007, Pradhan, Smith et al. 2012, Schonegge, Gallion et al. 2017). One way ligands can have a bias towards specific signaling is through the selective recruitment of arrestins (Violin and Lefkowitz

2007). Arrestins are scaffolding proteins that bind phosphorylated GPCRs to regulate their receptor fate. The DOR can adopt different receptor conformations in response to different agonists. For example, SNC80, a hallmark DOR agonist, is a high-internalizing DOR agonist (Pradhan, Becker et al. 2009, Pradhan, Perroy et al. 2016). SNC80 is shown to preferentially interact with arrestin 2, while a low-internalizing DOR agonist (ARM-390), preferentially interacts with arrestin 3 (Pradhan, Perroy et al. 2016). Agonist-specific recruitment of arrestins can differentially modify the function of the DOR, as arrestin 3 is shown to facilitate the resensitization of the receptor and inhibit tolerance to DOR agonists (Pradhan, Perroy et al. 2016). The interaction between the DOR and arrestins could provide insight into how the DOR regulates migraines.

In the event of continued agonist stimulation, it is possible for receptor responsiveness to be decreased. This feedback regulatory process is called desensitization and occurs in DORs. Desensitization of the DOR is controlled via phosphorylation of the receptor followed by recruitment of arrestins (Hasbi, Polastron et al. 1998) . In particular, c-terminal phosphorylation of the Ser363, Thr353, Leu245, and Leu246 residues are important for regulation of the DOR (Bradbury, Zelnik et al. 2009). Phosphorylation of the DOR is primarily mediated by G-protein coupled receptor kinase 2 (GRK2) (Guo, Wu et al. 2000). Following phosphorylation, arrestins 2/3 are recruited to the receptor. Once internalized in clathrin-coated pits, the receptor may be headed towards degradation, or may be recycled (Lobingier and von Zastrow 2018). DORs are targeted towards the lysosome for degradation via the endosomal sorting complex required for transport (ESCRT) machinery using ubiquitination-dependent or –independent mechanisms; the interaction

of G-protein-coupled receptor associated sorting proteins (GASPs) also effect the receptor fate of the DOR (Henry, White et al. 2011, Pradhan, Befort et al. 2011).

1.9.2.2. BEHAVIORAL EFFECTS OF DOR ACTIVATION

DOR activation mediates many behavioral effects. Here, I will briefly discuss the DOR as it relates to reward and locomotion. DOR activation increases locomotion in rodents. Deltorphin II, a DOR agonist, was given to rats and this DOR activation resulted in increased locomotion, rearing, and sniffing in a dose-dependent manner (Negri, Noviello et al. 1991). In addition to locomotion, DOR activation also mediates reward (Longoni, Cadoni et al. 1998). DOR agonists BW373U86 (0.5-1.0 mg/kg, s.c.) and SNC80 (1.25-5.0 mg/kg, s.c.) elicited a preference in the place-conditioning paradigm in a dose-dependent manner. When pretreated with naltrindole (5.0 mg/kg, s.c.), a DOR antagonist, prevented this place preference, suggesting that DOR activation has rewarding properties (Longoni, Cadoni et al. 1998). However, this result has not been replicated by many other groups. Additionally, our group has shown that DOR activation does not result in conditioned place preference (Pradhan, Smith et al. 2014).

Also, DOR agonists have a low abuse liability (Negus, Gatch et al. 1998, Stevenson, Folk et al. 2005), as they are not self-administered in animal models and do not cause dependence (Brandt, Furness et al. 2001, Pradhan, Smith et al. 2012). Considering the rewarding properties of MOR-based opioids, DOR agonists have relatively less adverse effects. Additionally, DOR activation results in limited respiratory depression, sedation, and constipation when compared to MOR activation (Stenberg, Ovlisen et al. 2005, Codd,

Carson et al. 2009). It is important to note that there is controversy around the level of respiratory depression caused by DOR activation. While some agonists like DPDPE may cause respiratory depression, other DOR agonists like SNC80 will only cause respiratory depression at high doses (Codd, Carson et al. 2009). In this thesis, I use the hallmark DOR agonist SNC80 to activate the DOR.

The DOR may also play a role in anxiety and depression. By knocking out the DOR gene, it is possible to determine the role of the DOR in anxiogenic and depressant-mediated behaviors. In 2000, a DOR KO mouse was generated by deleting exon 1 of the *Oprd1* gene (Filliol, Ghozland et al. 2000). This DOR KO mouse showed no binding to the DOR antagonist [^{3}H]Naltrindole, or the DOR agonists [^{3}H]DPDPE and [^{3}H]Deltorphin in the brain or periphery. Interestingly, quantitative receptor autoradiography showed a down-regulation of MORs and KORs in the homozygous mutant mice, but not heterozygous controls (Goody, Oakley et al. 2002). This DOR KO mice was used to show that DORs regulate emotional affect. Specifically, the DOR KO mice have enhanced anxiety and depressive-like behaviors, increased locomotor activity, and impaired learning (Filliol, Ghozland et al. 2000, Kieffer and Gaveriaux-Ruff 2002, Le Merrer, Plaza-Zabala et al. 2011). Additionally, these DOR KO mice also show enhanced sensitivity in models of chronic pain (Kieffer and Gaveriaux-Ruff 2002, Le Merrer, Becker et al. 2009, Gaveriaux-Ruff, Nozaki et al. 2011). Another way to show that the DOR is involved in emotional affect is to use conditional knock out mice by deleting the DOR in specific brain regions, and determining the effect of removing the gene in behavior. Using conditional knock out mice where the DOR is only expressed in forebrain GABAergic neurons, we can further

see the role of DOR in anxiety and depression (Chu Sin Chung, Boehrer et al. 2014, Chu Sin Chung, Keyworth et al. 2015). Specifically, DLX5/6 mice express Cre in forebrain GABAergic neurons and crossing these mice to a floxed DOR mouse deletes DOR in these forebrain GABAergic cells (Ruest, Hammer et al. 2003, Monory, Massa et al. 2006). There is almost a complete deletion of DORs in the olfactory bulb, nucleus accumbens and caudate putamen, and a ~50% loss in the hippocampus. There is a relatively unchanged DOR expression in the midbrain, brain stem, and spinal cord (Chu Sin Chung, Boehrer et al. 2014, Chu Sin Chung, Keyworth et al. 2015). These DLX5/6 cKO mice have reduced levels of anxiety and depression, which is in direct contrast to the KO mouse mentioned above (Chu Sin Chung, Keyworth et al. 2015). Overall, DORs positively modulate affective state. Genetic deletion of the DOR or enkephalin, its endogenous ligand, promotes anxiogenic and depressant-like behaviors in animal models (Konig, Zimmer et al. 1996, Filliol, Ghozland et al. 2000). In contrast, DOR agonists produce anxiolytic and antidepressant like effects in mice (Broom, Jutkiewicz et al. 2002, Saitoh, Kimura et al. 2004, Jutkiewicz 2006, Perrine, Hoshaw et al. 2006, Dripps, Wang et al. 2017, Dripps, Boyer et al. 2018, Dripps and Jutkiewicz 2018). As there is a high comorbidity between migraine and mood disorders, the positive aspects of the DOR make it a promising target.

A problem with using DOR agonists is that they cause convulsions. Prior work in the lab has shown that DOR-induced convulsions may be dependent on internalization of the receptor (Pradhan, Becker et al. 2009). These DLX5/6 cKO mice have also been used to show that SNC80 produces convulsions by disinhibiting forebrain GABAergic neurons

(Chu Sin Chung, Boehrer et al. 2014). One way to avoid convulsions may be to use non-internalizing DOR agonists, like ARM390 (Pradhan, Becker et al. 2009). Headaches that have a migraine-like phenotype are often comorbid with depression, anxiety, and stress (Silberstein, Dodick et al. 2007). The capability of DOR agonists to be effective analgesics and to modulate emotion may be particularly important for the treatment of chronic migraine and its comorbidities.

Additionally, DOR activation has anti-allodynic effects. Spinal administration of deltorphin II in the rat produced a dose-dependent inhibition of the tail-flick response, which was completely abolished by naltrindole (Improta and Broccardo 1992). DOR activation also mediates mechanical and thermal analgesia (Porreca, Mosberg et al. 1984). When injected in the thalamus, the DOR peptide agonist DADLE has resulted in increased latencies in the hot plate and tail flick tests, and these results have demonstrated the important role of DORs in supraspinal brain regions. Compared to MOR agonists, DOR agonists are relatively ineffective in treating acute pain (Gallantine and Meert 2005). However, they have been shown to have increased functionality in chronic pain states, specifically in models of inflammatory and neuropathic pain (Fraser, Gaudreau et al. 2000, Hurley and Hammond 2000, Cahill, Morinville et al. 2003, Nadal, Banos et al. 2006, Gaveriaux-Ruff, Karchewski et al. 2008, Pradhan, Becker et al. 2009, Pradhan, Smith et al. 2012). In a conditional knock out mouse where DORs are knocked out in peripheral nociceptors, one can determine the role of peripheral DORs in the regulation of pain (Gaveriaux-Ruff, Nozaki et al. 2011). These $Na_v1.8$ cKO mice have ~60-70% decrease in DORs in small- and medium-sized dorsal root ganglia and trigeminal ganglia. However,

brain and spinal cord DOR expression remains intact. These $Na_v1.8$ cKO mice have a similar response to acute noxious heat and mechanical stimuli when compared to floxed controls. In an inflammatory pain model, mechanical, but not thermal, hypersensitivity is was increased. DOR agonists, including SNC80, reverse NTG-induced hypersensitivity in the NTG model of chronic migraine (Pradhan, Smith et al. 2014). Interestingly, SNC80 loses its anti-nociceptive effects in inflammatory and neuropathic pain models, but not formalin-induced pain models (Gaveriaux-Ruff, Nozaki et al. 2011).

1.9.2.3. USING THE DOREGFP KNOCK IN MOUSE MODEL

The immunohistochemical characterization of the DOR is controversial, as many DOR antibodies have shown staining in DOR knock out animals. To better investigate the DOR, genetic mouse models have been developed and they are a useful tool for exploring trafficking and functionality of the DOR. The DOReGFP knockin (KI) mouse model has been crucial to understanding the role of DOR trafficking and functioning (Scherrer, Tryoen-Toth et al. 2006). In the DOReGFP KI mouse, an enhanced green fluorescent protein (eGFP) is fused to the C-terminus of the DOR, which allows visualization of the DOR (Scherrer, Tryoen-Toth et al. 2006). These mice have been critical to determining DOR expression (Scherrer, Imamachi et al. 2009, Poole, Pelayo et al. 2011, Erbs, Faget et al. 2012, Bardoni, Tawfik et al. 2014), and *in vivo* receptor trafficking to both exogenous (Pradhan, Becker et al. 2009, Pradhan, Walwyn et al. 2010) and endogenous (Poole, Pelayo et al. 2011, Faget, Erbs et al. 2012, Bertran-Gonzalez, Laurent et al. 2013) stimuli. In these mice, DOR transcription remains functional. However, quantitative mRNA

analyses show a ~50% increase of *Oprd1* transcription when compared to wild-type littermates (Scherrer, Tryoen-Toth et al. 2006). This increase in transcription has yielded concerns within the opioid community, as results using solely the DOReGFP KI mouse could differ from a nonmutant mouse line. Chapter 4 of this thesis relies heavily on the DOReGFP knockin mouse to visualize the DOR in a migraine-associated pain state.

1.10. SUMMARY AND DISSERTATION ORGANIZATION

In summary, characterization of DOR activation in animal models of chronic migraine may promote the development of much-needed migraine pharmacotherapies. In addition to having anti-hypersensitive effects, the DOR has antidepressant and anxiolytic characteristics that make it a promising target for treating migraine-associated pain as well as comorbid psychiatric illnesses. In this thesis, I carry out a thorough behavioral and molecular characterization of the DOR in models of migraine-associated pain. This dissertation is presented in manuscript format, and each chapter embodies a series of experiments that highlight the potential of DOR agonists in models of pain. Chapter 2 has been previously published in *Cephalalgia*, and this work demonstrates the development and thorough characterization of a novel mouse model of post-traumatic headache. Within Chapter 2, chronic DOR activation prevents the development of post-traumatic headache-associated pain. In Chapter 3, which has been peer-reviewed and published, I show that DOR activation can reverse established pain in models of chronic migraine, post-traumatic headache, medication overuse headache, and the accepted model of opioid-induced hyperalgesia. In Chapter 4, I explore the molecular interactions between DOR, CGRP, and the CGRP receptor in the NTG model of chronic migraine using

C57Bl6/J mice and DOReGFP KI mice. Within this last chapter, results show a novel way in which DOR regulates migraine-associated pain.

CHAPTER 2 RATIONALE

After reviewing the literature on headaches with a migraine-like phenotype, we wanted to expand the role of the delta opioid receptor (DOR) in another form of headache, post-traumatic headache (post-traumatic headache). There was a need to develop a novel mouse model of post-traumatic headache, as most of the models available at the time were based on moderate to severe traumatic brain injuries (TBIs). Since post-traumatic headache most often develops after mTBI, it was necessary to incorporate a model of mTBI, the closed-head weight drop model, with the nitroglycerin (NTG) model of chronic migraine that was readily available in the lab. In addition to developing a model of post-traumatic headache, I also pharmacologically validated the model so that the general research community can further use it as a tool to explore the mechanisms underlying mTBI-induced pain. This model also expanded the lab's techniques in measuring cephalic allodynia, which will help the lab conduct headache-related studies in the future.

The following chapter has been previously published in *Cephalalgia*, and a detailed break down of the role of each author is also included at the beginning of this thesis.

2. THE DEVELOPMENT OF A MOUSE MODEL OF MTBI-INDUCED POST-TRAUMATIC MIGRAINE, AND IDENTIFICATION OF THE DELTA OPIOID RECEPTOR AS A NOVEL THERAPEUTIC TARGET

2.1. INTRODUCTION

Post-traumatic headache is a debilitating secondary headache disorder which occurs after traumatic brain injury (TBI) (Headache Classification Committee of the International Headache 2013, Theeler, Lucas et al. 2013, Moye and Pradhan 2017). Within the United States, more than 1 million Americans experience mild TBIs (mTBIs), and a follow-up study indicated that up to 58% of mild traumatic brain injury patients developed chronic post-traumatic headache which persisted 1 year after injury (Couch and Bearss 2001, National Center for Injury Prevention and Control 2003, Management of Concussion/m 2009, Vargas and Dodick 2012, Lucas, Hoffman et al. 2014). The most severe post-traumatic headache has a migraine-like phenotype, develops within seven days to a year after injury, and typically progresses to a chronic condition (Headache Classification Committee of the International Headache 2013). Chronic migraine associated with post-traumatic headache is defined as 15 headache days or more per month, and lasts for three or more months (Headache Classification Committee of the International Headache 2013), and is not easily resolved.

To date, there are no post-traumatic headache-specific pharmacotherapies. In general, post-traumatic migraine is clinically similar to atraumatic migraine, and many post-traumatic headache patients are highly dependent on migraine therapies for acute and preventive treatment. However, these medications do not provide sufficient pain relief in

all patients (Visser, de Vriend et al. 1996), and similar to migraineurs, post-traumatic headache patients continue to have unmet medical needs (Bigal, Serrano D Fau - Reed et al. , Lipton, Buse et al. 2013). Although migraine is commonly observed following TBI, the central mechanisms by which brain trauma leads to migraine is unclear. A predictive model of post-traumatic headache, especially one highlighting the more severe migraine-like phenotype, would aid in understanding the mechanisms regulating this disorder, and would also provide a tool to screen novel pharmacotherapies.

Post-traumatic headache is typically induced by mTBI, however many of the preclinical TBI models involve craniotomy and/or penetrative brain injuries, such as controlled cortical impact (CCI) (Elliott, Oshinsky et al. 2012). The weight-drop model produces a non-invasive closed-head injury similar to a concussive injury observed in humans (Zohar, Schreiber et al. 2003, Zohar, Rubovitch et al. 2011). This mouse model of mild traumatic brain injury does not induce substantial anatomical damage to the brain nor is there notable damage to the blood-brain barrier (Zohar, Schreiber et al. 2003). In addition, this model has been used in rats to model cephalic pain associated with post-traumatic headache (Bree and Levy 2016). Considering that the most debilitating post-traumatic headache has a chronic migraine phenotype (Hoffman, Lucas et al. 2011, Theeler, Lucas et al. 2013), the aim of our study was to combine the closed head weight drop model with the nitroglycerin (NTG) model of chronic migraine-associate allodynia. NTG is a reliable human migraine trigger (Iversen, Olesen et al. 1989, Christiansen, Thomsen et al. 1999); and has been shown to evoke allodynia in mice (Bates, Nikai et al. 2010, Pradhan, Smith et al. 2014, Pradhan, Smith et al. 2014, Tipton, Tarash et al. 2015), an effect that is

amplified in a genetic model of familial migraine (Brennan, Bates et al. 2013). In addition, NTG produces light-aversive behavior (Sufka, Staszko et al. 2016, Farajdokht, Babri et al. 2017), and increased meningeal blood flow (Greco, Meazza et al. 2011). We have shown previously that chronic intermittent NTG produces both acute allodynia and a basal hypersensitivity which acts as a model of migraine chronification (Pradhan, Smith et al. 2014, Tipton, Tarash et al. 2015). In this study we examined the effect of mild traumatic brain injury on NTG-induced acute and chronic allodynia, and validated this model using established migraine pharmacotherapies. We also tested an agonist for the delta opioid receptor (DOR), which we have previously identified as a novel target for migraine (Pradhan, Smith et al. 2014). Additionally, we examined the effect of mild traumatic brain injury on expression of the pro-migraine neuropeptide, calcitonin gene related peptide (CGRP), thus providing a link between head trauma and post-traumatic headache.

2.2. MATERIALS AND METHODS

2.2.1. ANIMALS

All experiments used male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA; Charles River Laboratories, Wilmington, MA, USA), weighing 25-30g. Mice were group housed in a 12-12 light-dark cycle, where the lights were on from 07:00-19:00. Food and water were available ad libitum. All animals were randomly assigned to either sham or mild traumatic brain injury groups, and then randomly to the different treatment groups. All responses were collected in a blinded fashion by 1-2 experimenters. Weight was recorded at time of mTBI, and on each test day for all experiments. mild traumatic brain injury did not significantly affect weight gain and did not affect mortality. All experimental

procedures were approved by the University of Illinois at Chicago Office of Animal Care and Institutional Biosafety Committee, in accordance with Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) guidelines and the Animal Care Policies of the University of Illinois at Chicago. All results are reported according to Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

2.2.2. MILD TRAUMATIC BRAIN INJURY

Mild traumatic brain injury (mTBI) was induced using the closed head weight-drop method, as described previously (Zohar, Schreiber et al. 2003). Briefly, mice were mildly anesthetized with 2.5% isoflurane with an oxygen flow rate of 0.6-0.8 liters per minute. Mice were placed chest down on a foam sponge (dimensions: 7-1/2 in. x 5-1/2 in. x 1-7/8 in) to support the head and body, which allowed for anterior-posterior motion without any rotational movement at the moment of impact. The mouse and sponge were placed directly underneath the weight-drop device. The weight-drop device consisted of a hollow cylindrical tube (inner diameter 2.54 cm, 80 cm height) placed approximately 1cm vertically over the mouse's head, in between the ear and eye. To induce mTBI, a 30g weight (13 mm diameter, 34 mm height) was dropped through the tube, striking the mouse and causing a closed head injury. Immediately after mTBI, mice were returned to their home cages for recovery for 3 days, 2, 4, or 12 weeks. All mice regained consciousness and were ambulatory within five minutes of mTBI. Sham animals were anesthetized but not subjected to the weight-drop. Sham animals regained consciousness and were ambulatory within a minute of the sham procedure.

2.2.3. DRUG PREPARATION AND EXPERIMENTAL OUTLINE

All drug injections were 10 ml/kg. Nitroglycerin (NTG) was purchased at a concentration of 5.0 mg/mL, in 30% alcohol, 30% propylene glycol and water (American Reagent, NY, USA). NTG was freshly diluted on each test day in 0.9% saline to a concentration of 1mg/mL for high dose (10 mg/kg), and 0.01 mg/mL (0.1 mg/kg) for a low dose. The vehicle (VEH) used in these experiments was 0.9% saline. We previously found that there was no significant difference in mechanical thresholds between 0.9% saline, and the solution in which high dose NTG was dissolved (6% propylene glycol, 6% alcohol, 0.9% saline)(Pradhan, Smith et al. 2014).

An experimental outline is depicted in Figure 5. After mTBI, mice were returned to their home cage. After recovery, sham and mild traumatic brain injury mice were randomly assigned to different treatment groups. To induce chronic migraine-associated pain, mice were treated with NTG or vehicle every over day for nine days (5 treatment days total). On a test day basal responses were determined, NTG/vehicle injected, and post-treatment responses determined 2h later. For experiments in Figures 6 and 7, animals were tested with vehicle, a low/subthreshold dose of NTG (0.1 mg/kg, ip), or a high dose of NTG (10 mg/kg, ip), and after the final treatment day, they were tested every other day until basal thresholds recovered to post-treatment levels. For experiments in Figures 7-11, animals were tested with NTG 2 weeks post-mTBI/sham. On test days, basal responses were determined, and animals were given a low dose of NTG (0.1 mg/kg, ip) or vehicle. One hour and 15 minutes post-NTG mice were injected with vehicle, sumatriptan (0.6 mg/kg, ip), topiramate (30 mg/kg, ip), or SNC80 (10 mg/kg, ip), and were

tested 45 min later (2h post-NTG). For topiramate experiments, mice were also pretreated with topiramate for 2 day before NTG treatment, and also on the days in between test days. To determine CGRP expression (Figure 12), mice underwent mild traumatic brain injuryor sham, and 2 weeks post-injury tissue was collected for immunohistochemical analysis.

Sumatriptan was purchased at a concentration of 12 mg/mL and was diluted to 0.06 mg/mL in 0.9% saline (Sandoz, NC, USA). Topiramate (Johnson & Johnson) and SNC80 (Tocris Bioscience) were made fresh on each test day in saline, or 0.33% 1N HCl/0.9% saline, respectively.

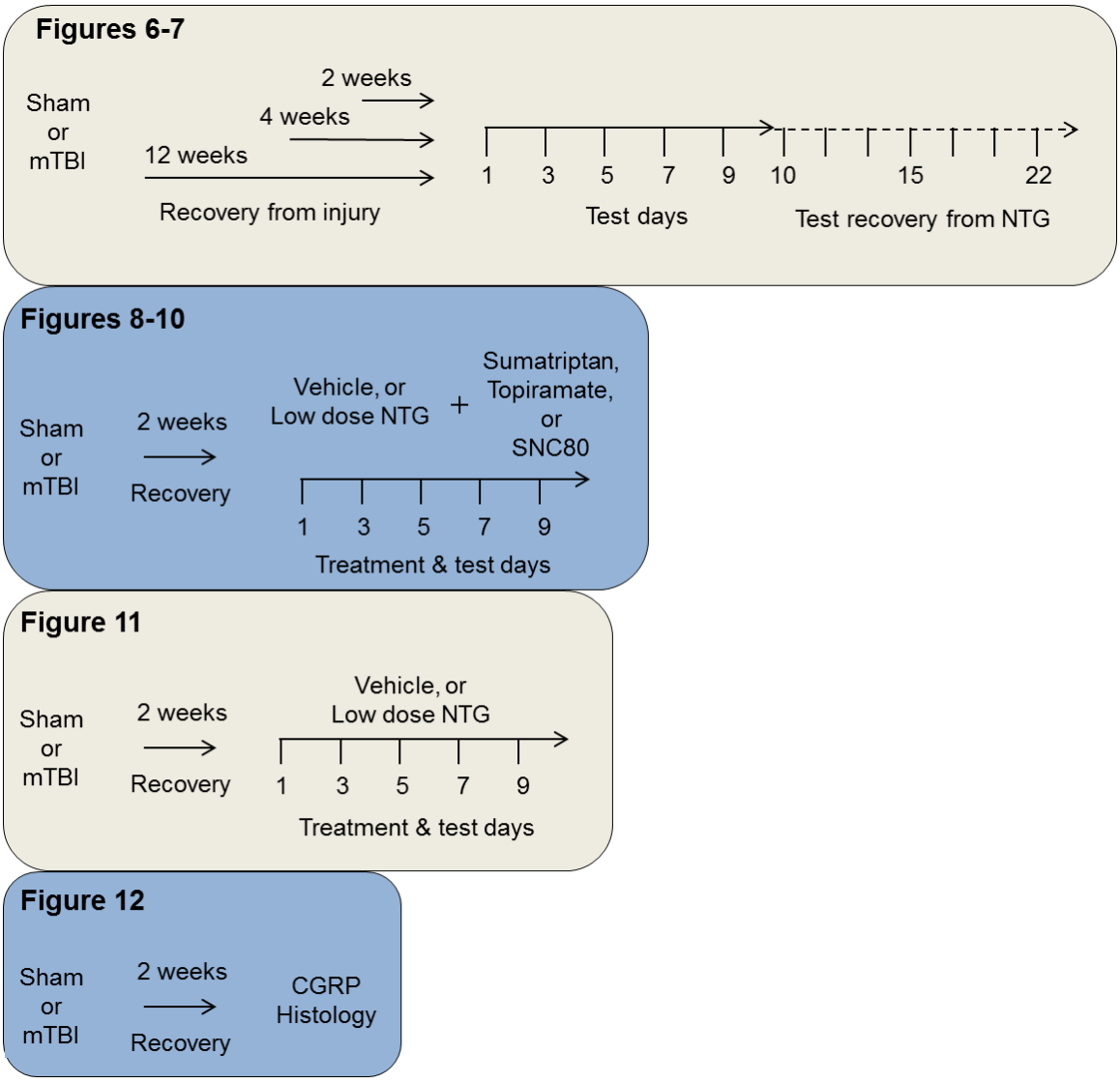


Figure 5: Schematic of experimental outline

2.2.4. SENSORY SENSITIVITY TESTING

For all behavioral experiments, mice were counterbalanced into groups following the first basal test for mechanical sensitivity. The experimenter was blinded to the injury condition of the animal and the drug condition being tested. No adverse events were observed in any of the experiments. All mice were tested in a separate behavior room with low-light (~35-50 lux) and low-noise conditions, between 09:00 and 16:00. For hind paw sensitivity,

the threshold for responses to punctate mechanical stimuli (mechanical allodynia) was tested according to the up-and-down method (Chaplan, Bach et al. 1994, Moye and Pradhan 2017). Briefly, the plantar surface of the mouse hind paw was stimulated with a series of eight von Frey hair filaments (bending force ranging from 0.008g to 2g). A response was defined as a lifting, shaking, or licking of the paw upon stimulation. The first filament tested was 0.4g. In the absence of a response, a heavier filament (up) was tried, and in the presence of a response, a lighter filament (down) was tested. This pattern was followed for a maximum of four filaments following the first response. Mice were tested as follows: 20 minutes habituation on testing rack, measurement of basal mechanical responses to von Frey hair filaments, administration of VEH/NTG, home cage for 1 hour and 40 minutes, 20 minutes habituation on testing rack, measurement of post-treatment mechanical responses to von Frey hair filaments. For cephalic sensitivity, mice were tested in 4 oz. paper cups, to which they had been previously habituated for 1h/day for 2 days. The periorbital region caudal to the eyes and near the midline was tested, similar to the up-down method described above, and herein (Ben Aissa, Tipton et al. 2017).

2.2.5. IMMUNOHISTOCHEMISTRY

Trigeminal ganglia (TG) were collected 2 weeks after mild traumatic brain injuryor sham. Mice were anesthetized with Somnasol (100 µL/mouse; 390 mg/mL pentobarbital sodium; Henry Schein, SKU#024352), and perfused intracardially with 15 mL of ice-cold phosphate buffer (0.1M PB, pH 7.2) and subsequently 50mL of ice-cold 4% paraformaldehyde (PFA)/0.1M PB (pH 7.4). TG was harvested from the mice and post-

fixed overnight in 4% PFA/0.1M PB at 4ºC. Tissue was cryoprotected in 30% sucrose/0.1M PB for 24-36 hours, or until it sank. TG was flash frozen in 2-methyl butane on dry ice, and sections of the TG were sliced at 16 µM. Upon slicing, TG sections were immediately mounted onto slides. Slides were blocked with 5% normal donkey serum in 0.1M phosphate-buffered saline with 0.3% Triton X-100 (NDST) for 1 hour at room temperature. Slides were incubated overnight at room temperature with primary sheep anti-CGRP antibody (RRID AB_725809; ab22560; Abcam; 1:1000 dilution) made in 1% NDST. Slides were washed with 1%NDST before incubating with a secondary antibody solution (Alexa Fluor 555 Donkey anti-Sheep; Life Technologies; 1:1000) made in 1% NDST for 2 hours at room temperature. Slides were washed with 0.1M phosphate buffer, and cover slipped with Mowiol-DAPI mounting solution. Images for quantification were taken by 2 observers in a blinded manner using an EVOS FL Auto Cell Imaging System, using a 20X objective. All images collected were used for analysis. Expression of CGRP was quantified by observers blinded to treatment groups. All CGRP-positive cells from all sections containing both right and left ganglia per mouse were analyzed (n=8/group). Confocal images were taken by a Zeiss Laser Scanning Microscope (LSM) 710 using a 25X objective.

2.2.6. STATISTICAL ANALYSIS

Data are expressed as mean $\pm$ s.e.m. All mice tested were included in the analysis. All statistical analyses were performed by SigmaStat software, and graphs were generated using GraphPad Prism. For all behavioral experiments, a two-way repeated-measures analysis of variance (ANOVA) was performed, with injury (sham/mTBI) and time (days)

as factors. For experiments with sumatriptan, topiramate, or SNC80, a 2-way repeated-measures ANOVA was performed, with drug and time as factors. When a significant interaction occurred, subsequent Holm-Sidak post-hoc analysis was performed. In this case, all groups were compared to thresholds for sham mice on day 1, and to sham-vehicle groups. For CGRP experiments, a Student's t-test was performed. A significance level of $p<0.05$ was used throughout this study. For the proposed experiments, we performed the following power analysis: Minimal detectable differences in means=0.3, expected standard deviation of residuals=0.4, desired power=0.8, alpha=0.05, n=15/group. Based on experience, we decreased this number accordingly.

2.3. RESULTS

A detailed description of the experiments performed in this study are outlined in Figure 5. We initially tested the effect of mild traumatic brain injuryalone on basal allodynia and observed that 3 days post-injury mild traumatic brain injurycaused a significant decrease in hind paw (Mean ± SEM, sham vs. mTBI; 1.28 ± 0.16 vs. 0.78 ± 0.14), and cephalic (sham vs. mBTI; 0.58 ± 0.04 vs. 0.27 ± 0.05) responses. As post-traumatic migraine can develop and persist long after initial injury, we wanted to test at a time when animals had recovered from the pain induced by injury alone. We therefore tested at least 2 weeks post-injury, a time at which mild traumatic brain injuryalone no longer affected basal thresholds in hind paw or cephalic regions.

2.3.1. MTBI INCREASES SENSITIVITY TO LOW-DOSE NTG

To determine the effect of mild traumatic brain injuryon susceptibility to develop migraine-associated pain mice were tested 2 weeks post-injury in the chronic NTG model. Varying doses of NTG (1-10 mg/kg, ip) have been shown previously to produce acute allodynia, and only higher doses of NTG (3-10 mg/kg, ip) produced chronic basal hypersensitivity (Bates, Nikai et al. 2010, Pradhan, Smith et al. 2014). To determine whether mild traumatic brain injuryincreased the susceptibility to developing migraine-associated pain 2 weeks post-injury, we tested a subthreshold dose of NTG (0.1 mg/kg, ip) to evoke acute but not chronic allodynia, as well as the standard high dose NTG (10 mg/kg, ip). Vehicle (VEH), low, or high dose NTG was administered every other day for 9 days (5 total test days). Mechanical thresholds were tested before (basal threshold) and 2 hours after (post-treatment threshold) VEH/NTG administration on each test day. At this 2 week post-injury time point, mild traumatic brain injuryalone did not produce a significant decrease in basal hind paw mechanical thresholds (Figure 6A, day 1). In both sham and mild traumatic brain injurygroups, a high-dose of NTG evoked both a progressive and sustained basal hypersensitivity (Figure 6A), and acute allodynia 2h post-injection (Figure 6B). Interestingly, a low-dose of NTG only produced a significant decrease in basal responses in the mild traumatic brain injurygroup, an effect not observed in the sham controls (Figure 6A); while both groups showed a significant acute allodynia to this low-dose (Figure 6B). Following the final treatment day (day 9), recovery from NTG-induced basal hypersensitivity was determined, and animals were followed until their mechanical responses returned to pre-NTG thresholds as determined on day 1. There was no significant effect of mild traumatic brain injuryon recovery time after NTG administration

(Figure 6C). These results indicate that mild traumatic brain injuryincreases sensitivity to chronic migraine-associated pain induced by repeated administration of NTG.

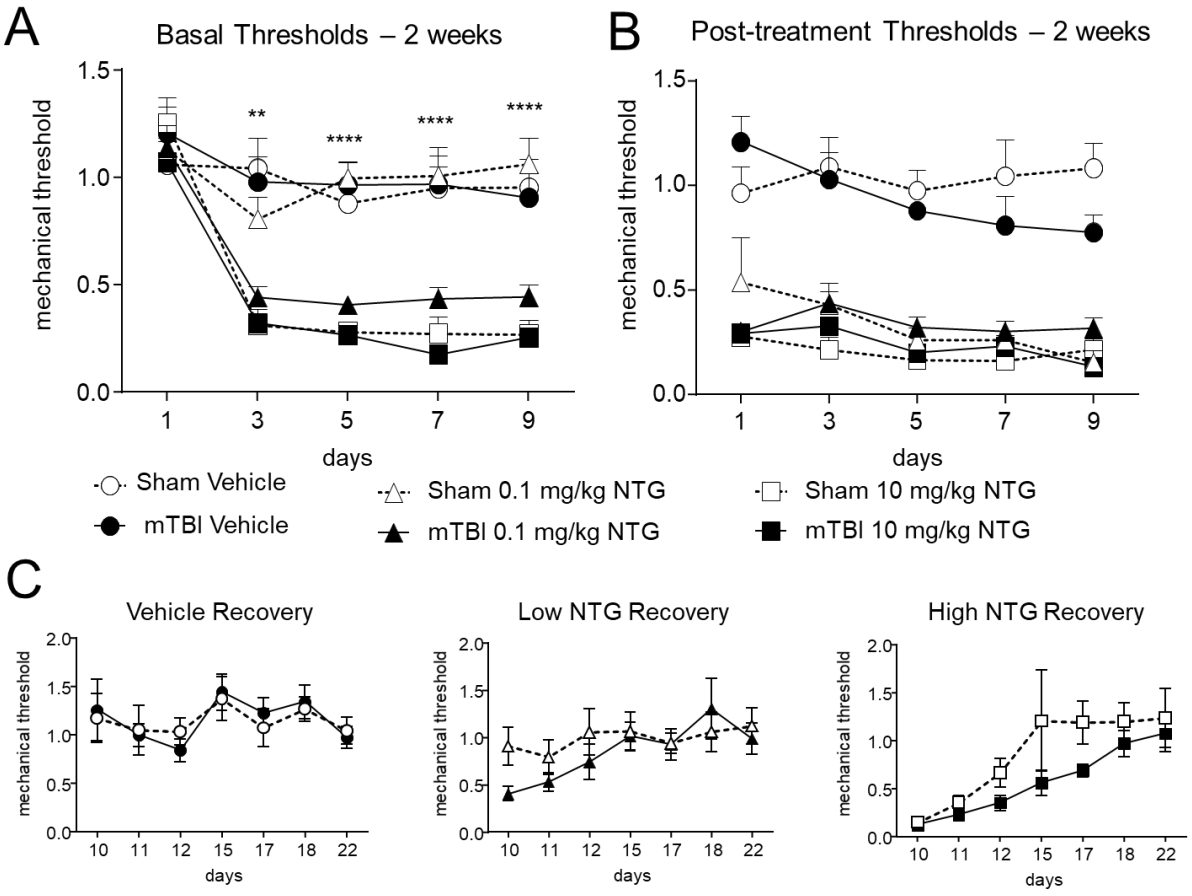


Figure 6: Mild traumatic brain injury increases mechanical hypersensitivity to a low-dose of NTG 2 weeks after closed head injury. Post-sham or injury, C57BL/6J mice received vehicle, low (0.1 mg/kg, ip), or high (10 mg/kg, ip) dose NTG every other day for 9 days (5 test days total). A) Basal mechanical thresholds, assessed prior to vehicle or NTG administration, revealed that mild traumatic brain injury animals treated with low dose NTG had significantly lower basal thresholds compared to corresponding sham controls. $p<0.01$ treatment, time, and interaction; two-way RM ANOVA and Holm-Sidak post hoc analysis. **$p<0.01$, ***$p<0.001$, n=8-12/group. B). In the same mice tested 2h post-NTG/VEH, both low- and high-dose NTG evoked hyperalgesia which did not differ between sham and mild traumatic brain injury groups. C) Recovery from NTG did not differ between mild traumatic brain injury and sham animals for any of the groups. mild traumatic brain injury animals are more susceptible to developing NTG-induced chronic pain.

2.3.2. MTBI HAS A LONG-LASTING EFFECT ON SENSITIVITY TO NTG-INDUCED CHRONIC PAIN

To determine whether the sensitivity induced by mild traumatic brain injury persists beyond 2 weeks, sham and mild traumatic brain injury groups were tested 4 and 12 weeks post-mTBI. As in Figure 6, animals were treated with vehicle, low-, or high-dose NTG every second day for 9 days (5 total test days). Again, mild traumatic brain injury alone did not alter basal responses on day 1 (Figure 7A and C). Similar to 2 weeks post-injury, both sham and mild traumatic brain injury groups developed basal hypersensitivity and acute allodynia to a high dose of NTG, and an acute response to low-dose NTG (Figure 7B and D). However, only the mild traumatic brain injury group developed a basal hypersensitivity to the low dose of NTG, an effect not observed in shams (Figure 7A and C). We tested mice following the final injection of NTG/vehicle to determine when their baselines returned to post-NTG levels. There was no difference in recovery time between sham and mild traumatic brain injury groups at 4 or 12 weeks post-injury (data not shown). The effects of mild traumatic brain injury are long-lasting, and even 12 weeks following injury mice were more susceptible to develop chronic NTG-induced pain.

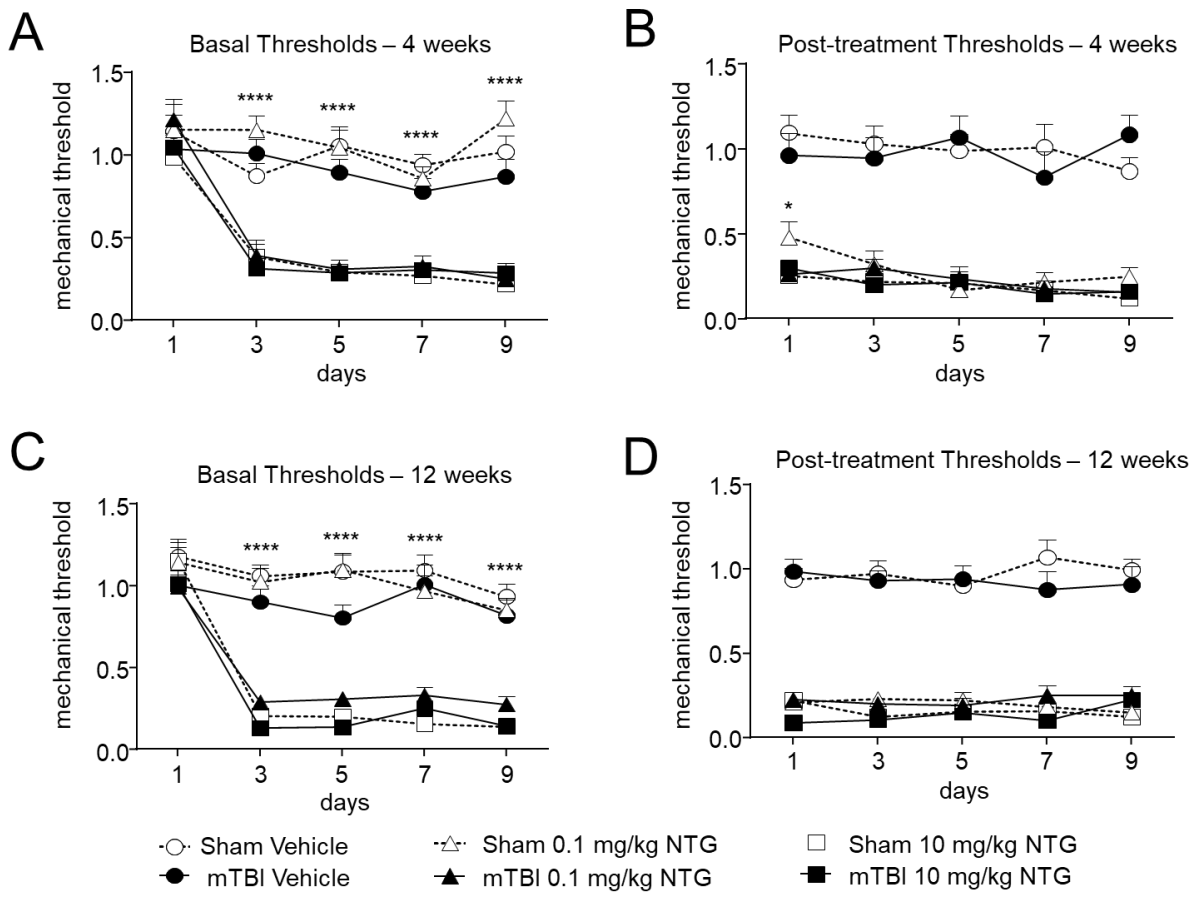


Figure 7: Mild traumatic brain injury increases mechanical hypersensitivity to a low dose of NTG 4 and 12 weeks after closed head injury. Post-sham or injury, C57BL/6J male mice received vehicle, low (0.1 mg/kg, ip), or high (10 mg/kg, ip) dose NTG every other day for 9 days (5 test days total). A & C) In both 4 and 12 week groups, assessed prior to vehicle or NTG administration, revealed that mild traumatic brain injury animals treated with low dose NTG had significantly lower basal thresholds compared to corresponding shams. $p<0.01$ treatment, time, and interaction; two-way RM ANOVA and Holm-Sidak post hoc analysis. $^{**}p<0.01$, $^{***}p<0.001$, n=11-18/group. B & D) In the same animals tested 2h post-NTG/VEH, both low- and high-dose NTG evoked hyperalgesia which did not differ between sham and mild traumatic brain injury groups. Even after 12 weeks post-injury, mild traumatic brain injury animals are more susceptible to developing NTG-induced chronic pain.

2.3.3. SUMATRIPTAN ALLEVIATES ACUTE, BUT NOT CHRONIC, ALLODYNIA WITHIN THE post-traumatic headache MODEL

To pharmacologically validate this model, we investigated the effects of the migraine abortive sumatriptan on post-traumatic headache-associated pain. At 2 weeks post-mild traumatic brain injury or sham, all animals were tested every other day for 9 days with a low-dose of NTG (0.1 mg/kg, ip), followed by vehicle or sumatriptan (0.6 mg/kg, ip; SUMA). In vehicle controls, low dose NTG induced basal allodynia in mild traumatic brain injury animals, but not sham controls (Figure 8A); and produced acute allodynia in both groups (Figure 8B). Sumatriptan significantly inhibited the post-treatment allodynia induced by NTG in both sham and mild traumatic brain injury mice (Figure 8B). Consistent with our previous findings using chronic high-dose NTG (Tipton, Tarash et al. 2015), sumatriptan did not affect the development of basal hypersensitivity to chronic low-dose NTG treatment in mild traumatic brain injury animals. However, we also observed that in sham animals, sumatriptan administration progressively lowered the basal mechanical thresholds (Figure 8A). These results indicate that while sumatriptan can reverse the acute effects of NTG after mild traumatic brain injury, it does not affect the progression of basal hypersensitivity that occurs with repeated NTG exposure. Furthermore, chronic treatment with sumatriptan alone could potentially synergize with NTG to worsen chronic migraine-associated pain.

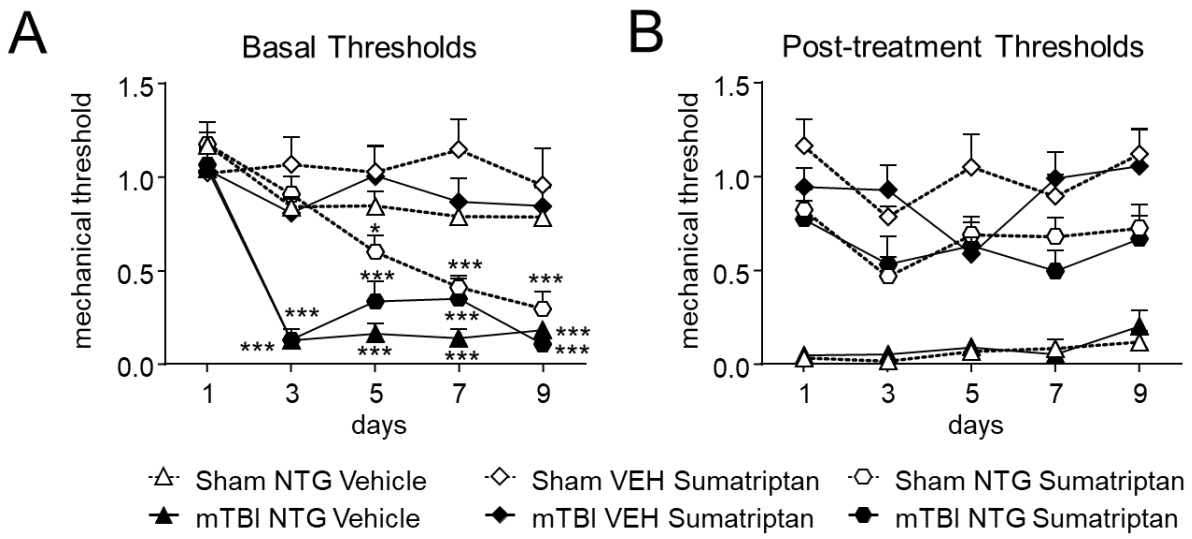


Figure 8: Sumatriptan inhibits acute post-traumatic headache-associated allodynia. Two weeks post-injury, C57BL/6J malemmice were injected every second day with low-dose NTG (0.1 mg/kg, ip), and 1h15min later with vehicle or sumatriptan (SUMA, 0.6 mg/kg, ip). A) Basal mechanical thresholds, assessed prior to drug administration, were significantly decreased in mild traumatic brain injuryanimals regardless of drug treatment. $p<0.001$ drug, time, and interaction, 2-way RM ANOVA as compared to sham-NTG-vehicle on day 1. There was also a time-dependent effect of sumatriptan on sham animals, and sumatriptan decreased the basal threshold by day 5 when compared to sham-NTG-vehicle controls; $p<0.01$ effect of drug, time, and interaction two-way RM ANOVA, Holm-Sidak post hoc analysis. n=8/group, *$p<0.05$, ***$p<0.001$ as compared to sham-NTG-veh on day 1. B) Regardless of injury, low dose NTG produced acute hyperalgesia 2 hours post-NTG, which was significantly attenuated by sumatriptan. 2-way RM ANOVA, $p<0.001$ for drug only.

2.3.4. TOPIRAMATE ATTENUATES ACUTE AND CHRONIC ALLODYNIA WITHIN THE post-traumatic headache MODEL

To further validate our model, we investigated the effects of the migraine preventive topiramate on post-traumatic headache-associated pain. At 2 weeks post-mild traumatic brain injury or sham, mice were injected with either vehicle or topiramate (TOPI, 30 mg/kg, ip) every day for 11 days. On days 3, 5, 7, 9 and 11 all animals were tested

with vehicle or low-dose NTG (0.1 mg/kg, ip). As above, low-dose NTG induced basal hypersensitivity in mTBI-vehicle treated animals, but not sham-vehicle treated controls (Figure 9A). Low-dose NTG produced acute allodynia in all vehicle controls (Figure 9B). Topiramate significantly attenuated the basal hypersensitivity to chronic low-dose NTG treatment in mild traumatic brain injury animals (Figure 9A). Furthermore, topiramate significantly inhibited post-NTG evoked allodynia in both sham and mild traumatic brain injury mice (Figure 9B). These results suggest that topiramate can reverse the acute effects of NTG after mild traumatic brain injury, and partially reduce the progression of basal hypersensitivity that occurs with repeated NTG exposure.

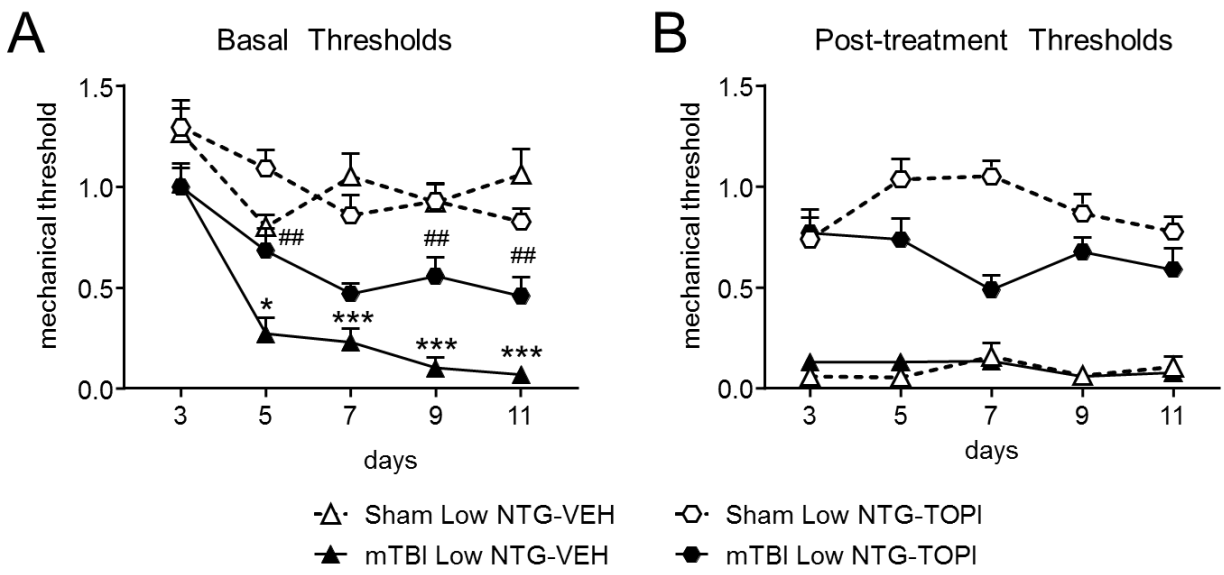


Figure 9: Topiramate inhibits both acute and chronic hyperalgesia induced by NTG. Two weeks post-injury, C57BL/6J male mice were injected every day with vehicle or topiramate (TOPI, 30 mg/kg, ip). On days 3, 5, 7, 9 and 11 mice were treated with low-dose NTG (0.1 mg/kg, ip), and 1h15min later with vehicle or topiramate. A) Basal mechanical thresholds, assessed prior to drug administration, were significantly decreased in the mild traumatic brain injurygroup treated with vehicle compared to their sham counterparts, and that effect was attenuated by topiramate. $p<0.001$, effect of injury, time and interaction two-way RM ANOVA as compared to sham-vehicle, Holm-Sidak post hoc analysis, *$p<0.05$, ***$p<0.001$ as compared to sham-vehicle day 1; mTBI-veh vs. mTBI-topiramate $p<0.05$ drug, time, interaction, two-way RM ANOVA, ##$p<0.01$ as compared to mTBI-vehicle day 1. n=8/group. B) Regardless of injury, low-dose NTG produced acute allodynia 2 hours post-NTG, which was significantly inhibited by topiramate. $p<0.001$ effect of drug only, 2-way RM ANOVA.

2.3.5. SNC80 INHIBITS ACUTE AND CHRONIC ALLODYNIA WITHIN THE post-traumatic headache MODEL

We next tested the delta opioid receptor (DOR) agonist, SNC80, within this model of post-traumatic headache-associated pain. As above, at 2 weeks post-injury all animals were treated every other day for 9 days with low-dose NTG (0.1 mg/kg, ip), and subsequently with vehicle or SNC80 (10 mg/kg, ip). Again low-dose NTG evoked basal hypersensitivity only in mild traumatic brain injury animals (Figure 10A); and acute post-treatment allodynia in both sham and mild traumatic brain injury mice (Figure 10B). Treatment with SNC80 significantly attenuated chronic basal hypersensitivity induced by low-dose NTG in the mild traumatic brain injury group (Figure 10A). Furthermore, SNC80 reversed NTG-induced acute allodynia in both sham and mild traumatic brain injury groups on each test day (Figure 10B). These data indicate that SNC80 may not only inhibit acute post-traumatic headache-associated pain but may also restrict the development of chronic post-traumatic headache-associated pain.

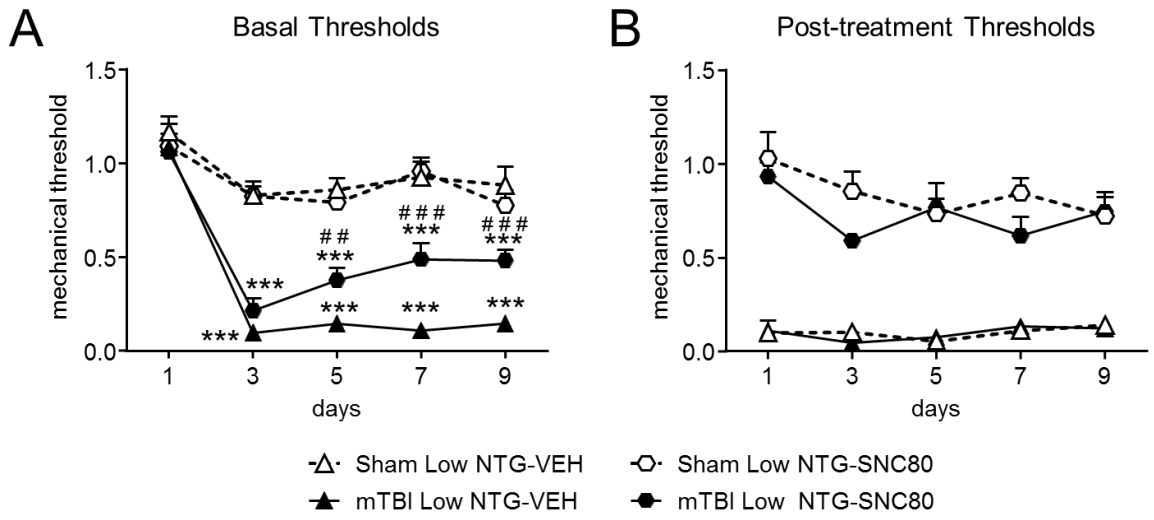


Figure 10: SNC80 inhibits both acute and chronic allodynia induced by NTG. Two weeks post-injury, C57BL/6J male mice were injected every second day with low-dose NTG (0.1 mg/kg, ip), and 1h15min later with vehicle or SNC80 (10 mg/kg, ip). A) Basal mechanical thresholds, assessed prior to drug administration, were significantly decreased in mild traumatic brain injury groups treated with vehicle compared to their sham counterparts, an effect that was attenuated by SNC80. $p<0.05$, effect of injury, time and interaction two-way RM ANOVA, Holm-Sidak post hoc analysis, ***$p<0.001$ as compared to sham-vehicle. $p<0.001$ drug, time, interaction, two-way RM ANOVA mTBI-veh vs. mTBI-SNC80, ## $p<0.01$, ###$p<0.001$ as compared to vehicle day 1. n=8-14/group. B) Regardless of injury, low-dose NTG produced acute hyperalgesia as determined 2 hours post-NTG, which was significantly inhibited by SNC80. $p<0.001$ effect of drug only, 2-way RM ANOVA.

2.3.6. MTBI INCREASES CEPHALIC HYPERSENSITIVITY TO LOW-DOSE NTG

To determine whether cephalic responses differed from hind paw responses, we tested the effect of low dose NTG on cephalic allodynia. At 2 weeks post-injury, mild traumatic brain injury and sham mice had similar cephalic mechanical thresholds on day 1 (Figure 11A, day 1). On each test day, low-dose NTG produced acute periorbital allodynia 2h post-administration regardless of injury (Figure 11B). In addition, mild traumatic brain injury mice treated with low-dose NTG also developed a profound basal hypersensitivity, an effect which was not seen in sham mice (Figure 11A). These results indicate that

similar to the hind paw, mild traumatic brain injury results in heightened sensitivity to the development of chronic migraine-associated pain.

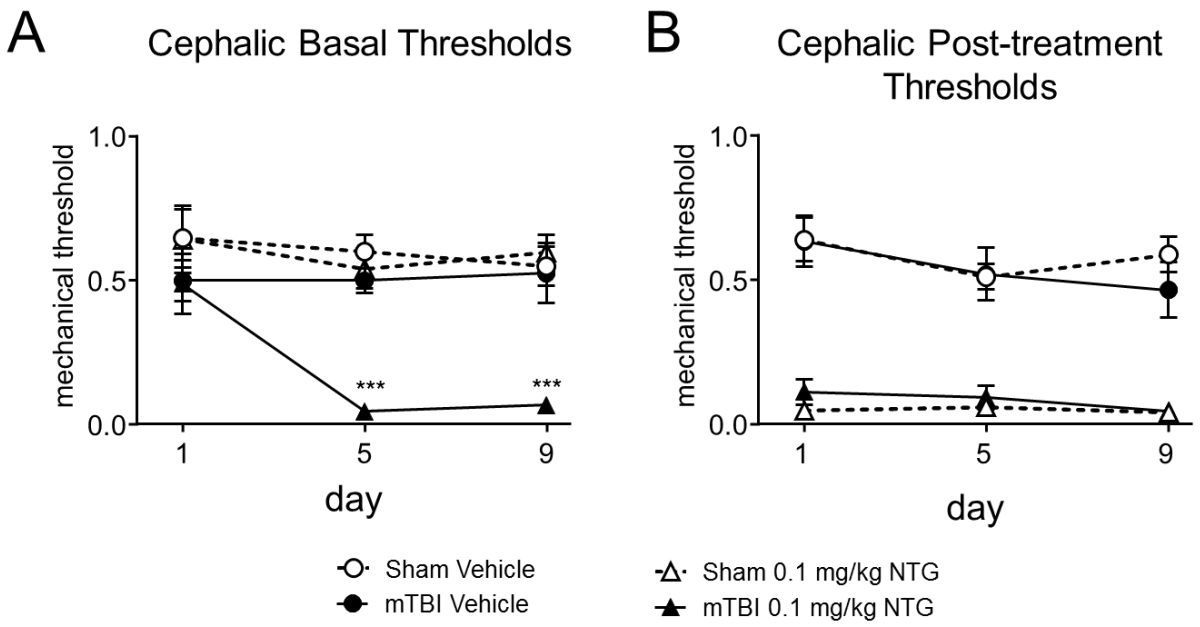


Figure 11: Mild traumatic brain injury increases cephalic mechanical hypersensitivity to a low-dose of NTG 2 weeks after closed head injury. Post-sham or injury, C57BL/6J male mice received either a vehicle or low dose NTG (0.1 mg/kg, ip) every day over 9 days, and tested every 4th day (days 1, 5, 9). A) Basal thresholds, assessed prior to vehicle or NTG administration, revealed that mild traumatic brain injury animals treated with low dose NTG had significantly lower basal cephalic thresholds than their sham counterparts. $p<0.001$ treatment, time and interaction; two-way RM ANOVA, Holm-Sidak post hoc analysis, ***$p<0.001$, n=8/group. B) In the same mice tested 2h post NTG/VEH, NTG evoked hyperalgesia which did not differ between sham and mild traumatic brain injury groups. mild traumatic brain injury increases the development of cephalic hypersensitivity to a low dose of NTG.

2.3.7. MTBI INCREASES EXPRESSION OF CGRP WITHIN THE TRIGEMINAL GANGLIA 2 WEEKS POST-INJURY

CGRP is considered to be an endogenous migraine generator, and plays a critical role in the regulation of migraine pain (Bigal, Walter et al. 2013). Trigeminal ganglia (TG) are first order cells that regulate head-specific pain, and we determined immunohistochemically if mild traumatic brain injury affected the amount and number of

CGRP expressing (CGRP+) cells within this region. We observed that 2 weeks post-injury, mild traumatic brain injury produced a significant increase in the overall expression of CGRP in each cell (Figure 12A), as well as an increase in the total number of CGRP+ cells (Figure 12B) relative to sham controls. Our results indicate that this mild traumatic brain injury procedure dynamically alters the expression of CGRP within the TG.

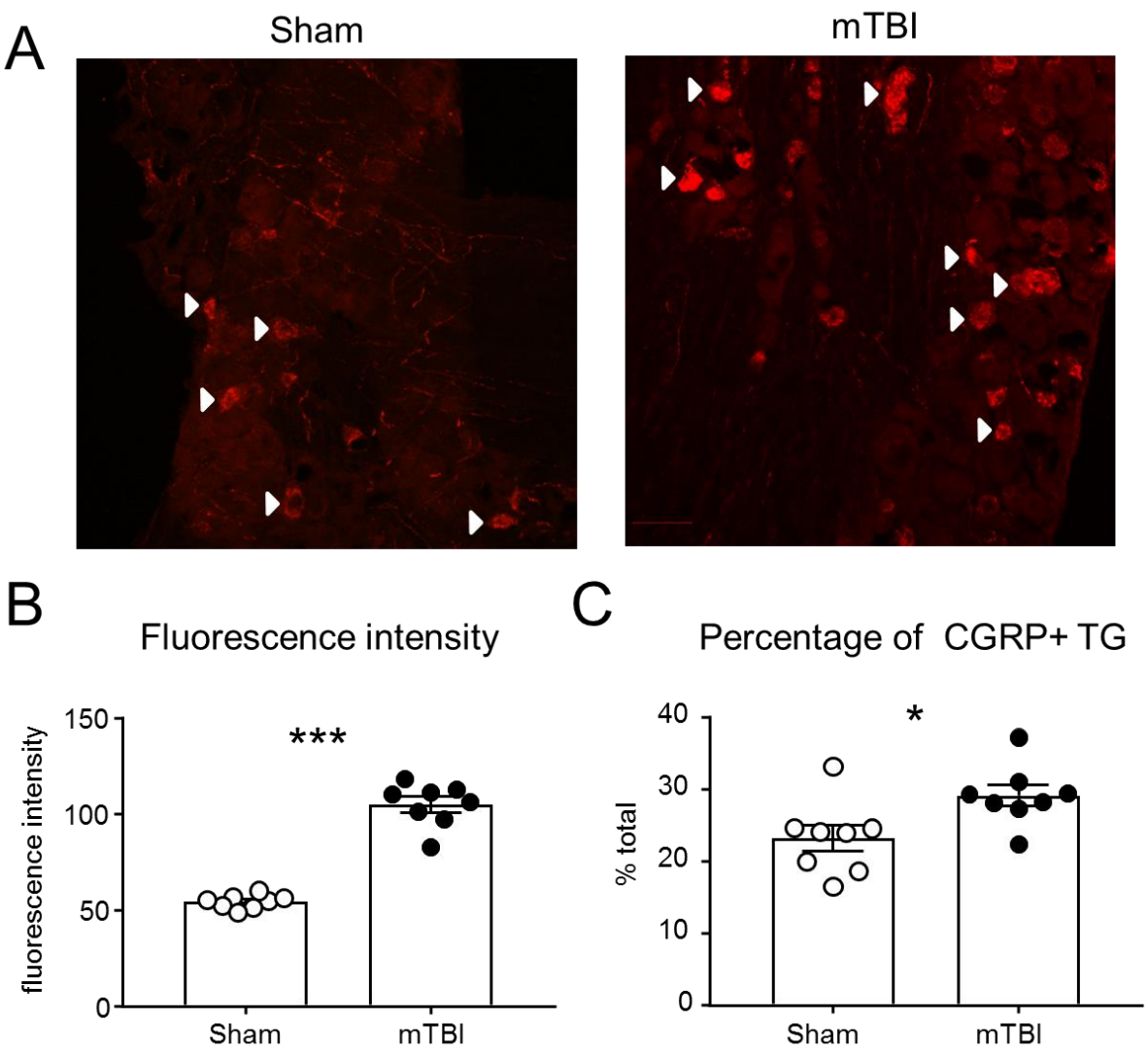


Figure 12: mild traumatic brain injurycauses an increase in the expression of the pro-migraine neuropeptide, CGRP, in the trigeminal ganglia. C57BL/6J male mice underwent a sham/mild traumatic brain injuryprocedure, and trigeminal ganglia was analyzed for CGRP quantification at 2 weeks post-injury. A) Representative images of trigeminal ganglia from sham and mild traumatic brain injurymice. White arrow heads indicate some, but not all CGRP+ ganglia. B) Quantification of the fluorescent intensity of CGRP positive cells shows that mild traumatic brain injurysignificantly increases the amount of CGRP in the TG. ***$p<0.001$, t-test, n=8 mice/group. C) Quantification of the percentage of CGRP positive cells show that mild traumatic brain injurysignificantly increased the overall number of TGs expressing CGRP. $p<0.05$, t-test, n=8 mice/group.

2.3.8. DISCUSSION

Despite the high prevalence of post-traumatic headache, the mechanisms underlying the progression from head trauma to post-traumatic headache remain unclear. A primary goal of this study was to characterize a mouse model of post-traumatic headache which combined published models of closed head injury and chronic migraine (Zohar, Schreiber et al. 2003, Pradhan, Smith et al. 2014). We demonstrate that this mild traumatic brain injury procedure alone produced mechanical allodynia at 3 days post-injury, but that hypersensitivity was resolved by 2 weeks post-injury. However, mild traumatic brain injury mice were more sensitive to the development of chronic migraine-associated pain as induced by low dose NTG, an effect observed in both cephalic and somatic regions. Acute allodynia within this model was blocked by the migraine abortive, sumatriptan; and acute and chronic post-traumatic headache-associated pain was inhibited by the migraine preventive topiramate. We also found that the selective delta opioid receptor agonist, SNC80, inhibited acute and chronic allodynia in this model, identifying this receptor as a novel therapeutic target for post-traumatic headache. Additionally, 2 weeks following closed head injury we observed an increase in the expression of the migraine-associated neuropeptide, CGRP in the trigeminal ganglia, which provides a potential mechanism for the heighted sensitivity to the development of chronic migraine associated with mild traumatic brain injury.

We have previously shown that chronic intermittent treatment with higher doses of NTG (3-10 mg/kg) can produce a progressive basal hypersensitivity in mice (Pradhan, Smith et al. 2014). In this study we tested a lower dose of NTG, which did not cause basal

hypersensitivity in sham controls, but significantly reduced mechanical thresholds in mild traumatic brain injury animals. This effect was long lasting, as sensitivity to low-dose NTG was still seen 12 weeks post-injury. Our findings are in keeping with the original characterization of the closed head weight-drop model in which long-term cognitive deficits were observed in the absence of structural damage to the brain (Zohar, Schreiber et al. 2003). However, this relatively mild TBI can still cause adaptations, especially at the level of inflammatory responses. Increased gene expression of the cytokine CCL13 was observed up to 7 days post-injury in this model (Israelsson, Wang et al. 2009), and increased dural mast cell degranulation was also found up to 30 days post-injury (Levy, Edut et al. 2016). Furthermore, closed head injury models using a heavier weight (50g, as compared to 30g used herein) have resulted in elevated levels of tumor necrosis factor-alpha (TNF-α) post-TBI (Baratz, Tweedie et al. 2015). One possibility is that neuroinflammation induced by mild traumatic brain injury can ultimately trigger sensitization of the trigeminovascular complex resulting in post-traumatic headache (Moye and Pradhan 2017). In patients, post-traumatic headache can develop 1 week to 1 year after injury, and may even manifest outside of this time frame (National Center for Injury Prevention and Control 2003, Headache Classification Committee of the International Headache 2013, Moye and Pradhan 2017). The mild nature of the injury used in this model may reflect sensitization to sub-concussive head trauma and may contribute to the major inflammatory changes shown in previous studies. Future studies will focus on characterizing the effect of anti-inflammatory agents within our model of post-traumatic migraine. Our results reflect the finding that a single mild traumatic brain injury can have long-term effects on the susceptibility to developing chronic post-traumatic

headache. It should be noted that we only used C57BL/6J mice, as the NTG dosing regimen has been well characterized in this mouse strain (20). Other mouse strains may respond differently to mild traumatic brain injury, and/or have a different dose response to NTG.

To determine the predictive validity of this model of post-traumatic migraine, we tested the migraine abortive, sumatriptan, and the preventive, topiramate. A clinical study examining the treatment of post-traumatic headache in soldiers found that triptans significantly alleviated post-traumatic headache, and topiramate could act as an effective preventive (Erickson 2011). In our study, sumatriptan significantly inhibited the acute allodynia induced by low dose NTG in mild traumatic brain injury animals, which is consistent with previous work using high doses of NTG (Bates, Nikai et al. 2010, Pradhan, Smith et al. 2014, Pradhan, Smith et al. 2014). We were surprised to find that in sham animals treated with low dose NTG and sumatriptan there was a decrease in basal responses. Chronic daily treatment with sumatriptan can be used to model medication overuse headache (De Felice, Ossipov et al. 2010, Tipton, Tarash et al. 2015), although in our study sumatriptan was only administered every other day. Chronic treatment with sumatriptan alone may act with low-dose NTG to exacerbate migraine-associated pain through a yet undetermined mechanism. Chronic daily administration of the migraine preventive, topiramate, alleviated both the acute allodynia and chronic basal hypersensitivity induced by low-dose NTG in the mild traumatic brain injury animals. These results are consistent with clinical reports which show that topiramate can be effective in the treatment of chronic post-traumatic headache (Erickson 2011, Minen,

Boubour et al. 2016), and has been used extensively as a migraine preventive (Diener, Bussone et al. 2007). These experiments were performed in the periphery. We have previously shown that sumatriptan and topiramate (Pradhan, Smith et al. 2014), along with the migraine preventive propranolol (Tipton, Tarash et al. 2015) can block migraine-associated pain induced by high dose NTG, also assessed in the periphery. In addition, in the dural inflammation model, application of inflammatory mediators to the dura produced mechanical sensitivity in both cephalic and hind paw regions (Edelmayer, Le et al. 2012, Edelmayer, Ossipov et al. 2012), similar to the effects observed in our study; and these results likely reflect the development of central sensitization which may be mediated through neurons within the thalamus (Burstein, Jakubowski et al. 2010). Together, our pharmacological results support the use of this mouse post-traumatic headache model as a pharmacological screening tool.

There are limited therapeutic options for the treatment of post-traumatic headache, and many patients use established migraine therapies which do not provide sufficient pain relief in all patients (Visser, de Vriend et al. 1996). We have previously shown in preclinical models that DOR activation can inhibit multiple migraine-associated symptoms, including allodynia, negative affect, and aura (Pradhan, Smith et al. 2014). In addition, anatomical studies have shown that DOR can be co-expressed with CGRP in the TG (Rice, Xie et al. 2016), thus further supporting the role of DOR as a potential therapy for migraine-associated pain. In our study we found that SNC80 could block post-traumatic headache-related acute allodynia, and that it had a protective effect on the mTBI-NTG induced basal hypersensitivity. DOR may be a particularly promising target for TBI-associated

pathologies. For example, DOR agonists are effective in models of peripheral hyperalgesia (Pradhan, Befort et al. 2011, Charles and Pradhan 2016), and chronic pain conditions, including headache, are a major source of disability following TBI (Nampiaparampil 2008). Importantly, DOR agonists produce anxiolytic and antidepressant effects (Filliol, Ghozland et al. 2000, Pradhan, Befort et al. 2011, Lutz and Kieffer 2012). Emotional dysregulation is often comorbid with chronic pain and migraine and contribute to a feed forward cycle of disability. Post-traumatic stress disorder is especially comorbid with post-traumatic headache, and its presence is associated with increased severity of post-traumatic headache (Theeler, Mercer et al. 2008, O'Neil, Carlson et al. 2013, Theeler, Lucas et al. 2013, Scofield, Proctor et al. 2017). The ability of DOR agonists to alleviate negative emotional states, would be beneficial in these more complicated clinical situations. The delta opioid receptor may be uniquely positioned to alleviate multiple aspects of mTBI-related pathologies, including post-traumatic headache.

We observed that CGRP expression was significantly increased in TG following mTBI, and we postulate that this augmentation likely promotes the development of post-traumatic headache from traumatic brain injury (Moye and Pradhan 2017). This increase was observed 2 weeks post-injury, a time at which allodynia induced by mild traumatic brain injury alone was already resolved. CGRP is an endogenous migraine generator, and this neuropeptide plays a critical role in migraine pathophysiology. CGRP infusion can induce headache (Lassen, Haderslev et al. 2002), and levels of CGRP in the circulation are upregulated during acute migraine attacks (Goadsby,

Edvinsson et al. 1990). Additionally, CGRP receptor antagonists are effective in aborting migraine (Olesen, Diener et al. 2004); and antibodies targeting CGRP and its receptor are currently in drug development with promising results in late stage clinical trials (Hou, Xing et al. 2017, Tso and Goadsby 2017). In terms of mTBI-related pain, experiments performed in rats found that both TBI by controlled cortical impact (Elliott, Oshinsky et al. 2012, Theeler, Lucas et al. 2013, Daiutolo, Tyburski et al. 2016) and repeated mild head injury (Tyburski, Cheng et al. 2017) resulted in increased CGRP expression in the trigeminal nucleus caudalis as compared to controls. The TG is a major source of CGRP to the trigeminal nucleus caudalis (Edvinsson 2017, Goadsby, Holland et al. 2017), and together these structures form part of the trigeminovascular complex which regulate head-specific pain. The CGRP antagonist MK8825 was also found to attenuate both periorbital allodynia and photosensitivity evoked by controlled cortical impact injury (Daiutolo, Tyburski et al. 2016). Furthermore, in a rat model CGRP inhibition blocked increased sensitivity to NTG (Bree and Levy 2016). In this study, weight-drop increased acute periorbital allodynia evoked by NTG up to 30 days post-injury, and this allodynia was blocked by a CGRP antibody (Bree and Levy 2016). NTG also increased conditioned place aversion in mild traumatic brain injuryrats, an effect that was blocked by CGRP antibody treatment (Bree and Levy 2016). Our study further supports the role of CGRP as a link for the development of post-traumatic headache following mild traumatic brain injury and expands the role of this neuropeptide for post-traumatic headache with a chronic migraine-like phenotype. Taken together, these results suggest that our model reflects the role of CGRP in the development to post-

traumatic headache and support the notion that upcoming CGRP-targeted therapies will be promising for the treatment of this disorder.

post-traumatic headache is a debilitating disorder which can result in chronic disability and decreased quality of life. A better understanding of the mechanisms that regulate post-traumatic headache would allow for the discovery of more targeted approaches to treat this disorder. Here, we have characterized a novel mouse model of post-traumatic headache, one which specifically reflects the more severe post-traumatic chronic migraine phenotype. The development of this model opens up the possibility for investigators to easily use genetic, opto- and chemogenetic approaches which have been optimized for use in mice. In addition, this model can be used to screen novel therapies for post-traumatic headache, and we have used it to identify the delta opioid receptor as a promising target. We also recapitulate findings that CGRP is an important facilitator between mild traumatic brain injury and the development of post-traumatic headache. Future studies will use this model to further identify the molecular mechanisms regulating post-traumatic headache.

CHAPTER 3 RATIONALE

The previous chapter focused on the characterization of a novel model of post-traumatic headache, and I used this model as a tool to screen the DOR as a promising therapeutic. After demonstrating the anti-allodynic properties of DOR activation in a model of post-traumatic headache, I wanted to expand and determine whether DOR activation produced similar results in other models of headache. I also was interested to see whether chronic DOR would result in hyperalgesia, much like chronic use of sumatriptan/opioids result in hyperalgesia. These questions shaped the aims that would become the next chapter.

This next chapter has also been peer-reviewed and published in *Neuropharmacology*. A detailed break down of the role of each author is also included at the beginning of this thesis.

3. DELTA OPIOID RECEPTOR AGONISTS ARE EFFECTIVE FOR MULTIPLE TYPES OF HEADACHE DISORDERS

3.1. INTRODUCTION

Headache disorders are ranked as the third highest worldwide for years lost to disability (Burstein, Noseda et al. 2015, 2017). Primary headaches are due to the headache condition itself and include migraine. Although episodic migraine is more common, chronic migraine is more debilitating, and these patients experience at least 15 or more headache days/month (Headache Classification Committee of the International Headache 2013). While a number of preventatives are available, they are not highly effective and have low tolerability (Blumenfeld, Bloudek et al. 2013). Secondary headaches are defined as headaches that are due to another medical condition (Headache Classification Committee of the International Headache 2013), and common causes include traumatic brain injury and medication overuse. Post-traumatic headache is highly prevalent, and more than 50% of mild traumatic brain injury (mTBI) patients go on to develop post-traumatic headache, which can last for up to 5 years post-injury (Stacey, Lucas et al. 2017). Medication overuse headache (medication overuse headache) is observed following chronic use of medications prescribed for headache which paradoxically exacerbate and increase the frequency of headache (Headache Classification Committee of the International Headache 2013). For example, medication overuse headache has been reported for triptan overuse, a commonly prescribed class of acute migraine medications (Limmroth, Katsarava et al. 2002, Katsarava, Schneeweiss et al. 2004). Similarly, chronic use of opioids results in opioid induced hyperalgesia, a phenomenon where pain severity increases beyond the original pain, and expands in area

(Hayhurst and Durieux 2016). Opioids are commonly prescribed for migraine, and can produce headache that is more frequent and severe, and refractory to other treatment (Bigal and Lipton 2009, Buse, Pearlman et al. 2012, Thorlund, Sun-Edelstein et al. 2016). Currently, the first line of treatment for medication overuse headache and opioid-induced hyperalgesia is withdrawal of the overused drug (Diener, Holle et al. 2016), but this has low patient compliance, and adjunct therapies that are mechanistically distinct from the medication overuse headache-causing drug would be helpful.

Despite the high prevalence of headache disorders, patients have limited therapeutic options. Our group recently identified the delta opioid receptor (DOR) as a promising target for migraine (Pradhan, Smith et al. 2014, Charles and Pradhan 2016). We found that in a nitroglycerin (NTG) preclinical model of migraine, DOR agonists significantly inhibited migraine-associated allodynia and conditioned place aversion, a correlate of migraine-associated negative affect (Pradhan, Smith et al. 2014). In addition, we also observed that DOR activation decreased the number of cortical spreading depression events in a model of migraine aura (Pradhan, Smith et al. 2014). Our group also recently developed a model of post-traumatic migraine (Moye, Novack et al. 2018), the most severe form of post-traumatic headache (Theeler, Lucas et al. 2013); and again DOR activation effectively prevented the development of chronic migraine induced by mild traumatic brain injury.

Clinically, headache disorder patients present with already established headache. To date, we have shown that DOR agonists can alleviate the development of migraine or

post-traumatic headache, but it is unknown if DOR activation can effectively block already established pain associated with primary and secondary headaches. The aim of this study was to determine if the DOR agonist, SNC80, could alleviate established cephalic and peripheral allodynia in models of chronic migraine, post-traumatic headache, medication overuse headache associated with triptans, and opioid-induced hyperalgesia. Further, we also determined if chronic DOR activation could itself produce a medication overuse headache/opioid-induced hyperalgesia state.

3.2. MATERIALS AND METHODS

3.2.1. ANIMALS

Experiments were performed on male and female C57BL6/J mice (Jackson Laboratories, Bar Harbor, ME. USA), weighing 20-30g, and no sex differences were observed. Mice were group housed in a 12h-12h light-dark cycle, where the lights were turned on at 07:00 and turned off at 19:00. Food and water were available ad libitum. All responses were conducted in a blinded fashion by 1-3 experimenters. Weight was recorded on each test day for all experiments. Neither treatments nor drugs significantly affected weight gain or mortality. All experimental procedures were approved by the University of Illinois at Chicago Animal Care and Institutional Biosafety Committees, in accordance with Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) guidelines and the Animal Care Policies of the University of Illinois at Chicago. All results are reported according to Animal Research: reporting of In Vivo Experiments (ARRIVE) guidelines.

3.2.2. SENSORY SENSITIVITY TESTING

Separate groups of animals were used for hind paw and cephalic experiments. For all behavioral experiments, mice were counterbalanced into groups following the first basal test for mechanical sensitivity. The experimenter was blinded to the drug condition being tested. No adverse effects were observed in any of the experiments. All mice were tested in a separate behavior room with low-light (~35-50 lux) and low-noise conditions, between 09:00 and 16:00. For all behavioral tests, mice were habituated to the testing rack for 2 days prior to the first test day, and on each test day for 20 minutes prior to the first measurement. For peripheral measurements, the plantar surface of the mouse hind paw was tested. For cephalic testing, mice were tested in 4 oz paper cups, to which they had been previously habituated for 1 hour over 2 days. The periorbital region caudal to the eyes and near the midline was tested. To assess mechanical sensitivity, the threshold for responses to punctate mechanical stimuli (mechanical allodynia) was tested according to the up-and-down method (Chaplan, Bach et al. 1994). The region of interest was stimulated with a series of eight von Frey hair filaments (bending force ranging from 0.00g to 2g). A response was defined as a lifting, shaking, or licking of the hind paw or head, depending on the region tested. The first filament tested was 0.4g. In the absence of a response, a heavier filament (up) was tried, and in the presence of a response, a lighter filament (down) was tested. This pattern was followed for a maximum of four filaments following the first response.

3.2.3. NTG MODEL OF CHRONIC MIGRAINE

NTG was purchased at a concentration of 5.0 mg/mL, in 30% alcohol, 30% propylene glycol and water (American Reagent, NY, USA). NTG was freshly diluted on each test day in 0.9% saline to a concentration of 1mg/mL for a dose of 10 mg/kg. The vehicle (VEH) used in these experiments was 0.9% saline. We previously found that there was no significant difference in mechanical thresholds between 0.9% saline, and the solution in which NTG was dissolved in (6% propylene glycol, 6% alcohol, 0.9% saline) (Pradhan, Smith et al. 2014). Mice were treated every second day for 9 days with vehicle or NTG (10 mg/kg, ip). For hind paw experiments, basal thresholds were assessed on days 1, 3, 5, 7, and 9. For cephalic experiments, basal thresholds were assessed on days 1, 5, and 9. On test days, mechanical thresholds were measured prior to vehicle/NTG injection.

3.2.4. MODEL OF POST-TRAUMATIC HEADACHE

Mild traumatic brain injury (mTBI) was induced by using the closed head weight-drop method, as described previously (Zohar, Schreiber et al. 2003, Moye, Novack et al. 2018). Briefly, mice were mildly anesthetized with 2.5% isoflurane with an oxygen flow rate of 0.6-0.8 liters per minute. Mice were placed chest down on a foam sponge (dimensions: 7-1/2 in. x 5-1/2 in. x 1-7/8 in) to support the head and body, which allowed for anterior-posterior motion without any rotational movement at the moment of impact. The mouse and sponge were placed directly underneath the weight-drop device which consisted of a hollow cylindrical tube (inner diameter 2.54 cm, 80 cm height) placed approximately 1cm vertically over the mouse’s head, in between the ear and eye. To induce mTBI, a 30g weight (13 mm diameter, 34 mm height) was dropped through the tube, striking the

mouse and causing a closed head injury. Immediately after mTBI, mice were returned to their home cages for recovery for 2 weeks. Sham animals were anesthetized but not subjected to the weight-drop. To model post-traumatic headache, a low dose of NTG (0.1 mg/kg IP) was used relative to the chronic model describe above. We have previously shown that this dose does not cause basal hypersensitivity in intact or sham mice but does in mild traumatic brain injuryanimals (Pradhan, Smith et al. 2014, Moye, Novack et al. 2018). NTG was freshly diluted on each test day in 0.9% saline to a concentration of 0.01mg/mL for a dose of 0.1 mg/kg. Two weeks following mild traumatic brain injuryor a sham procedure, mice were treated every other day for 9 days with vehicle or low dose NTG (0.1 mg/kg, ip), and tested in the same time frames as described above.

3.2.5. MODEL OF MEDICATION OVERUSE HEADACHE (medication overuse headache)

Sumatriptan (SUMA; 0.6 mg/kg, ip; Sandoz, NC, USA) was purchased at a concentration of 12 mg/mL and diluted to 0.06 mg/mL in 0.9% saline. Mice were treated once daily with vehicle or SUMA (0.6 mg/kg, ip) over 11 days, and tested on days 1, 3, 5, 7, 9, and 11 for hind paw testing, and on days 1, 5, and 9 for cephalic testing. On test days, mechanical thresholds were measured 30 minutes after injection.

3.2.6. MODEL OF OPIOID INDUCED HYPERALGESIA (opioid-induced hyperalgesia)

A stock concentration of 10 mg//ml morphine (MORPH) was diluted fresh daily with saline. Mice were treated twice daily with vehicle or MORPH over 4 days (20 mg/kg s.c. days 1-3, 40 mg/kg s.c. day 4), and tested on days 1-4 for hind paw testing, and on days 1 and

3 for cephalic testing. Basal mechanical thresholds were measured daily prior to MORPH injections.

3.2.7. TESTING EFFECT OF DELTA OPIOID RECEPTOR ACTIVATION

Eighteen to twenty-four hours after the last drug administration day, we determined the effect of SNC80. On this challenge test day, basal hind paw and cephalic mechanical thresholds were determined, after which mice received either vehicle (VEH) or SNC80 (10 mg/kg, ip; Tocris Bioscience, Bristol, UK). SNC80 was diluted to 1 mg/mL in 0.33% 1N HCl/0.9% saline. Post-SNC80 thresholds were assessed 2 hours after basal testing, and 45 minutes after SNC80 injection.

3.2.8. MODELING EFFECT OF CHRONIC DOR ACTIVATION

To determine whether chronic DOR activation caused hypersensitivity similar to medication overuse headache, Mice were treated once daily with vehicle, SUMA (0.6 mg/kg, ip), or SNC80 (10 mg/kg, ip) over 11 days. For hind paw experiments, mice were tested on days 1, 3, 5, 7, 9, and 11, and another cohort of mice were tested on days 1 and 11. For cephalic experiments, mice were tested on days 1 and 11.

3.2.9. STATISTICAL ANALYSIS

Data are expressed as mean $\pm$ s.e.m. All mice tested were included in the analysis. All statistical analyses were performed by SigmaStat software, and graphs were generated using GraphPad Prism. For all behavioral experiments, a two-way repeated-measures analysis of variance (ANOVA) was performed, with treatment

(vehicle/SUMA/MORPH/NTG/SNC80) and time (days) as factors. When a significant interaction occurred, subsequent Holm-Sidak post-hoc analysis was performed. A significance level of $p<0.05$ was used.

3.3. RESULTS

3.3.1. DOR ACTIVATION INHIBITS ESTABLISHED CHRONIC MIGRAINE-ASSOCIATED PAIN

To determine the effect of DOR activation in a model of chronic migraine, we tested whether an acute dose of SNC80 could reverse established mechanical allodynia to chronic intermittent administration of the human migraine trigger NTG (Pradhan, Smith et al. 2014, Moye and Pradhan 2017). To model chronic migraine, NTG (10 mg/kg, ip) or a vehicle was given every other day for 9 days (5 test days total). Hind paw thresholds were taken prior to NTG administration on days 1, 3, 5, 7, and 9, and in a separate group of mice, cephalic thresholds were taken on days 1, 5, and 9 (Figure 13A). Mice chronically treated with NTG developed a basal peripheral and cephalic hypersensitivity, an effect not seen in the VEH treated groups (Figure 13B and D). Twenty-four hours after the final NTG/VEH administration (day 10), basal responses were measured, and NTG-treated mice continued to show peripheral and cephalic allodynia (Figure 13C and E: basal). Mice were treated acutely with vehicle or SNC80 (10 mg/kg IP), and post-treatment thresholds were measured 45 minutes later. SNC80 had no effect on mechanical responses in animals chronically treated with vehicle (Figure 13C,E: post-drug). However, SNC80 significantly attenuated peripheral and cephalic allodynia induced by chronic NTG. These

data demonstrate that DOR activation can block established chronic migraine-associated pain.

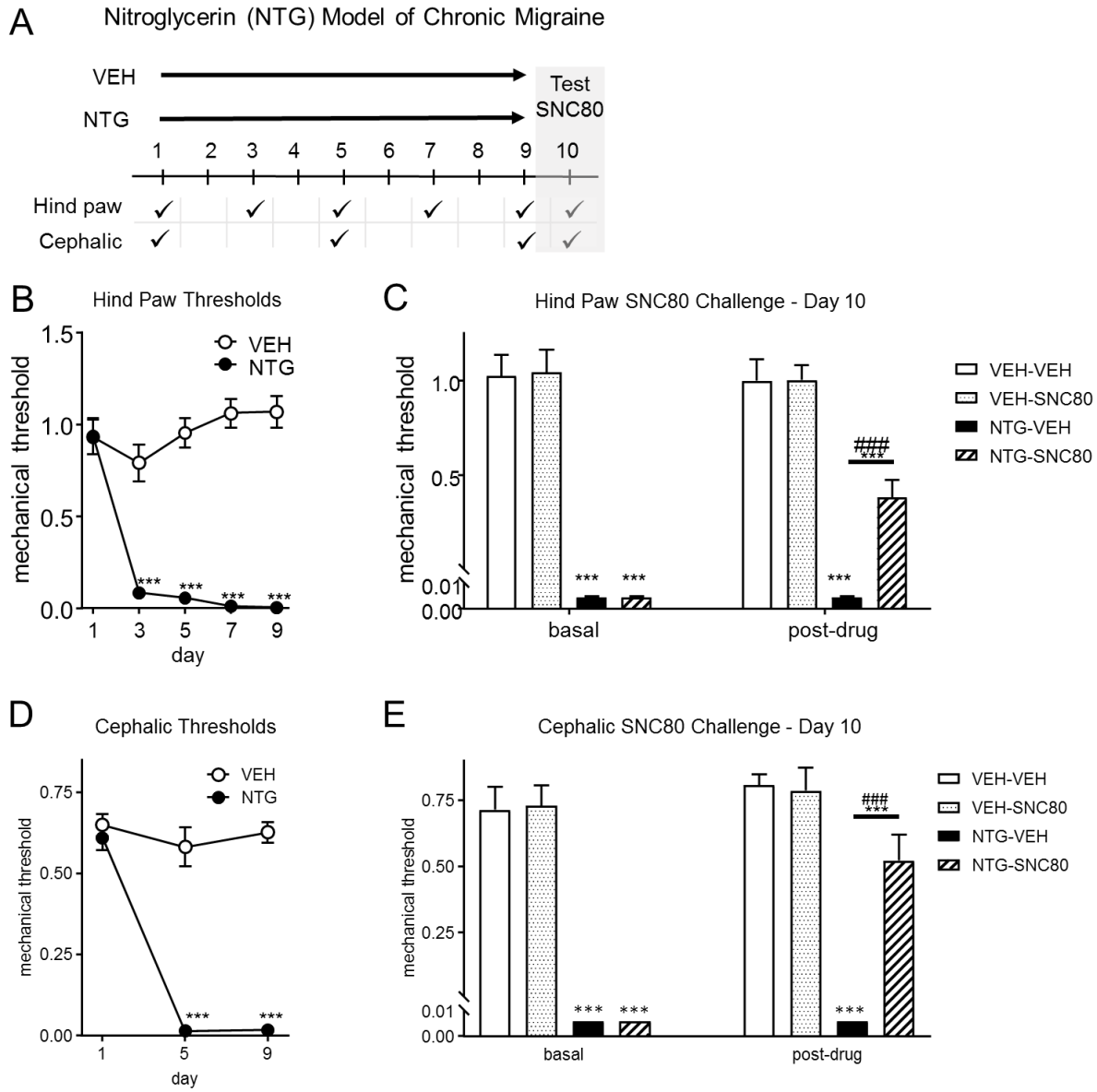


Figure 13: SNC80 treatment attenuates chronic NTG-induced allodynia. (a) Experimental outline. Separate groups of mice were tested for hind paw or cephalic allodynia. Male and female mice were used for hind paw testing, and as no differences were observed only male mice were used for cephalic testing. C57BL6/J mice were treated with vehicle (0.9% NaCl, VEH) or NTG (10 mg/kg, IP) every second day for 9 days. Baselines were measured prior to VEH/NTG administration. NTG produced a basal hypersensitivity in hind paw (b) and cephalic (d) regions, an effect not observed in vehicle (VEH) treated mice. $p<0.001$ effect of drug, time, and interaction, two-way RM ANOVA and Holm-Sidak post hoc analysis. ***$p<0.001$ relative to vehicle on day 1. On day 10, hind paw (c) or cephalic (e) basal responses were measured and NTG-treated mice had significantly

lower thresholds compared to VEH (basal). SNC80 (10 mg/kg IP, post-drug) was administered and animals were tested 45 min later. SNC80 significantly inhibited allodynia in both regions. p<0.001 05 effect of treatment, drug, and interaction, two-way ANOVA and Holm-Sidak post hoc analysis. ***p<0.001 relative to veh-veh, ## p<0.01, ###p<0.001 relative to NTG-VEH. n=6/group. DOR activation blocks chronic migraine-associated pain.

3.3.2. DOR ACTIVATION INHIBITS POST-TRAUMATIC HEADACHE

We have previously developed a model of post-traumatic headache by combining the closed head weight drop method and the NTG model of chronic migraine (Moye, Novack et al. 2018). Mice underwent a closed head injury or sham procedure followed by a 2 week recovery period. At 2 weeks post-injury, low dose NTG (0.1 mg/kg, ip) or a vehicle was administered every other day over 9 days (5 test days total). Similar to the chronic migraine model, hind paw thresholds were taken prior to NTG administration every other day, and in a separate group of mice cephalic thresholds were taken on days 1, 5, and 9 (Figure 14A). mild traumatic brain injurymice developed a basal peripheral and cephalic hypersensitivity following chronic intermittent treatment with low dose NTG, an effect not observed in the sham controls (Figure 14B and D). On day 10, 24h after the final NTG/VEH administration, mTBI-NTG-treated mice continued to show peripheral and cephalic allodynia (Figure 14C and E: basal). Animals were treated with either vehicle or SNC80 (10 mg/kg IP), and post-treatment thresholds were measured 45 minutes later. Acute treatment with SNC80 effectively inhibited hind paw and cephalic allodynia (Figure 14C and E: post-drug); and did not affect general nociception in the mice chronically treated with vehicle. These data demonstrate that DOR activation can alleviate established post-traumatic headache-associated pain.

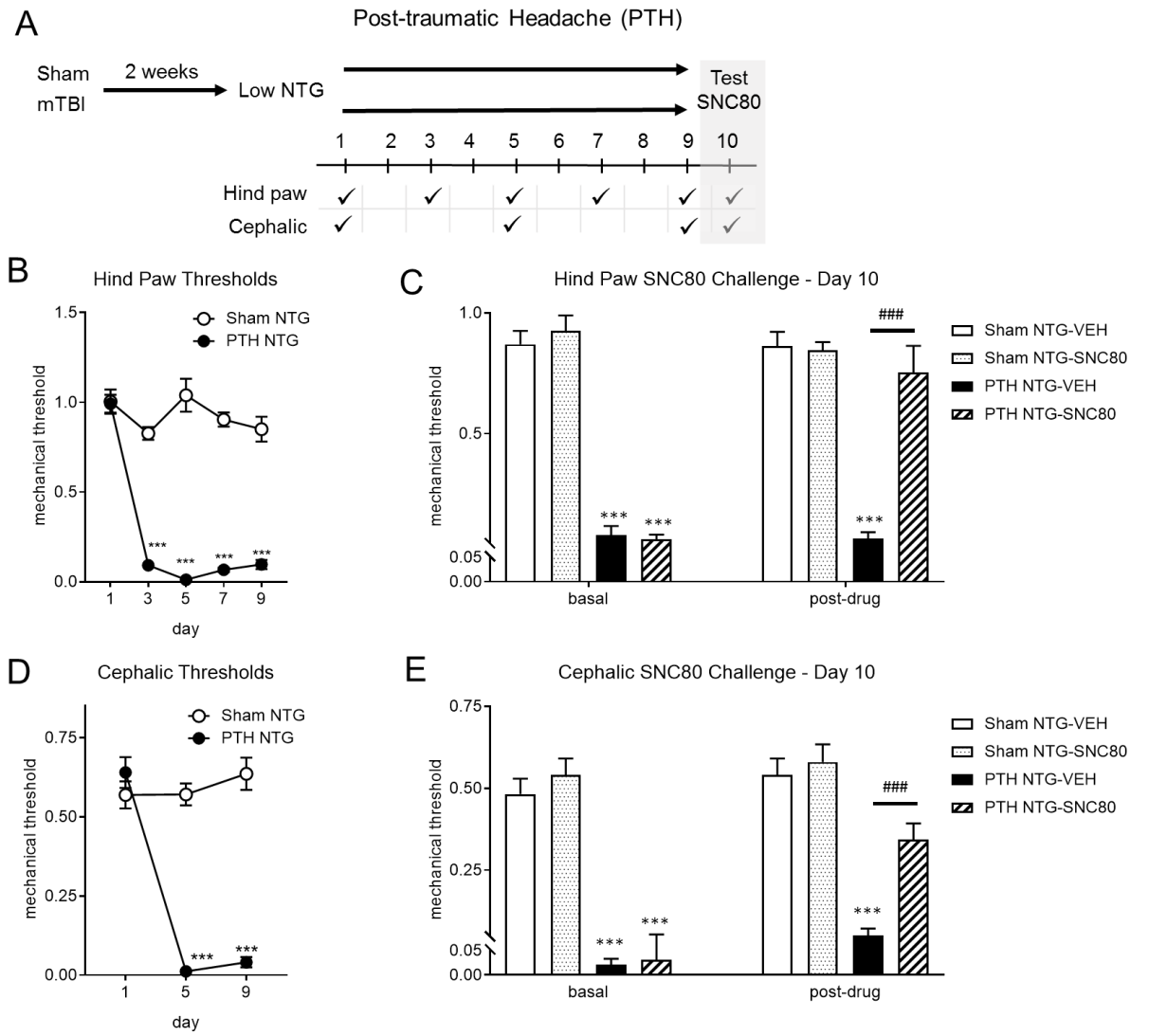


Figure 14: SNC80 treatment acutely reverses allodynia in a model of post-traumatic headache. (a) Experimental outline. Separate groups of mice were tested for hind paw or cephalic allodynia. Male and female mice were used for hind paw testing, and as no differences were observed only male mice were used for cephalic testing. C57BL6/J mice either underwent a closed head weight drop (mTBI) or sham procedure, and 2 weeks later were treated with vehicle (0.9% NaCl, VEH) or low dose NTG (0.1 mg/kg, IP) every second day for 9 days. Baselines were measured prior to VEH/NTG administration. NTG produced a basal hypersensitivity in hind paw (b) and cephalic (d) regions, an effect not observed in vehicle (VEH) treated mice. $p<0.001$ effect of drug, time, and interaction, two-way RM ANOVA and Holm-Sidak post hoc analysis. ***$p<0.001$ relative to vehicle on day 1. On day 10, hind paw (c) or cephalic (e) basal responses were measured and NTG-treated mice had significantly lower thresholds compared to VEH (basal). SNC80 (10 mg/kg IP, post-drug) was administered and animals were tested 45 min later. SNC80 significantly inhibited allodynia in both regions. $p<0.001$ 05 effect of treatment, drug, and interaction, two-way ANOVA and Holm-Sidak post hoc analysis. **$p<0.01$, ***$p<0.001$

relative to VEH-VEH, ###p<0.001 relative to NTG-VEH. n=/group (hind paw), n=6/group (cephalic). DOR activation blocks pain associated with post-traumatic headache.

3.3.3. DOR ACTIVATION INHIBITS MEDICATION OVERUSE HEADACHE TO SUMATRIPTAN

Overuse of sumatriptan (SUMA), an acute migraine medication, can lead to medication overuse headache. We tested whether an acute dose of SNC80 would inhibit chronic SUMA-induced allodynia. To model medication overuse headache, SUMA or a vehicle was given once daily for 11 days (Tipton, Tarash et al. 2015). Hind paw thresholds were tested 30 minutes before VEH or SUMA injection every other day, and cephalic thresholds on days 1, 5, and 9 (Figure 15A). Mice chronically treated specifically with SUMA developed basal hind paw and cephalic hypersensitivity (Figure 15B and D). Twenty-four hours after the last SUMA/VEH injection (day 12), basal hind paw and cephalic thresholds continued to be low in the SUMA treated groups (Figure 15C and E; basal). Mice were treated with SNC80 (10 mg/kg IP) or vehicle, and SNC80 significantly attenuated this allodynia (Figure 15C and E; post-drug). These data suggest that DOR activation can inhibit medication overuse headache caused by overuse of sumatriptan.

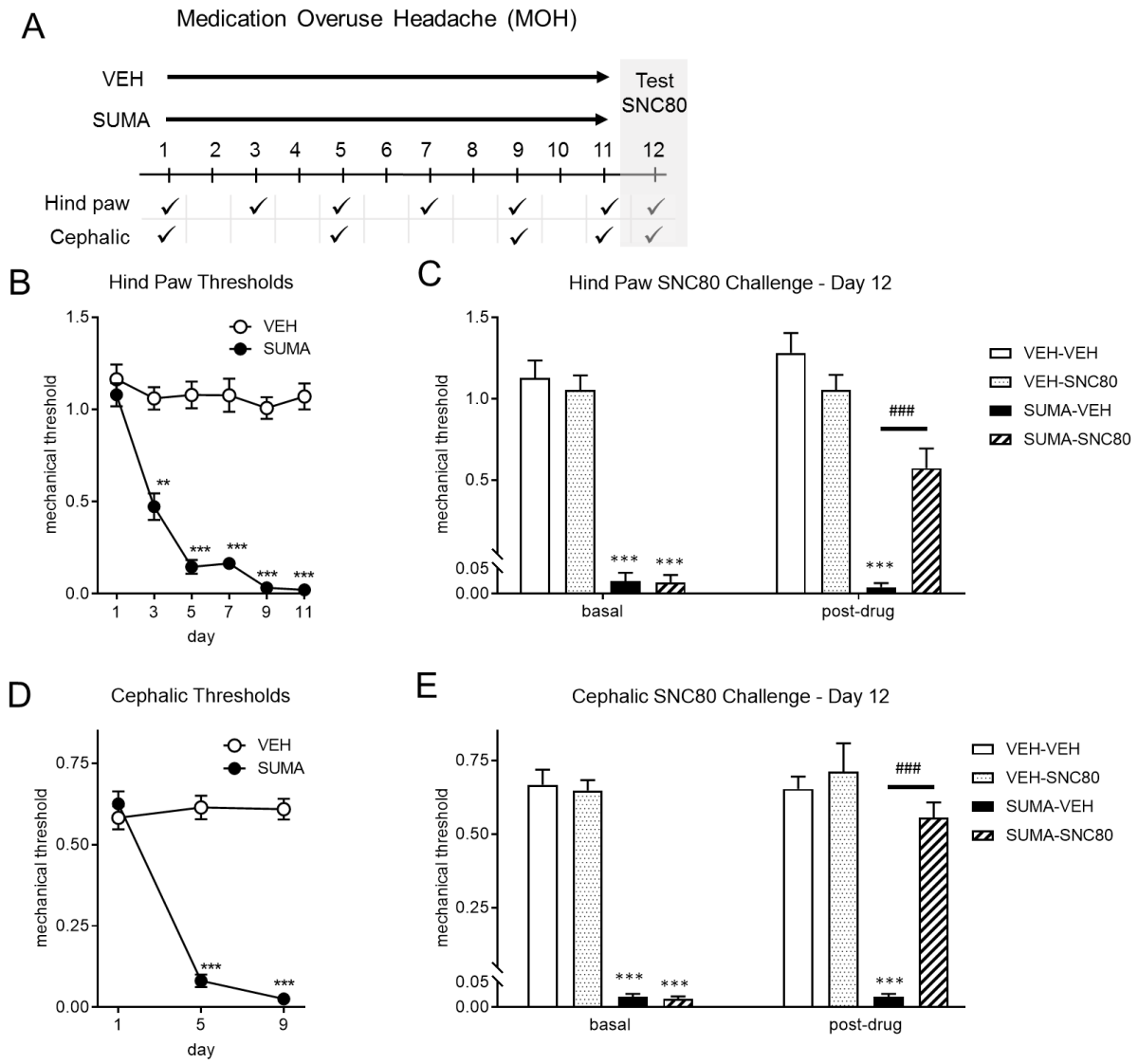


Figure 15: SNC80 treatment attenuates hind paw and cephalic allodynia induced by chronic sumatriptan. (a) Experimental outline. Separate groups of mice were tested for hind paw or cephalic allodynia. Male and female mice were used for hind paw testing, and as no differences were observed only male mice were used for cephalic testing. C57BL6/J mice were treated with vehicle (0.9% NaCl, VEH) or sumatriptan (SUMA, 0.6 mg/kg, IP) every day for 11 days. Baselines were measured prior to VEH/SUMA administration. SUMA produced a basal hypersensitivity in hind paw (b) and cephalic (d) regions, an effect not observed in vehicle (VEH) treated mice. $p<0.001$ effect of drug, time, and interaction, two-way RM ANOVA and Holm-Sidak post hoc analysis. ***$p<0.001$ relative to vehicle on day 1. On day 10, hind paw (c) or cephalic (e) basal responses were measured and SUMA-treated mice had significantly lower thresholds compared to VEH (basal). SNC80 (10 mg/kg IP, post-drug) was administered and animals were tested 45 min later. SNC80 significantly inhibited allodynia in both regions. $p<0.0501$ effect of treatment, drug, and interaction, two-way ANOVA and Holm-Sidak post hoc analysis. ***$p<0.001$ relative to VEH-VEH, ###$p<0.001$ relative to SUMA-VEH. n=8-9/group (hind

paw), n=11/group (cephalic). DOR activation blocks pain associated with medication overuse headache.

3.3.4. DOR ACTIVATION INHIBITS OPIOID-INDUCED HYPERALGESIA TO MORPHINE

To determine the effect of DOR activation on opioid-induced hyperalgesia, we tested whether an acute dose of SNC80 would reverse established peripheral and cephalic allodynia induced by chronic morphine (MORPH). To model opioid-induced hyperalgesia, MORPH (20 mg/kg, SC days 1-3; 40 mg/kg, SC day 4) or vehicle was given twice a day for 4 days. All basal responses were determined in the AM before the morning injection which occurred 2 hours after testing (Figure 16A). Only mice chronically treated with morphine developed a basal hind paw or cephalic hypersensitivity (Figure 16B and D), an effect that was still observed 18-24h after the final VEH/MORPH injection (Figure 16C and E; basal). Animals were treated with either vehicle or SNC80 (10 mg/kg IP), and SNC80 significantly attenuated hind paw and cephalic allodynia in chronic morphine treated animals (Figure 16C and E: post-drug). These data demonstrate that DOR activation can inhibit opioid-induced hyperalgesia-associated pain and indicates that DOR and MOR regulate pain through different mechanisms.

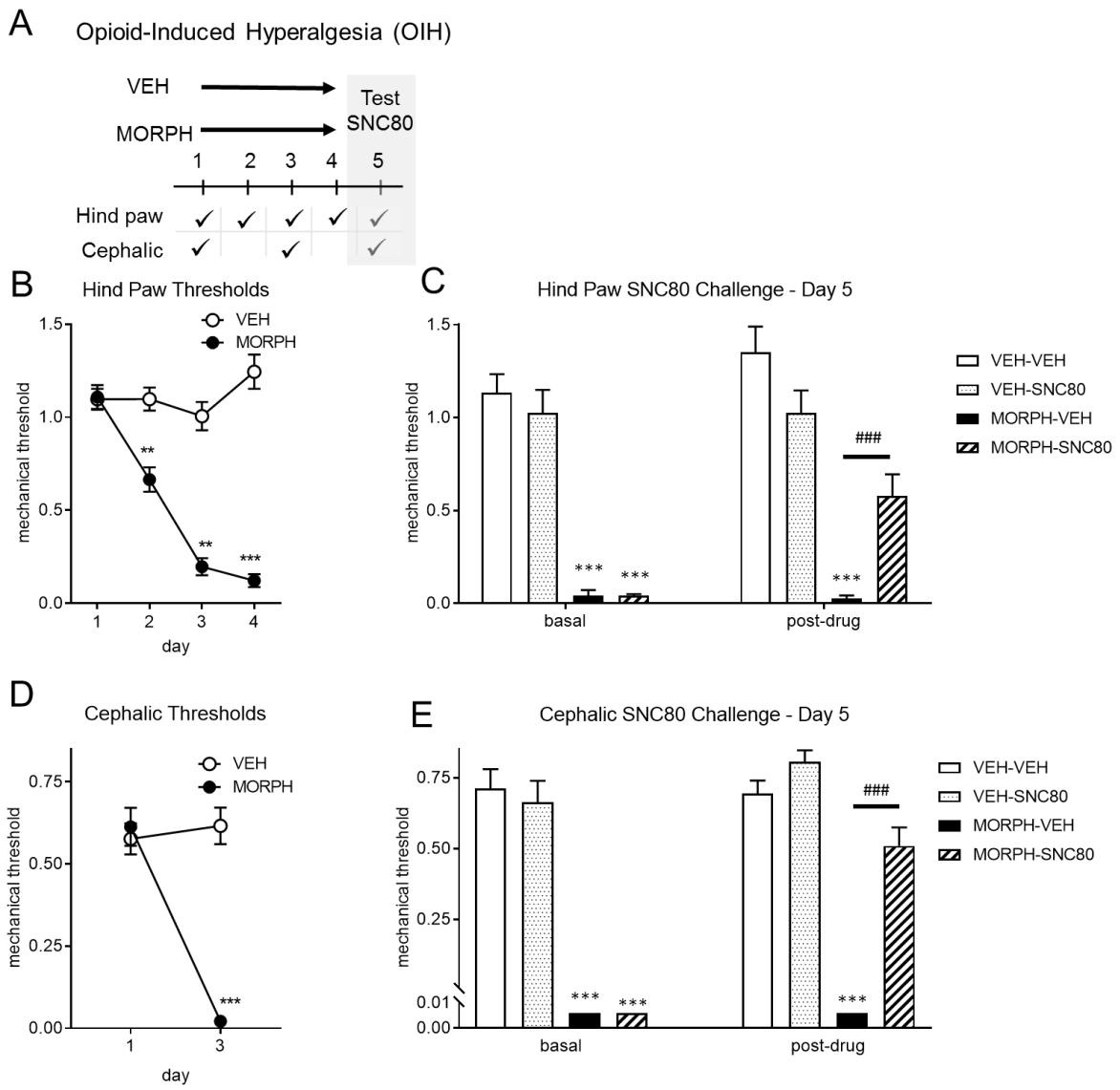


Figure 16: SNC80 treatment attenuates allodynia induced by chronic morphine. (a) Experimental outline. Separate groups of mice were tested for hind paw or cephalic allodynia. Male and female mice were used for hind paw testing, and as no differences were observed only male mice were used for cephalic testing. C57BL6/J mice were treated with vehicle (0.9% NaCl, VEH) or morphine (MORPH, 20 mg/kg SC days 1-3; 40 mg/kg SC day 4) twice a day for 4 days. Injections occurred in the morning and late afternoon. Baselines were measured prior to the VEH/MORPH administration in the morning. MORPH produced a basal hypersensitivity in hind paw (b) and cephalic (d) regions, an effect not observed in vehicle (VEH) treated mice. $p<0.001$ effect of drug, time, and interaction, two-way RM ANOVA and Holm-Sidak post hoc analysis. ***$p<0.001$ relative to vehicle on day 1. On day 10, hind paw (c) or cephalic (e) basal responses were measured and MORPH-treated mice had significantly lower thresholds compared to VEH (basal). SNC80 (10 mg/kg IP, post-drug) was administered and animals were tested 45 min later. SNC80 significantly inhibited allodynia in both regions. $p<0.001$ effect of treatment, drug, and interaction, two-way ANOVA and Holm-Sidak post hoc analysis. *$p<0.05$, ***$p<0.001$ relative to

VEH-VEH, ###p<0.001 relative to MORPH-VEH. n=8-9/group (hind paw), n=6/group (cephalic). DOR activation blocks pain associated with opioid induced hyperalgesia.

3.3.5. CHRONIC DOR ACTIVATION PRODUCES LIMITED opioid-induced hyperalgesia

We determined if chronic daily administration of SNC80 would cause opioid-induced hyperalgesia-associated allodynia. As a positive control, we concurrently tested a group of mice with sumatriptan (Figure 17A). Mice were given vehicle, SUMA or SNC80 once a day for 11 days, and initially tested on days 1, 3, 5, 7, 9, and 11 for the development of peripheral allodynia. Mice chronically treated with SUMA or SNC80 developed a basal hind paw hypersensitivity, an effect not seen in the vehicle control group (Figure 17B). Along with the pharmacological effects, repeated testing can produce associative learning, which can be a major component of drug tolerance and hyperalgesia. To determine whether the basal hypersensitivity in SUMA- and SNC80-treated mice was pharmacologically induced or learned, mice were given vehicle, SUMA, or SNC80 once a day for 11 days, but only tested on days 1 and 11. Mice chronically treated with SUMA developed a basal peripheral allodynia, an effect not seen in vehicle or SNC80-treated mice (Figure 17C). To determine if chronic DOR activation would cause cephalic allodynia, a separate group of mice were similarly treated daily with SNC80 for 11 days and cephalic thresholds were measured on days 1 and 11. There was no significant difference between VEH- and SNC80-treated mice (Figure 17D). To further characterize when mice would develop medication overuse headache to SNC80, we injected mice with vehicle or SNC80 every day for 11 days, and tested on days 1, 5 and 11. On day 5, there was no significant difference between VEH- and SNC80-treated mice, in contrast to VEH-

and SUMA-treated mice. However, by the third test day (day 11) SNC80 and SUMA-treated animals both showed periorbital allodynia relative to controls (Figure 17E). These data indicate that repeated use of a DOR agonist produces a limited form of medication overuse headache that is less severe than sumatriptan.

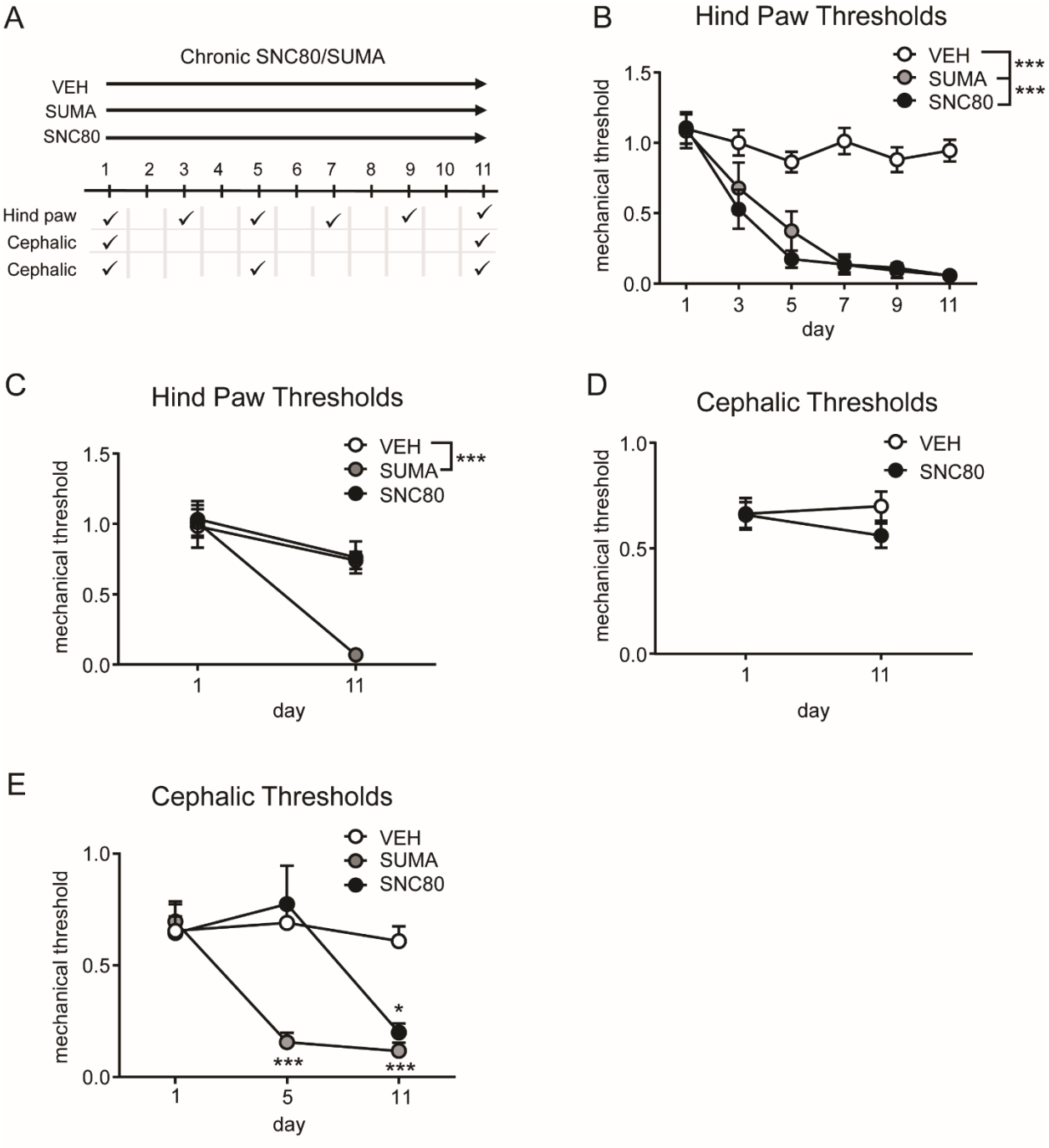


Figure 17: Chronic SNC80 administration induces limited opioid-induced hyperalgesia/medication overuse headache. (a) Experimental outline. Separate groups of mice were tested for hind paw or cephalic allodynia. Male and female mice were used for hind paw testing, and as no differences were observed only male mice were used for cephalic testing. C57BL6/J mice were treated with vehicle (0.9% NaCl, VEH), SNC80 (10 mg/kg IP), or SUMA (0.6 mg/kg IP) daily for 11 days. Baselines were measured prior to the VEH/SNC80/SUMA administration. (b) SUMA- and SNC80-treated animals had significantly lower hind paw mechanical responses relative to VEH controls. $p<0.001$ effect of drug, time, and interaction, two-way RM ANOVA and Holm-Sidak post-hoc analysis. n=8-12/group. (c) Male C57Bl/6J mice were treated with VEH, SUMA, or SNC80 every day for 11 days, but only tested on days 1 and 11. SUMA-treated animals had significantly lower hind paw mechanical responses on day 11, an effect not observed in VEH- or SNC80-treated mice. $p<0.001$ effect of drug, time, and interaction, two-way RM ANOVA and Holm-Sidak post-hoc analysis. n=8-10/group. (d) Mice were treated with VEH or SNC80 every day for 11 days and tested on days 1 and 11 for cephalic responses. SNC80 did not induce cephalic hypersensitivity, $p=0.359$ effect of drug and time, two-way RM ANOVA, n=8/group. (e) Mice were treated with VEH, SUMA, or SNC80 every day for 11 days, and tested on days 1, 5 and 11 for cephalic responses. SNC80 induced cephalic hypersensitivity, but at a slower rate to sumatriptan. $p<0.001$ effect of drug, time, and interaction, two-way RM ANOVA and Holm-Sidak post-hoc analysis. n=8-12/group. Chronic DOR activation does not induce opioid-induced hyperalgesia/medication overuse headache as rapidly as sumatriptan, and this effect may be through increased associative learning.

3.4. DISCUSSION

Despite the extraordinary disability caused by headache disorders, these patients have remarkably few effective treatment options. Chronic migraine poses a significant clinical burden, and is experienced by 1-2% of the population (May and Schulte 2016), with approximately 3% of episodic migraine patients converting to chronic migraine per year (Scher, Stewart et al. 2003). Chronic migraine is treated with preventives from varying drug classes, including tricyclic antidepressants, anti-convulsants and beta blockers (Charles 2017). However, these treatments only work for a subset of patients and are associated with a number of adverse effects resulting in less than 50% of patients being satisfied with their treatment (Bigal, Serrano et al. 2008). Patients suffering from post-

traumatic headache face similar limitations. There are no specific pharmacological treatments for post-traumatic headache, and there has never been a large scale clinical trial to test post-traumatic headache-specific therapies (Monteith and Borsook 2014, Moye and Pradhan 2017). post-traumatic headache with a migraine-like phenotype is usually treated with the same category of drugs as migraine without injury, with comparably poor success rates (Moye and Pradhan 2017). A further complication associated with the treatment of headache is the phenomenon of medication overuse headache. Overuse of medications prescribed to treat migraine, such as triptans and opioids, have been associated with medication overuse headache, and an estimated 15% of migraine patients go on to develop this disorder (Schwedt, Alam et al. 2018). The primary treatment for medication overuse headache is withdrawal from the overused medication, however, clinical studies have reported a 20-40% relapse rate of detoxified patients within the first year of withdrawal, with most patients relapsing within the first 6 months of withdrawal (Katsarava, Limmroth et al. 2003). There is clearly a need to diversify the tool box of pharmacotherapies available for the treatment of primary and secondary disorders. The results from our study indicate that DOR could be a promising addition to this tool box and could be effective for multiple types of headache disorders.

We have previously identified DOR as a novel therapeutic target for migraine (Charles and Pradhan 2016); and shown that DOR agonists, when administered shortly after NTG administration, block acute NTG-evoked peripheral allodynia (Pradhan, Smith et al. 2014). We have also previously demonstrated that when SNC80 is administered with low-dose NTG in a model of post-traumatic migraine, DOR activation can prevent the

development to chronic post-traumatic headache (Moye, Novack et al. 2018). Patients usually present with well-established headache, and the goal of this study was to determine the utility of DOR agonists in models that reflect this clinically significant state. We found that the DOR agonist, SNC80, blocked established pain associated with chronic migraine, post-traumatic headache, and medication overuse headache to chronic sumatriptan or morphine. In all models, we measured mechanical allodynia in both peripheral and cephalic regions, and DOR activation was anti-allodynic regardless of area tested. In addition, we observed that unlike sumatriptan or morphine, chronic DOR stimulation did not pharmacologically result in medication overuse headache/opioid-induced hyperalgesia, thus strengthening the case for DOR as a promising target for drug development.

In the United States, MOR-based therapies such as morphine, hydrocodone, and oxycodone, are still regularly prescribed for headache (Bigal and Lipton 2009, Buse, Pearlman et al. 2012, Thorlund, Sun-Edelstein et al. 2016). Paradoxically, while opioids provide acute relief, chronic use results in refractory headache and contributes to the progression of migraine from an episodic to a chronic state (Bigal and Lipton 2009, Buse, Pearlman et al. 2012, Thorlund, Sun-Edelstein et al. 2016), a condition associated with opioid-induced hyperalgesia (Chu, Angst et al. 2008, Roeckel, Le Coz et al. 2016). Additionally, MOR agonists are highly addictive, and over-prescription has led to a devastating public health crisis. In order to circumvent this cycle of sensitization and drug abuse, pharmacotherapies that are mechanistically different from MOR are required. Although DOR is a member of the opioid receptor family and has pain relieving effects, it

is physically and functionally a distinct protein. MOR and DOR are expressed in different cellular and anatomical brain regions. For example, MOR is highly expressed in pain processing regions such as the thalamus and periaqueductal grey, while DOR expression is higher in the striatum and cortical regions (Mansour, Fox et al. 1995, Le Merrer, Becker et al. 2009). Even in areas that express both receptors, MOR and DOR are often expressed on different cell types. Only a small percentage of cells in pain processing regions such as the dorsal root ganglia, spinal cord, and lateral parabrachial nucleus showed co-expression of MOR and DOR (Scherrer, Imamachi et al. 2009, Bardoni, Tawfik et al. 2014, Wang, Tawfik et al. 2018); and MOR and DOR were differentially expressed on dural projections from the trigeminal ganglia (Rice, Xie et al. 2017). The distinct roles of MOR and DOR are supported by our finding that SNC80 can inhibit opioid-induced hyperalgesia-associated pain. opioid-induced hyperalgesia is caused by maladaptations in pain circuits induced by chronic morphine treatment. Our findings would suggest that despite this chronic exposure to morphine, DOR functionality remains intact and could be an effective treatment for opioid-induced hyperalgesia, acting as an adjunct therapy during opioid withdrawal.

DOR agonists are not effective in most models of acute pain, but rather gain functionality in chronic conditions. Multiple publications show that DOR is dynamic, and increased functionality is observed following chronic stimuli such as pain (Cahill, Morinville et al. 2003, Kabli and Cahill 2007, Pradhan, Smith et al. 2013, Huang, Lv et al. 2015), intestinal inflammation (DiCello, Saito et al. 2018), morphine administration (Cahill, Morinville et al. 2001), and ethanol exposure (van Rijn, Brissett et al. 2012). In keeping with these

findings, our results also show that DOR agonists are effective in multiple models of headache-associated pain, in which each model requires long term exposure to NTG, morphine, or sumatriptan. How DOR functionality increases in chronic pain states is a topic of intense study (Vicente-Sanchez, Segura et al. 2016). DOR mRNA levels have been found to be upregulated in animal models of peripheral inflammatory pain (Cahill, Morinville et al. 2003) and acute pulpitis (Huang, Lv et al. 2015), indicating that pain can cause increased DOR expression. In addition, microscopic studies indicate that DORs are predominantly located on intracellular compartments, and that tissue injury redistributes these receptors to the cell membrane (Zhang, Bao et al. 1998, Bao, Jin et al. 2003, Cahill, Morinville et al. 2003, Guan, Xu et al. 2005, Patwardhan, Berg et al. 2005, Gendron, Lucido et al. 2006). However, there is some debate regarding the specificity of the DOR antibodies used in these studies (Scherrer, Imamachi et al. 2009, Wang, Zhao et al. 2010, Bardoni, Tawfik et al. 2014). Electrophysiological studies have also revealed that chronic peripheral pain can also increase DOR coupling to Ca2+ channels in dorsal root ganglia (Pradhan, Smith et al. 2013). There are a number of lines of evidence, including the findings presented herein, to indicate that DOR functionality is upregulated following chronic pain; and future studies will focus on where and how this increase occurs.

One of our aims was to determine if chronic treatment with a DOR agonist would result in opioid-induced hyperalgesia/medication overuse headache. Interestingly, we found that daily treatment and testing with SNC80 resulted in subsequent hyperalgesia, but that daily treatment alone did not result in increased pain sensitivity, unlike sumatriptan. These

results suggest that pharmacological activation of DOR does not produce opioid-induced hyperalgesia/medication overuse headache, however, if chronic SNC80 is paired with repeated testing then DOR activation might facilitate associative learning resulting in behavioral sensitization. The DOR is expressed in a number of brain regions that can regulate different kinds of learning; including the hippocampus, amygdala, and striatum (Le Merrer, Becker et al. 2009, Pradhan, Befort et al. 2011, Pellissier, Pujol et al. 2016). Knockout of DOR results in impairment in object recognition tasks (Le Merrer, Rezai et al. 2013), as well as deficiencies in place conditioning tasks (Le Merrer, Plaza-Zabala et al. 2011). In addition, DORs in the nucleus accumbens shell have been shown to modulate predictive learning (Bertran-Gonzalez, Laurent et al. 2013, Laurent, Bertran-Gonzalez et al. 2014, Laurent, Morse et al. 2015, Laurent, Wong et al. 2015). We have also previously demonstrated that tolerance to SNC80 is significantly dependent on associative learning (Pradhan, Walwyn et al. 2010, Vicente-Sanchez, Dripps et al. 2018), and environmental cues related to memory and learning can modulate behavioral outcomes to repeated exposure to opioids (Gamble et al., 1989; Mitchell et al., 2000). Our results should be considered during the development of DOR agonists for headache, as chronic DOR activation could facilitate associated learned behavior in migraineurs.

We demonstrate that DOR is a promising therapeutic for several established headache disorders, including medication overuse headache, opioid-induced hyperalgesia and post-traumatic headache. Unlike the mu opioid receptor, DOR agonists have low abuse liability as they are not readily self-administered in animal models and do not cause physical dependence (Negus, Gatch et al. 1998, Brandt, Furness et al. 2001, Stevenson,

Folk et al. 2005). DOR agonists also do not produce significant adverse effects such as respiratory depression or constipation (Gallantine and Meert 2005, Codd, Carson et al. 2009). An additional benefit of DOR-based therapeutics is that activation of DOR can positively regulate emotional tone and have been previously developed for the treatment of anxiety and depression (Lutz and Kieffer 2013). This effect may be particularly important considering the high co-morbidity between headache disorders and psychiatric conditions (Minen, Begasse De Dhaem et al. 2016), and the negative emotional state induced by withdrawal from opioids (Koob and Volkow 2016). Headache disorders are incredibly disabling, and DOR is a novel mechanistically-distinct option that could expand the treatment portfolio.

CHAPTER 4 RATIONALE

The previous chapter demonstrated the anti-allodynic characteristics of a hallmark DOR agonist in multiple models of headache. These results, in particular the way that DOR activation is especially anti-allodynic in the periorbital region in all of the models of headache, shed light on the critical role of DOR in pain. It is possible that the DOR is an untapped target and that DOR agonists could yield powerful anti-migraine therapeutics. In order to further explore how DOR regulates migraine-associated pain, I dedicated the last chapter of my thesis to investigating the colocalization of the DOR with the pro-migraine peptide calcitonin gene-related peptide (CGRP) and the CGRP receptor within the trigeminovascular pathway. I had initially hypothesized that the DOR was colocalized with CGRP in TG neurons and expected to see results similar to those done with 5HT1B and 5HT1D receptors and CGRP in the sumatriptan studies. However, my results did not agree with my initial hypothesis, and I began to explore the colocalization of DOR with the CGRP receptor instead. This last chapter of my thesis is dedicated to that exploration and has also opened the doors to future directions in the DOR field. The chapter has been written in manuscript form and is in the process of being submitted for publication.

4. DELTA OPIOID RECEPTOR ACTIVATION PREVENTS CHRONIC MIGRAINE-ASSOCIATED PAIN AND IS ASSOCIATED WITH THE CGRP RECEPTOR IN THE TRIGEMINOVASCULAR COMPLEX

4.1. INTRODUCTION

Migraine is the 3rd most disabling illness in the world among people aged 15 to 49 years, and this debilitating disease affects 12% of the population worldwide (WHO 2011, Woldeamanuel and Cowan 2017). Chronic migraineurs suffer from unilateral, throbbing headache, disruptions to sensory and motor systems, and up to one third of migraineurs also experience aura (Russell, Rasmussen et al. 1995, Headache Classification Committee of the International Headache 2013). This neurological disorder causes functional impairment during and between migraine attacks, and it is common for comorbidities such as depression, anxiety, and sleep disturbances to manifest and further decrease quality of life (Buse, Rupnow et al. 2009). Despite the severe negative effects of migraine on patients, their families, and the economy, this public health issue remains understudied and migraine therapeutics remain limited. To promote the development of novel treatments for chronic migraine, we propose the delta opioid receptor (DOR) as a promising migraine pharmacotherapy.

We test the effect of SNC80, a hallmark DOR agonist, in the nitroglycerin (NTG) model of chronic migraine, which is an archetype representative of migraine symptomology (Pradhan, Smith et al. 2014). Intravenous NTG reliably induces migraine in migraineurs, and headaches in non-migraineurs (Iversen, Olesen et al. 1989, Christiansen, Thomsen et al. 1999). In animal models, chronic NTG induces migraine-associated mechanical

allodynia, conditioned place aversion, and photophobia (Markovics, Kormos et al. 2012, Pradhan, Smith et al. 2014). Here, we explore the effect of chronic NTG, as well as chronic SNC80 following NTG, in the trigeminovascular complex, a region that has been highly implicated in migraine pathophysiology. Specifically, we characterize how DOR activation in this chronic pain state modulates expression of calcitonin gene-related peptide (CGRP) and the CGRP receptor (CGRPR) in the trigeminovascular complex.

CGRP and CGRPR have been highly implicated in the pathogenesis of migraine. Pioneering clinical studies showed increased CGRP in the jugular outflow during a severe migraine attack (Goadsby, Edvinsson et al. 1990, Goadsby and Edvinsson 1993). The latest clinical studies have confirmed the interdependence between CGRP, CGRPR, and migraine, as the injection of CGRP into the cubital vein of migraineurs causes a delayed headache, and CGRPR antagonists abort migraine (Lassen, Haderslev et al. 2002, Goadsby, Reuter et al. 2017). Migraine-associated pain modulates expression of CGRP and the CGRPR in the trigeminovascular complex, and we hypothesized that DOR may regulate this peptide-receptor complex.

The DOR is emerging as a promising target for the treatment of migraine, and has been recently shown to prevent the development of pain associated with post-traumatic headache and atraumatic migraine-associated allodynia (Pradhan, Smith et al. 2014, Moye, Novack et al. 2018). Chronic stimuli such as pain has been shown to increase DOR functionality (Cahill, Morinville et al. 2003, Pradhan, Smith et al. 2013), and this receptor plasticity may enhance the pain-relieving effects of DOR agonists. Here, we further add

to growing data showcasing the anti-allodynic effects of DOR activation. We show that on a behavioral and molecular level, DOR activation prevents the development of acute and chronic migraine-associated pain, and that this augmented behavior correlates with suppressed CGRP expression in the trigeminovascular complex.

4.2. MATERIALS AND METHODS

4.2.1. ANIMALS

Experiments were performed on male and female C57BL6/J mice (Jackson Laboratories, Bar Harbor, ME. USA) and DOReGFP knockin mice weighing 20-30g, and no sex differences were observed. Mice were group housed in a 12h-12h light-dark cycle, where the lights were turned on at 07:00 and turned off at 19:00. Food and water were available ad libitum. All responses were conducted in a blinded fashion by 1-3 experimenters. Weight was recorded on each test day for all experiments. Neither treatments nor drugs significantly affected weight gain or mortality. All experimental procedures were approved by the University of Illinois at Chicago Animal Care and Institutional Biosafety Committees, in accordance with Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC) guidelines and the Animal Care Policies of the University of Illinois at Chicago. All results are reported according to Animal Research: reporting of In Vivo Experiments (ARRIVE) guidelines.

4.2.2. SENSORY SENTITIVITY TRAINING

Separate groups of animals were used for all experiments. For all behavioral experiments, mice were counterbalanced into groups following the first basal test for mechanical sensitivity. The experimenter was blinded to the drug condition being tested. No adverse effects were observed in any of the experiments. All mice were tested in a separate behavior room with low-light (~35-50 lux) and low-noise conditions, between 09:00 and 16:00. For all behavioral tests, mice were habituated to the testing rack for 2 days prior to the first test day, and on each test day for 20 minutes prior to the first measurement. For peripheral measurements, the plantar surface of the mouse hind paw was tested. For cephalic testing, mice were tested in 4 oz paper cups, to which they had been previously habituated for 1 hour over 2 days. The periorbital region caudal to the eyes and near the midline was tested. To assess mechanical sensitivity, the threshold for responses to punctate mechanical stimuli (mechanical allodynia) was tested according to the up-and-down method (Chaplan et al., 1994). The region of interest was stimulated with a series of eight von Frey hair filaments (bending force ranging from 0.00g to 2g). A response was defined as a lifting, shaking, or licking of the hind paw or head, depending on the region tested. The first filament tested was 0.4g. In the absence of a response, a heavier filament (up) was tried, and in the presence of a response, a lighter filament (down) was tested. This pattern was followed for a maximum of four filaments following the first response.

4.2.3. NTG MODEL OF CHRONIC MIGRAINE

NTG was purchased at a concentration of 5.0 mg/mL, in 30% alcohol, 30% propylene glycol and water (American Reagent, NY, USA). NTG was freshly diluted on each test day in 0.9% saline to a concentration of 1mg/mL for a dose of 10 mg/kg. The vehicle (VEH) used in these experiments was 0.9% saline. We previously found that there was no significant difference in mechanical thresholds between 0.9% saline, and the solution in which NTG was dissolved in (6% propylene glycol, 6% alcohol, 0.9% saline) (Pradhan et al., 2014a). Mice were treated every second day for 9 days with vehicle or NTG (10 mg/kg, ip). For hind paw experiments, basal thresholds were assessed on days 1, 3, 5, 7, and 9. For cephalic experiments, basal thresholds were assessed on days 1, 5, and 9. On test days, mechanical thresholds were measured prior to vehicle/NTG injection.

4.2.4. CHRONIC TREATMENT WITH SNC80

To determine whether chronic DOR activation prevented the development of NTG-induced hypersensitivity, mice were treated with SNC80 (10 mg/kg, ip) 75 minutes after NTG administration on days 1, 3, 5, 7 and 9. For hind paw experiments, mice were tested on days 1, 3, 5, 7, and 9. For cephalic experiments, mice were tested on days 1, 5, and 9.

4.2.5. IMMUNOHISTOCHEMISTRY AND IMAGING

Trigeminal ganglia (TG) and brains containing the trigeminal nucleus caudalis (TNC) were collected on day 10 of the NTG paradigm. Mice were anesthetized with Somnasol (100 µL/mouse; 390 mg/mL pentobarbital sodium; Henry Schein, SKU#024352), and perfused

intracardially with 15 mL of ice-cold phosphate buffer (0.1M PB, pH 7.2) and subsequently 50mL of ice-cold 4% paraformaldehyde (PFA)/0.1M PB (pH 7.4). TG and brains were harvested from the mice and post-fixed overnight in 4% PFA/0.1M PB at 4ºC. Tissue was cryoprotected in 30% sucrose/0.1M PB for 24-36 hours, or until it sank. Sections of the TG and TNC were sliced at 14 µM. Upon slicing, TG sections were immediately mounted onto slides, and TNC sections free-floated in 0.01M PB. Slides were blocked with 5% normal donkey serum in 0.1M phosphate-buffered saline with 0.3% Triton X-100 (NDST) for 1 hour at room temperature. Slides were incubated overnight at room temperature with primary sheep anti-CGRP antibody (RRID AB_725809; ab22560; Abcam; 1:1000 dilution), primary chicken anti-eGFP antibody, primary rabbit anti-eGFP antibody, and primary rabbit anti-RAMP1 antibody, made in 1% NDST. Slides were washed with 1%NDST before incubating with a secondary antibody solution (Alexa Fluor 555 Donkey anti-Sheep; Life Technologies; 1:1000, Alexa Fluor 350 Donkey anti-Sheep; Alexa Fluor 488 Donkey anti-Chicken; Alexa Fluor 488 Donkey anti-Rabbit; Alexa Fluor 555 Donkey anti-Rabbit) made in 1% NDST for 2 hours at room temperature. Slides were washed with 0.1M phosphate buffer, and cover slipped with Fluoromount G mounting solution. Images for quantification were taken by 2 observers in a blinded manner using an EVOS FL Auto Cell Imaging System, using a 20X objective.

All images collected were used for analysis. Expression of CGRP, DOReGFP, and RAMP1 was quantified by observers blinded to treatment groups. All CGRP-positive, DOReGFP-positive, and RAMP1-positive cells from all sections were analyzed. Confocal images from Figures 19, 22, and 23 were taken by a Zeiss Laser Scanning Microscope

(LSM) 710 using a 25X objective. Remaining figures were taken by an EVOS FL Auto Cell Imaging System, using a 20X objective and a 45X objective. For all images, only one plane is being shown, and contrast, brightness, and exposure were kept constant in order to be able to compare any experimental group with its vehicle counterpart.

4.2.6. RNASCOPE FLUORESCENT IN SITU HYBRIDIZATION

RNAscope kit was purchased from Advanced Cell Diagnostics RNAScope Technology (ACD Bioscience). Briefly, C57Bl6/J mice were anesthetized, brain and trigeminal ganglia were collected and immediately frozen. Frozen tissue was cut at 14 µm and processed per the manufacturer's protocol. The probes used were targeted against the genes for DOR, RAMP1, and CRLR.

4.2.7. STATISTICAL ANALYSES

Data are expressed as mean + s.e.m. All mice tested were included in the analysis. All statistical analyses were performed by SigmaStat software, and graphs were generated using GraphPad Prism. For all behavioral experiments, a two-way repeated-measures analysis of variance (ANOVA) was performed, with treatment (VEH/NTG), drug (VEH/SNC80) and time (days) as factors. For immunohistochemical experiments comparing two groups, a Student's t-test was performed. For immunohistochemical experiments comparing more than two groups, a one-way ANOVA was performed. When a significant interaction occurred, subsequent Holm-Sidak post-hoc analysis was performed. A significance level of $p<0.05$ was used.

4.3. RESULTS

4.3.1. DOR ACTIVATION FOLLOWING NTG PREVENTS THE DEVELOPMENT OF MIGRAINE-ASSOCIATED PAIN

To determine whether DOR activation prevents the development of NTG-induced migraine-associated pain, we tested the effect of SNC80 following systemic chronic, intermittent NTG injections. We used separate groups of mice for hind paw (peripheral) and cephalic (periorbital) mechanical sensitivity testing. On days 1, 3, 5, 7, and 9, we treated the mice with either VEH or NTG, followed by VEH or SNC80 (Figure 18A). We tested peripheral regions on days 1, 3, 5, 7, and 9, and cephalic regions on days 1, 5, and 9 (Figure 18A). On each test day, mice habituated to the testing rack for peripheral testing, and cups on racks for cephalic testing, for 20 minutes before baseline testing (Figure 18B). After baseline testing on day 1, we counterbalanced treatment groups based on basal threshold response, and then treated with either VEH or NTG (Figure 18B). After 1h and 15 mins, we randomly assigned drug groups and administered either VEH or SNC80 to each treatment group (Figure 18B). After 45 mins, we tested post-drug mechanical thresholds and returned the mice to their home cage (Figure 18B). Mice chronically treated with NTG-VEH had significantly lower peripheral basal thresholds to mechanical pain on each test day, an effect not seen in VEH-VEH mice (Figure 18C). Interestingly, NTG-treated mice that were subsequently treated with SNC80 showed significantly higher peripheral mechanical thresholds than NTG-VEH mice on each test day (Figure 18C). NTG-SNC80 thresholds were similar to VEH-VEH thresholds, and only differed on day 3 ($p<0.001$) and day 7 ($p<0.05$) (Figure 18C. In addition to preventing the development of NTG-induced peripheral basal hypersensitivity, SNC80 also reversed

NTG-evoked hypersensitivity on each test day (Figure 18D). Similar to peripheral thresholds, cephalic testing revealed that chronic NTG induced a basal level of cephalic hypersensitivity which was blocked by chronic, intermittent SNC80 administration (Figure 18E). SNC80 also inhibited NTG-evoked cephalic hypersensitivity on each test day (Figure 18F). Altogether, these results demonstrate that activating the DOR following NTG injections can inhibit the development of NTG-induced basal hypersensitivity in peripheral and cephalic regions.

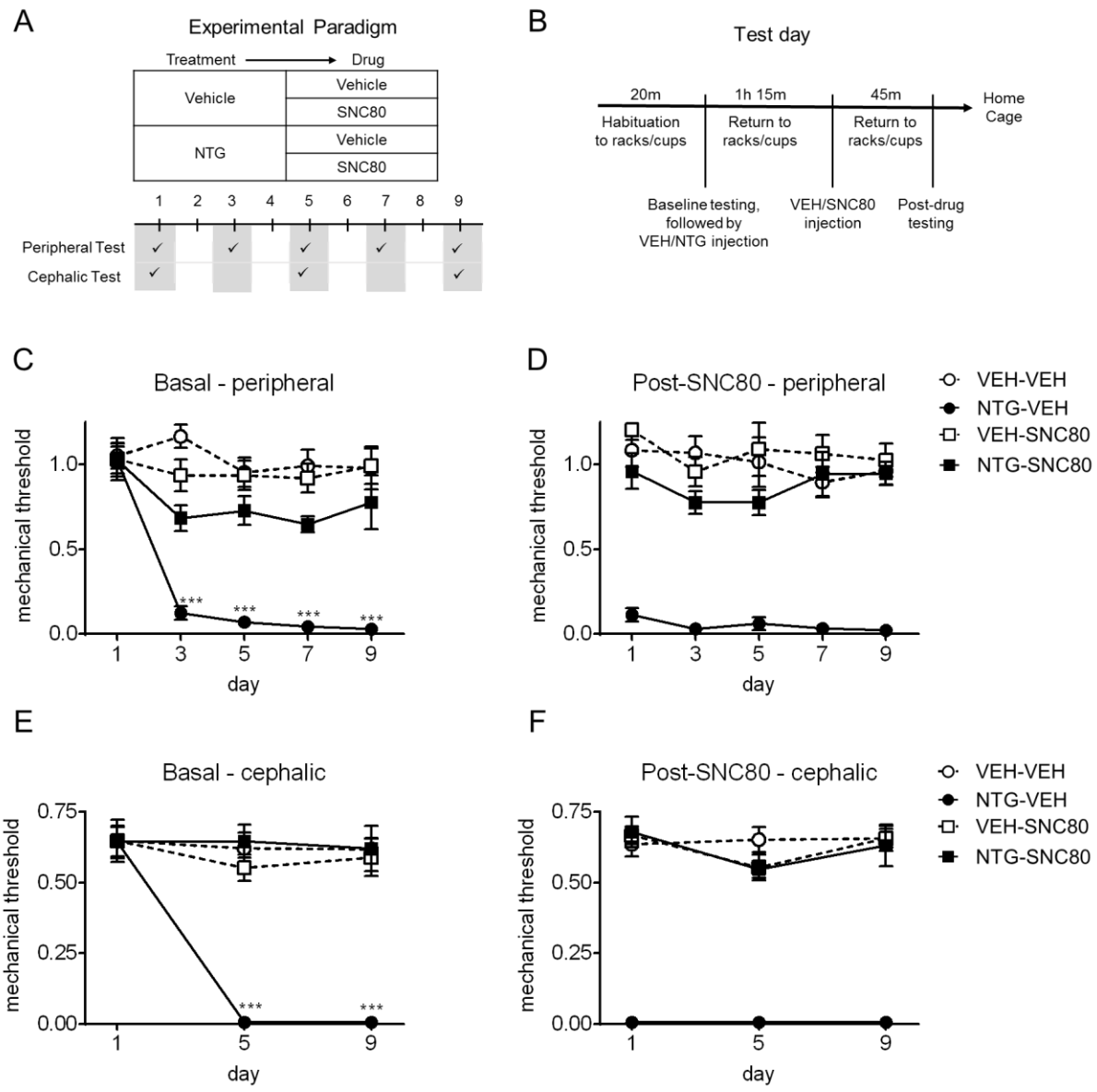


Figure 18: Chronic, intermittent SNC80 administered subsequently to NTG prevents the development of NTG-induced mechanical allodynia. (a) Experimental paradigm. Male and female C57BL6/J mice were treated with vehicle (0.9% NaCl; VEH) or NTG (10 mg/kg, ip), followed by VEH or SNC80 (10 mg/kg, ip), every second day for 9 days. Separate groups of mice were tested for peripheral (days 1, 3, 5, 7, and 9) and cephalic (days 1, 5, and 9) allodynia. (b) Test day schematic. Baselines were measured prior to VEH/NTG administration. Post-drug thresholds were measured 45 min. after VEH/SNC80 administration. (c) Over the course of 9 days, NTG-VEH mice developed a basal level of peripheral hypersensitivity, an effect that was reversed in NTG-SNC80 mice. $p<0.0001$ effect of treatment group, time, and interaction, two-way RM ANOVA, Holm-Sidak post hoc analysis, n=8/group, *$p<0.05$, **$p<0.01$***$p<0.001$ relative to VEH-VEH. (d) On each test day, NTG evoked peripheral hypersensitivity, and SNC80 reversed this acute allodynia. $p<0.0001$ effect of treatment group. (e) Over the course of 9 days, NTG mice

developed a basal level of cephalic hypersensitivity, an effect that was reversed in NTG-SNC80 mice. $p<0.0001$ effect of treatment group, day, and interaction, two-way RM ANOVA, Holm-Sidak post hoc analysis, n=6/group, ***$p<0.001$ relative to VEH-VEH. (f) On each test day, NTG evoked cephalic hypersensitivity, and SNC80 reversed this acute allodynia. $p<0.0001$ effect of treatment group. DOR activation after NTG administration prevents the development of acute and chronic migraine-associated hypersensitivity in peripheral and cephalic regions.

4.3.2. DOR ACTIVATION SUPPRESSES NTG-INDUCED CGRP INCREASE IN THE TRIGEMINAL GANGLIA AND ITS MAIN OUTPUT REGION, THE TRIGEMINAL NUCLEUS CAUDALIS

To determine whether migraine-associated pain via chronic, intermittent injections of NTG could result in increased levels of CGRP in migraine-associated regions, we visualized and measured CGRP within the trigeminal ganglia (TG) and trigeminal nucleus caudalis (TNC). We also determined the effect of NTG-SNC80 on CGRP expression within the TG and TNC. Separate groups of mice were used for behavioral experiments (Figure 18C-F) and immunohistological experiments (Figure 19), and we followed the same treatment and drug paradigm as mentioned prior (Figure 18A). Outlined arrowheads in the representative TG images show CGRP+ cells within the TG (Figure 19A). Mice chronically treated with NTG-VEH had significantly more CGRP+ cells in the TG as compared to VEH-VEH (Figure 19B). Interestingly, NTG-SNC80 mice had CGRP+ cell counts comparable to those seen in the VEH-VEH group (Figure 19B). Using fluorescence intensity as a proxy for how much CGRP is being expressed within each CGRP+ cell, we measured fluorescence intensity of each CGRP+ cell within the TG and compared across all groups. NTG-VEH mice showed more fluorescent CGRP+ cells than VEH-VEH, and NTG-SNC80 CGRP+ cells had fluorescence intensity levels comparable to VEH-VEH

(Figure 19C). Preliminary studies also suggest that a majority of the cells in the TG are myelinated (NF200+, a marker of myelination), and confirm that CGRP+ cells in the TG are not nonpeptidergic (IB4+) (Figure 20). To determine whether this effect was propagated downstream to the trigeminocervical complex, we visualized and measured CGRP expression in the TNC. Immunohistological staining showed CGRP afferent endings in the superficial laminae of the TNC (Figure 19D; dashed lines outline the lateral edge of the superficial laminae of the TNC), and no CGRP cell bodies. By using fluorescence intensity of the superficial laminae of the TNC as a proxy for how much CGRP was being released, we measured and compared CGRP levels among all groups. NTG-VEH mice had significantly more fluorescent CGRP expression as compared to VEH-VEH mice (Figure 19E). NTG-SNC80 mice had CGRP fluorescence comparable to VEH-VEH mice (Figure 19E). Overall, chronic, intermittent SNC80 administration suppressed the NTG-induced increase of CGRP within the TG and TNC.

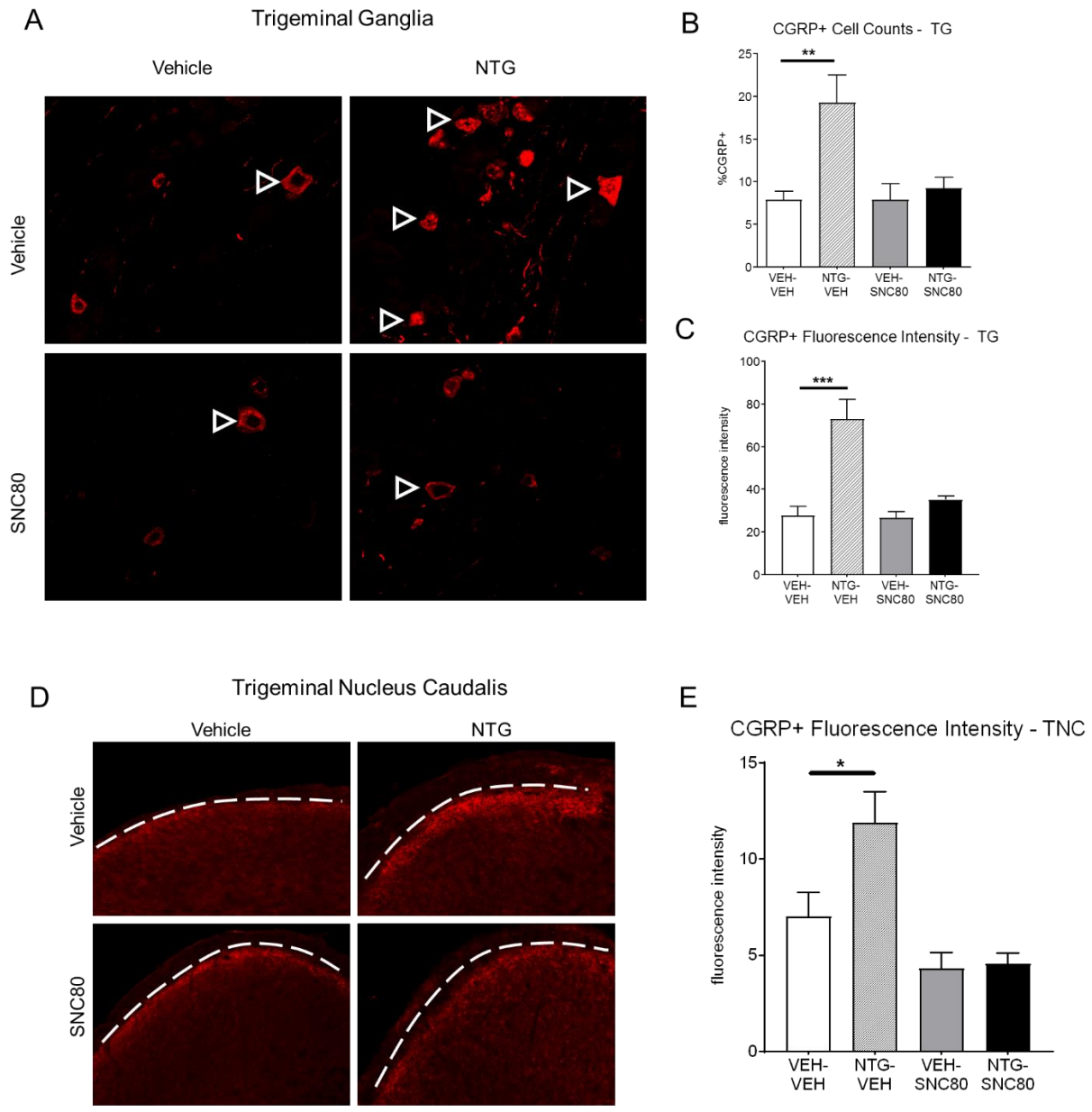


Figure 19: Chronic, intermittent SNC80 administered subsequently to NTG suppresses NTG-induced CGRP increase in the trigeminal ganglia and trigeminal nucleus caudalis. (a) Representative images of CGRP expression in the trigeminal ganglia of treatment groups. Outlined arrowheads point to CGRP+ cells in the trigeminal ganglia. (b) Chronic NTG administration increases CGRP expression in the trigeminal ganglia, an effect that was reversed in NTG-SNC80 mice. $p<0.05$ treatment, drug, and interaction, n=4/group, two-way ANOVA, Holm-Sidak post hoc analysis, **p=0.05 relative to VEH-VEH. (c) Chronic NTG administration increased the amount of CGRP expressed within each CGRP+ cell in the trigeminal ganglia, as detected by fluorescence intensity. NTG mice concurrently treated with SNC80 showed suppressed levels of CGRP, similar to VEH mice. $p<0.001$ treatment, drug, and interaction, n=5/group, two-way ANOVA, Holm-Sidak post hoc analysis, ***$p<0.001$ relative to VEH-VEH. (d) Representative images of CGRP expression in the trigeminal nucleus caudalis of test groups. Dashed lines outline the

lateral edge of the superficial laminae of the trigeminal nucleus caudalis. (e) Chronic NTG administration increased CGRP expression in the superficial layers of the trigeminal nucleus caudalis, an effect that was reversed in NTG-SNC80 mice. $p=0.01$ effect of treatment group, n=4/group, one-way ANOVA, Holm-Sidak post hoc analysis, $*p<0.05$ relative to VEH. Chronic, intermittent NTG increases CGRP expression in the trigeminal ganglia and trigeminal nucleus caudalis, an effect that is suppressed by chronic, intermittent DOR activation.

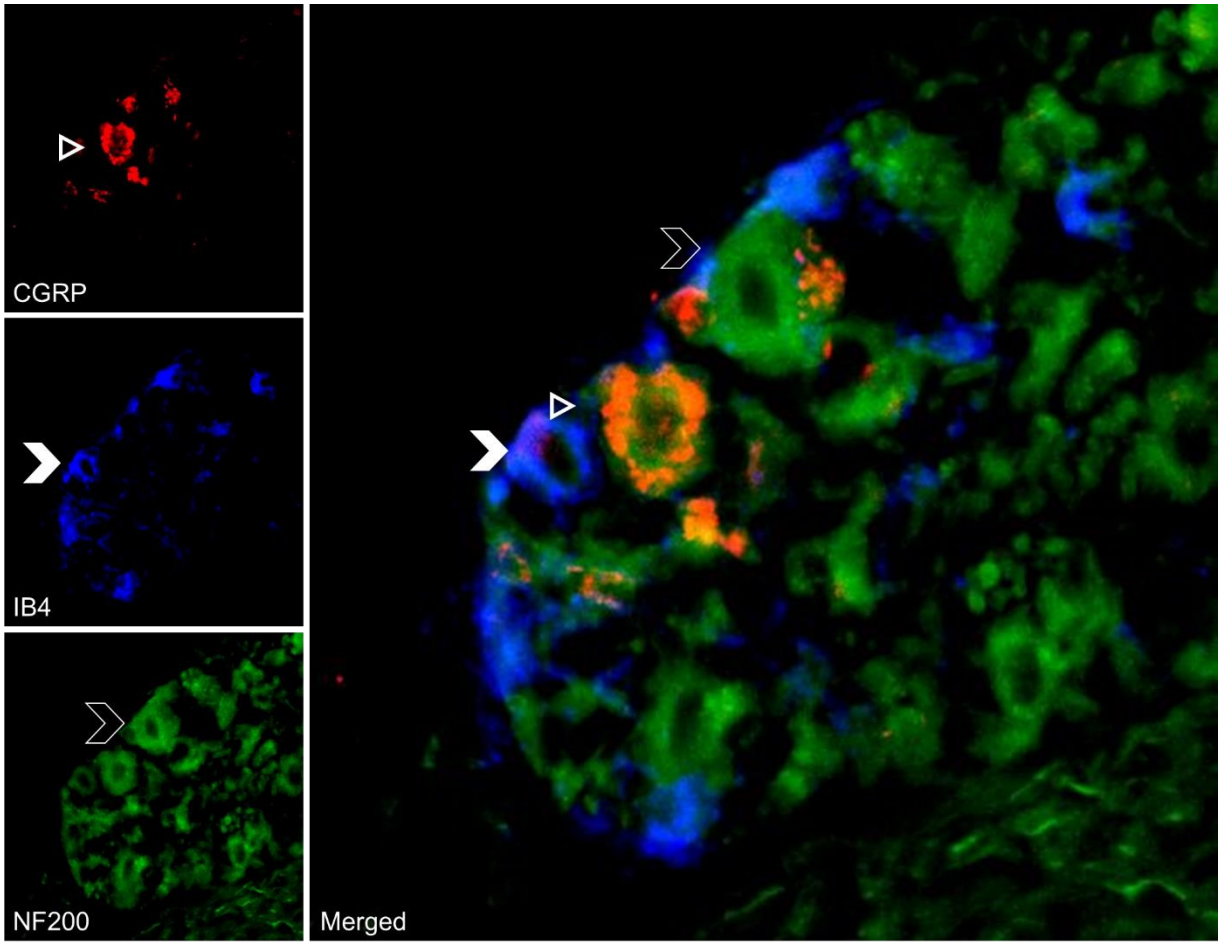


Figure 20: Representative image of CGRP, IB4, and NF200 in the trigeminal ganglia. (a) In the left panel, outlined arrowhead shows a CGRP+ cell, filled in chevron shows an IB4+ cell, and an outlined chevron shows a NF200+ cell. In the right panel, the merged image shows the 3 markers for cell types in the trigeminal ganglia.

4.3.3. NTG INCREASES CGRP AND DOR EXPRESSION, BUT NOT CO-EXPRESSION, IN THE TRIGEMINAL GANGLIA

To determine whether NTG-induced CGRP increase was reproducible in DOReGFP knockin mice, we used immunohistochemistry to visualize and measure CGRP expression in the TG. Similar to C57BL6/J mice, DOReGFP knockin mice also develop a basal level of cephalic hypersensitivity to chronic, intermittent NTG (Figure 22). Additionally, we also wanted to determine whether chronic, intermittent NTG altered DOR

expression, as well as co-expression of CGRP and DOR, in the TG. Representative images of VEH (Figure 21A) and NTG (Figure 21B) TG demonstrate DOR+ cells with filed in arrowheads, CGRP+ cells with outlined arrowheads, and merged images with DAPI have outlined boxes around DOR+ and CGRP+ cells (Figure 21A). An outlined chevron points to a cell which co-expresses CGRP and DOR in the NTG TG (Figure 21B). Chronic NTG also results in increased CGRP expression in DOReGFP knockin TG, similar to that seen in C57BL6/J mice (Figure 21C, D). Interestingly, chronic, intermittent NTG resulted in increased number of DOR+ cells in the TG (Figure 21E). Using fluorescence intensity as a proxy for DOR functionality, we measured and compared fluorescence intensity of DOR+ cells in the VEH and NTG groups. Chronic, intermittent NTG resulted in increased fluorescence intensity of the DOR+ population relative to VEH groups (Figure 21F). We also measured how many cells co-expressed both CGRP and DOR and found that NTG did not affect the percentage of co-expressed cells within the DOR+ and CGRP+ population (Figure 21G-H). Ultimately, NTG resulted in increased CGRP and DOR expression, but not co-expression, in the TG.

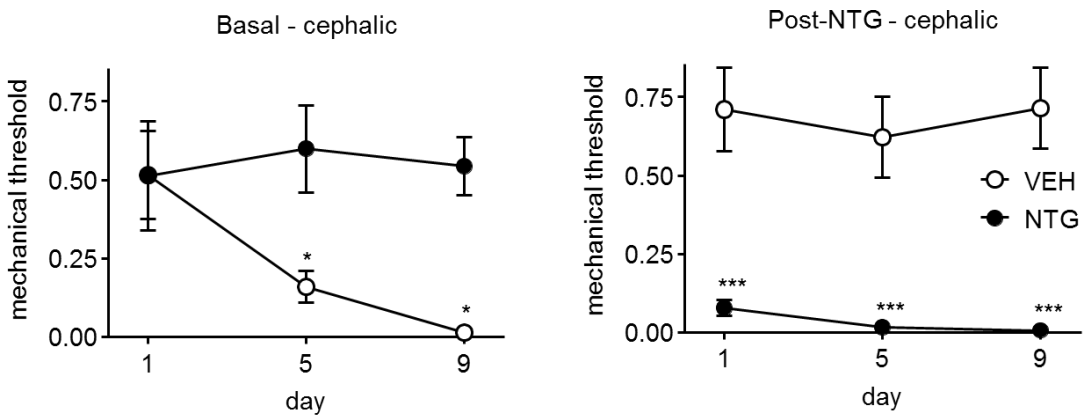


Figure 21: Chronic NTG results in the development of basal hypersensitivity similar to that seen in male and female C57Bl6/J mice. (a) Chronic NTG results in the development of basal cephalic hypersensitivity, an effect not seen in VEH mice. *p<0.05 effect of treatment group, t-test per day, n=5/group. (b) NTG evokes acute cephalic hypersensitivity similar to C57Bl6/J mice. ***p<0.001 effect of treatment group, t-test per day, n=5/group.

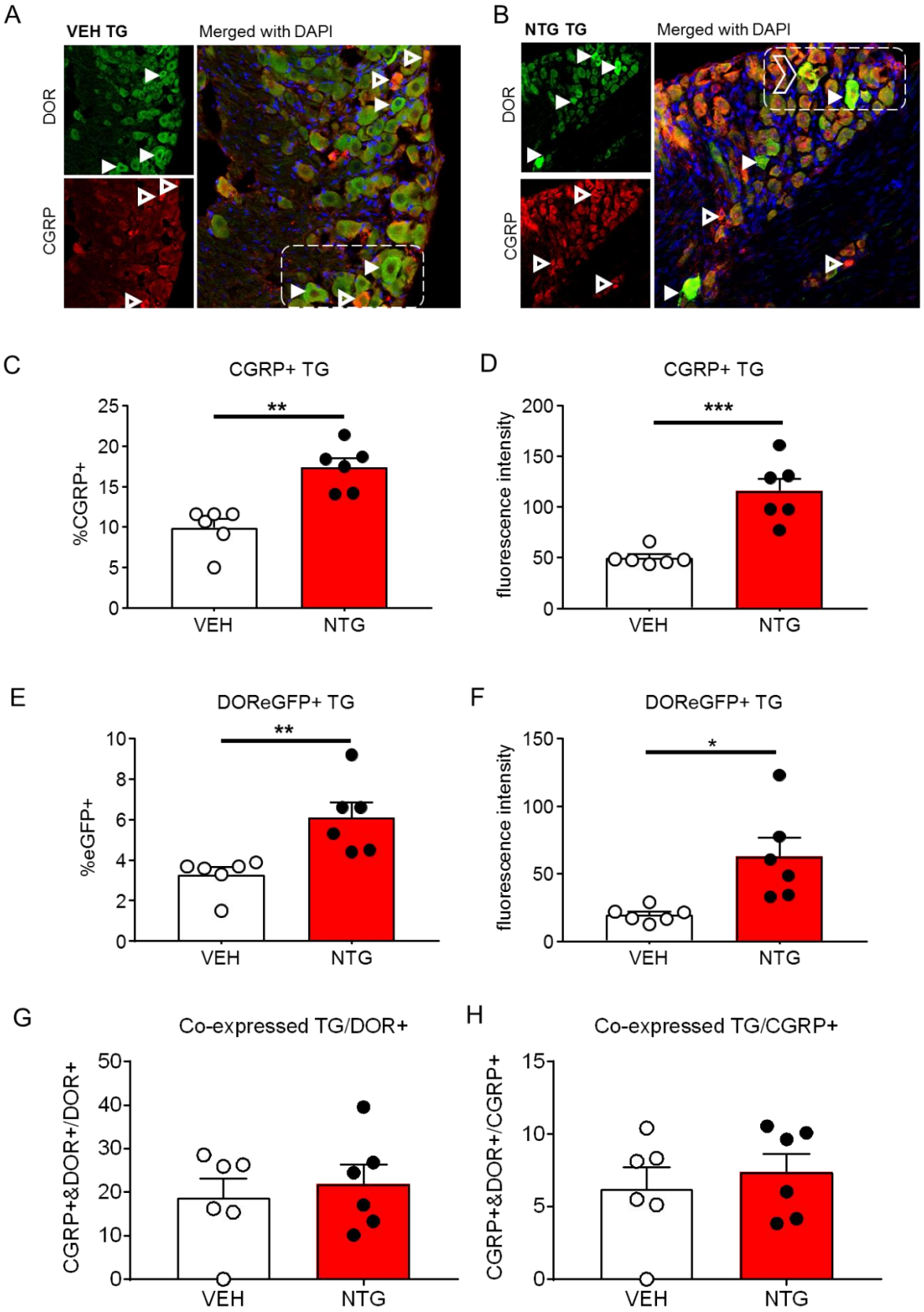

A
VEH TG
Merged with DAPI
DOR
CGRP
B
NTG TG
Merged with DAPI
DOR
CGRP
C
CGRP+ TG
%CGRP+
**
VEH
NTG
D
CGRP+ TG
fluorescence intensity
***
VEH
NTG
E
DOReGFP+ TG
%eGFP+
**
VEH
NTG
F
DOReGFP+ TG
fluorescence intensity
*
VEH
NTG
G
Co-expressed TG/DOR+
CGRP+&DOR+/DOR+
VEH
NTG
H
Co-expressed TG/CGRP+
CGRP+&DOR+/CGRP+
VEH
NTG

Figure 22: Chronic, intermittent NTG increases CGRP and DOR expression, but not co-expression, in the trigeminal ganglia. (a, b) Representative images of DOReGFP+ and CGRP+ cells in the trigeminal ganglia after chronic VEH and NTG. Outlined arrowheads point to CGRP+ cells and filled in arrowheads point to DOR+ cells in the trigeminal ganglia. Dashed lines in the merged images outline DOR+ and CGRP+ cells. An outlined chevron points to a cell co-expressing CGRP and DOR in the NTG group. (c) NTG increases the percentage of CGRP+ cells in the TG of DOReGFP knockin mice. ***p<0.001, t-test, n=6/group. (d) NTG increases fluorescence intensity in each CGRP+ trigeminal ganglia in DOReGFP knockin mice. ***p<0.001, t-test, n=6/group. (e) NTG increases the percentage of DOR+ cells in the TG of DOReGFP knockin mice. **p=0.01, t-test, n=6/group. (f) NTG increases fluorescence intensity in each DOR+ trigeminal ganglia in DOReGFP knockin mice. *p=0.05, t-test, n=6/group. (g) NTG does not alter co-expression of CGRP+ and DOR+ cells within the DOR+ population in the trigeminal ganglia. p=0.6202, t-test, n=6/group. (h) NTG does not alter co-expression of CGRP+ and DOR+ cells within the CGRP+ population in the trigeminal ganglia. p=0.5715, t-test, n=6/group. Chronic NTG increases CGRP+ and DOR+ cells, but not co-expression, in the trigeminal ganglia.

4.3.4. CHRONIC, INTERMITTENT NTG INCREASES EXPRESSION OF CGRP AND DOR WITHIN THE SUPERFICIAL LAMINAE OF THE TRIGEMINAL NUCLEUS CAUDALIS

To determine whether systemic NTG also modulates CGRP and DOR expression in migraine-associated areas downstream from the TG, we used immunohistochemistry to visualize and measure CGRP and DOR expression in the TNC. Dashed lines in representative images represent the lateral edge of the superficial laminae of the TNC (Figure 23A). While DOR expression seemed diffusely spread across the TNC (Figure 23A; top panel), CGRP expression was robustly expressed in the superficial laminae of the TNC (Figure 23A; middle panel). We compared the fluorescence intensity of DOR and CGRP in the superficial laminae of the TNC and found that NTG did not alter the expression of DOR (Figure 23B), but reproducibly increased CGRP expression in the TNC (Figure 23C). A plot profile of the fluorescence across the TNC shows that CGRP

expression is most robust in the transition from the trigeminal spinal tract through lamina I of the TNC, and that DOR expression begins to increase in the transition from lamina 1 through the deeper laminae of the TNC (Figure 23D). One way to better visualize how NTG affects DOR expression on a cellular level is to administer SNC80, which will cause internalization of the DOR. Filled in arrowheads in representative images show DOR+ cells in the superficial laminae of the TNC in both VEH and NTG groups. NTG caused an increase in the percentage of DOR+ cells in the superficial laminae of the TNC (Figure 23F), as well as an increase in fluorescence intensity of each DOR+ cell (Figure 23G). Altogether, chronic, intermittent NTG results in the increased expression of CGRP and DOR within the TNC.

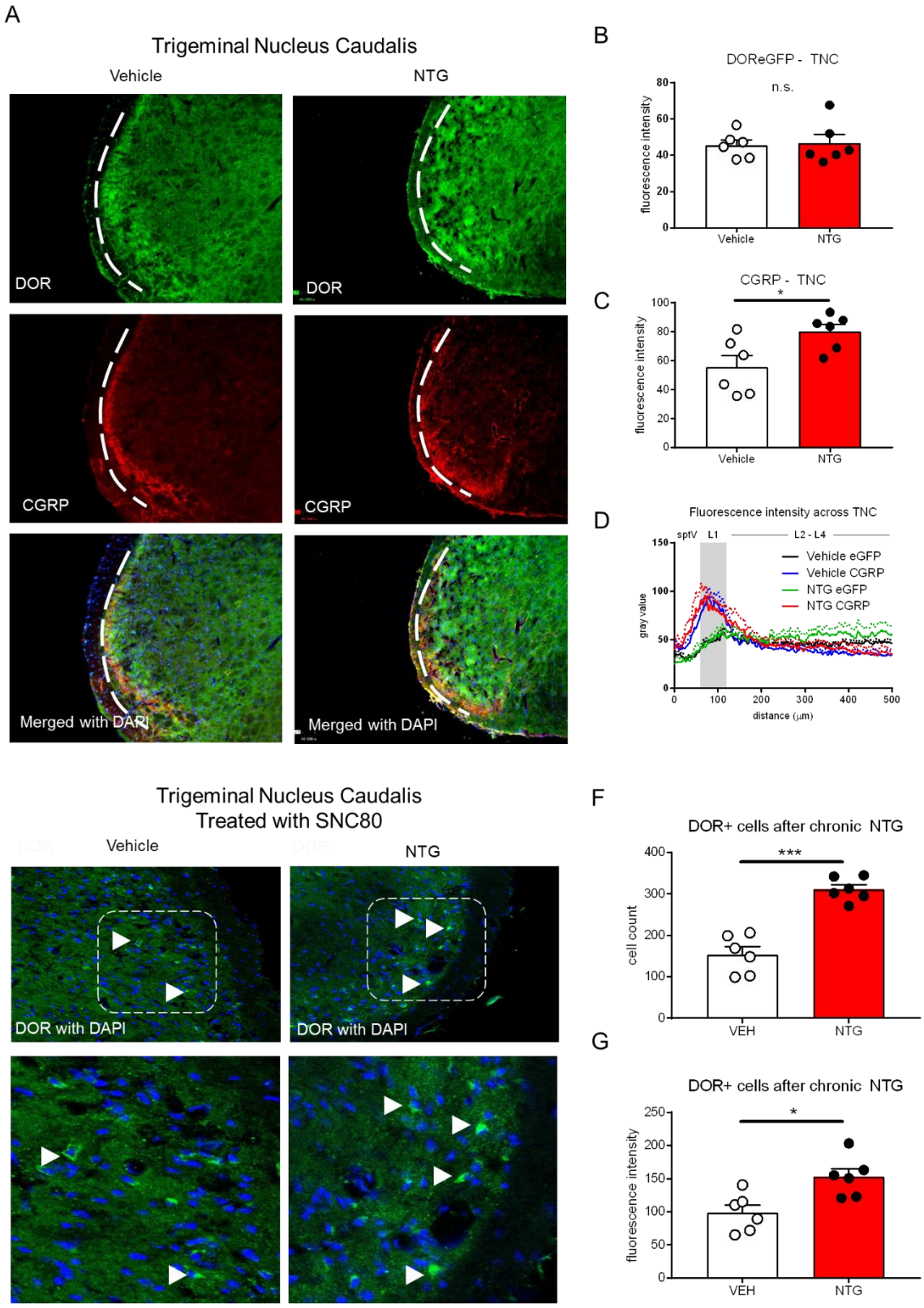
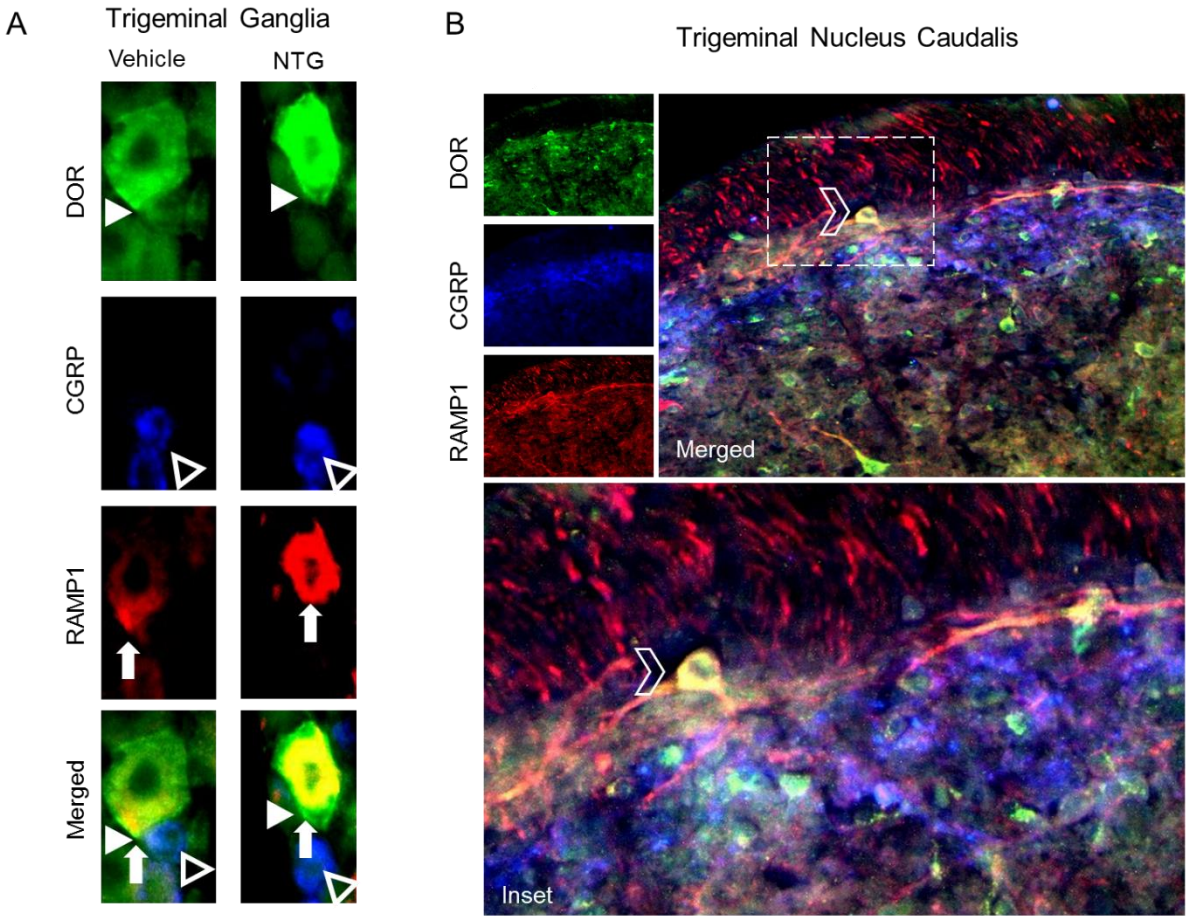

A
Trigeminal Nucleus Caudalis
Vehicle
NTG
DOR
DOR
CGRP
CGRP
Merged with DAPI
Merged with DAPI
B
DOReGFP - TNC
n.s.
fluorescence intensity
Vehicle
NTG
C
CGRP - TNC
*
fluorescence intensity
Vehicle
NTG
D
Fluorescence intensity across TNC
sptV
L1
L2 - L4
Vehicle eGFP
Vehicle CGRP
NTG eGFP
NTG CGRP
gray value
distance (μm)
E
Trigeminal Nucleus Caudalis
Treated with SNC80
Vehicle
NTG
DOR with DAPI
DOR with DAPI
F
DOR+ cells after chronic NTG
***
cell count
VEH
NTG
G
DOR+ cells after chronic NTG
*
fluorescence intensity
VEH
NTG

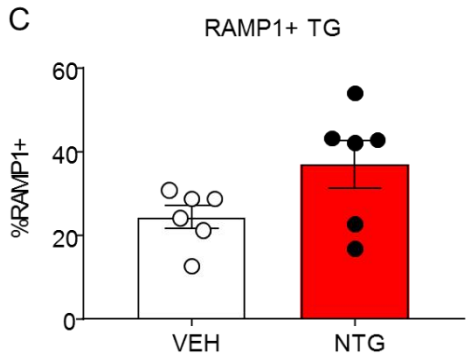
Figure 23: NTG increases CGRP+ expression in the superficial laminae of the trigeminal nucleus caudalis in DOReGFP knockin mice. (a) Representative images of DOR+ and CGRP+ expression in DOReGFP knockin mice after chronic VEH or NTG. Dashed lines outline the lateral edge of the superficial laminae of the trigeminal nucleus caudalis. (b) NTG does not appear to cause a diffuse increase of DOR+ expression in the superficial laminae of the trigeminal nucleus caudalis. p=0.8532, t-test, n=6/group. (c) NTG increases CGRP expression in superficial laminae of the trigeminal nucleus caudalis. *p=0.05, t-test, n=6/group. (d) Plot profile detailing fluorescence intensity from the trigeminal tract through the deeper laminae of the trigeminal nucleus caudalis. CGRP and DOR fluorescence peak in different laminae of the trigeminal nucleus caudalis. (e) Representative images of internalized DOR+ cells in DOReGFP knockin mice after chronic VEH or NTG. Filled in arrowheads point to DOR+ cells in the superficial laminae of the trigeminal nucleus caudalis. The bottom panel focuses on the inset outlined in the top panel. (f) NTG increases the percentage of DOR+ cells in the superficial laminae of the trigeminal nucleus caudalis. ***p<0.0001, t-test, n=6/group. (g) NTG increases the fluorescence intensity of each DOR+ cell in the superficial laminae of the trigeminal nucleus caudalis. *p<0.05, t-test, n=6/group. Chronic NTG increases CGRP and DOR expression in the superficial laminae of the trigeminal nucleus caudalis.

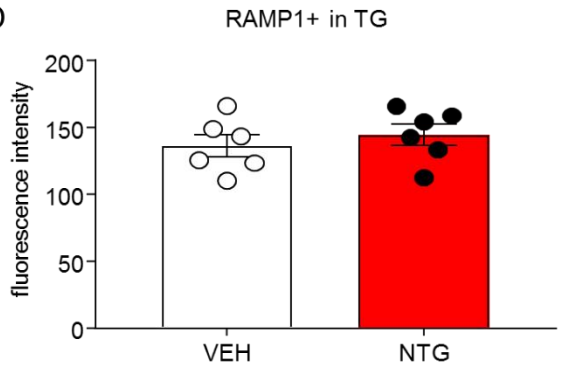
4.3.5. THE CGRP RECEPTOR IS ROBUSTLY CO-EXPRESSED WITH DOR IN THE TRIGEMINAL GANGLIA AND TRIGEMINAL NUCLEUS CAUDALIS

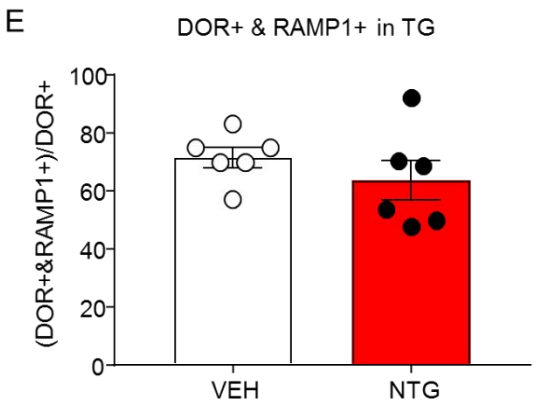
To discover a possible mechanism by which DOR regulates NTG-induced migraine-associated pain, we used immunohistochemistry to visualize RAMP1, the rate limiting molecule within the CGRP receptor (CGRPR), in the TG and TNC. Filled in arrowheads in the representative images show DOR+ large cells (top panel), outlined arrowheads show CGRP+ small cells (2nd to top panel), filled in arrows RAMP1+ large cells (2nd to bottom panel), and a merged image demonstrating a cell which co-expresses DOR and RAMP1 and is in close proximity to a CGRP+ small cell (bottom panel) (Figure 24A). NTG resulted in increased RAMP1 expression in the TG (Figure 24C), without any change in cellular fluorescence between groups (Figure 24D). These results suggest that there is a high co-expression between RAMP1 and DOR in the TG, which remains stable after chronic, intermittent NTG (Figure 24D). Preliminary studies also suggest that in the

DOReGFP knockin mouse, DOR+ and CGRP+ cells are not IB4+ (Figure 25). IB4 is a marker for non-peptidergic cells, and these results suggest that DOR+ cells are mainly peptidergic. To determine whether this co-expression is present in migraine-associated areas downstream from the TG, we internalized the DOR via SNC80 administration, and visualized DOR, RAMP1, and CGRP in the TNC. Based on prior results suggesting that DOR and CGRP are not co-expressed in the TNC, we measured co-expression of DOR and RAMP1 in the superficial laminae of the TNC. Similar to the TG, there is a high co-expression of DOR and RAMP1 in the TNC (Figure 24E), which remains stable after chronic, intermittent NTG (Figure 24F). To confirm that we were examining the CGRP receptor, we also used RNAScope to visualize the calcitonin receptor like receptor (CRLR), a key component of the CGRP receptor (Figure 25). There are issues with the antibodies currently available for CRLR, which led us to using RNAScope to determine colocalization of the DOR and CRLR. There was a high coexpression of CRLR and DOR in the superficial laminae of the TNC (Figure 25). Interestingly, chronic NTG did not significantly alter gene expression of DOR or CRLR (Figure 25 B-E). Additionally, preliminary studies suggest that a majority of these DOR+ cells in the superficial laminae of the TNC are GAD65/67+, are specifically parvalbumin+ (Figure 26A). In addition to their GABAergic profile, DOR+ cells highly express RAMP1, a key molecule in the CGRPR complex.


A
Trigeminal Ganglia
Vehicle
NTG
DOR
CGRP
RAMP1
Merged
B
Trigeminal Nucleus Caudalis
DOR
CGRP
RAMP1
Merged
Inset


C
RAMP1+ TG
%RAMP1+
60
40
20
0
VEH
NTG


D
RAMP1+ in TG
fluorescence intensity
200
150
100
50
0
VEH
NTG


E
DOR+ & RAMP1+ in TG
(DOR+&RAMP1+)/DOR+
100
80
60
40
20
0
VEH
NTG

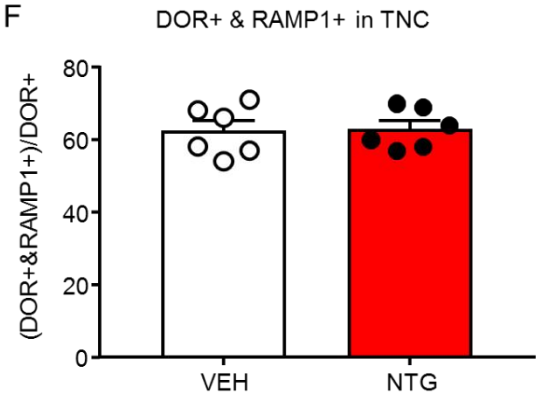

F
DOR+ & RAMP1+ in TNC
(DOR+&RAMP1+)/DOR+
80
60
40
20
0
VEH
NTG

Figure 24: DOR is highly co-expressed with RAMP1 in the trigeminal ganglia and the trigeminal nucleus caudalis. (a) Representative images of DOR, CGRP, and RAMP1 in the trigeminal ganglia after chronic VEH or NTG. Filled in arrowheads point to a DOR+ cell, outlined arrowheads point to a CGRP+ cell, and a filled in arrow points to a RAMP1+ cell. (b) Representative image of DOR, CGRP, and RAMP1 in the trigeminal nucleus caudalis after chronic NTG. Outlined chevron points to a DOR+ and RAMP1+ cell in the superficial laminae of the trigeminal nucleus caudalis (c) NTG increases the percentage of RAMP1+ cells in the trigeminal ganglia. *p=0.05, t-test, n=6/group. (d) NTG does not alter fluorescence intensity of RAMP1 within RAMP1+ trigeminal ganglia. p=0.4797, t-test, n=6/group. (e) NTG does not alter co-expression of RAMP1+ and DOR+ trigeminal ganglia within the DOR+ population. p=0.3347, t-test, n=6/group. (f) NTG does not alter co-expression of RAMP1+ and DOR+ cells within the DOR+ population in the superficial laminae of the trigeminal nucleus caudalis. p=0.6564, t-test, n=6/group. There is a high co-expression of DOR+ and RAMP1+ cells in the trigeminal ganglia and superficial layers of the trigeminal nucleus caudalis.

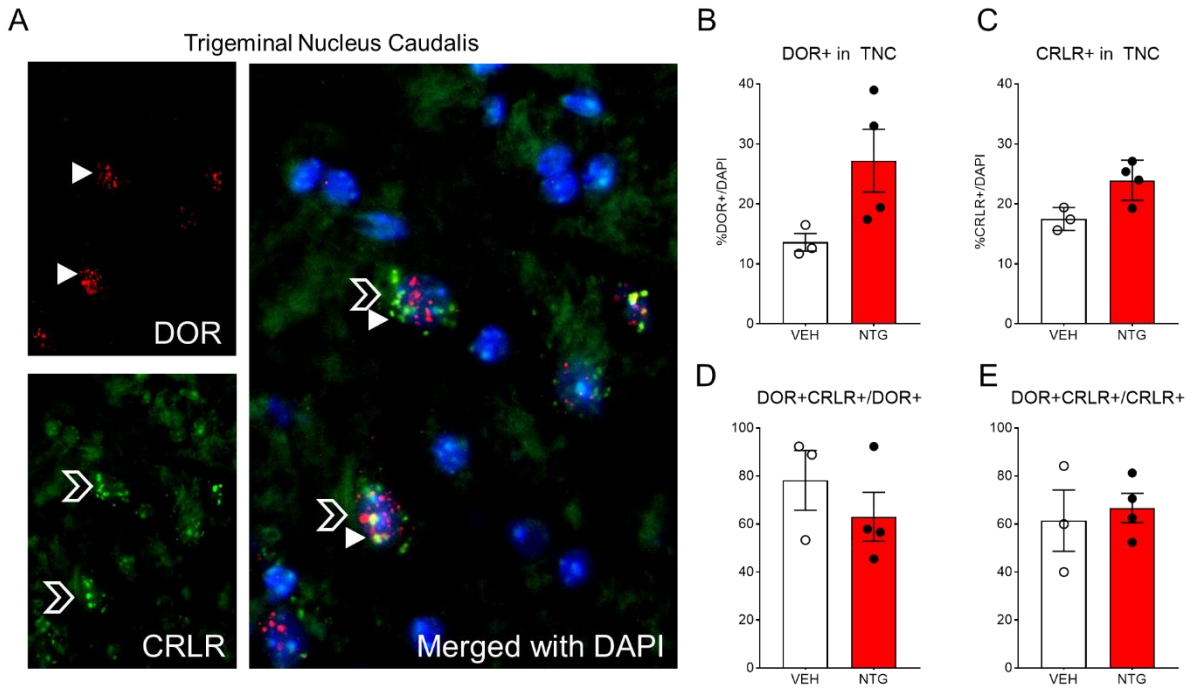


Figure 25: DOR is highly co-expressed with CRLR in the superficial laminae of the trigeminal nucleus caudalis (TNC). (a) Representative images of DOR and CRLR in the TNC in naïve mice. Filled in arrowheads point to a DOR+ cell, outlined chevrons point to a CRLR+ cell, and the merged image shows colocalization of both markers (c) NTG does not cause a significant increase in CRLR+ cells in the TNC (d) NTG does not alter co-expression of CRLR+ and DOR+ trigeminal ganglia within the DOR+ population. (E) NTG does not alter co-expression of CRLR+ and DOR+ cells within the CRLR+ population in the superficial laminae of the TNC. There is a high co-expression of DOR+ and CRLR+ cells in the superficial layers of the trigeminal nucleus caudalis that remain unchanged after chronic NTG.

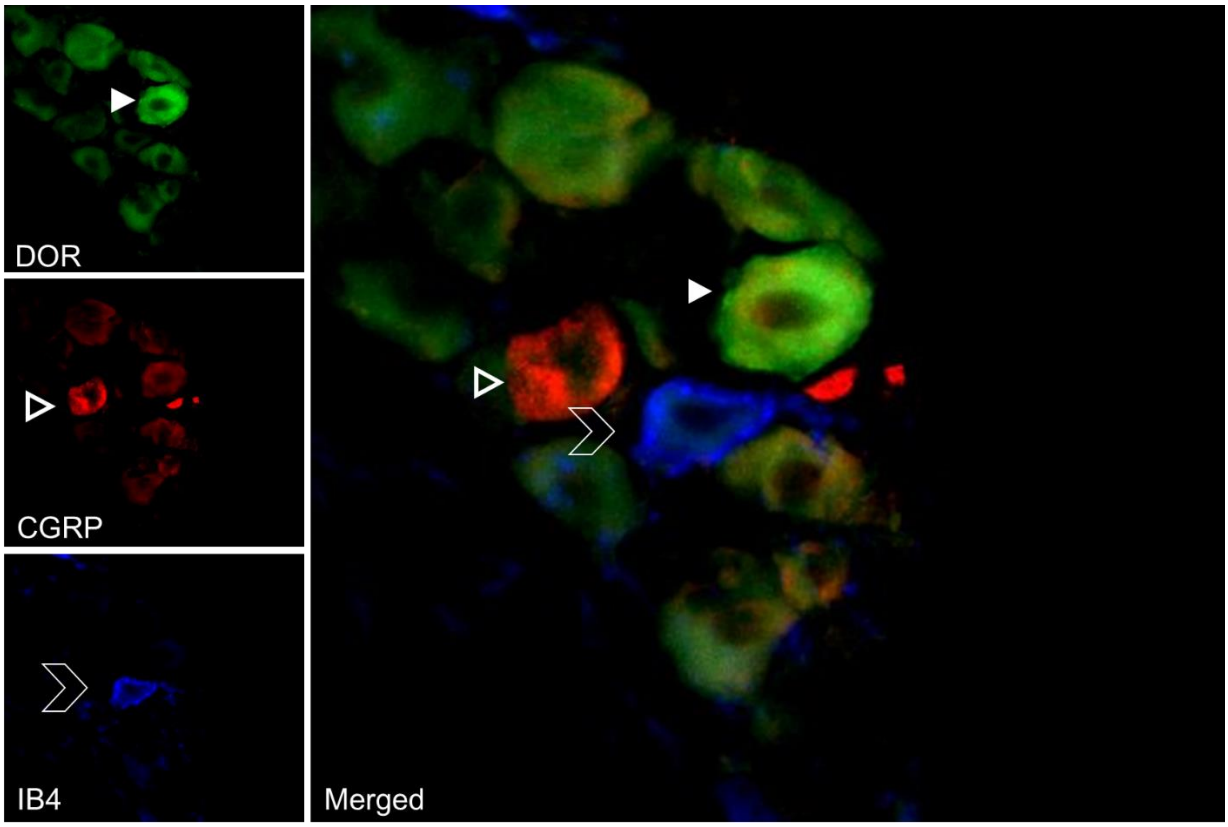


Figure 26: Representative image of DOR, CGRP, and IB4 in the trigeminal ganglia. (a) In the left panel, filled in arrowhead shows a DOR+ cell, an outlined arrowhead shows a CGRP+ cell, and an outlined chevron shows an IB4+ cell. In the right panel, the merged image shows the 3 markers for cell types in the trigeminal ganglia.

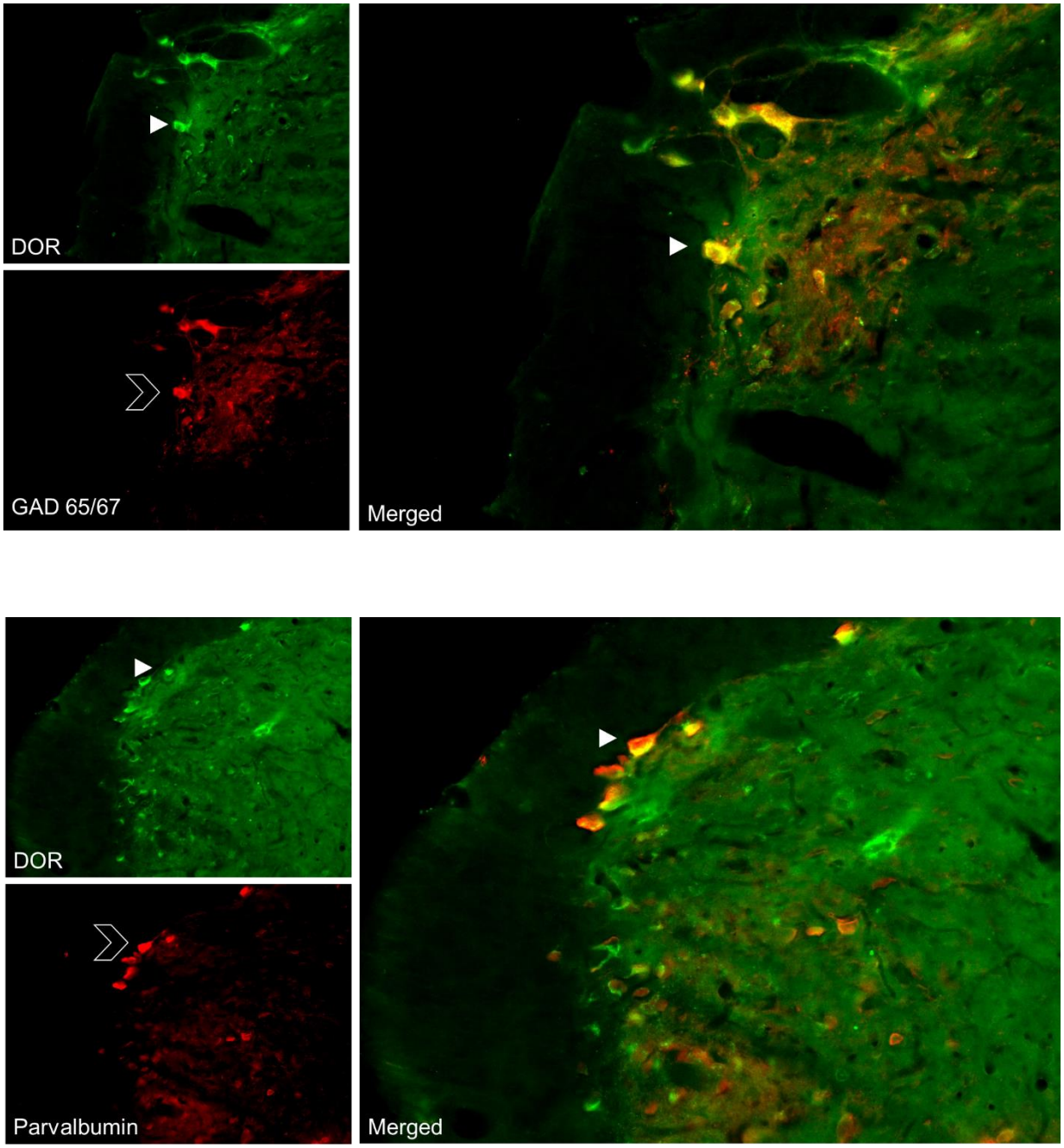
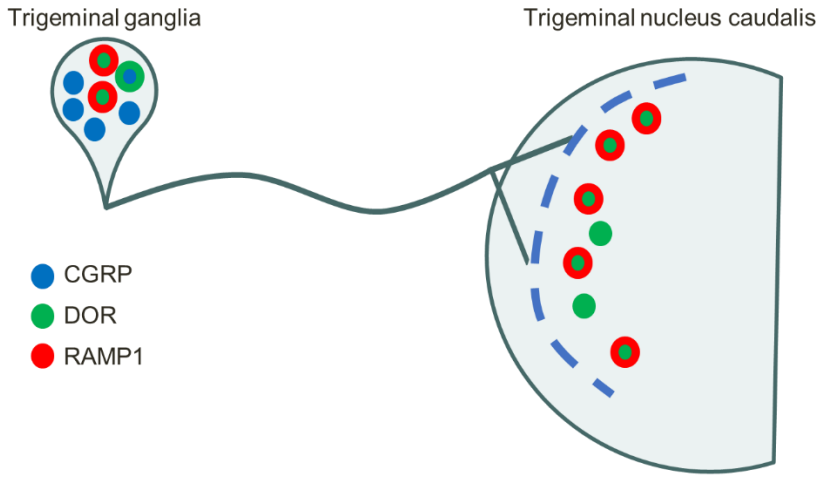


Figure 27: Representative image of DOR and GABAergic markers in the trigeminal ganglia. (a) In the left panel, filled in arrowhead shows a DOR+ cell, and an outlined chevron shows a GAD65/76+ cell. In the right panel, the merged image shows the 2 markers for cell types in the trigeminal ganglia. (b) In the left panel, filled in arrowhead shows a DOR+ cell, and an outlined chevron shows a Parvalbumin+ cell. In the right panel, the merged image shows the 2 markers for cell types in the trigeminal ganglia.

4.3.6. DOR ACTIVATION COULD INHIBIT THE ABILITY OF THE CGRP TO BIND TO THE CGRP RECEPTOR

The aim of this study was to determine a mechanism by which DOR activation regulates migraine-associated pain. We found that CGRP and DOR are minimally expressed in the TG and TNC (Figure 27A), and that DOR is highly co-expressed with RAMP1, the rate limiting molecule of the CGRPR (Figure 27A). Interestingly, we found that chronic NTG increases CGRP and DOR expression, but not co-expression, in the TG and TNC (Figure 27B). We also found that the co-expression of DOR and RAMP1 does not change after chronic NTG (Figure 27B), possibly due to the already high co-expression of the two molecules. Our results suggest that after chronic NTG, CGRP could bind to the CGRPR and result in migraine-associated pain (Figure 27C). By activating the DOR, inhibition of the cell prevents CGRP binding to the CGRPR, resulting in the prevention of migraine-associated hypersensitivity (Figure 27C). Further studies will focus on the GABAergic profile of these DOR+ cells in the TG and TNC and will also probe the interplay between the DOR and the CGRPR.

A



B

Migraine-associated pain (NTG)			
Trigeminal Ganglia		Trigeminal Nucleus Caudalis	
↑ CGRP	• Low CGRP & DOR co-expression • High RAMP1 & DOR co-expression	↑ CGRP	• No CGRP/DOR co-expression • High RAMP1 & DOR co-expression
↑ DOR		↑ DOR	
↑ RAMP1		RAMP1	

C

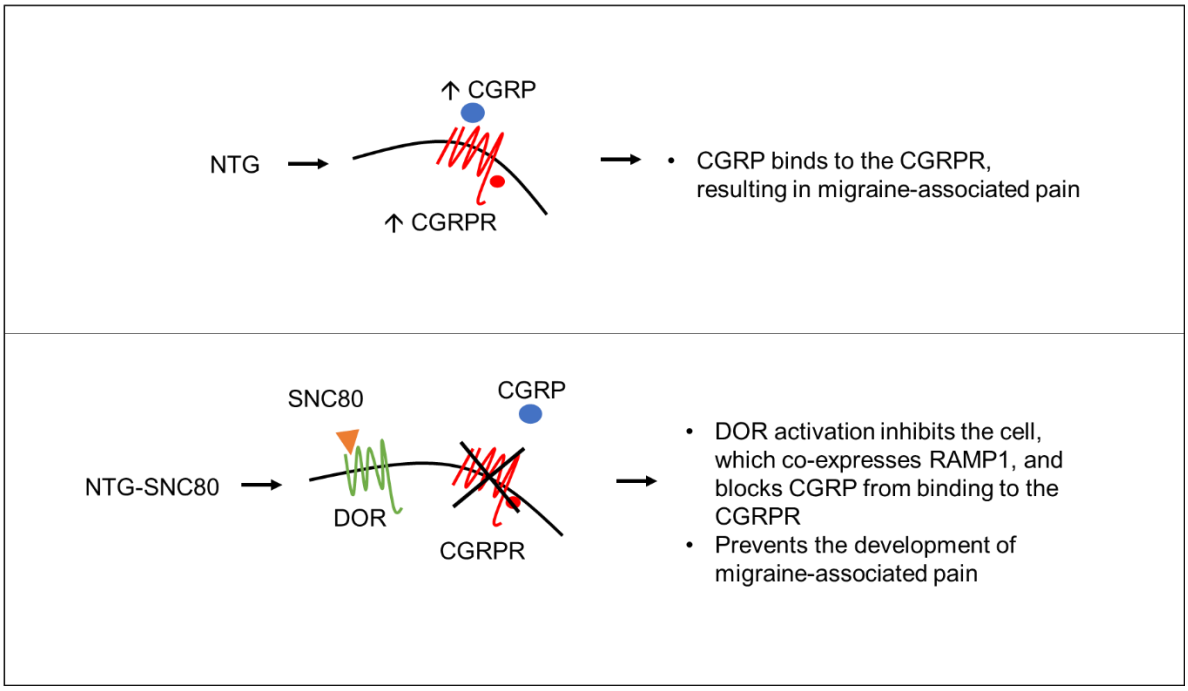


Figure 28: Schematics summarizing paper's findings. (a) Schematic depicting CGRP, DOR, and RAMP1 expression in the trigeminal ganglia and the trigeminal nucleus caudalis. (b) Table summarizing the effect of NTG on CGRP, DOR, and RAMP1 in the trigeminal ganglia and trigeminal nucleus caudalis. (c) Schematic depicting a proposed mechanism of action by which DOR regulates CGRP in migraine-associated pain.

4.4. DISCUSSION

Migraine is an incredibly debilitating disorder, and migraineurs have remarkably few treatment options. Migraines are recurrent, often life-long, and tend to be highly associated with psychiatric illnesses (ICHD3b 2013, Minen, Begasse De Dhaem et al. 2016). Comorbid psychiatric illnesses are risk factors for chronic migraine, and migraineurs with a psychiatric comorbidity have up to six times more hospital visits than migraineurs without a psychiatric comorbidity (Minen and Tanev 2014). The progression from episodic to chronic migraine is a clinical concern, and approximately 3% of migraineurs progress from episodic to chronic migraine per year (Scher, Stewart et al. 2003). First-line treatment for chronic migraine is preventatives from varying drug classes, including tricyclic antidepressants, anti-convulsants, and beta blockers (Charles 2017). These preventatives are associated with several adverse effects, are only effective in a subset of patients, and ultimately result in less than 50% of patients being satisfied with their treatment (Bigal, Serrano et al. 2008). As dissatisfaction grows, patients may overuse their medications to relieve their migraine pain. Paradoxically, medication overuse worsens headache and leads to a phenomenon called medication overuse headache (medication overuse headache). An estimated 15% of migraineurs go on to develop medication overuse headache, which further exacerbates and increases the frequency of migraine (ICHD3b 2013, Schwedt, Alam et al. 2018). To date, the primary treatment for this medication overuse headache is withdrawal from the overused medication, which leaves the patient without a viable migraine therapeutic and thus no way to relieve their pain (Katsarava, Limmroth et al. 2003). Clearly, there are multiple risk factors for chronic migraine and there is a need to expand the migraine treatment portfolio

with novel pharmacotherapies. The results from this study suggest that delta opioid receptor (DOR) activation regulates migraine-associated pain and may be a promising treatment for this debilitating neurological disorder.

We have previously identified the DOR as an emerging target for the treatment of migraine-like headaches (Pradhan, Smith et al. 2014, Charles and Pradhan 2016, Moye, Novack et al. 2018). In the nitroglycerin (NTG) mouse model of chronic migraine, chronic, intermittent NTG resulted in a basal level of hypersensitivity and DOR agonists blocked this NTG-evoked allodynia (Pradhan, Smith et al. 2014). Similarly, SNC80, a hallmark DOR agonist, blocked peripheral and cephalic NTG-induced allodynia in a model of post-traumatic headache (post-traumatic headache) (Moye, Novack et al. 2018). When SNC80 was administered subsequent to NTG in this model of post-traumatic headache, DOR activation prevented the development of chronic post-traumatic headache (Moye, Novack et al. 2018). The goal of this study was to behaviorally and molecularly characterize how DOR activation prevents the chronification of migraine. We found that SNC80 blocked and prevented the development of NTG-induced migraine associated pain, and that chronic SNC80 also suppressed NTG-induced expression of the pro-migraine regulator, calcitonin gene-related peptide (CGRP) in the trigeminovascular complex. Our results indicate that DOR is minimally co-expressed with CGRP, and highly co-expressed with the CGRP receptor complex in the trigeminovascular complex, suggesting a possible mechanism by which DOR regulates migraine-associated pain.

Where and how migraine chronification occurs is unclear. However, there is growing evidence implicating CGRP as a critical peptide regulating migraine-associated pain. Studies now confirm the critical importance of CGRP and the CGRP receptor in migraine pathophysiology (Dodick, Goadsby et al. 2014, Dodick, Goadsby et al. 2014, Bigal, Edvinsson et al. 2015, Giamberardino, Affaitati et al. 2016, Sun, Dodick et al. 2016, Voss, Lipton et al. 2016, Khan, Olesen et al. 2017). In this study, migraine-associated pain via chronic systemic injections of NTG alters CGRP and DOR function, and this augmented functionality of the DOR may regulate NTG-induced cephalic allodynia. Here, we reconfirm prior data showing that chronic NTG results in the development of basal hypersensitivity (Pradhan, Smith et al. 2014), and further show that chronic SNC80 can prevent this NTG-induced basal hypersensitivity. We have previously shown that DOR agonists can block NTG-induced peripheral hypersensitivity (Pradhan, Smith et al. 2014), and that chronic DOR activation can prevent post-traumatic headache-associated pain (Moye, Novack et al. 2018). Here, we show that chronic DOR activation can also prevent the development of atraumatic NTG-induced cephalic allodynia, which is in line with prior results. In addition to preventing the development of NTG-induced basal hypersensitivity, chronic DOR activation also suppresses the expression of NTG-induced CGRP in the trigeminovascular complex. While chronic NTG increases CGRP expression in the trigeminal ganglia (TG) and trigeminal nucleus caudalis (TNC), chronic DOR activation prevents or normalizes the increase of this pro-migraine peptide.

Since chronic NTG modulated expression of CGRP, we rationalized that chronic NTG may also alter DOR. To determine the effect of NTG on DOR, we used the DOReGFP

knockin mouse in the NTG model of chronic migraine. We reconfirmed that chronic NTG increased CGRP expression in the TG and TNC, and also found that chronic NTG increased cellular fluorescence of each CGRP+ cell in the TG. Interestingly, there were no CGRP cell bodies in the TNC. Chronic NTG also increased DOR cell count and fluorescence in the TG and TNC. This fluorescent increase may suggest that the DOR is more functional in this migraine-associated state. We initially hypothesized that DOR may be inhibiting CGRP release. However, there is minimal co-expression between CGRP and DOR in the TG, and no overlap between CGRP and DOR expression in the TNC. Another way that the DOR may be disrupting CGRP signaling is via the CGRP receptor. We found that DOR is highly co-expressed with the CGRP receptor in both the TG and TNC, suggesting a possible mechanism by which DOR activation regulates migraine-associated pain. DOR activation may block the ability of CGRP to bind to the CGRP receptor in the trigeminovascular complex, a migraine-associated region. It is also possible that CGRP will still bind to its receptor, but that DOR activation will inhibit the cell and then prevent the propagation of the CGRP signal. In either scenario, DOR activation could prevent the positive feedback loop in which CGRP is increased with NTG, which would normalize CGRP levels in the NTG model. To the best of our knowledge, this is the first time that DOR has been shown to be co-expressed with the CGRP receptor.

CGRP receptor antagonists are at the forefront of migraine medicine (Goadsby, Reuter et al. 2017). Our work shows that chronic DOR activation can modulate NTG-induced CGRP increase, and that DOR may be more functional in this chronic pain state. The lack of effective treatment options has had detrimental societal consequences, such as the

dependence and overuse of medications, including opioids, which has contributed to the national opioid epidemic (Reid, Engles-Horton et al. 2002, Colas, Munoz et al. 2004). It is imperative that researchers and physicians better understand the molecular mechanisms underlying migraine, and that this understanding lay the foundation for the development of effective and safer migraine therapeutics. In this study, we demonstrate that the DOR is a promising target for the treatment of chronic migraine. We show that DOR activation reverses migraine-associated mechanical allodynia, and that chronic DOR activation can modulate the expression of CGRP, a pro-migraine peptide, in the NTG model of chronic migraine. For the first time, we also show that DOR is co-expressed with the CGRP receptor, demonstrating a possible mechanism of action by which DOR regulates migraine-associated pain. Altogether, our results show that the DOR could be a pharmacotherapy that would adequately broaden the migraine treatment portfolio.

5. CONCLUSION

5.1. INTRODUCTION

In this thesis, I demonstrate the potential of DOR activation as a therapeutic target for the treatment of headache disorders. Overall, I show that DOR activation can attenuate established cephalic pain and prevent the development of chronic migraine-associated cephalic pain. The DOR may regulate cephalic pain by modulating levels of the pro-migraine peptide CGRP within the trigeminovascular pathway. Through immunohistological staining and RNAScope in situ hybridization, I show that the DOR is co-expressed with components of the CGRP receptor, RAMP1 and CRLR, in the TG and TNC. This finding was incredibly exciting, as co-expression of the DOR and CGRP receptor has never been shown before. The interplay between the DOR and CGRP receptor may correspond with the modulated CGRP levels seen in NTG-SNC80 mice, and the DOR may regulate migraine-associated cephalic pain via the CGRP receptor. To conclude my thesis, I will summarize the main findings from each chapter, discuss methodological limitations, highlight key aspects from this work, and discuss future directions.

5.2. SUMMARY

In Chapter 2, I developed, characterized, and pharmacologically validated a mouse model of post-traumatic headache. Prior to this publication, there were limited models of mTBI-induced migraine, and the addition of this mouse model will allow researchers to further explore the progression from mild traumatic brain injury to post-traumatic headache. Interestingly, I also found that mild traumatic brain injury alone increased the expression

of CGRP within the TG and TNC. This finding provides insight into how mild traumatic brain injury could increase susceptibility to developing migraine-associated pain. If mild traumatic brain injury increases CGRP expression in the TG and TNC, then the trigeminovascular pathway may already be sensitized to sensory stimuli. The sensitization of this system could promote the development of a migraine-like headache. I also pharmacologically validated this model of post-traumatic headache and showed that chronic DOR activation can attenuate the development of post-traumatic headache-associated pain similar to the preventive migraine therapeutic topirimate in the hind paw and periorbital region. The preventive effect of DOR activation in this mouse model led to the second chapter of this thesis. Although chronic DOR activation inhibited the development of post-traumatic headache-associated pain, it was unclear whether acute DOR activation could reverse established pain. Within the models of pain like the NTG model of chronic migraine, the mouse model of post-traumatic headache, medication overuse headache, and opioid-induced hyperalgesia, acute DOR activation reversed peripheral and cephalic hypersensitivity. During the development of these studies, we observed that SNC80, the hallmark DOR agonist, completely reversed peripheral hypersensitivity and partially attenuated cephalic hypersensitivity. This observation is in line with other results showing that DOR agonists may be relatively ineffective as abortive therapeutics in acute pain states, although those studies specifically focused on comparing DOR agonists and MOR agonists (Gallantine and Meert 2005). The preventive effects of chronic DOR activation were incredibly promising, and these results led me to the last chapter of this thesis. To better understand how chronic DOR activation may regulate migraine-associated pain, I focused the last chapter of this thesis to exploring

the molecular interplay between DOR and the pro-migraine peptide, CGRP. I initially hypothesized that CGRP and DOR were co-expressed, and that DOR activation inhibited the neural activity of the cell and thus prevented CGRP release. However, I found that there was little co-expression of DOR and CGRP in the TG, and no CGRP+ cell bodies in the TNC. In the TNC, there was a stark difference in CGRP and DOR expression. I observed CGRP+ afferent endings in lamina I of the TNC, and scattered DOR+ cell bodies in laminae I-II of the TNC. These findings suggest that DOR is not directly inhibiting CGRP release, and that the DOR may regulate CGRP via a different mechanism. In the final series of experiments of Chapter 4, components of the CGRP receptor, RAMP1 and CRLR, were examined in the TG and TNC. RAMP1 was present throughout the TG and TNC, and there was a high co-expression of RAMP1 and DOR in both regions. To confirm that these were cells positive for the CGRP receptor, CRLR was also examined. There was a high co-expression between DOR+ and CRLR+ cells in the TNC, suggesting that the TNC contains cells that express DOR and the CGRP receptor. This co-expression was not significantly changed after chronic NTG, and this lack of change may be due to the already high co-expression between both receptors. Also, it is possible that chronic NTG alters signaling in these regions, and thus future experiments should focus on exploring the effect of DOR activation on CGRP signaling. Taken together, the results presented in this thesis suggest that the DOR may be a promising target for the treatment of headache disorders. If DOR agonists are to be used clinically, further experiments should focus on determining the mechanism of action by which DOR regulates CGRP signaling. Additionally, the long-term effect of chronic DOR activation should be explored,

specifically to determine if chronic DOR activation would reduce the frequency and/or severity of recurrent headache attacks.

5.3. LIMITATIONS

Throughout this thesis, von Frey hair filaments were used to detect thresholds to mechanical stimuli. This sensory sensitivity test was used to determine changes in mechanical thresholds in the hind paw and the periorbital region. While this pain assay provides valuable information related to the response to mechanical stimuli, the results do not fully convey the cohesive pain state of the animal. Specifically, a major limitation to this pain assay is that this threshold response only provides information specific to mechanical stimuli. Threshold responses could differ depending on the region being tested. As seen in Chapter 3, SNC80 completely reversed cephalic hypersensitivity in models of chronic migraine, post-traumatic headache, medication overuse headache, and opioid-induced hyperalgesia. However, SNC80 only attenuated peripheral hypersensitivity, suggesting that DOR activation may affect peripheral and central systems at a different rate. In Chapter 4, chronic SNC80 prevented the development of NTG-induced hypersensitivity in the hind paw and in the periorbital region. It is possible that acute DOR activation may reverse hypersensitivity at a different rate depending on the region and may not be the most beneficial therapy as an acute treatment. However, in Chapter 4 chronic DOR activation prevented the development of migraine-associated pain and may be the most beneficial as prophylactic therapy in patients with established pain. To fully characterize DOR agonists, future experiments should also examine other behavioral changes like facial grimacing and change in emotional affect. Migraine

symptomology extends beyond cutaneous allodynia and analyzing a range of migraine-associated behaviors would strengthen the rationale for developing DOR agonists as migraine therapies.

In addition to sensory sensitivity testing, the conclusions in this thesis rely heavily on the results from immunohistochemistry (IHC) and RNAScope fluorescent in situ hybridization (FISH). Although IHC and FISH may be relatively simple techniques, they can produce varying results depending on the tissue being studied. For example, the quality of images produced using IHC and FISH are dependent on fixation time, tissue processing, and antigen retrieval. In this thesis, all the IHC experiments used paraformaldehyde (PFA) to fix tissue. PFA can mask, or even damage, antibody binding sites (Rickert and Maliniak 1989). None of the tissues used in this thesis underwent an epitope retrieval method because each antibody produced a strong signal. Additionally, other IHC studies examining CGRP and DOR yielded similar expression profiles, and two different antibodies were used to compare RAMP1 expression in the TG and TNC (Scherrer, Tryoen-Toth et al. 2006, Eftekhari 2013). While optimizing the RNAScope FISH technique, it became apparent that the TNC and TG would require different fixation times. FISH images from the TNC were consistently robust in fresh frozen and fixed tissue, but there were weak signals in images from the TG. Future RNAScope FISH studies should focus on optimizing this technique for the TG, and possibly the dorsal root ganglia (DRG).

Finally, interpretations about DOR regulation are, in part, dependent on the DOReGFP KI mouse model. As there are no antibodies specific to the DOR, the DOReGFP KI mouse

was used to visualize the DOR. However, there is approximately a 50% increase in DOR mRNA in these mice, which alters the net amount of DOR (Scherrer, Tryoen-Toth et al. 2006). Also, the eGFP tag could possibly alter the cellular distribution, subcellular compartmentalization, and overall trafficking of the DOR due to its relatively large size (Gendron, Mittal et al. 2015, Zhang, Bao et al. 2015). A major concern with the DOReGFP KI mouse is that the DORs are expressed on the cell membrane, while multiple studies have shown that DORs are primarily located (Gendron, Mittal et al. 2015) on intracellular vesicles that are trafficked to the cell surface (Bao, Jin et al. 2003, Guan, Xu et al. 2005, Cahill, Holdridge et al. 2007, Gendron, Mittal et al. 2015, Charfi, Abdallah et al. 2018). While it is possible that the DORs in the DOReGFP KI mouse are disproportionately localized to the cell membrane in a naïve state, multiple studies have shown that the DOR has robust receptor plasticity. Specifically, the DOR is dynamic and chronic stimuli like pain can increase DOR functionality (Cahill, Morinville et al. 2003, Kabli and Cahill 2007, Pradhan, Smith et al. 2013). My results show increased DOR expression after chronic NTG, which is in line with these prior findings. Furthermore, behavioral and IHC results from DOReGFP KI mice are similar to those seen in C57Bl6/J mice. Overall, the DOReGFP KI mouse is an innovative tool that has allowed researchers to visualize the DOR. Similarly, the translational impact of these findings may be more important than identically replicating the cellular profile of the DOR.

5.4. REGULATION OF HEADACHE-ASSOCIATED PAIN WITHIN THE TRIGEMINOVASCULAR PATHWAY

Painful stimuli are transmitted from primary afferents to the spinal cord, and then to the brain via ascending pain pathways, and descending pain pathways send inhibitory signals back to the spinal cord (Millan 1999, Millan 2002). These primary afferents can be small, unmyelinated C fibers, thinly myelinated Aδ fibers, or large myelinated Aβ fibers. C and Aδ fibers primarily terminate in the superficial laminae of the dorsal horn, and Aβ fibers terminate in the deeper laminae. In the spinal cord, afferents terminate on projection neurons and excitatory or inhibitory interneurons. By comparing the spinal cord to TNC, we can postulate that primary afferents from the trigeminal ganglia (TG) are also terminating on projection neurons or interneurons. If this is true, then the projection neurons that transmit information further in to the brain stem can be modulated. There are also brain regions that send descending inhibitory signals to the spinal cord, and this circuitry could also further modulate the perception of pain. Chronic migraine could result in the dysregulation of this pain circuitry, and DOR activation may normalize the net excitatory or inhibitory tone needed for analgesia. My data show that chronic NTG results in increased expression of CGRP within the TG and TNC of C57Bl6/J and DOReGFP knockin mice. How chronic NTG upregulates CGRP needs further elucidation. However, it is possible that this upregulation is reflective of the relationship between CGRP and nitric oxide. It has been proposed that CGRP may be acting upstream of nitric oxide (Bellamy, Bowen et al. 2006). Previous evidence shows that the mitogen-activated protein kinases (MAPK) regulate the gene for CGRP (Thiagalingam, De Bustros et al. 1996, Durham and Russo 1998, Durham and Russo 2000). Specifically, the promoter region of

CGRP is activated by the MAP kinase kinase (MEK1), and is it thought that the 5-HT1_1 receptor agonist, CGS 12066A, acts directly on MEK1 to decrease CGRP (Durham and Russo 1998). These sets of experiments suggest that NTG, a nitic oxide donor, may feed into this feedback loop by promoting the increased expression of MAP kinases, which can lead to downstream increases of CGRP. Interestingly, CGRP may further feed into this loop by promoting the increase of certain immediate early genes, like CREB, that may promote enhanced activity of the downstream signaling cascades implicated in migraine, or by activating other MAP kinases that may ultimately contribute to the production of nitric oxide (Figure 29).

Chronic, intermittent SNC80 administration following NTG administration normalized this CGRP expression, suggesting that chronic DOR activation normalizes the net peptidergic tone of the trigeminovascular pathway. Overall, it is unknown what threshold is necessary to promote a migraine attack. In the experiments outline in this thesis, we see increased levels of CGRP 2 weeks after closed head injury, but this increased CGRP expression does not directly correlate to decreased mechanical threshold. In the NTG model of chronic migraine, we see increased CGRP levels after chronic NTG, and these increased thresholds do correspond with decreased mechanical thresholds. These results raise the question of whether a certain threshold of CGRP is necessary to promote a migraine-like phenotype. The complex interplay between CGRP, nitric oxide, and other molecules within the signaling cascades suggest that there are other players that contribute to a migraine-like phenotype. As mentioned previously, MAP kinases regulate CGRP, and it is possible that while increased levels of CGRP are associated with the migraine-like

phenotype, many other peptides outside the scope of this thesis may also be involved in the migraine-like phenotype. For future experiments, it would be interesting to delve outside of CGRP and look at other peptides such as PACAP-38 that are thought to be involved in migraine. By looking at peptides outside of CGRP, we may be able to begin to form a network of peptides, receptors, and signaling cascades that could possibly be involved in the pathogenesis of migraine.

As the DOR can regulate migraine-associated pain, it is also possible that this pain and accompanying pro-pain peptides can influence the DOR. Specifically, it is possible that pro-migraine peptides like CGRP can sensitize the DOR and promote its increased functionality in a chronic pain state. My results show that there is increased DOR expression after chronic NTG. In addition to the evidence showing increased functionality of the DOR after chronic stimuli, we see a similar phenomenon in the MOR literature. For example, substance P, a neuropeptide involved in inflammatory pain, can increase the recycling of the MOR in TG neurons, and thus enhance the antinociceptive properties of MOR (Bowman, Soohoo et al. 2015). My results also show that the DOR colocalizes with components of the CGRP receptor. While I initially hypothesized that the DOR may directly inhibit CGRP release, it is possible that the DOR interacts with the CGRP receptor and this interplay blunts CGRP propagation. Similar results have also been seen in the MOR field. The substance P receptor (NK1) and MOR have been studied in the trigeminal dorsal horn, and approximately 32% of MOR-immunoreactive dendrites contained NK1 (Aicher, Punnoose et al. 2000). These results show that both MOR and DOR may

modulate nociceptive responses at postsynaptic sites instead of presynaptically inhibiting peptide release.

5.5. FUTURE DIRECTIONS

Based on my results from this thesis, DOR activation has anti-allodynic properties that are attractive for the treatment of headache disorders. Behaviorally, acute and chronic DOR activation results in the attenuation or reversal of headache-associated pain in multiple models of headache. To further build on these results, future experiments could examine the long-term effects of DOR activation in headache. For example, certain preventive medications can reduce the frequency and severity of recurrent headache attacks. It is possible that DOR agonists could also have a protective effect, and it would be relatively simple to test. Within the NTG-SNC80 paradigm, mice would be allowed to recover after being chronically treated with VEH or NTG, followed by VEH or SNC80. After recovery, mice would be exposed again to NTG or VEH to determine whether there is a reduced response to NTG in SNC80-treated mice. A similar experimental setup could also be tested in the model of post-traumatic headache, medication overuse headache, and opioid-induced hyperalgesia for comparison. These results would determine whether chronic DOR activation has a protective effect on future recurrent headache attacks.

Additionally, my results showed that the DOR is minimally co-expressed with CGRP, and highly co-expressed with the CGRP receptor. Based on these results, I hypothesize that activating the DOR leads to reduced activity of the cell, which could prevent the function of the CGRP receptor (Figure 29). To further determine whether this is true,

electrophysiological studies could examine the activity of a DOR+ cell. It is difficult to record from TG and TNC, and only a few electrophysiologists have recorded from the TNC (Oshinsky and Luo 2006, Davies and North 2009). However, recording from a DOR+ cell would yield interesting results. Specifically, one slice preparation could sit in a saline bath, and recordings could be examined after CGRP is applied to the bath, and then also in another slice preparation after SNC80 is applied to the bath. If the DOR regulates CGRP via the CGRP receptor, then application of SNC80 should limit the effect of CGRP on the cell's activity. These results would provide mechanistic insight into how the DOR regulates CGRP within the TNC.

To further probe the interplay between the DOR and the CGRP receptor, it would be interesting to explore the possibility of heterodimerization between the DOR and the CGRP receptor. As mentioned previously, the CGRP receptor is a complex that is comprised of RAMP1, CRLR, and RCP. There is growing evidence to believe that of these 3 molecules, the RAMP family may bind to other receptors and influence downstream signaling pathways. RAMP1 partners with CRLR to form the CGRP receptor, and RAMP1 also partners with the calcitonin receptor to form the amylin receptors (J, Simms et al. 2016). RAMPs, in general, are widely distributed throughout the nervous system (Roux and Cottrell 2014). RAMP1 knockout mice are viable, have hypertension, and appear to have a dysregulated immune system, suggesting that RAMP1 may partner with other molecules to regulate these processes (Tsujikawa, Yayama et al. 2007). Interestingly, RAMPs may interact with other GPCRs, specifically other members of the secretin GPCR family (Roux and Cottrell 2014). Of specific interest to migraine, it has been recently

shown that all 3 members of the RAMP family bind to the $VPAC_1$ receptor, which is the receptor for vasoactive intestinal peptide (VIP) and PACAP (Christopoulos, Christopoulos et al. 2003). PACAP has been recently implicated in migraine, and the lab is currently putting effort into characterizing the role of PACAP in the chronic NTG model of migraine. Since RAMP1 partners with the CRLR to form the CGRP receptor, and all RAMPs may partner with the $VPAC_1$ receptor to regulate VIP and PACAP, it is possible that RAMP1 also partners with other GPCRs in some capacity. It would be interesting to determine whether RAMP1 partners with the DOR, considering that they are co-expressed within a majority of the cells in the TNC. If RAMP1 does partner with the DOR in at least some cells within the trigeminovascular pathway, there would be evidence suggesting that DOR trafficking may play a part in its regulation of CGRP. Within the experiments in this thesis, SNC80, a hallmark DOR internalizing agonist, was used. If RAMP1 were interacting with the DOR in some capacity, internalization of the DOR in the presence of SNC80 may also influence the fate of RAMP1. In this hypothetical scenario, internalized DOR may internalize RAMP1, which would interfere with RAMP1's ability to partner with CRLR to form the CGRP receptor. As CGRP will not bind to an incomplete receptor complex, the pro-migraine peptide will not be able to bind and exert any further downstream effects. There is still much to learn about the role of RAMPs and DORs in migraine, and the possible interaction between these 2 molecules may lead to the discovery of improved therapies for migraine. In Figure 29, I revisit the schematic from the beginning of this thesis and have placed the DOR upstream of nitric oxide. If CGRP acts upstream of nitric oxide, then it is possible that the DOR may interact with the CGRP receptor, which would affect the ability of CGRP to bind and thus limit its contribution to the production of nitric

oxide. There is much to learn about the network of molecules underlying the pathophysiology of migraine, and the results form this thesis are only at the forefront of possibly novel therapeutics for migraine.

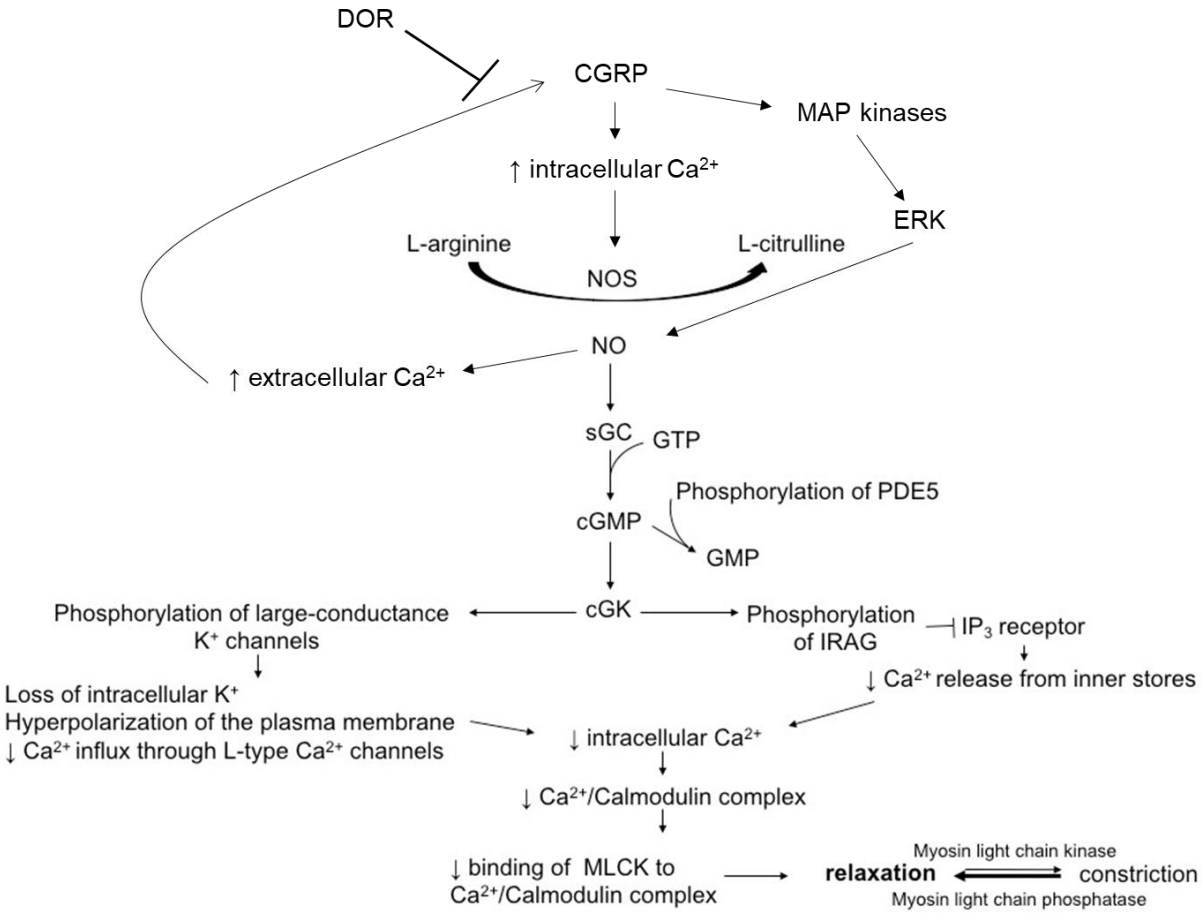


Figure 29: Schematic of where DOR may exert its anti-migraine effects. In this schematic, which was initially introduced at the beginning of this thesis, the DOR exerts its anti-migraine effects by interacting with the CGRP receptor, possibly RAMP1. By disrupting the CGRP receptor complex, it is possible that DOR may limit the ability of CGRP to bind to the CGRP receptor, and thus blunt any downstream effects CGRP may have on nitric oxide.

5.6. CONCLUDING REMARKS

The primary aim of this thesis is to highlight the analgesic properties of the DOR. In Chapter 2, SNC80, a DOR agonist, was used to pharmacologically validate a novel mouse model of post-traumatic headache. In Chapter 3, SNC80 was used to show that acute DOR activation can reverse headache-associated pain. In Chapter 4, the DOR was visualized and examined using the DOReGFP knockin mouse model. Specifically, the DOR was examined in relation to CGRP and the CGRP receptor. Altogether, this thesis showcases a thorough characterization of DOR activation in multiple models of headache. This detailed screening of DOR agonists could promote the development of these compounds for the treatment of migraine. The results from this thesis clearly show the anti-hypersensitive effect of DOR activation in multiple models of headache, and also provide a possible explanation as to how DOR regulates headache-associated pain. Further experiments may provide information on the long-term effects of DOR activation on headache disorders, and the effect of DOR activation on peptidergic signaling within migraine.

REFERENCES

(1712). Bibliotheca, Anatomica, Medica, Chirurgica, Etc,. London.

(2017). "Global, regional, and national burden of neurological disorders during 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015." Lancet Neurol **16**(11): 877-897.

Adelman, J. U., L. C. Adelman, M. C. Freeman, R. L. Von Seggern and J. Drake (2004). "Cost considerations of acute migraine treatment." Headache **44**(3): 271-285.

Adly, C., J. Straumanis and A. Chesson (1992). "Fluoxetine prophylaxis of migraine." Headache **32**(2): 101-104.

Afridi, S. K., M. S. Matharu, L. Lee, H. Kaube, K. J. Friston, R. S. Frackowiak and P. J. Goadsby (2005). "A PET study exploring the laterality of brainstem activation in migraine using glyceryl trinitrate." Brain **128**(Pt 4): 932-939.

Ahn, A. H. and A. I. Basbaum (2005). "Where do triptans act in the treatment of migraine?" Pain **115**(1-2): 1-4.

Aicher, S. A., A. Punnoose and A. Goldberg (2000). "mu-Opioid receptors often colocalize with the substance P receptor (NK1) in the trigeminal dorsal horn." J Neurosci **20**(11): 4345-4354.

Akerman, S., P. R. Holland and J. Hoffmann (2013). "Pearls and pitfalls in experimental in vivo models of migraine: dural trigeminovascular nociception." Cephalalgia **33**(8): 577-592.

Al-Hasani, R. and M. R. Bruchas (2011). "Molecular mechanisms of opioid receptor-dependent signaling and behavior." Anesthesiology **115**(6): 1363-1381.

Alevizaki, M., A. Shiraishi, F. V. Rassool, G. J. Ferrier, I. MacIntyre and S. Legon (1986). "The calcitonin-like sequence of the beta CGRP gene." FEBS Lett **206**(1): 47-52.

Amara, S. G., J. L. Arriza, S. E. Leff, L. W. Swanson, R. M. Evans and M. G. Rosenfeld (1985). "Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide." Science **229**(4718): 1094-1097.

Amara, S. G., V. Jonas, M. G. Rosenfeld, E. S. Ong and R. M. Evans (1982). "Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products." Nature **298**(5871): 240-244.

Amin, F. M., A. Hougaard, H. W. Schytz, M. S. Asghar, E. Lundholm, A. I. Parvaiz, P. J. de Koning, M. R. Andersen, H. B. Larsson, J. Fahrenkrug, J. Olesen and M. Ashina (2014). "Investigation of the pathophysiological mechanisms of migraine attacks induced by pituitary adenylate cyclase-activating polypeptide-38." Brain **137**(Pt 3): 779-794.

Antonaci, F., N. Ghiotto, S. Wu, E. Pucci and A. Costa (2016). "Recent advances in migraine therapy." Springerplus **5**: 637.

Arvidsson, U., R. J. Dado, M. Riedl, J. H. Lee, P. Y. Law, H. H. Loh, R. Elde and M. W. Wessendorf (1995). "delta-Opioid receptor immunoreactivity: distribution in brainstem and spinal cord, and relationship to biogenic amines and enkephalin." J Neurosci **15**(2): 1215-1235.

Arvidsson, U., M. Riedl, S. Chakrabarti, J. H. Lee, A. H. Nakano, R. J. Dado, H. H. Loh, P. Y. Law, M. W. Wessendorf and R. Elde (1995). "Distribution and targeting of a mu-opioid receptor (MOR1) in brain and spinal cord." J Neurosci **15**(5 Pt 1): 3328-3341.

Asakura, M., T. Tsukamoto, H. Kubota, J. Imafuku, M. Ino, J. Nishizaki, A. Sato, K. Shinbo and K. Hasegawa (1987). "Role of serotonin in the regulation of beta-adrenoceptors by antidepressants." Eur J Pharmacol **141**(1): 95-100.

Ashina, M., L. Bendtsen, R. Jensen, L. H. Lassen, F. Sakai and J. Olesen (1999). "Possible mechanisms of action of nitric oxide synthase inhibitors in chronic tension-type headache." Brain **122 (Pt 9)**: 1629-1635.

Ashtari, F., V. Shaygannejad and M. Akbari (2008). "A double-blind, randomized trial of low-dose topiramate vs propranolol in migraine prophylaxis." Acta Neurol Scand **118**(5): 301-305.

Aurora, S. K., D. W. Dodick, C. C. Turkel, R. E. DeGryse, S. D. Silberstein, R. B. Lipton, H. C. Diener and M. F. Brin (2010). "OnabotulinumtoxinA for treatment of chronic migraine: results from the double-blind, randomized, placebo-controlled phase of the PREEMPT 1 trial." Cephalalgia **30**(7): 793-803.

Bagdy, G., P. Riba, V. Kecskeméti, D. Chase and G. Juhász (2010). "Headache-type adverse effects of NO donors: vasodilation and beyond." Br J Pharmacol **160**(1): 20-35.

Baillie, L. D., A. H. Ahn and S. J. Mulligan (2012). "Sumatriptan inhibition of N-type calcium channel mediated signaling in dural CGRP terminal fibres." Neuropharmacology **63**(3): 362-367.

Bao, L., S. X. Jin, C. Zhang, L. H. Wang, Z. Z. Xu, F. X. Zhang, L. C. Wang, F. S. Ning, H. J. Cai, J. S. Guan, H. S. Xiao, Z. Q. Xu, C. He, T. Hokfelt, Z. Zhou and X. Zhang (2003). "Activation of delta opioid receptors induces receptor insertion and neuropeptide secretion." Neuron **37**(1): 121-133.

Baratz, R., D. Tweedie, J. Y. Wang, V. Rubovitch, W. Luo, B. J. Hoffer, N. H. Greig and C. G. Pick (2015). "Transiently lowering tumor necrosis factor-alpha synthesis ameliorates neuronal cell loss and cognitive impairments induced by minimal traumatic brain injury in mice." J Neuroinflammation **12**: 45.

Bardoni, R., V. L. Tawfik, D. Wang, A. Francois, C. Solorzano, S. A. Shuster, P. Choudhury, C. Betelli, C. Cassidy, K. Smith, J. C. de Nooij, F. Mennicken, D. O'Donnell, B. L. Kieffer, C. J. Woodbury, A. I. Basbaum, A. B. MacDermott and G. Scherrer (2014). "Delta opioid receptors presynaptically regulate cutaneous mechanosensory neuron input to the spinal cord dorsal horn." Neuron **81**(6): 1312-1327.

Bartsch, T. and P. J. Goadsby (2003). "The trigeminocervical complex and migraine: current concepts and synthesis." Curr Pain Headache Rep **7**(5): 371-376.

Basbaum, A. I., D. M. Bautista, G. Scherrer and D. Julius (2009). "Cellular and molecular mechanisms of pain." Cell **139**(2): 267-284.

Bates, E. A., T. Nikai, K. C. Brennan, Y. H. Fu, A. C. Charles, A. I. Basbaum, L. J. Ptacek and A. H. Ahn (2010). "Sumatriptan alleviates nitroglycerin-induced mechanical and thermal allodynia in mice." Cephalalgia **30**(2): 170-178.

Bates, E. A., T. Nikai, K. C. Brennan, Y. H. Fu, A. C. Charles, A. I. Basbaum, L. J. Ptacek and A. H. Ahn (2010). "Sumatriptan alleviates nitroglycerin-induced mechanical and thermal allodynia in mice." Cephalalgia **30**(2): 170-178.

Bellamy, J., E. J. Bowen, A. F. Russo and P. L. Durham (2006). "Nitric oxide regulation of calcitonin gene-related peptide gene expression in rat trigeminal ganglia neurons." Eur J Neurosci **23**(8): 2057-2066.

Ben Aissa, M., A. F. Tipton, Z. Bertels, R. Gandhi, L. S. Moye, M. Novack, B. M. Bennett, Y. Wang, V. Litosh, S. H. Lee, I. N. Gaisina, G. R. Thatcher and A. A. Pradhan (2017). "Soluble guanylyl cyclase is a critical regulator of migraine-associated pain." Cephalalgia: 333102417737778.

Berman, N. E., V. Puri, S. Chandrala, S. Puri, R. Macgregor, C. S. Liverman and R. M. Klein (2006). "Serotonin in trigeminal ganglia of female rodents: relevance to menstrual migraine." Headache **46**(8): 1230-1245.

Bertran-Gonzalez, J., V. Laurent, B. C. Chieng, M. J. Christie and B. W. Balleine (2013). "Learning-related translocation of delta-opioid receptors on ventral striatal cholinergic interneurons mediates choice between goal-directed actions." J Neurosci **33**(41): 16060-16071.

Bigal, M. E., S. Ashina, R. Burstein, M. L. Reed, D. Buse, D. Serrano, R. B. Lipton and A. Group (2008). "Prevalence and characteristics of allodynia in headache sufferers: a population study." Neurology **70**(17): 1525-1533.

Bigal, M. E., L. Edvinsson, A. M. Rapoport, R. B. Lipton, E. L. Spierings, H. C. Diener, R. Burstein, P. S. Loupe, Y. Ma, R. Yang and S. D. Silberstein (2015). "Safety, tolerability, and efficacy of TEV-48125 for preventive treatment of chronic migraine: a multicentre, randomised, double-blind, placebo-controlled, phase 2b study." Lancet Neurol **14**(11): 1091-1100.

Bigal, M. E. and R. B. Lipton (2008). "Excessive acute migraine medication use and migraine progression." Neurology **71**(22): 1821-1828.

Bigal, M. E. and R. B. Lipton (2009). "Excessive opioid use and the development of chronic migraine." Pain **142**(3): 179-182.

Bigal, M. E. and R. B. Lipton (2011). "Migraine chronification." Curr Neurol Neurosci Rep **11**(2): 139-148.

Bigal, M. E., D. Serrano, D. Buse, A. Scher, W. F. Stewart and R. B. Lipton (2008). "Acute migraine medications and evolution from episodic to chronic migraine: a longitudinal population-based study." Headache **48**(8): 1157-1168.

Bigal, M. E., M. Serrano D Fau - Reed, R. B. Reed M Fau - Lipton and R. B. Lipton "Chronic migraine in the population: burden, diagnosis, and satisfaction with treatment." (1526-632X (Electronic)).

Bigal, M. E., D. Serrano, M. Reed and R. B. Lipton (2008). "Chronic migraine in the population: burden, diagnosis, and satisfaction with treatment." Neurology **71**(8): 559-566.

Bigal, M. E., S. Walter and A. M. Rapoport (2013). "Calcitonin gene-related peptide (CGRP) and migraine current understanding and state of development." Headache **53**(8): 1230-1244.

Blackburn, T. P., A. J. Cross, C. Hille and P. Slater (1988). "Autoradiographic localization of delta opiate receptors in rat and human brain." Neuroscience **27**(2): 497-506.

Blau, J. N. (1991). "Migraine postdromes: symptoms after attacks." Cephalalgia **11**(5): 229-231.

Blumenfeld, A. M., L. M. Bloudek, W. J. Becker, D. C. Buse, S. F. Varon, G. A. Maglinte, T. K. Wilcox, A. K. Kawata and R. B. Lipton (2013). "Patterns of use and reasons for discontinuation of prophylactic medications for episodic migraine and chronic migraine: results from the second international burden of migraine study (IBMS-II)." Headache **53**(4): 644-655.

Boden, W. E., S. K. Padala, K. P. Cabral, I. R. Buschmann and M. S. Sidhu (2015). "Role of short-acting nitroglycerin in the management of ischemic heart disease." Drug Des Devel Ther **9**: 4793-4805.

Bowman, S. L., A. L. Soohoo, D. J. Shiwarski, S. Schulz, A. A. Pradhan and M. A. Puthenveedu (2015). "Cell-autonomous regulation of Mu-opioid receptor recycling by substance P." Cell Rep **10**(11): 1925-1936.

Boyer, N., J. Signoret-Genest, A. Artola, R. Dallel and L. Monconduit (2017). "Propranolol treatment prevents chronic central sensitization induced by repeated dural stimulation." Pain **158**(10): 2025-2034.

Bradbury, F. A., J. C. Zelnik and J. R. Traynor (2009). "G protein independent phosphorylation and internalization of the delta-opioid receptor." J Neurochem **109**(5): 1526-1535.

Brain, S. D. and A. D. Grant (2004). "Vascular actions of calcitonin gene-related peptide and adrenomedullin." Physiol Rev **84**(3): 903-934.

Brandes, J. L., J. R. Saper, M. Diamond, J. R. Couch, D. W. Lewis, J. Schmitt, W. Neto, S. Schwabe and D. Jacobs (2004). "Topiramate for migraine prevention: a randomized controlled trial." Jama **291**(8): 965-973.

Brandt, M. R., M. S. Furness, K. C. Rice, B. D. Fischer and S. S. Negus (2001). "Studies of tolerance and dependence with the delta-opioid agonist SNC80 in rhesus monkeys responding under a schedule of food presentation." J Pharmacol Exp Ther **299**(2): 629-637.

Bree, D. and D. Levy (2016). "Development of CGRP-dependent pain and headache related behaviours in a rat model of concussion: Implications for mechanisms of post-traumatic headache." Cephalalgia.

Breimer, L. H., I. MacIntyre and M. Zaidi (1988). "Peptides from the calcitonin genes: molecular genetics, structure and function." Biochem J **255**(2): 377-390.

Brennan, K. C., E. A. Bates, R. E. Shapiro, J. Zyuzin, W. C. Hallows, Y. Huang, H. Y. Lee, C. R. Jones, Y. H. Fu, A. C. Charles and L. J. Ptacek (2013). "Casein kinase idelta mutations in familial migraine and advanced sleep phase." Sci Transl Med **5**(183): 183ra156, 181-111.

Broom, D. C., E. M. Jutkiewicz, K. C. Rice, J. R. Traynor and J. H. Woods (2002). "Behavioral effects of delta-opioid receptor agonists: potential antidepressants?" Jpn J Pharmacol **90**(1): 1-6.

Burgos-Vega, C. C., L. D. Quigley, G. Trevisan Dos Santos, F. Yan, M. Asiedu, B. Jacobs, M. Motina, N. Safdar, H. Yousuf, A. Avona, T. J. Price and G. Dussor (2018). "Non-invasive dural stimulation in mice: A novel preclinical model of migraine." Cephalalgia: 333102418779557.

Burstein, R., B. Collins and M. Jakubowski (2004). "Defeating migraine pain with triptans: a race against the development of cutaneous allodynia." Ann Neurol **55**(1): 19-26.

Burstein, R. and M. Jakubowski (2005). "Unitary hypothesis for multiple triggers of the pain and strain of migraine." J Comp Neurol **493**(1): 9-14.

Burstein, R., M. Jakubowski, E. Garcia-Nicas, V. Kainz, Z. Bajwa, R. Hargreaves, L. Becerra and D. Borsook (2010). "Thalamic sensitization transforms localized pain into widespread allodynia." Ann Neurol **68**(1): 81-91.

Burstein, R., R. Noseda and D. Borsook (2015). "Migraine: multiple processes, complex pathophysiology." J Neurosci **35**(17): 6619-6629.

Burstein, R., H. Yamamura, A. Malick and A. M. Strassman (1998). "Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons." J Neurophysiol **79**(2): 964-982.

Buse, D. C., S. H. Pearlman, M. L. Reed, D. Serrano, D. S. Ng-Mak and R. B. Lipton (2012). "Opioid use and dependence among persons with migraine: results of the AMPP study." Headache **52**(1): 18-36.

Buse, D. C., M. F. Rupnow and R. B. Lipton (2009). "Assessing and managing all aspects of migraine: migraine attacks, migraine-related functional impairment, common comorbidities, and quality of life." Mayo Clin Proc **84**(5): 422-435.

Cahill, C. M., S. V. Holdridge and A. Morinville (2007). "Trafficking of delta-opioid receptors and other G-protein-coupled receptors: implications for pain and analgesia." Trends Pharmacol Sci **28**(1): 23-31.

Cahill, C. M., K. A. McClellan, A. Morinville, C. Hoffert, D. Hubatsch, D. O'Donnell and A. Beaudet (2001). "Immunohistochemical distribution of delta opioid receptors in the rat central nervous system: evidence for somatodendritic labeling and antigen-specific cellular compartmentalization." J Comp Neurol **440**(1): 65-84.

Cahill, C. M., A. Morinville, C. Hoffert, D. O'Donnell and A. Beaudet (2003). "Up-regulation and trafficking of delta opioid receptor in a model of chronic inflammation: implications for pain control." Pain **101**(1-2): 199-208.

Cahill, C. M., A. Morinville, M. C. Lee, J. P. Vincent, B. Collier and A. Beaudet (2001). "Prolonged morphine treatment targets delta opioid receptors to neuronal plasma membranes and enhances delta-mediated antinociception." J.Neurosci. **21**(19): 7598-7607.

Capuano, A., M. C. Greco, P. Navarra and G. Tringali (2014). "Correlation between algogenic effects of calcitonin-gene-related peptide (CGRP) and activation of trigeminal vascular system, in an in vivo experimental model of nitroglycerin-induced sensitization." Eur J Pharmacol **740**: 97-102.

CDC (2016). Wide-ranging online data for epidemiologic research (WONDER). Atlanta, GA, National Center for Health Statistics.

Cernak, I. and L. J. Noble-Haeusslein (2010). "Traumatic brain injury: an overview of pathobiology with emphasis on military populations." J Cereb Blood Flow Metab **30**(2): 255-266.

Cernuda-Morollon, E., D. Larrosa, C. Ramon, J. Vega, P. Martinez-Camblor and J. Pascual (2013). "Interictal increase of CGRP levels in peripheral blood as a biomarker for chronic migraine." Neurology **81**(14): 1191-1196.

Cevoli, S., E. Sancisi, D. Grimaldi, G. Pierangeli, S. Zanigni, M. Nicodemo, P. Cortelli and P. Montagna (2009). "Family history for chronic headache and drug overuse as a risk factor for headache chronification." Headache **49**(3): 412-418.

Chang, H. Y., M. Daubresse, S. P. Kruszewski and G. C. Alexander (2014). "Prevalence and treatment of pain in EDs in the United States, 2000 to 2010." Am J Emerg Med **32**(5): 421-431.

Chaplan, S. R., F. W. Bach, J. W. Pogrel, J. M. Chung and T. L. Yaksh (1994). "Quantitative assessment of tactile allodynia in the rat paw." J.Neurosci.Methods **53**(1): 55-63.

Charfi, I., K. Abdallah, L. Gendron and G. Pineyro (2018). "Delta opioid receptors recycle to the membrane after sorting to the degradation path." Cell Mol Life Sci **75**(12): 2257-2271.

Charles, A. (2017). "Migraine." N Engl J Med **377**(6): 553-561.

Charles, A. and K. Brennan (2009). "Cortical spreading depression-new insights and persistent questions." Cephalalgia **29**(10): 1115-1124.

Charles, A. and K. C. Brennan (2008). "A touch of increased pain: cutaneous allodynia in migraine." Ann Neurol **63**(2): 130-132.

Charles, A. and A. A. Pradhan (2016). "Delta-opioid receptors as targets for migraine therapy." Curr Opin Neurol **29**(3): 314-319.

Cherian, L., R. Hlatky and C. S. Robertson (2004). "Nitric oxide in traumatic brain injury." Brain Pathol **14**(2): 195-201.

Chou, D. E., M. Shnayderman Yugrakh, D. Winegarner, V. Rowe, D. Kuruvilla and J. Schoenen (2018). "Acute migraine therapy with external trigeminal neurostimulation (ACME): A randomized controlled trial." Cephalalgia: 333102418811573.

Christiansen, I., L. L. Thomsen, D. Daugaard, V. Ulrich and J. Olesen (1999). "Glyceryl trinitrate induces attacks of migraine without aura in sufferers of migraine with aura." Cephalalgia **19**(7): 660-667.

Christopoulos, A., G. Christopoulos, M. Morfis, M. Udawela, M. Laburthe, A. Couvineau, K. Kuwasako, N. Tilakaratne and P. M. Sexton (2003). "Novel receptor partners and function of receptor activity-modifying proteins." J Biol Chem **278**(5): 3293-3297.

Chu, L. F., M. S. Angst and D. Clark (2008). "Opioid-induced hyperalgesia in humans: molecular mechanisms and clinical considerations." Clin J Pain **24**(6): 479-496.

Chu Sin Chung, P., A. Boehrer, A. Stephan, A. Matifas, G. Scherrer, E. Darcq, K. Befort and B. L. Kieffer (2014). "Delta opioid receptors expressed in forebrain GABAergic neurons are responsible for SNC80-induced seizures." Behav Brain Res **278C**: 429-434.

Chu Sin Chung, P., H. L. Keyworth, E. Martin-Garcia, P. Charbogne, E. Darcq, A. Bailey, D. Filliol, A. Matifas, G. Scherrer, A. M. Ouagazzal, C. Gaveriaux-Ruff, K. Befort, R. Maldonado, I. Kitchen and B. L. Kieffer (2015). "A novel anxiogenic role for the delta opioid receptor expressed in GABAergic forebrain neurons." Biol Psychiatry **77**(4): 404-415.

Codd, E. E., J. R. Carson, R. W. Colburn, D. J. Stone, C. R. Van Besien, S. P. Zhang, P. R. Wade, E. L. Gallantine, T. F. Meert, L. Molino, S. Pullan, C. M. Razler, S. L. Dax and C. M. Flores (2009). "JNJ-20788560 [9-(8-azabicyclo[3.2.1]oct-3-ylidene)-9H-xanthene-3-carboxylic acid diethylamide], a selective delta opioid receptor agonist, is a potent and efficacious antihyperalgesic agent that does not produce respiratory depression, pharmacologic tolerance, or physical dependence." J.Pharmacol.Exp.Ther. **329**(1): 241-251.

Colas, R., P. Munoz, R. Temprano, C. Gomez and J. Pascual (2004). "Chronic daily headache with analgesic overuse: epidemiology and impact on quality of life." Neurology **62**(8): 1338-1342.

Colombo, B., P. O. Annovazzi and G. Comi (2004). "Therapy of primary headaches: the role of antidepressants." Neurol Sci **25 Suppl 3**: S171-175.

Cornelisse, L. N., J. E. Van der Harst, J. C. Lodder, P. J. Baarendse, A. J. Timmerman, H. D. Mansvelder, B. M. Spruijt and A. B. Brussaard (2007). "Reduced 5-HT1A- and GABAB receptor function in dorsal raphe neurons upon chronic fluoxetine treatment of socially stressed rats." J Neurophysiol **98**(1): 196-204.

Coronado, V. G., L. Xu, S. V. Basavaraju, L. C. McGuire, M. M. Wald, M. D. Faul, B. R. Guzman and J. D. Hemphill (2011). Surveillance for traumatic brain injury-related deaths--United States, 1997-2007. MMWR Surveill Summ. **60:** 1-32.

Couch, J. R. and C. Bearss (2001). "Chronic daily headache in the posttrauma syndrome: relation to extent of head injury." Headache **41**(6): 559-564.

D'Onofrio, F., A. Russo, F. Conte, G. Casucci, A. Tessitore and G. Tedeschi (2014). "Post-traumatic headaches: an epidemiological overview." Neurol Sci **35 Suppl 1**: 203-206.

Dado, R. J., P. Y. Law, H. H. Loh and R. Elde (1993). "Immunofluorescent identification of a delta (delta)-opioid receptor on primary afferent nerve terminals." Neuroreport **5**(3): 341-344.

Daiutolo, B. V., A. Tyburski, S. W. Clark and M. B. Elliott (2016). "Trigeminal Pain Molecules, Allodynia, and Photosensitivity Are Pharmacologically and Genetically Modulated in a Model of Traumatic Brain Injury." J Neurotrauma **33**(8): 748-760.

Daubresse, M., H. Y. Chang, Y. Yu, S. Viswanathan, N. D. Shah, R. S. Stafford, S. P. Kruszewski and G. C. Alexander (2013). "Ambulatory diagnosis and treatment of nonmalignant pain in the United States, 2000-2010." Med Care **51**(10): 870-878.

Davies, A. J. and R. A. North (2009). "Electrophysiological and morphological properties of neurons in the substantia gelatinosa of the mouse trigeminal subnucleus caudalis." Pain **146**(1-2): 214-221.

De Agostino, R., B. Federspiel, E. Cesnulis and P. S. Sandor (2015). "High-cervical spinal cord stimulation for medically intractable chronic migraine." Neuromodulation **18**(4): 289-296; discussion 296.

De Felice, M., M. H. Ossipov, R. Wang, G. Dussor, J. Lai, I. D. Meng, J. Chichorro, J. S. Andrews, S. Rakhit, S. Maddaford, D. Dodick and F. Porreca (2010). "Triptan-induced enhancement of neuronal nitric oxide synthase in trigeminal ganglion dural afferents

underlies increased responsiveness to potential migraine triggers." Brain **133**(Pt 8): 2475-2488.

De Felice, M., M. H. Ossipov, R. Wang, J. Lai, J. Chichorro, I. Meng, D. W. Dodick, T. W. Vanderah, G. Dussor and F. Porreca (2010). "Triptan-induced latent sensitization: a possible basis for medication overuse headache." Ann Neurol **67**(3): 325-337.

Demarque, M., A. Represa, H. Becq, I. Khalilov, Y. Ben-Ari and L. Aniksztejn (2002). "Paracrine intercellular communication by a Ca2+- and SNARE-independent release of GABA and glutamate prior to synapse formation." Neuron **36**(6): 1051-1061.

Denninger, J. W. and M. A. Marletta (1999). "Guanylate cyclase and the .NO/cGMP signaling pathway." Biochim Biophys Acta **1411**(2-3): 334-350.

Dhawan, B. N., F. Cesselin, R. Raghubir, T. Reisine, P. B. Bradley, P. S. Portoghese and M. Hamon (1996). "International Union of Pharmacology. XII. Classification of opioid receptors." Pharmacol Rev **48**(4): 567-592.

Diamond, S., M. E. Bigal, S. Silberstein, E. Loder, M. Reed and R. B. Lipton (2007). "Patterns of diagnosis and acute and preventive treatment for migraine in the United States: results from the American Migraine Prevalence and Prevention study." Headache **47**(3): 355-363.

DiCello, J. J., A. Saito, P. Rajasekhar, E. M. Eriksson, R. M. McQuade, C. J. Nowell, B. W. Sebastian, J. Fichna, N. A. Veldhuis, M. Canals, N. W. Bunnett, S. E. Carbone and D. P. Poole (2018). "Inflammation-associated changes in DOR expression and function in the mouse colon." Am J Physiol Gastrointest Liver Physiol.

Diener, H. C., P. Barbanti, C. Dahlof, U. Reuter, J. Habeck and J. Podhorna (2011). "BI 44370 TA, an oral CGRP antagonist for the treatment of acute migraine attacks: results from a phase II study." Cephalalgia **31**(5): 573-584.

Diener, H. C., G. Bussone, J. C. Van Oene, M. Lahaye, S. Schwalen and P. J. Goadsby (2007). "Topiramate reduces headache days in chronic migraine: a randomized, double-blind, placebo-controlled study." Cephalalgia **27**(7): 814-823.

Diener, H. C., E. Hartung, J. Chrubasik, S. Evers, J. Schoenen, A. Eikermann, G. Latta and W. Hauke (2001). "A comparative study of oral acetylsalicyclic acid and metoprolol for the prophylactic treatment of migraine. A randomized, controlled, double-blind, parallel group phase III study." Cephalalgia **21**(2): 120-128.

Diener, H. C., D. Holle, K. Solbach and C. Gaul (2016). "Medication-overuse headache: risk factors, pathophysiology and management." Nat Rev Neurol **12**(10): 575-583.

Dodick, D. W., F. Freitag, J. Banks, J. Saper, J. Xiang, M. Rupnow, D. Biondi, S. J. Greenberg and J. Hulihan (2009). "Topiramate versus amitriptyline in migraine prevention: a 26-week, multicenter, randomized, double-blind, double-dummy, parallel-group noninferiority trial in adult migraineurs." Clin Ther **31**(3): 542-559.

Dodick, D. W., P. J. Goadsby, S. D. Silberstein, R. B. Lipton, J. Olesen, M. Ashina, K. Wilks, D. Kudrow, R. Kroll, B. Kohrman, R. Bargar, J. Hirman, J. Smith and A. L. D. s. investigators (2014). "Safety and efficacy of ALD403, an antibody to calcitonin gene-related peptide, for the prevention of frequent episodic migraine: a randomised, double-blind, placebo-controlled, exploratory phase 2 trial." Lancet Neurol **13**(11): 1100-1107.

Dodick, D. W., P. J. Goadsby, E. L. Spierings, J. C. Scherer, S. P. Sweeney and D. S. Grayzel (2014). "Safety and efficacy of LY2951742, a monoclonal antibody to calcitonin gene-related peptide, for the prevention of migraine: a phase 2, randomised, double-blind, placebo-controlled study." Lancet Neurol **13**(9): 885-892.

Dodick, D. W., C. C. Turkel, R. E. DeGryse, S. K. Aurora, S. D. Silberstein, R. B. Lipton, H. C. Diener and M. F. Brin (2010). "OnabotulinumtoxinA for treatment of chronic migraine: pooled results from the double-blind, randomized, placebo-controlled phases of the PREEMPT clinical program." Headache **50**(6): 921-936.

Dripps, I. J., B. T. Boyer, R. R. Neubig, K. C. Rice, J. R. Traynor and E. M. Jutkiewicz (2018). "Role of signalling molecules in behaviours mediated by the delta opioid receptor agonist SNC80." Br J Pharmacol **175**(6): 891-901.

Dripps, I. J. and E. M. Jutkiewicz (2018). "Delta Opioid Receptors and Modulation of Mood and Emotion." Handb Exp Pharmacol **247**: 179-197.

Dripps, I. J., Q. Wang, R. R. Neubig, K. C. Rice, J. R. Traynor and E. M. Jutkiewicz (2017). "The role of regulator of G protein signaling 4 in delta-opioid receptor-mediated behaviors." Psychopharmacology (Berl) **234**(1): 29-39.

Durham, P. L. and C. G. Masterson (2013). "Two mechanisms involved in trigeminal CGRP release: implications for migraine treatment." Headache **53**(1): 67-80.

Durham, P. L. and A. F. Russo (1998). "Serotonergic repression of mitogen-activated protein kinase control of the calcitonin gene-related peptide enhancer." Mol Endocrinol **12**(7): 1002-1009.

Durham, P. L. and A. F. Russo (1999). "Regulation of calcitonin gene-related peptide secretion by a serotonergic antimigraine drug." J Neurosci **19**(9): 3423-3429.

Durham, P. L. and A. F. Russo (2000). "Differential regulation of mitogen-activated protein kinase-responsive genes by the duration of a calcium signal." Mol Endocrinol **14**(10): 1570-1582.

Edelmayer, R. M., L. N. Le, J. Yan, X. Wei, R. Nassini, S. Materazzi, D. Preti, G. Appendino, P. Geppetti, D. W. Dodick, T. W. Vanderah, F. Porreca and G. Dussor (2012). "Activation of TRPA1 on dural afferents: a potential mechanism of headache pain." Pain **153**(9): 1949-1958.

Edelmayer, R. M., M. H. Ossipov and F. Porreca (2012). "An experimental model of headache-related pain." Methods Mol Biol **851**: 109-120.

Edvinsson, L. (2015). "The Journey to Establish CGRP as a Migraine Target: A Retrospective View." Headache **55**(9): 1249-1255.

Edvinsson, L. (2017). "The Trigeminovascular Pathway: Role of CGRP and CGRP Receptors in Migraine." Headache **57 Suppl 2**: 47-55.

Edvinsson, L., R. Ekman, I. Jansen, A. Ottosson and R. Uddman (1987). "Peptide-containing nerve fibers in human cerebral arteries: immunocytochemistry, radioimmunoassay, and in vitro pharmacology." Ann Neurol **21**(5): 431-437.

Eftekhari, S. (2013). Distribution of CGRP and CGRP receptor in the trigeminovascular system and CNS. PhD, Lund University.

Elliott, M. B., M. L. Oshinsky, P. S. Amenta, O. O. Awe and J. I. Jallo (2012). "Nociceptive neuropeptide increases and periorbital allodynia in a model of traumatic brain injury." Headache **52**(6): 966-984.

Erbs, E., L. Faget, G. Scherrer, P. Kessler, D. Hentsch, J. L. Vonesch, A. Matifas, B. L. Kieffer and D. Massotte (2012). "Distribution of delta opioid receptor-expressing neurons in the mouse hippocampus." Neuroscience **221**: 203-213.

Erbs, E., L. Faget, G. Scherrer, A. Matifas, D. Filliol, J. L. Vonesch, M. Koch, P. Kessler, D. Hentsch, M. C. Birling, M. Koutsourakis, L. Vasseur, P. Veinante, B. L. Kieffer and D. Massotte (2015). "A mu-delta opioid receptor brain atlas reveals neuronal co-occurrence in subcortical networks." Brain Struct Funct **220**(2): 677-702.

Erdener, S. E. and T. Dalkara (2014). "Modelling headache and migraine and its pharmacological manipulation." Br J Pharmacol **171**(20): 4575-4594.

Erickson, J. C. (2011). "Treatment outcomes of chronic post-traumatic headaches after mild head trauma in US soldiers: an observational study." Headache **51**(6): 932-944.

Eriksen, M. K., L. L. Thomsen, I. Andersen, F. Nazim and J. Olesen (2004). "Clinical characteristics of 362 patients with familial migraine with aura." Cephalalgia **24**(7): 564-575.

Escher, C. M., L. Paracka, D. Dressler and K. Kollewe (2017). "Botulinum toxin in the management of chronic migraine: clinical evidence and experience." Ther Adv Neurol Disord **10**(2): 127-135.

Evans, B. N., M. I. Rosenblatt, L. O. Mnayer, K. R. Oliver and I. M. Dickerson (2000). "CGRP-RCP, a novel protein required for signal transduction at calcitonin gene-related peptide and adrenomedullin receptors." J Biol Chem **275**(40): 31438-31443.

Evans, R. M., H. You, S. Hameed, C. Altier, A. Mezghrani, E. Bourinet and G. W. Zamponi (2010). "Heterodimerization of ORL1 and opioid receptors and its consequences for N-type calcium channel regulation." J Biol Chem **285**(2): 1032-1040.

Faget, L., E. Erbs, J. Le Merrer, G. Scherrer, A. Matifas, N. Benturquia, F. Noble, M. Decossas, M. Koch, P. Kessler, J. L. Vonesch, Y. Schwab, B. L. Kieffer and D. Massotte (2012). "In vivo visualization of delta opioid receptors upon physiological activation uncovers a distinct internalization profile." J.Neurosci. **32**(21): 7301-7310.

Farajdokht, F., S. Babri, P. Karimi, M. R. Alipour, R. Bughchechi and G. Mohaddes (2017). "Chronic ghrelin treatment reduced photophobia and anxiety-like behaviors in nitroglycerin- induced migraine: role of pituitary adenylate cyclase-activating polypeptide." Eur J Neurosci **45**(6): 763-772.

Faul M, X. L., Wald MM, Coronado V (2010). "Traumatic brain injury in the United States: emergency department visits, hospitalizations and deaths, 2002-2006. ." Atlanta,

Georgia: Centers for Disease Control and Prevention, National Center for Injury Prevention and Control.

Feil, R. and T. Kleppisch (2008). "NO/cGMP-dependent modulation of synaptic transmission." Handb Exp Pharmacol(184): 529-560.

Ferguson, J. M. (2001). "SSRI Antidepressant Medications: Adverse Effects and Tolerability." Prim Care Companion J Clin Psychiatry **3**(1): 22-27.

Ferrari, M. D., J. Odink, C. Tapparelli, G. M. Van Kempen, E. J. Pennings and G. W. Bruyn (1989). "Serotonin metabolism in migraine." Neurology **39**(9): 1239-1242.

Ferrari, M. D. and P. R. Saxena (1992). "Clinical effects and mechanism of action of sumatriptan in migraine." Clin Neurol Neurosurg **94 Suppl**: S73-77.

Filliol, D., S. Ghozland, J. Chluba, M. Martin, H. W. Matthes, F. Simonin, K. Befort, C. Gaveriaux-Ruff, A. Dierich, M. LeMeur, O. Valverde, R. Maldonado and B. L. Kieffer (2000). "Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses." Nat Genet **25**(2): 195-200.

Filliol, D., S. Ghozland, J. Chluba, M. Martin, H. W. Matthes, F. Simonin, K. Befort, C. Gaveriaux-Ruff, A. Dierich, M. LeMeur, O. Valverde, R. Maldonado and B. L. Kieffer (2000). "Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses." Nat.Genet. **25**(2): 195-200.

Florin, S., J. Meunier and J. Costentin (2000). "Autoradiographic localization of [3H]nociceptin binding sites in the rat brain." Brain Res **880**(1-2): 11-16.

Fraser, G. L., G. A. Gaudreau, P. B. Clarke, D. P. Menard and M. N. Perkins (2000). "Antihyperalgesic effects of delta opioid agonists in a rat model of chronic inflammation." Br J Pharmacol **129**(8): 1668-1672.

Freeland, K., Y. Z. Liu and D. S. Latchman (2000). "Distinct signalling pathways mediate the cAMP response element (CRE)-dependent activation of the calcitonin gene-related peptide gene promoter by cAMP and nerve growth factor." Biochem J **345 Pt 2**: 233-238.

Friedman, B. W., J. West, D. R. Vinson, M. T. Minen, A. Restivo and E. J. Gallagher (2015). "Current management of migraine in US emergency departments: an analysis of the National Hospital Ambulatory Medical Care Survey." Cephalalgia **35**(4): 301-309.

Gallantine, E. L. and T. F. Meert (2005). "A comparison of the antinociceptive and adverse effects of the mu-opioid agonist morphine and the delta-opioid agonist SNC80." Basic Clin Pharmacol Toxicol **97**(1): 39-51.

Garza, I. and J. W. Swanson (2006). "Prophylaxis of migraine." Neuropsychiatr Dis Treat **2**(3): 281-291.

Garzon, M. and V. M. Pickel (2001). "Plasmalemmal mu-opioid receptor distribution mainly in nondopaminergic neurons in the rat ventral tegmental area." Synapse **41**(4): 311-328.

Gaveriaux-Ruff, C., L. A. Karchewski, X. Hever, A. Matifas and B. L. Kieffer (2008). "Inflammatory pain is enhanced in delta opioid receptor-knockout mice." Eur.J.Neurosci **27**(10): 2558-2567.

Gaveriaux-Ruff, C. and B. L. Kieffer (2011). "Delta opioid receptor analgesia: recent contributions from pharmacology and molecular approaches." Behav Pharmacol **22**(5-6): 405-414.

Gaveriaux-Ruff, C., C. Nozaki, X. Nadal, X. C. Hever, R. Weibel, A. Matifas, D. Reiss, D. Filliol, M. A. Nassar, J. N. Wood, R. Maldonado and B. L. Kieffer (2011). "Genetic ablation of delta opioid receptors in nociceptive sensory neurons increases chronic pain and abolishes opioid analgesia." Pain.

Gendron, L., C. M. Cahill, M. von Zastrow, P. W. Schiller and G. Pineyro (2016). "Molecular Pharmacology of delta-Opioid Receptors." Pharmacol Rev **68**(3): 631-700.

Gendron, L., A. L. Lucido, F. Mennicken, D. O'Donnell, J. P. Vincent, T. Stroh and A. Beaudet (2006). "Morphine and pain-related stimuli enhance cell surface availability of somatic delta-opioid receptors in rat dorsal root ganglia." J.Neurosci. **26**(3): 953-962.

Gendron, L., N. Mittal, H. Beaudry and W. Walwyn (2015). "Recent advances on the delta opioid receptor: from trafficking to function." Br J Pharmacol **172**(2): 403-419.

Giamberardino, M. A., G. Affaitati, M. Curto, A. Negro, R. Costantini and P. Martelletti (2016). "Anti-CGRP monoclonal antibodies in migraine: current perspectives." Intern Emerg Med **11**(8): 1045-1057.

Giffin, N. J., R. B. Lipton, S. D. Silberstein, J. Olesen and P. J. Goadsby (2016). "The migraine postdrome: An electronic diary study." Neurology **87**(3): 309-313.

Giffin, N. J., L. Ruggiero, R. B. Lipton, S. D. Silberstein, J. F. Tvedskov, J. Olesen, J. Altman, P. J. Goadsby and A. Macrae (2003). "Premonitory symptoms in migraine: an electronic diary study." Neurology **60**(6): 935-940.

Goadsby, P. J. and L. Edvinsson (1993). "The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats." Ann Neurol **33**(1): 48-56.

Goadsby, P. J., L. Edvinsson and R. Ekman (1988). "Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system." Ann Neurol **23**(2): 193-196.

Goadsby, P. J., L. Edvinsson and R. Ekman (1990). "Vasoactive peptide release in the extracerebral circulation of humans during migraine headache." Ann Neurol **28**(2): 183-187.

Goadsby, P. J., P. R. Holland, M. Martins-Oliveira, J. Hoffmann, C. Schankin and S. Akerman (2017). "Pathophysiology of Migraine: A Disorder of Sensory Processing." Physiol Rev **97**(2): 553-622.

Goadsby, P. J., U. Reuter, Y. Hallstrom, G. Broessner, J. H. Bonner, F. Zhang, S. Sapra, H. Picard, D. D. Mikol and R. A. Lenz (2017). "A Controlled Trial of Erenumab for Episodic Migraine." N Engl J Med **377**(22): 2123-2132.

Goldberg, L. D. (2005). "The cost of migraine and its treatment." Am J Manag Care **11**(2 Suppl): S62-67.

Goldstein, A., S. Tachibana, L. I. Lowney, M. Hunkapiller and L. Hood (1979). "Dynorphin-(1-13), an extraordinarily potent opioid peptide." Proc Natl Acad Sci U S A **76**(12): 6666-6670.

Gomes, I., A. P. Ijzerman, K. Ye, E. L. Maillet and L. A. Devi (2011). "G protein-coupled receptor heteromerization: a role in allosteric modulation of ligand binding." Mol Pharmacol **79**(6): 1044-1052.

Gomes, I., B. A. Jordan, A. Gupta, N. Trapaidze, V. Nagy and L. A. Devi (2000). "Heterodimerization of mu and delta opioid receptors: A role in opiate synergy." J Neurosci **20**(22): Rc110.

Goodman, R. R., S. H. Snyder, M. J. Kuhar and W. S. Young, 3rd (1980). "Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography." Proc Natl Acad Sci U S A **77**(10): 6239-6243.

Goody, R. J., S. M. Oakley, D. Filliol, B. L. Kieffer and I. Kitchen (2002). "Quantitative autoradiographic mapping of opioid receptors in the brain of delta-opioid receptor gene knockout mice." Brain Res. **945**(1): 9-19.

Gouarderes, C., S. Tellez, J. A. Tafani and J. M. Zajac (1993). "Quantitative autoradiographic mapping of delta-opioid receptors in the rat central nervous system using [125I][D.Ala2]deltorphin-I." Synapse **13**(3): 231-240.

Grafstein, B. (1956). "Mechanism of spreading cortical depression." J Neurophysiol **19**(2): 154-171.

Graham, J. R. and H. G. Wolff (1938). "Mechanism of migraine headache and action of ergotamine tartrate." Archives of Neurology & Psychiatry **39**(4): 737-763.

Gray, J. A. and A. R. Green (1987). "Increased GABAB receptor function in mouse frontal cortex after repeated administration of antidepressant drugs or electroconvulsive shocks." Br J Pharmacol **92**(2): 357-362.

Greco, R., A. S. Mangione, F. Siani, F. Blandini, M. Vairetti, G. Nappi, G. Sandrini, M. G. Buzzi and C. Tassorelli (2014). "Effects of CGRP receptor antagonism in nitroglycerin-induced hyperalgesia." Cephalalgia **34**(8): 594-604.

Greco, R., C. Meazza, A. S. Mangione, M. Allena, M. Bolla, D. Amantea, H. Mizoguchi, G. Sandrini, G. Nappi and C. Tassorelli (2011). "Temporal profile of vascular changes induced by systemic nitroglycerin in the meningeal and cortical districts." Cephalalgia **31**(2): 190-198.

Guan, J. S., Z. Z. Xu, H. Gao, S. Q. He, G. Q. Ma, T. Sun, L. H. Wang, Z. N. Zhang, I. Lena, I. Kitchen, R. Elde, A. Zimmer, C. He, G. Pei, L. Bao and X. Zhang (2005). "Interaction with vesicle luminal protachykinin regulates surface expression of delta-opioid receptors and opioid analgesia." Cell **122**(4): 619-631.

Guo, J., Y. Wu, W. Zhang, J. Zhao, L. A. Devi, G. Pei and L. Ma (2000). "Identification of G protein-coupled receptor kinase 2 phosphorylation sites responsible for agonist-stimulated delta-opioid receptor phosphorylation." Mol Pharmacol **58**(5): 1050-1056.

Gupta, P., S. Singh, V. Goyal, G. Shukla and M. Behari (2007). "Low-dose topiramate versus lamotrigine in migraine prophylaxis (the Lotolamp study)." Headache **47**(3): 402-412.

Hadjikhani, N., M. Sanchez Del Rio, O. Wu, D. Schwartz, D. Bakker, B. Fischl, K. K. Kwong, F. M. Cutrer, B. R. Rosen, R. B. Tootell, A. G. Sorensen and M. A. Moskowitz (2001). "Mechanisms of migraine aura revealed by functional MRI in human visual cortex." Proc Natl Acad Sci U S A **98**(8): 4687-4692.

Halker, R. B., A. J. Starling, B. B. Vargas and T. J. Schwedt (2016). "ACE and ARB Agents in the Prophylactic Therapy of Migraine-How Effective Are They?" Curr Treat Options Neurol **18**(4): 15.

Harrison, C. (2013). "Trial watch: opioid receptor blocker shows promise in Phase II depression trial." Nat Rev Drug Discov **12**(6): 415.

Hasbi, A., J. Polastron, S. Allouche, L. Stanasila, D. Massotte and P. Jauzac (1998). "Desensitization of the delta-opioid receptor correlates with its phosphorylation in SK-N-BE cells: involvement of a G protein-coupled receptor kinase." J Neurochem **70**(5): 2129-2138.

Haut, S. R., M. E. Bigal and R. B. Lipton (2006). "Chronic disorders with episodic manifestations: focus on epilepsy and migraine." Lancet Neurol **5**(2): 148-157.

Hayhurst, C. J. and M. E. Durieux (2016). "Differential Opioid Tolerance and Opioid-induced Hyperalgesia: A Clinical Reality." Anesthesiology **124**(2): 483-488.

Headache Classification Committee of the International Headache, S. (2013). "The International Classification of Headache Disorders, 3rd edition (beta version)." Cephalalgia **33**(9): 629-808.

Henry, A. G., I. J. White, M. Marsh, M. von Zastrow and J. N. Hislop (2011). "The role of ubiquitination in lysosomal trafficking of delta-opioid receptors." Traffic **12**(2): 170-184.

Hewitt, D. J., S. K. Aurora, D. W. Dodick, P. J. Goadsby, Y. J. Ge, R. Bachman, D. Taraborelli, X. Fan, C. Assaid, C. Lines and T. W. Ho (2011). "Randomized controlled trial of the CGRP receptor antagonist MK-3207 in the acute treatment of migraine." Cephalalgia **31**(6): 712-722.

Ho, T. W., M. D. Ferrari, D. W. Dodick, V. Galet, J. Kost, X. Fan, H. Leibensperger, S. Froman, C. Assaid, C. Lines, H. Koppen and P. K. Winner (2008). "Efficacy and tolerability of MK-0974 (telcagepant), a new oral antagonist of calcitonin gene-related peptide receptor, compared with zolmitriptan for acute migraine: a randomised, placebo-controlled, parallel-treatment trial." Lancet **372**(9656): 2115-2123.

Hoffman, J. M., S. Lucas, S. Dikmen, C. A. Braden, A. W. Brown, R. Brunner, R. Diaz-Arrastia, W. C. Walker, T. K. Watanabe and K. R. Bell (2011). "Natural history of headache after traumatic brain injury." J Neurotrauma **28**(9): 1719-1725.

Horasanli, B., F. B. Atac, I. Coven, B. Karakurum Goksel and S. Benli (2013). "Angiotensin I-converting enzyme gene (I/D) polymorphism in patients with migraine." Headache **53**(1): 161-164.

Hou, M., H. Xing, Y. Cai, B. Li, X. Wang, P. Li, X. Hu and J. Chen (2017). "The effect and safety of monoclonal antibodies to calcitonin gene-related peptide and its receptor on migraine: a systematic review and meta-analysis." J Headache Pain **18**(1): 42.

Hsieh, C. L., C. C. Kim, B. E. Ryba, E. C. Niemi, J. K. Bando, R. M. Locksley, J. Liu, M. C. Nakamura and W. E. Seaman (2013). "Traumatic brain injury induces macrophage subsets in the brain." Eur J Immunol **43**(8): 2010-2022.

Huang, J., Y. Lv, Y. Fu, L. Ren, P. Wang, B. Liu, K. Huang and J. Bi (2015). "Dynamic Regulation of Delta-Opioid Receptor in Rat Trigeminal Ganglion Neurons by Lipopolysaccharide-induced Acute Pulpitis." J Endod **41**(12): 2014-2020.

Hughes, J., H. W. Kosterlitz and T. W. Smith (1997). "The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. 1977." Br J Pharmacol **120**(4 Suppl): 428-436; discussion 426-427.

Hughes, J., T. Smith, B. Morgan and L. Fothergill (1975). "Purification and properties of enkephalin - the possible endogenous ligand for the morphine receptor." Life Sci **16**(12): 1753-1758.

Hurley, R. W. and D. L. Hammond (2000). "The analgesic effects of supraspinal mu and delta opioid receptor agonists are potentiated during persistent inflammation." J Neurosci **20**(3): 1249-1259.

ICHD3b (2013). "The International Classification of Headache Disorders, 3rd edition (beta version)." Cephalalgia **33**(9): 629-808.

Improta, G. and M. Broccardo (1992). "Spinal antinociceptive effects of [D-Ala2]deltorphin II, a novel and highly selective delta-opioid receptor agonist." Peptides **13**(6): 1123-1126.

Israelsson, C., Y. Wang, A. Kylberg, C. G. Pick, B. J. Hoffer and T. Ebendal (2009). "Closed head injury in a mouse model results in molecular changes indicating inflammatory responses." J Neurotrauma **26**(8): 1307-1314.

Iversen, H. K., J. Olesen and P. Tfelt-Hansen (1989). "Intravenous nitroglycerin as an experimental model of vascular headache. Basic characteristics." Pain **38**(1): 17-24.

Iyengar, S., M. H. Ossipov and K. W. Johnson (2017). "The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine." Pain **158**(4): 543-559.

J, J. G., J. Simms, J. Barwell, D. R. Poyner, H. A. Watkins, A. A. Pioszak, P. M. Sexton and D. L. Hay (2016). "An allosteric role for receptor activity-modifying proteins in defining GPCR pharmacology." Cell Discov **2**: 16012.

Jacobs, B. A., M. M. Pando, E. Jennings, T. A. Chavera, W. P. Clarke and K. A. Berg (2018). "Allosterism within delta Opioid-kappa Opioid Receptor Heteromers in Peripheral Sensory Neurons: Regulation of kappa Opioid Agonist Efficacy." Mol Pharmacol **93**(4): 376-386.

Justic, U. D. o. (2011). Automation of Reports and Consolidate Orders System (ARCOS). D. E. A. D. US Department of Justic. Springfield, VA.

Jutkiewicz, E. M. (2006). "The antidepressant -like effects of delta-opioid receptor agonists." Mol Interv **6**(3): 162-169.

Kabli, N. and C. M. Cahill (2007). "Anti-allodynic effects of peripheral delta opioid receptors in neuropathic pain." Pain **127**(1-2): 84-93.

Karatas, H., S. E. Erdener, Y. Gursoy-Ozdemir, S. Lule, E. Eren-Kocak, Z. D. Sen and T. Dalkara (2013). "Spreading depression triggers headache by activating neuronal Panx1 channels." Science **339**(6123): 1092-1095.

Katsarava, Z., V. Limmroth, M. Finke, H. C. Diener and G. Fritsche (2003). "Rates and predictors for relapse in medication overuse headache: a 1-year prospective study." Neurology **60**(10): 1682-1683.

Katsarava, Z., S. Schneeweiss, T. Kurth, U. Kroener, G. Fritsche, A. Eikermann, H. C. Diener and V. Limmroth (2004). "Incidence and predictors for chronicity of headache in patients with episodic migraine." Neurology **62**(5): 788-790.

Kelman, L. (2006). "The postdrome of the acute migraine attack." Cephalalgia **26**(2): 214-220.

Keskinbora, K. and I. Aydinli (2008). "A double-blind randomized controlled trial of topiramate and amitriptyline either alone or in combination for the prevention of migraine." Clin Neurol Neurosurg **110**(10): 979-984.

Khan, S., A. Olesen and M. Ashina (2017). "CGRP, a target for preventive therapy in migraine and cluster headache: Systematic review of clinical data." Cephalalgia: 333102417741297.

Kieffer, B. L. and C. J. Evans (2009). "Opioid receptors: from binding sites to visible molecules in vivo." Neuropharmacology **56 Suppl 1**: 205-212.

Kieffer, B. L. and C. Gaveriaux-Ruff (2002). "Exploring the opioid system by gene knockout." Prog Neurobiol **66**(5): 285-306.

Knoll, A. T. and W. A. Carlezon, Jr. (2010). "Dynorphin, stress, and depression." Brain Res **1314**: 56-73.

Konig, M., A. M. Zimmer, H. Steiner, P. V. Holmes, J. N. Crawley, M. J. Brownstein and A. Zimmer (1996). "Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin." Nature **383**(6600): 535-538.

Koob, G. F. and N. D. Volkow (2016). "Neurobiology of addiction: a neurocircuitry analysis." Lancet Psychiatry **3**(8): 760-773.

Kowa, H., E. Fusayasu, T. Ijiri, K. Ishizaki, K. Yasui, K. Nakaso, M. Kusumi, T. Takeshima and K. Nakashima (2005). "Association of the insertion/deletion polymorphism of the angiotensin I-converting enzyme gene in patients of migraine with aura." Neurosci Lett **374**(2): 129-131.

Kristoffersen, E. S. and C. Lundqvist (2014). "Medication-overuse headache: epidemiology, diagnosis and treatment." Ther Adv Drug Saf **5**(2): 87-99.

Lamping, K. (2001). "Interactions between NO and cAMP in the regulation of vascular tone." Arterioscler Thromb Vasc Biol **21**(5): 729-730.

Lassen, L. H., P. A. Haderslev, V. B. Jacobsen, H. K. Iversen, B. Sperling and J. Olesen (2002). "CGRP may play a causative role in migraine." Cephalalgia **22**(1): 54-61.

Laurent, V., J. Bertran-Gonzalez, B. C. Chieng and B. W. Balleine (2014). "delta-opioid and dopaminergic processes in accumbens shell modulate the cholinergic control of predictive learning and choice." J Neurosci **34**(4): 1358-1369.

Laurent, V., A. K. Morse and B. W. Balleine (2015). "The role of opioid processes in reward and decision-making." Br J Pharmacol **172**(2): 449-459.

Laurent, V., F. L. Wong and B. W. Balleine (2015). "delta-Opioid receptors in the accumbens shell mediate the influence of both excitatory and inhibitory predictions on choice." Br J Pharmacol **172**(2): 562-570.

Lauritzen, M. (1994). "Pathophysiology of the migraine aura. The spreading depression theory." Brain **117 (Pt 1)**: 199-210.

Lauritzen, M., J. P. Dreier, M. Fabricius, J. A. Hartings, R. Graf and A. J. Strong (2011). "Clinical relevance of cortical spreading depression in neurological disorders: migraine, malignant stroke, subarachnoid and intracranial hemorrhage, and traumatic brain injury." J Cereb Blood Flow Metab **31**(1): 17-35.

Le Merrer, J., J. A. Becker, K. Befort and B. L. Kieffer (2009). "Reward processing by the opioid system in the brain." Physiol Rev **89**(4): 1379-1412.

Le Merrer, J., A. Plaza-Zabala, C. D. Boca, A. Matifas, R. Maldonado and B. L. Kieffer (2011). "Deletion of the delta Opioid Receptor Gene Impairs Place Conditioning But Preserves Morphine Reinforcement." Biol.Psychiatry.

Le Merrer, J., X. Rezai, G. Scherrer, J. A. Becker and B. L. Kieffer (2013). "Impaired hippocampus-dependent and facilitated striatum-dependent behaviors in mice lacking the delta opioid receptor." Neuropsychopharmacology **38**(6): 1050-1059.

Lee, M., S. M. Silverman, H. Hansen, V. B. Patel and L. Manchikanti (2011). "A comprehensive review of opioid-induced hyperalgesia." Pain Physician **14**(2): 145-161.

Lee, W. I., Y. Xu, S. M. Fung and H. L. Fung (2003). "eNOS-dependent vascular interaction between nitric oxide and calcitonin gene-related peptide in mice: gender selectivity and effects on blood aggregation." Regul Pept **110**(2): 115-122.

Leo, A. A. P. (1944). "Spreading Depression of Activity in the Cerebral Cortex." Journal of Neurophysiology **7**(6): 359-390.

Leroux, E. (2016). Migraines: More than a headache, Dundurn Press.

Levy, D., S. Edut, R. Baraz-Goldstein, V. Rubovitch, R. Defrin, D. Bree, H. Gariepy, J. Zhao and C. G. Pick (2016). "Responses of dural mast cells in concussive and blast models of mild traumatic brain injury in mice: Potential implications for post-traumatic headache." Cephalalgia **36**(10): 915-923.

Levy, D., V. Kainz, R. Burstein and A. M. Strassman (2012). "Mast cell degranulation distinctly activates trigemino-cervical and lumbosacral pain pathways and elicits widespread tactile pain hypersensitivity." Brain Behav Immun **26**(2): 311-317.

Li, J., C. V. Vause and P. L. Durham (2008). "Calcitonin gene-related peptide stimulation of nitric oxide synthesis and release from trigeminal ganglion glial cells." Brain Res **1196**: 22-32.

Limmroth, V., Z. Katsarava, G. Fritsche, S. Przywara and H. C. Diener (2002). "Features of medication overuse headache following overuse of different acute headache drugs." Neurology **59**(7): 1011-1014.

Lipton, R. B., M. E. Bigal, S. Ashina, R. Burstein, S. Silberstein, M. L. Reed, D. Serrano and W. F. Stewart (2008). "Cutaneous allodynia in the migraine population." Ann Neurol **63**(2): 148-158.

Lipton, R. B., M. E. Bigal, M. Diamond, F. Freitag, M. L. Reed, W. F. Stewart and A. A. Group (2007). "Migraine prevalence, disease burden, and the need for preventive therapy." Neurology **68**(5): 343-349.

Lipton, R. B., D. C. Buse, D. Serrano, S. Holland and M. L. Reed (2013). "Examination of unmet treatment needs among persons with episodic migraine: results of the American Migraine Prevalence and Prevention (AMPP) Study." Headache **53**(8): 1300-1311.

Lipton, R. B., S. W. Hamelsky, K. B. Kolodner, T. J. Steiner and W. F. Stewart (2000). "Migraine, quality of life, and depression: a population-based case-control study." Neurology **55**(5): 629-635.

Lipton, R. B., S. Munjal, A. Alam, D. C. Buse, K. M. Fanning, M. L. Reed, T. J. Schwedt and D. W. Dodick (2018). "Migraine in America Symptoms and Treatment (MAST) Study: Baseline Study Methods, Treatment Patterns, and Gender Differences." Headache.

Lipton, R. B., W. F. Stewart and A. I. Scher (2001). "Epidemiology and economic impact of migraine." Curr Med Res Opin **17 Suppl 1**: s4-12.

Lloyd, K. G., F. Thuret and A. Pilc (1985). "Upregulation of gamma-aminobutyric acid (GABA) B binding sites in rat frontal cortex: a common action of repeated administration of different classes of antidepressants and electroshock." J Pharmacol Exp Ther **235**(1): 191-199.

Lobingier, B. T. and M. von Zastrow (2018). "When trafficking and signaling mix: how subcellular location shapes GPCR activation of heterotrimeric G proteins." Traffic.

Loh, H. H., L. F. Tseng, E. Wei and C. H. Li (1976). "beta-endorphin is a potent analgesic agent." Proceedings of the National Academy of Sciences **73**(8): 2895-2898.

Longoni, R., C. Cadoni, A. Mulas, G. Di Chiara and L. Spina (1998). "Dopamine-dependent behavioural stimulation by non-peptide delta opioids BW373U86 and SNC 80: 2. Place-preference and brain microdialysis studies in rats." Behav Pharmacol **9**(1): 9-14.

Lucas, S., J. M. Hoffman, K. R. Bell and S. Dikmen (2014). "A prospective study of prevalence and characterization of headache following mild traumatic brain injury." Cephalalgia **34**(2): 93-102.

Lutz, P. E. and B. L. Kieffer (2012). "Opioid receptors: distinct roles in mood disorders." Trends Neurosci.

Lutz, P. E. and B. L. Kieffer (2013). "Opioid receptors: distinct roles in mood disorders." Trends Neurosci **36**(3): 195-206.

Ma, Q. P., R. Hill and D. Sirinathsinghji (2001). "Colocalization of CGRP with 5-HT1B/1D receptors and substance P in trigeminal ganglion neurons in rats." Eur J Neurosci **13**(11): 2099-2104.

Malick, A., R. M. Strassman and R. Burstein (2000). "Trigeminohypothalamic and reticulohypothalamic tract neurons in the upper cervical spinal cord and caudal medulla of the rat." J Neurophysiol **84**(4): 2078-2112.

Management of Concussion/m, T. B. I. W. G. (2009). "VA/DoD Clinical Practice Guideline for Management of Concussion/Mild Traumatic Brain Injury." J Rehabil Res Dev **46**(6): CP1-68.

Maniyar, F. H., T. Sprenger, T. Monteith, C. Schankin and P. J. Goadsby (2014). "Brain activations in the premonitory phase of nitroglycerin-triggered migraine attacks." Brain **137**(Pt 1): 232-241.

Manni, E., B. Bagolini, V. E. Pettorossi and P. Errico (1989). "Effect of botulinum toxin on extraocular muscle proprioception." Doc Ophthalmol **72**(2): 189-198.

Mansour, A., C. A. Fox, H. Akil and S. J. Watson (1995). "Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications." Trends Neurosci. **18**(1): 22-29.

Mansour, A., H. Khachaturian, M. E. Lewis, H. Akil and S. J. Watson (1988). "Anatomy of CNS opioid receptors." Trends Neurosci **11**(7): 308-314.

Marcus, R., P. J. Goadsby, D. Dodick, D. Stock, G. Manos and T. Z. Fischer (2014). "BMS-927711 for the acute treatment of migraine: a double-blind, randomized, placebo controlled, dose-ranging trial." Cephalalgia **34**(2): 114-125.

Markovics, A., V. Kormos, B. Gaszner, A. Lashgarara, E. Szoke, K. Sandor, K. Szabadfi, B. Tuka, J. Tajti, J. Szolcsanyi, E. Pinter, H. Hashimoto, J. Kun, D. Reglodi and Z. Helyes (2012). "Pituitary adenylate cyclase-activating polypeptide plays a key role in nitroglycerol-induced trigeminovascular activation in mice." Neurobiol.Dis. **45**(1): 633-644.

Marques-Lopes, J., M. Pinto, D. Pinho, M. Morato, D. Patinha, A. Albino-Teixeira and I. Tavares (2009). "Microinjection of angiotensin II in the caudal ventrolateral medulla induces hyperalgesia." Neuroscience **158**(4): 1301-1310.

Martin, P., P. Pichat, J. Massol, P. Soubrie, K. G. Lloyd and A. J. Puech (1989). "Decreased GABA B receptors in helpless rats: reversal by tricyclic antidepressants." Neuropsychobiology **22**(4): 220-224.

Martin, T. J. and J. C. Eisenach (2001). "Pharmacology of opioid and nonopioid analgesics in chronic pain states." J Pharmacol Exp Ther **299**(3): 811-817.

Mathew, N. T., A. Rapoport, J. Saper, L. Magnus, J. Klapper, N. Ramadan, B. Stacey and S. Tepper (2001). "Efficacy of gabapentin in migraine prophylaxis." Headache **41**(2): 119-128.

Matsuzawa, Y., Y. S. C. Lee, F. Fraser, D. Langenbahn, A. Shallcross, S. Powers, R. Lipton, N. Simon and M. Minen (2018). "Barriers to Behavioral Treatment Adherence for Headache: An Examination of Attitudes, Beliefs, and Psychiatric Factors." Headache.

May, A. and L. H. Schulte (2016). "Chronic migraine: risk factors, mechanisms and treatment." Nat Rev Neurol **12**(8): 455-464.

Mennicken, F., J. Zhang, C. Hoffert, S. Ahmad, A. Beaudet and D. O'Donnell (2003). "Phylogenetic changes in the expression of delta opioid receptors in spinal cord and dorsal root ganglia." J Comp Neurol **465**(3): 349-360.

Messlinger, K., M. J. Fischer and J. K. Lennerz (2011). "Neuropeptide effects in the trigeminal system: pathophysiology and clinical relevance in migraine." Keio J Med **60**(3): 82-89.

Messlinger, K., U. Hanesch, M. Baumgartel, B. Trost and R. F. Schmidt (1993). "Innervation of the dura mater encephali of cat and rat: ultrastructure and calcitonin gene-related peptide-like and substance P-like immunoreactivity." Anat Embryol (Berl) **188**(3): 219-237.

Meunier, J. C., C. Mollereau, L. Toll, C. Suaudeau, C. Moisand, P. Alvinerie, J. L. Butour, J. C. Guillemot, P. Ferrara, B. Monsarrat and et al. (1995). "Isolation and structure of the endogenous agonist of opioid receptor-like ORL1 receptor." Nature **377**(6549): 532-535.

Mihalik, J. P., J. Register-Mihalik, Z. Y. Kerr, S. W. Marshall, M. C. McCrea and K. M. Guskiewicz (2013). "Recovery of posttraumatic migraine characteristics in patients after mild traumatic brain injury." Am J Sports Med **41**(7): 1490-1496.

Millan-Guerrero, R. O., R. Isais-Millan, S. Barreto-Vizcaino, I. Gutierrez, L. Rivera-Castano, B. Trujillo-Hernandez and L. M. Baltazar (2008). "Subcutaneous histamine versus topiramate in migraine prophylaxis: a double-blind study." Eur Neurol **59**(5): 237-242.

Millan, M. J. (1999). "The induction of pain: an integrative review." Prog Neurobiol **57**(1): 1-164.

Millan, M. J. (2002). "Descending control of pain." Prog Neurobiol **66**(6): 355-474.

Minen, M. T., O. Begasse De Dhaem, A. Kroon Van Diest, S. Powers, T. J. Schwedt, R. Lipton and D. Silbersweig (2016). "Migraine and its psychiatric comorbidities." J Neurol Neurosurg Psychiatry **87**(7): 741-749.

Minen, M. T., A. Boubour, H. Walia and W. Barr (2016). "Post-Concussive Syndrome: a Focus on Post-Traumatic Headache and Related Cognitive, Psychiatric, and Sleep Issues." Curr Neurol Neurosci Rep **16**(11): 100.

Minen, M. T., K. Lindberg, R. E. Wells, J. Suzuki, C. Grudzen, L. Balcer and E. Loder (2015). "Survey of Opioid and Barbiturate Prescriptions in Patients Attending a Tertiary Care Headache Center." Headache **55**(9): 1183-1191.

Minen, M. T. and K. Tanev (2014). "Influence of psychiatric comorbidities in migraineurs in the emergency department." Gen Hosp Psychiatry **36**(5): 533-538.

Monory, K., F. Massa, M. Egertova, M. Eder, H. Blaudzun, R. Westenbroek, W. Kelsch, W. Jacob, R. Marsch, M. Ekker, J. Long, J. L. Rubenstein, S. Goebbels, K. A. Nave, M. During, M. Klugmann, B. Wolfel, H. U. Dodt, W. Zieglgansberger, C. T. Wotjak, K. Mackie, M. R. Elphick, G. Marsicano and B. Lutz (2006). "The endocannabinoid system controls key epileptogenic circuits in the hippocampus." Neuron **51**(4): 455-466.

Monteith, T. S. and D. Borsook (2014). "Insights and advances in post-traumatic headache: research considerations." Curr Neurol Neurosci Rep **14**(2): 428.

Morris, H. R., M. Panico, T. Etienne, J. Tippins, S. I. Girgis and I. MacIntyre (1984). "Isolation and characterization of human calcitonin gene-related peptide." Nature **308**(5961): 746-748.

Moye, L. S., M. L. Novack, A. F. Tipton, H. Krishnan, S. C. Pandey and A. A. Pradhan (2018). "The development of a mouse model of mTBI-induced post-traumatic migraine, and identification of the delta opioid receptor as a novel therapeutic target." Cephalalgia: 333102418777507.

Moye, L. S. and A. A. Pradhan (2017). "From blast to bench: A translational mini-review of posttraumatic headache." J Neurosci Res **95**(6): 1347-1354.

Moye, L. S. and A. A. A. Pradhan (2017). "Animal Model of Chronic Migraine-Associated Pain." Curr Protoc Neurosci **80**: 9 60 61-69 60 69.

Mulderry, P. K., M. A. Ghatei, A. E. Bishop, Y. S. Allen, J. M. Polak and S. R. Bloom (1985). "Distribution and chromatographic characterisation of CGRP-like immunoreactivity in the brain and gut of the rat." Regul Pept **12**(2): 133-143.

Murray, C. J., T. Vos, R. Lozano, M. Naghavi, A. D. Flaxman, C. Michaud, M. Ezzati, K. Shibuya, J. A. Salomon, S. Abdalla, V. Aboyans, J. Abraham, I. Ackerman, R. Aggarwal, S. Y. Ahn, M. K. Ali, M. Alvarado, H. R. Anderson, L. M. Anderson, K. G. Andrews, C. Atkinson, L. M. Baddour, A. N. Bahalim, S. Barker-Collo, L. H. Barrero, D. H. Bartels, M. G. Basanez, A. Baxter, M. L. Bell, E. J. Benjamin, D. Bennett, E. Bernabe, K. Bhalla, B. Bhandari, B. Bikbov, A. Bin Abdulhak, G. Birbeck, J. A. Black, H. Blencowe, J. D. Blore, F. Blyth, I. Bolliger, A. Bonaventure, S. Boufous, R. Bourne, M. Boussinesq, T. Braithwaite, C. Brayne, L. Bridgett, S. Brooker, P. Brooks, T. S. Brugha, C. Bryan-Hancock, C. Bucello, R. Buchbinder, G. Buckle, C. M. Budke, M. Burch, P. Burney, R. Burstein, B. Calabria, B. Campbell, C. E. Canter, H. Carabin, J. Carapetis, L. Carmona, C. Cella, F. Charlson, H. Chen, A. T. Cheng, D. Chou, S. S. Chugh, L. E. Coffeng, S. D. Colan, S. Colquhoun, K. E. Colson, J. Condon, M. D. Connor, L. T. Cooper, M. Corriere, M. Cortinovis, K. C. de Vaccaro, W. Couser, B. C. Cowie, M. H. Criqui, M. Cross, K. C. Dabhadkar, M. Dahiya, N. Dahodwala, J. Damsere-Derry, G. Danaei, A. Davis, D. De Leo, L. Degenhardt, R. Dellavalle, A. Delossantos, J. Denenberg, S. Derrett, D. C. Des Jarlais, S. D. Dharmaratne, M. Dherani, C. Diaz-Torne, H. Dolk, E. R. Dorsey, T. Driscoll, H. Duber, B. Ebel, K. Edmond, A. Elbaz, S. E. Ali, H. Erskine, P. J. Erwin, P. Espindola, S. E. Ewoigbokhan, F. Farzadfar, V. Feigin, D. T. Felson, A. Ferrari, C. P. Ferri, E. M. Fevre, M. M. Finucane, S. Flaxman, L. Flood, K. Foreman, M. H. Forouzanfar, F. G. Fowkes, M. Fransen, M. K. Freeman, B. J. Gabbe,

S. E. Gabriel, E. Gakidou, H. A. Ganatra, B. Garcia, F. Gaspari, R. F. Gillum, G. Gmel, D. Gonzalez-Medina, R. Gosselin, R. Grainger, B. Grant, J. Groeger, F. Guillemin, D. Gunnell, R. Gupta, J. Haagsma, H. Hagan, Y. A. Halasa, W. Hall, D. Haring, J. M. Haro, J. E. Harrison, R. Havmoeller, R. J. Hay, H. Higashi, C. Hill, B. Hoen, H. Hoffman, P. J. Hotez, D. Hoy, J. J. Huang, S. E. Ibeanusi, K. H. Jacobsen, S. L. James, D. Jarvis, R. Jasrasaria, S. Jayaraman, N. Johns, J. B. Jonas, G. Karthikeyan, N. Kassebaum, N. Kawakami, A. Keren, J. P. Khoo, C. H. King, L. M. Knowlton, O. Kobusingye, A. Koranteng, R. Krishnamurthi, F. Laden, R. Lalloo, L. L. Laslett, T. Lathlean, J. L. Leasher, Y. Y. Lee, J. Leigh, D. Levinson, S. S. Lim, E. Limb, J. K. Lin, M. Lipnick, S. E. Lipshultz, W. Liu, M. Loane, S. L. Ohno, R. Lyons, J. Mabweijano, M. F. MacIntyre, R. Malekzadeh, L. Mallinger, S. Manivannan, W. Marcenes, L. March, D. J. Margolis, G. B. Marks, R. Marks, A. Matsumori, R. Matzopoulos, B. M. Mayosi, J. H. McAnulty, M. M. McDermott, N. McGill, J. McGrath, M. E. Medina-Mora, M. Meltzer, G. A. Mensah, T. R. Merriman, A. C. Meyer, V. Miglioli, M. Miller, T. R. Miller, P. B. Mitchell, C. Mock, A. O. Mocumbi, T. E. Moffitt, A. A. Mokdad, L. Monasta, M. Montico, M. Moradi-Lakeh, A. Moran, L. Morawska, R. Mori, M. E. Murdoch, M. K. Mwaniki, K. Naidoo, M. N. Nair, L. Naldi, K. M. Narayan, P. K. Nelson, R. G. Nelson, M. C. Nevitt, C. R. Newton, S. Nolte, P. Norman, R. Norman, M. O'Donnell, S. O'Hanlon, C. Olives, S. B. Omer, K. Ortblad, R. Osborne, D. Ozgediz, A. Page, B. Pahari, J. D. Pandian, A. P. Rivero, S. B. Patten, N. Pearce, R. P. Padilla, F. Perez-Ruiz, N. Perico, K. Pesudovs, D. Phillips, M. R. Phillips, K. Pierce, S. Pion, G. V. Polanczyk, S. Polinder, C. A. Pope, 3rd, S. Popova, E. Porrini, F. Pourmalek, M. Prince, R. L. Pullan, K. D. Ramaiah, D. Ranganathan, H. Razavi, M. Regan, J. T. Rehm, D. B. Rein, G. Remuzzi, K. Richardson, F. P. Rivara, T. Roberts, C. Robinson, F. R. De Leon, L. Ronfani, R. Room, L. C. Rosenfeld, L. Rushton, R. L. Sacco, S. Saha, U. Sampson, L. Sanchez-Riera, E. Sanman, D. C. Schwebel, J. G. Scott, M. Segui-Gomez, S. Shahraz, D. S. Shepard, H. Shin, R. Shivakoti, D. Singh, G. M. Singh, J. A. Singh, J. Singleton, D. A. Sleet, K. Sliwa, E. Smith, J. L. Smith, N. J. Stapelberg, A. Steer, T. Steiner, W. A. Stolk, L. J. Stovner, C. Sudfeld, S. Syed, G. Tamburlini, M. Tavakkoli, H. R. Taylor, J. A. Taylor, W. J. Taylor, B. Thomas, W. M. Thomson, G. D. Thurston, I. M. Tleyjeh, M. Tonelli, J. A. Towbin, T. Truelsen, M. K. Tsilimbaris, C. Ubeda, E. A. Undurraga, M. J. van der Werf, J. van Os, M. S. Vavilala, N. Venketasubramanian, M. Wang, W. Wang, K. Watt, D. J. Weatherall, M. A. Weinstock, R. Weintraub, M. G. Weisskopf, M. M. Weissman, R. A. White, H. Whiteford, N. Wiebe, S. T. Wiersma, J. D. Wilkinson, H. C. Williams, S. R. Williams, E. Witt, F. Wolfe, A. D. Woolf, S. Wulf, P. H. Yeh, A. K. Zaidi, Z. J. Zheng, D. Zonies, A. D. Lopez, M. A. AlMazroa and Z. A. Memish (2012). "Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010." Lancet **380**(9859): 2197-2223.

Nadal, X., J. E. Banos, B. L. Kieffer and R. Maldonado (2006). "Neuropathic pain is enhanced in delta-opioid receptor knockout mice." Eur.J.Neurosci. **23**(3): 830-834.

Nakamura-Craig, M. and B. K. Gill (1991). "Effect of neurokinin A, substance P and calcitonin gene related peptide in peripheral hyperalgesia in the rat paw." Neurosci Lett **124**(1): 49-51.

Nampiaparampil, D. E. (2008). "Prevalence of chronic pain after traumatic brain injury: a systematic review." Jama **300**(6): 711-719.

Narita, M., M. Funada and T. Suzuki (2001). "Regulations of opioid dependence by opioid receptor types." Pharmacol Ther **89**(1): 1-15.

National Center for Injury Prevention and Control (2003). Report to Congress on Mild Traumatic Brain Injury in the United States: Steps to Prevent a Serious Public Health Problem. Atlanta, GA: Centers for Disease Control and Prevention.

Neal, C. R., Jr., A. Mansour, R. Reinscheid, H. P. Nothacker, O. Civelli, H. Akil and S. J. Watson, Jr. (1999). "Opioid receptor-like (ORL1) receptor distribution in the rat central nervous system: comparison of ORL1 receptor mRNA expression with (125)I-[(14)Tyr]-orphanin FQ binding." J Comp Neurol **412**(4): 563-605.

Negri, L., V. Noviello and F. Angelucci (1991). "Behavioural effects of deltorphins in rats." Eur J Pharmacol **209**(3): 163-168.

Negus, S. S., M. B. Gatch, N. K. Mello, X. Zhang and K. Rice (1998). "Behavioral effects of the delta-selective opioid agonist SNC80 and related compounds in rhesus monkeys." J Pharmacol Exp Ther **286**(1): 362-375.

Neumann, S., J. M. Braz, K. Skinner, I. J. Llewellyn-Smith and A. I. Basbaum (2008). "Innocuous, not noxious, input activates PKCgamma interneurons of the spinal dorsal horn via myelinated afferent fibers." J Neurosci **28**(32): 7936-7944.

Noseda, R. and R. 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, R., M. Jakubowski, V. Kainz, D. Borsook and R. Burstein (2011). "Cortical projections of functionally identified thalamic trigeminovascular neurons: implications for migraine headache and its associated symptoms." J Neurosci **31**(40): 14204-14217.

O'Neil, M. E., K. Carlson, D. Storzbach, L. Brenner, M. Freeman, A. Quinones, M. Motu'apuaka, M. Ensley and D. Kansagara (2013). Complications of Mild Traumatic Brain Injury in Veterans and Military Personnel: A Systematic Review. Washington DC.

Olesen, J. (2008). "The role of nitric oxide (NO) in migraine, tension-type headache and cluster headache." Pharmacol.Ther. **120**(2): 157-171.

Olesen, J. (2010). "Nitric oxide-related drug targets in headache." Neurotherapeutics **7**(2): 183-190.

Olesen, J., H. C. Diener, I. W. Husstedt, P. J. Goadsby, D. Hall, U. Meier, S. Pollentier, L. M. Lesko and B. B. C. P. o. C. S. Group (2004). "Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine." N Engl J Med **350**(11): 1104-1110.

Olesen, J. and I. Jansen-Olesen (2012). "Towards a reliable animal model of migraine." Cephalalgia **32**(7): 578-580.

Omori, K. and J. Kotera (2007). "Overview of PDEs and their regulation." Circ Res **100**(3): 309-327.

Oshinsky, M. L. and J. Luo (2006). "Neurochemistry of trigeminal activation in an animal model of migraine." Headache **46 Suppl 1**: S39-44.

Ozawa, A., G. Brunori, D. Mercatelli, J. Wu, A. Cippitelli, B. Zou, X. S. Xie, M. Williams, N. T. Zaveri, S. Low, G. Scherrer, B. L. Kieffer and L. Toll (2015). "Knock-In Mice with NOP-eGFP Receptors Identify Receptor Cellular and Regional Localization." J Neurosci **35**(33): 11682-11693.

Patel, N. V., M. E. Bigal, K. B. Kolodner, C. Leotta, J. E. Lafata and R. B. Lipton (2004). "Prevalence and impact of migraine and probable migraine in a health plan." Neurology **63**(8): 1432-1438.

Patwardhan, A. M., K. A. Berg, A. N. Akopain, N. A. Jeske, N. Gamper, W. P. Clarke and K. M. Hargreaves (2005). "Bradykinin-induced functional competence and trafficking of the delta-opioid receptor in trigeminal nociceptors." J Neurosci **25**(39): 8825-8832.

Paulozzi LJ, J. C., Mack KA, Rudd RA. (2011). Vital Signs: Overdoses of Prescription Opioid Pain Relievers--United States, 1999-2008., MMWR. **60 (43):** 1487-1492.

Pellissier, L. P., C. N. Pujol, J. A. Becker and J. Le Merrer (2016). "Delta Opioid Receptors: Learning and Motivation." Handb Exp Pharmacol.

Perrine, S. A., B. A. Hoshaw and E. M. Unterwald (2006). "Delta opioid receptor ligands modulate anxiety-like behaviors in the rat." Br J Pharmacol **147**(8): 864-872.

Phebus, L. A. and K. W. Johnson (2001). "Dural inflammation model of migraine pain." Curr Protoc Neurosci **Chapter 9**: Unit9.1.

Pick, C. G., D. Paul and G. W. Pasternak (1991). "Comparison of naloxonazine and beta-funaltrexamine antagonism of mu 1 and mu 2 opioid actions." Life Sci **48**(21): 2005-2011.

Pietrobon, D. and M. A. Moskowitz (2013). "Pathophysiology of migraine." Annu Rev Physiol **75**: 365-391.

Pietrobon, D. and M. A. Moskowitz (2014). "Chaos and commotion in the wake of cortical spreading depression and spreading depolarizations." Nat Rev Neurosci **15**(6): 379-393.

Pilc, A. and K. G. Lloyd (1984). "Chronic antidepressants and GABA "B" receptors: a GABA hypothesis of antidepressant drug action." Life Sci **35**(21): 2149-2154.

Poole, D. P., J. C. Pelayo, G. Scherrer, C. J. Evans, B. L. Kieffer and N. W. Bunnett (2011). "Localization and regulation of fluorescently labeled delta opioid receptor, expressed in enteric neurons of mice." Gastroenterology **141**(3): 982-991.

Porreca, F., H. I. Mosberg, R. Hurst, V. J. Hruby and T. F. Burks (1984). "Roles of mu, delta and kappa opioid receptors in spinal and supraspinal mediation of gastrointestinal transit effects and hot-plate analgesia in the mouse." J Pharmacol Exp Ther **230**(2): 341-348.

Pradhan, A., M. Smith, B. McGuire, C. Evans and W. Walwyn (2013). "Chronic inflammatory injury results in increased coupling of delta opioid receptors to voltage-gated Ca2+ channels." Mol Pain **9**: 8.

Pradhan, A., M. Smith, B. McGuire, C. Evans and W. Walwyn (2013). "Chronic inflammatory injury results in increased coupling of delta opioid receptors to voltage-gated Ca2+ channels." Mol Pain **9**(8): 8.

Pradhan, A. A., J. A. Becker, G. Scherrer, P. Tryoen-Toth, D. Filliol, A. Matifas, D. Massotte, C. Gaveriaux-Ruff and B. L. Kieffer (2009). "In vivo delta opioid receptor internalization controls behavioral effects of agonists." PLoS One **4**(5): e5425.

Pradhan, A. A., J. A. Becker, G. Scherrer, P. Tryoen-Toth, D. Filliol, A. Matifas, D. Massotte, C. Gaveriaux-Ruff and B. L. Kieffer (2009). "In vivo delta opioid receptor internalization controls behavioral effects of agonists." PLoS.One. **4**(5): e5425.

Pradhan, A. A., K. Befort, C. Nozaki, C. Gaveriaux-Ruff and B. L. Kieffer (2011). "The delta opioid receptor: an evolving target for the treatment of brain disorders." Trends Pharmacol Sci **32**(10): 581-590.

Pradhan, A. A., K. Befort, C. Nozaki, C. Gaveriaux-Ruff and B. L. Kieffer (2011). "The delta opioid receptor: an evolving target for the treatment of brain disorders." Trends Pharmacol.Sci.

Pradhan, A. A., Z. Bertels and S. Akerman (2018). "Targeted Nitric Oxide Synthase Inhibitors for Migraine." Neurotherapeutics **15**(2): 391-401.

Pradhan, A. A. and P. B. Clarke (2005). "Comparison between delta-opioid receptor functional response and autoradiographic labeling in rat brain and spinal cord." J.Comp Neurol. **481**(4): 416-426.

Pradhan, A. A. and P. B. Clarke (2005). "Comparison between delta-opioid receptor functional response and autoradiographic labeling in rat brain and spinal cord." J Comp Neurol **481**(4): 416-426.

Pradhan, A. A., J. Perroy, W. M. Walwyn, M. L. Smith, A. Vicente-Sanchez, L. Segura, A. Bana, B. L. Kieffer and C. J. Evans (2016). "Agonist-Specific Recruitment of Arrestin Isoforms Differentially Modify Delta Opioid Receptor Function." J Neurosci **36**(12): 3541-3551.

Pradhan, A. A., M. L. Smith, B. L. Kieffer and C. J. Evans (2012). "Ligand-directed signalling within the opioid receptor family." Br.J.Pharmacol. **167**(5): 960-969.

Pradhan, A. A., M. L. Smith, B. L. Kieffer and C. J. Evans (2012). "Ligand-directed signalling within the opioid receptor family." Br J Pharmacol **167**(5): 960-969.

Pradhan, A. A., M. L. Smith, B. McGuire, I. Tarash, C. J. Evans and A. Charles (2014). "Characterization of a novel model of chronic migraine." Pain **155**(2): 269-274.

Pradhan, A. A., M. L. Smith, J. Zyuzin and A. Charles (2014). "delta-Opioid receptor agonists inhibit migraine-related hyperalgesia, aversive state and cortical spreading depression in mice." Br J Pharmacol **171**(9): 2375-2384.

Pradhan, A. A., W. Walwyn, C. Nozaki, D. Filliol, E. Erbs, A. Matifas, C. Evans and B. L. Kieffer (2010). "Ligand-directed trafficking of the delta-opioid receptor in vivo: two paths toward analgesic tolerance." J.Neurosci. **30**(49): 16459-16468.

Pratt, G. D. and N. G. Bowery (1993). "Repeated administration of desipramine and a GABAB receptor antagonist, CGP 36742, discretely up-regulates GABAB receptor binding sites in rat frontal cortex." Br J Pharmacol **110**(2): 724-735.

Punay, N. C. and J. R. Couch (2003). "Antidepressants in the treatment of migraine headache." Curr Pain Headache Rep **7**(1): 51-54.

Purkiss, J., M. Welch, S. Doward and K. Foster (2000). "Capsaicin-stimulated release of substance P from cultured dorsal root ganglion neurons: involvement of two distinct mechanisms." Biochem Pharmacol **59**(11): 1403-1406.

Qian, J. and J. L. Noebels (2003). "Topiramate alters excitatory synaptic transmission in mouse hippocampus." Epilepsy Res **55**(3): 225-233.

Ramadan, N. M. (2004). "Prophylactic migraine therapy: mechanisms and evidence." Curr Pain Headache Rep **8**(2): 91-95.

Rao, B. S., D. G. Das, V. R. Taraknath and Y. Sarma (2000). "A double blind controlled study of propranolol and cyproheptadine in migraine prophylaxis." Neurol India **48**(3): 223-226.

Reid, M. C., L. L. Engles-Horton, M. B. Weber, R. D. Kerns, E. L. Rogers and P. G. O'Connor (2002). "Use of opioid medications for chronic noncancer pain syndromes in primary care." Journal of General Internal Medicine **17**(3): 173-179.

Reinscheid, R. K., H. P. Nothacker, A. Bourson, A. Ardati, R. A. Henningsen, J. R. Bunzow, D. K. Grandy, H. Langen, F. J. Monsma, Jr. and O. Civelli (1995). "Orphanin

FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor." Science **270**(5237): 792-794.

Reuter, U., A. Chiarugi, H. Bolay and M. A. Moskowitz (2002). "Nuclear factor-kappaB as a molecular target for migraine therapy." Ann Neurol **51**(4): 507-516.

Rice, F. L., J. Y. Xie, P. J. Albrecht, E. Acker, J. Bourgeois, E. Navratilova, D. W. Dodick and F. Porreca (2016). "Anatomy and immunochemical characterization of the non-arterial peptidergic diffuse dural innervation of the rat and Rhesus monkey: Implications for functional regulation and treatment in migraine." Cephalalgia.

Rice, F. L., J. Y. Xie, P. J. Albrecht, E. Acker, J. Bourgeois, E. Navratilova, D. W. Dodick and F. Porreca (2017). "Anatomy and immunochemical characterization of the non-arterial peptidergic diffuse dural innervation of the rat and Rhesus monkey: Implications for functional regulation and treatment in migraine." Cephalalgia **37**(14): 1350-1372.

Rickert, R. R. and R. M. Maliniak (1989). "Intralaboratory quality assurance of immunohistochemical procedures. Recommended practices for daily application." Arch Pathol Lab Med **113**(6): 673-679.

Roeckel, L. A., G. M. Le Coz, C. Gaveriaux-Ruff and F. Simonin (2016). "Opioid-induced hyperalgesia: Cellular and molecular mechanisms." Neuroscience **338**: 160-182.

Rosenthal, M. (1992). "Mild traumatic brain injury syndrome." Annals of Emergency Medicine **22**(6): 115-118.

Ross, M., McNaughton, FL (1945). "Chronic Posttraumatic Head Symptoms." Canad. M. A. J. **53**: 12-17.

Roux, B. T. and G. S. Cottrell (2014). "G protein-coupled receptors: what a difference a 'partner' makes." Int J Mol Sci **15**(1): 1112-1142.

Ruest, L. B., R. E. Hammer, M. Yanagisawa and D. E. Clouthier (2003). "Dlx5/6-enhancer directed expression of Cre recombinase in the pharyngeal arches and brain." Genesis. **37**(4): 188-194.

Russell, F. A., R. King, S. J. Smillie, X. Kodji and S. D. Brain (2014). "Calcitonin gene-related peptide: physiology and pathophysiology." Physiol Rev **94**(4): 1099-1142.

Russell, M. B., B. K. Rasmussen, P. Thorvaldsen and J. Olesen (1995). "Prevalence and sex-ratio of the subtypes of migraine." Int J Epidemiol **24**(3): 612-618.

Russwurm, M., C. Russwurm, D. Koesling and E. Mergia (2013). "NO/cGMP: the past, the present, and the future." Methods Mol Biol **1020**: 1-16.

Saitoh, A., Y. Kimura, T. Suzuki, K. Kawai, H. Nagase and J. Kamei (2004). "Potential anxiolytic and antidepressant-like activities of SNC80, a selective delta-opioid agonist, in behavioral models in rodents." J Pharmacol Sci **95**(3): 374-380.

Saper, J. R., S. D. Silberstein, A. E. Lake, 3rd and M. E. Winters (1994). "Double-blind trial of fluoxetine: chronic daily headache and migraine." Headache **34**(9): 497-502.

Sawynok, J., M. J. Esser and A. R. Reid (2001). "Antidepressants as analgesics: an overview of central and peripheral mechanisms of action." J Psychiatry Neurosci **26**(1): 21-29.

Schaeffer, C., D. Vandroux, L. Thomassin, P. Athias, L. Rochette and J. L. Connat (2003). "Calcitonin gene-related peptide partly protects cultured smooth muscle cells from apoptosis induced by an oxidative stress via activation of ERK1/2 MAPK." Biochim Biophys Acta **1643**(1-3): 65-73.

Schellenberg, R., A. Lichtenthal, H. Wohling, C. Graf and K. Brixius (2008). "Nebivolol and metoprolol for treating migraine: an advance on beta-blocker treatment?" Headache **48**(1): 118-125.

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.

Scherrer, G., N. Imamachi, Y. Q. Cao, C. Contet, F. Mennicken, D. O'Donnell, B. L. Kieffer and A. I. Basbaum (2009). "Dissociation of the opioid receptor mechanisms that control mechanical and heat pain." Cell **137**(6): 1148-1159.

Scherrer, G., P. Tryoen-Toth, D. Filliol, A. Matifas, D. Laustriat, Y. Q. Cao, A. I. Basbaum, A. Dierich, J. L. Vonesh, C. Gaveriaux-Ruff and B. L. Kieffer (2006). "Knockin mice expressing fluorescent delta-opioid receptors uncover G protein-coupled receptor dynamics in vivo." Proc Natl Acad Sci U S A **103**(25): 9691-9696.

Schonegge, A. M., J. Gallion, L. P. Picard, A. D. Wilkins, C. Le Gouill, M. Audet, W. Stallaert, M. J. Lohse, M. Kimmel, O. Lichtarge and M. Bouvier (2017). "Evolutionary action and structural basis of the allosteric switch controlling beta2AR functional selectivity." Nat Commun **8**(1): 2169.

Schrader, H., L. J. Stovner, G. Helde, T. Sand and G. Bovim (2001). "Prophylactic treatment of migraine with angiotensin converting enzyme inhibitor (lisinopril): randomised, placebo controlled, crossover study." Bmj **322**(7277): 19-22.

Schulte, L. H. and A. May (2016). "The migraine generator revisited: continuous scanning of the migraine cycle over 30 days and three spontaneous attacks." Brain **139**(Pt 7): 1987-1993.

Schwedt, T. J., A. Alam, M. L. Reed, K. M. Fanning, S. Munjal, D. C. Buse, D. W. Dodick and R. B. Lipton (2018). "Factors associated with acute medication overuse in people with migraine: results from the 2017 migraine in America symptoms and treatment (MAST) study." J Headache Pain **19**(1): 38.

Scofield, D. E., S. P. Proctor, J. R. Kardouni, O. T. Hill and C. J. McKinnon (2017). "Risk Factors for Mild Traumatic Brain Injury and Subsequent Post-traumatic Stress Disorder and Mental Health Disorders among US Army Soldiers." J Neurotrauma.

Seiler, K., J. I. Nusser, J. K. Lennerz, W. L. Neuhuber and K. Messlinger (2013). "Changes in calcitonin gene-related peptide (CGRP) receptor component and nitric oxide receptor (sGC) immunoreactivity in rat trigeminal ganglion following glyceroltrinitrate pretreatment." J Headache Pain **14**: 74.

Shahien, R. and K. Beiruti (2012). "Preventive agents for migraine: focus on the antiepileptic drugs." J Cent Nerv Syst Dis **4**: 37-49.

Silberstein, S. D. (2006). "Preventive treatment of migraine." Trends Pharmacol Sci **27**(8): 410-415.

Silberstein, S. D., D. Dodick, F. Freitag, S. H. Pearlman, S. R. Hahn, A. I. Scher and R. B. Lipton (2007). "Pharmacological approaches to managing migraine and associated comorbidities--clinical considerations for monotherapy versus polytherapy." Headache **47**(4): 585-599.

Silberstein, S. D., S. Holland, F. Freitag, D. W. Dodick, C. Argoff and E. Ashman (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): 1337-1345.

Smith, J. M., D. P. Bradley, M. F. James and C. L. Huang (2006). "Physiological studies of cortical spreading depression." Biol Rev Camb Philos Soc **81**(4): 457-481.

Somjen, G. G. (2001). "Mechanisms of spreading depression and hypoxic spreading depression-like depolarization." Physiol Rev **81**(3): 1065-1096.

Stacey, A., S. Lucas, S. Dikmen, N. Temkin, K. R. Bell, A. Brown, R. Brunner, R. Diaz-Arrastia, T. K. Watanabe, A. Weintraub and J. M. Hoffman (2017). "Natural History of Headache Five Years after Traumatic Brain Injury." J Neurotrauma **34**(8): 1558-1564.

Steenbergh, P. H., J. W. Hoppener, J. Zandberg, A. Visser, C. J. Lips and H. S. Jansz (1986). "Structure and expression of the human calcitonin/CGRP genes." FEBS Lett **209**(1): 97-103.

Stenberg, C., K. Ovlisen, O. Svendsen and B. Lauritzen (2005). "Effect of local anaesthesia on neuronal c-fos expression in the spinal dorsal horn and hypothalamic paraventricular nucleus after surgery in rats." Basic Clin Pharmacol Toxicol **96**(5): 381-386.

Stevenson, G. W., J. E. Folk, K. C. Rice and S. S. Negus (2005). "Interactions between delta and mu opioid agonists in assays of schedule-controlled responding, thermal nociception, drug self-administration, and drug versus food choice in rhesus monkeys: studies with SNC80 [(+)-4-[(alphaR)-alpha-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenz yl]-N,N-diethylbenzamide] and heroin." J.Pharmacol.Exp.Ther. **314**(1): 221-231.

Stewart, W. F., C. Wood, M. L. Reed, J. Roy, R. B. Lipton and A. A. Group (2008). "Cumulative lifetime migraine incidence in women and men." Cephalalgia **28**(11): 1170-1178.

Storey, J. R., C. S. Calder, D. E. Hart and D. L. Potter (2001). "Topiramate in migraine prevention: a double-blind, placebo-controlled study." Headache **41**(10): 968-975.

Stovner, L., K. Hagen, R. Jensen, Z. Katsarava, R. Lipton, A. Scher, T. Steiner and J. A. Zwart (2007). "The global burden of headache: a documentation of headache prevalence and disability worldwide." Cephalalgia **27**(3): 193-210.

Strassman, A. M. and R. Burstein (2013). "A new animal model of headache: Ongoing pain vs stimulus-evoked hypersensitivity." Cephalalgia **33**(13): 1073-1074.

Sufka, K. J., S. M. Staszko, A. P. Johnson, M. E. Davis, R. E. Davis and T. A. Smitherman (2016). "Clinically relevant behavioral endpoints in a recurrent nitroglycerin migraine model in rats." J Headache Pain **17**: 40.

Sulaiman, M. R., M. Niklasson, R. Tham and M. B. Dutia (1999). "Modulation of vestibular function by nociceptin/orphanin FQ: an in vivo and in vitro study." Brain Res **828**(1-2): 74-82.

Sun, H., D. W. Dodick, S. Silberstein, P. J. Goadsby, U. Reuter, M. Ashina, J. Saper, R. Cady, Y. Chon, J. Dietrich and R. Lenz (2016). "Safety and efficacy of AMG 334 for prevention of episodic migraine: a randomised, double-blind, placebo-controlled, phase 2 trial." Lancet Neurol **15**(4): 382-390.

Sun, Y. and T. J. Gan (2008). "Acupuncture for the management of chronic headache: a systematic review." Anesth Analg **107**(6): 2038-2047.

Suzdak, P. D. and G. Gianutsos (1986). "Effect of chronic imipramine or baclofen on GABA-B binding and cyclic AMP production in cerebral cortex." Eur J Pharmacol **131**(1): 129-133.

T Tanielian, L. J. (2008). "Invisible Wounds of War."

Taylor, A. M., K. W. Roberts, A. A. Pradhan, H. A. Akbari, W. Walwyn, K. Lutfy, F. I. Carroll, C. M. Cahill and C. J. Evans (2015). "Anti-nociception mediated by a kappa opioid receptor agonist is blocked by a delta receptor agonist." Br J Pharmacol **172**(2): 691-703.

Tempel, A. and R. S. Zukin (1987). "Neuroanatomical patterns of the mu, delta, and kappa opioid receptors of rat brain as determined by quantitative in vitro autoradiography." Proc Natl Acad Sci U S A **84**(12): 4308-4312.

The American Migraine Foundation. "The Timeline of a Migraine Attack." from https://americanmigrainefoundation.org/resource-library/timeline-migraine-attack/.

Theeler, B., S. Lucas, R. G. Riechers, 2nd and R. L. Ruff (2013). "Post-traumatic headaches in civilians and military personnel: a comparative, clinical review." Headache **53**(6): 881-900.

Theeler, B. J., R. Mercer and J. C. Erickson (2008). "Prevalence and impact of migraine among US Army soldiers deployed in support of Operation Iraqi Freedom." Headache **48**(6): 876-882.

Thiagalingam, A., A. De Bustros, M. Borges, R. Jasti, D. Compton, L. Diamond, M. Mabry, D. W. Ball, S. B. Baylin and B. D. Nelkin (1996). "RREB-1, a novel zinc finger protein, is involved in the differentiation response to Ras in human medullary thyroid carcinomas." Mol Cell Biol **16**(10): 5335-5345.

Thorlund, K., C. Sun-Edelstein, E. Druyts, S. Kanters, S. Ebrahim, R. Bhambri, E. Ramos, E. J. Mills, M. Lanteri-Minet and S. Tepper (2016). "Risk of medication overuse headache across classes of treatments for acute migraine." J Headache Pain **17**(1): 107.

Tipton, A. F., I. Tarash, B. McGuire, A. Charles and A. A. Pradhan (2015). "The effects of acute and preventive migraine therapies in a mouse model of chronic migraine." Cephalalgia.

Toll, L., M. R. Bruchas, G. Calo, B. M. Cox and N. T. Zaveri (2016). "Nociceptin/Orphanin FQ Receptor Structure, Signaling, Ligands, Functions, and Interactions with Opioid Systems." Pharmacol Rev **68**(2): 419-457.

Tronvik, E., L. J. Stovner, G. Helde, T. Sand and G. Bovim (2003). "Prophylactic treatment of migraine with an angiotensin II receptor blocker: a randomized controlled trial." Jama **289**(1): 65-69.

Tseng, L. F. and J. M. Fujimoto (1985). "Differential actions of intrathecal naloxone on blocking the tail-flick inhibition induced by intraventricular beta-endorphin and morphine in rats." J Pharmacol Exp Ther **232**(1): 74-79.

Tso, A. R. and P. J. Goadsby (2017). "Anti-CGRP Monoclonal Antibodies: the Next Era of Migraine Prevention?" Curr Treat Options Neurol **19**(8): 27.

Tsujikawa, K., K. Yayama, T. Hayashi, H. Matsushita, T. Yamaguchi, T. Shigeno, Y. Ogitani, M. Hirayama, T. Kato, S. Fukada, S. Takatori, H. Kawasaki, H. Okamoto, M. Ikawa, M. Okabe and H. Yamamoto (2007). "Hypertension and dysregulated

proinflammatory cytokine production in receptor activity-modifying protein 1-deficient mice." Proc Natl Acad Sci U S A **104**(42): 16702-16707.

Tvedskov, J. F., P. Tfelt-Hansen, K. A. Petersen, L. T. Jensen and J. Olesen (2010). "CGRP receptor antagonist olcegepant (BIBN4096BS) does not prevent glyceryl trinitrate-induced migraine." Cephalalgia **30**(11): 1346-1353.

Tyburski, A. L., L. Cheng, S. Assari, K. Darvish and M. B. Elliott (2017). "Frequent mild head injury promotes trigeminal sensitivity concomitant with microglial proliferation, astrocytosis, and increased neuropeptide levels in the trigeminal pain system." J Headache Pain **18**(1): 16.

U.S. Food & Drug Administration (2018). FDA approves novel preventive treatment for migraine.

Uddman, R., L. Edvinsson, R. Ekman, T. Kingman and J. McCulloch (1985). "Innervation of the feline cerebral vasculature by nerve fibers containing calcitonin gene-related peptide: trigeminal origin and co-existence with substance P." Neurosci Lett **62**(1): 131-136.

van Rijn, R. M., D. I. Brissett and J. L. Whistler (2012). "Emergence of functional spinal delta opioid receptors after chronic ethanol exposure." Biol Psychiatry **71**(3): 232-238.

Vargas, B. B. and D. W. Dodick (2012). "Posttraumatic headache." Curr Opin Neurol **25**(3): 284-289.

Vaughan, C. W., E. E. Bagley, G. M. Drew, A. Schuller, J. E. Pintar, S. P. Hack and M. J. Christie (2003). "Cellular actions of opioids on periaqueductal grey neurons from C57B16/J mice and mutant mice lacking MOR-1." Br J Pharmacol **139**(2): 362-367.

Vicente-Sanchez, A., I. J. Dripps, A. F. Tipton, H. Akbari, A. Akbari, E. M. Jutkiewicz and A. A. Pradhan (2018). "Tolerance to high-internalizing delta opioid receptor agonist is critically mediated by arrestin 2." Br J Pharmacol.

Vicente-Sanchez, A., L. Segura and A. A. Pradhan (2016). "The delta opioid receptor tool box." Neuroscience **338**: 145-159.

Victor, T. W., X. Hu, J. C. Campbell, D. C. Buse and R. B. Lipton (2010). "Migraine prevalence by age and sex in the United States: a life-span study." Cephalalgia **30**(9): 1065-1072.

Villalba, N., S. K. Sonkusare, T. A. Longden, T. L. Tran, A. M. Sackheim, M. T. Nelson, G. C. Wellman and K. Freeman (2014). "Traumatic brain injury disrupts cerebrovascular tone through endothelial inducible nitric oxide synthase expression and nitric oxide gain of function." J Am Heart Assoc **3**(6): e001474.

Violin, J. D. and R. J. Lefkowitz (2007). "Beta-arrestin-biased ligands at seven-transmembrane receptors." Trends Pharmacol Sci **28**(8): 416-422.

Visser, W. H., R. H. de Vriend, N. H. Jaspers and M. D. Ferrari (1996). "Sumatriptan-nonresponders: a survey in 366 migraine patients." Headache **36**(8): 471-475.

Vos, T., A. D. Flaxman, M. Naghavi, R. Lozano, C. Michaud, M. Ezzati, K. Shibuya, J. A. Salomon, S. Abdalla, V. Aboyans, J. Abraham, I. Ackerman, R. Aggarwal, S. Y. Ahn, M. K. Ali, M. Alvarado, H. R. Anderson, L. M. Anderson, K. G. Andrews, C. Atkinson, L. M. Baddour, A. N. Bahalim, S. Barker-Collo, L. H. Barrero, D. H. Bartels, M. G. Basanez, A. Baxter, M. L. Bell, E. J. Benjamin, D. Bennett, E. Bernabe, K. Bhalla, B. Bhandari, B. Bikbov, A. Bin Abdulhak, G. Birbeck, J. A. Black, H. Blencowe, J. D. Blore, F. Blyth, I. Bolliger, A. Bonaventure, S. Boufous, R. Bourne, M. Boussinesq, T. Braithwaite, C. Brayne, L. Bridgett, S. Brooker, P. Brooks, T. S. Brugha, C. Bryan-Hancock, C. Bucello, R. Buchbinder, G. Buckle, C. M. Budke, M. Burch, P. Burney, R. Burstein, B. Calabria, B. Campbell, C. E. Canter, H. Carabin, J. Carapetis, L. Carmona, C. Cella, F. Charlson, H. Chen, A. T. Cheng, D. Chou, S. S. Chugh, L. E. Coffeng, S. D. Colan, S. Colquhoun, K. E. Colson, J. Condon, M. D. Connor, L. T. Cooper, M. Corriere, M. Cortinovis, K. C. de Vaccaro, W. Couser, B. C. Cowie, M. H. Criqui, M. Cross, K. C. Dabhadkar, M. Dahiya, N. Dahodwala, J. Damsere-Derry, G. Danaei, A. Davis, D. De Leo, L. Degenhardt, R. Dellavalle, A. Delossantos, J. Denenberg, S. Derrett, D. C. Des Jarlais, S. D. Dharmaratne, M. Dherani, C. Diaz-Torne, H. Dolk, E. R. Dorsey, T. Driscoll, H. Duber, B. Ebel, K. Edmond, A. Elbaz, S. E. Ali, H. Erskine, P. J. Erwin, P. Espindola, S. E. Ewoigbokhan, F. Farzadfar, V. Feigin, D. T. Felson, A. Ferrari, C. P. Ferri, E. M. Fevre, M. M. Finucane, S. Flaxman, L. Flood, K. Foreman, M. H. Forouzanfar, F. G. Fowkes, R. Franklin, M. Fransen, M. K. Freeman, B. J. Gabbe, S. E. Gabriel, E. Gakidou, H. A. Ganatra, B. Garcia, F. Gaspari, R. F. Gillum, G. Gmel, R. Gosselin, R. Grainger, J. Groeger, F. Guillemin, D. Gunnell, R. Gupta, J. Haagsma, H. Hagan, Y. A. Halasa, W. Hall, D. Haring, J. M. Haro, J. E. Harrison, R. Havmoeller, R. J. Hay, H. Higashi, C. Hill, B. Hoen, H. Hoffman, P. J. Hotez, D. Hoy, J. J. Huang, S. E. Ibeanusi, K. H. Jacobsen, S. L. James, D. Jarvis, R. Jasrasaria, S. Jayaraman, N. Johns, J. B. Jonas, G. Karthikeyan, N. Kassebaum, N. Kawakami, A. Keren, J. P. Khoo, C. H. King, L. M. Knowlton, O. Kobusingye, A. Koranteng, R. Krishnamurthi, R. Lalloo, L. L. Laslett, T. Lathlean, J. L. Leasher, Y. Y. Lee, J. Leigh, S. S. Lim, E. Limb, J. K. Lin, M. Lipnick, S. E. Lipshultz, W. Liu, M. Loane, S. L. Ohno, R. Lyons, J. Ma, J.

Mabweijano, M. F. MacIntyre, R. Malekzadeh, L. Mallinger, S. Manivannan, W. Marcenes, L. March, D. J. Margolis, G. B. Marks, R. Marks, A. Matsumori, R. Matzopoulos, B. M. Mayosi, J. H. McAnulty, M. M. McDermott, N. McGill, J. McGrath, M. E. Medina-Mora, M. Meltzer, G. A. Mensah, T. R. Merriman, A. C. Meyer, V. Miglioli, M. Miller, T. R. Miller, P. B. Mitchell, A. O. Mocumbi, T. E. Moffitt, A. A. Mokdad, L. Monasta, M. Montico, M. Moradi-Lakeh, A. Moran, L. Morawska, R. Mori, M. E. Murdoch, M. K. Mwaniki, K. Naidoo, M. N. Nair, L. Naldi, K. M. Narayan, P. K. Nelson, R. G. Nelson, M. C. Nevitt, C. R. Newton, S. Nolte, P. Norman, R. Norman, M. O'Donnell, S. O'Hanlon, C. Olives, S. B. Omer, K. Ortblad, R. Osborne, D. Ozgediz, A. Page, B. Pahari, J. D. Pandian, A. P. Rivero, S. B. Patten, N. Pearce, R. P. Padilla, F. Perez-Ruiz, N. Perico, K. Pesudovs, D. Phillips, M. R. Phillips, K. Pierce, S. Pion, G. V. Polanczyk, S. Polinder, C. A. Pope, 3rd, S. Popova, E. Porrini, F. Pourmalek, M. Prince, R. L. Pullan, K. D. Ramaiah, D. Ranganathan, H. Razavi, M. Regan, J. T. Rehm, D. B. Rein, G. Remuzzi, K. Richardson, F. P. Rivara, T. Roberts, C. Robinson, F. R. De Leon, L. Ronfani, R. Room, L. C. Rosenfeld, L. Rushton, R. L. Sacco, S. Saha, U. Sampson, L. Sanchez-Riera, E. Sanman, D. C. Schwebel, J. G. Scott, M. Segui-Gomez, S. Shahraz, D. S. Shepard, H. Shin, R. Shivakoti, D. Singh, G. M. Singh, J. A. Singh, J. Singleton, D. A. Sleet, K. Sliwa, E. Smith, J. L. Smith, N. J. Stapelberg, A. Steer, T. Steiner, W. A. Stolk, L. J. Stovner, C. Sudfeld, S. Syed, G. Tamburlini, M. Tavakkoli, H. R. Taylor, J. A. Taylor, W. J. Taylor, B. Thomas, W. M. Thomson, G. D. Thurston, I. M. Tleyjeh, M. Tonelli, J. A. Towbin, T. Truelsen, M. K. Tsilimbaris, C. Ubeda, E. A. Undurraga, M. J. van der Werf, J. van Os, M. S. Vavilala, N. Venketasubramanian, M. Wang, W. Wang, K. Watt, D. J. Weatherall, M. A. Weinstock, R. Weintraub, M. G. Weisskopf, M. M. Weissman, R. A. White, H. Whiteford, S. T. Wiersma, J. D. Wilkinson, H. C. Williams, S. R. Williams, E. Witt, F. Wolfe, A. D. Woolf, S. Wulf, P. H. Yeh, A. K. Zaidi, Z. J. Zheng, D. Zonies, A. D. Lopez, C. J. Murray, M. A. AlMazroa and Z. A. Memish (2012). "Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010." Lancet **380**(9859): 2163-2196.

Voss, T., R. B. Lipton, D. W. Dodick, N. Dupre, J. Y. Ge, R. Bachman, C. Assaid, S. K. Aurora and D. Michelson (2016). "A phase IIb randomized, double-blind, placebo-controlled trial of ubrogepant for the acute treatment of migraine." Cephalalgia **36**(9): 887-898.

Walker, W. C., J. H. Marwitz, A. R. Wilk, J. M. Ketchum, J. M. Hoffman, A. W. Brown and S. Lucas (2013). "Prediction of headache severity (density and functional impact) after traumatic brain injury: A longitudinal multicenter study." Cephalalgia **33**(12): 998-1008.

Wang, D., V. L. Tawfik, G. Corder, S. A. Low, A. Francois, A. I. Basbaum and G. Scherrer (2018). "Functional Divergence of Delta and Mu Opioid Receptor Organization in CNS Pain Circuits." Neuron **98**(1): 90-108.e105.

Wang, H. B., B. Zhao, Y. Q. Zhong, K. C. Li, Z. Y. Li, Q. Wang, Y. J. Lu, Z. N. Zhang, S. Q. He, H. C. Zheng, S. X. Wu, T. G. Hokfelt, L. Bao and X. Zhang (2010). "Coexpression of delta- and mu-opioid receptors in nociceptive sensory neurons." Proc Natl Acad Sci U S A **107**(29): 13117-13122.

Wang, Z., L. R. Gardell, M. H. Ossipov, T. W. Vanderah, M. B. Brennan, U. Hochgeschwender, V. J. Hruby, T. P. Malan, Jr., J. Lai and F. Porreca (2001). "Pronociceptive actions of dynorphin maintain chronic neuropathic pain." J Neurosci **21**(5): 1779-1786.

Warden, D. (2006). "Military TBI during the Iraq and Afghanistan Wars." J Head Trauma Rehabil **21**(5): 398-402.

Waterfield, A. A., F. M. Leslie, J. A. Lord, N. Ling and H. W. Kosterlitz (1979). "Opioid activities of fragments of beta-endorphin and of its leucine65-analogue. Comparison of the binding properties of methionine- and leucine-enkephalin." Eur J Pharmacol **58**(1): 11-18.

Weatherall, M. W. (2015). "The diagnosis and treatment of chronic migraine." Ther Adv Chronic Dis **6**(3): 115-123.

Wee, S. and G. F. Koob (2010). "The role of the dynorphin-kappa opioid system in the reinforcing effects of drugs of abuse." Psychopharmacology (Berl) **210**(2): 121-135.

Wells, R. E. and E. Loder (2012). "Mind/Body and behavioral treatments: the evidence and approach." Headache **52 Suppl 2**: 70-75.

WHO, L. t. B. (2011). Atlas of headache disorders and resources in the world 2011. **72**.

Wimalawansa, S. J., H. R. Morris, A. Etienne, I. Blench, M. Panico and I. MacIntyre (1990). "Isolation, purification and characterization of beta-hCGRP from human spinal cord." Biochem Biophys Res Commun **167**(3): 993-1000.

Woldeamanuel, Y. W. and R. P. Cowan (2017). "Migraine affects 1 in 10 people worldwide featuring recent rise: A systematic review and meta-analysis of community-based studies involving 6 million participants." J Neurol Sci **372**: 307-315.

Yaksh, T. L., R. L. Kohl and T. A. Rudy (1977). "Induction of tolerance and withdrawal in rats receiving morphine in the spinal subarachnoid space." Eur J Pharmacol **42**(3): 275-284.

Yaksh, T. L. and T. A. Rudy (1976). "Chronic catheterization of the spinal subarachnoid space." Physiol Behav **17**(6): 1031-1036.

Yan, L., T. Yinghui, Y. Bo, Z. Gang, X. Xian and Z. Lu (2011). "Effect of calcitonin gene-related peptide on nitric oxide production in osteoblasts: an experimental study." Cell Biol Int **35**(8): 757-765.

Zaidi, M., L. H. Breimer and I. MacIntyre (1987). "Biology of peptides from the calcitonin genes." Q J Exp Physiol **72**(4): 371-408.

Zhang, X., L. Bao, U. Arvidsson, R. Elde and T. Hokfelt (1998). "Localization and regulation of the delta-opioid receptor in dorsal root ganglia and spinal cord of the rat and monkey: evidence for association with the membrane of large dense-core vesicles." Neuroscience **82**(4): 1225-1242.

Zhang, X., L. Bao and S. Li (2015). "Opioid receptor trafficking and interaction in nociceptors." Br J Pharmacol **172**(2): 364-374.

Zhang, X., D. Levy, R. Noseda, V. Kainz, M. Jakubowski and R. Burstein (2010). "Activation of meningeal nociceptors by cortical spreading depression: implications for migraine with aura." J Neurosci **30**(26): 8807-8814.

Zhang, X., A. A. Velumian, O. T. Jones and P. L. Carlen (2000). "Modulation of high-voltage-activated calcium channels in dentate granule cells by topiramate." Epilepsia **41 Suppl 1**: S52-60.

Zohar, O., V. Rubovitch, A. Milman, S. Schreiber and C. G. Pick (2011). "Behavioral consequences of minimal traumatic brain injury in mice." Acta Neurobiol Exp (Wars) **71**(1): 36-45.

Zohar, O., S. Schreiber, V. Getslev, J. P. Schwartz, P. G. Mullins and C. G. Pick (2003). "Closed-head minimal traumatic brain injury produces long-term cognitive deficits in mice." Neuroscience **118**(4): 949-955.

Zona, C., M. T. Ciotti and M. Avoli (1997). "Topiramate attenuates voltage-gated sodium currents in rat cerebellar granule cells." Neurosci Lett **231**(3): 123-126.

APPENDIX A: PERMISSION TO RE-USE ARTICLE IN THESIS FROM CEPHALALGIA

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Laura Moye <lsegur4@uic.edu>

RE: Re-use article in thesis

PermissionsUK <PermissionsUK@sagepub.com>
To: Laura Moye <lsegur4@uic.edu>

Wed, Sep 5, 2018 at 6:09 PM

Dear Laura Moye,

Thank you for your email. As you are a co-author of "The development of a mouse model of mTBI-induced post-traumatic migraine, and identification of the delta opioid receptor as a novel therapeutic target" in the SAGE journal *Cephalalgia*, I am pleased to report we can grant your request without a fee as part of your thesis.

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APPENDIX B: PERMISSION TO REUSE ARTICLE IN THESIS FROM NEUROPHARMACOLOGY

APPENDIX C: ANIMAL PROTOCOL 15-066

UIC UNIVERSITY OF ILLINOIS AT CHICAGO

Office of Animal Care and
Institutional Biosafety Committees (MC 672)
Office of the Vice Chancellor for Research
206 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

May 7, 2015

Amynah Pradhan
Psychiatry
M/C 912

Dear Dr. Pradhan:

The protocol indicated below was reviewed at a convened ACC meeting in accordance with the Animal Care Policies of the University of Illinois at Chicago on **4/21/2015**. *The protocol was not initiated until final clarifications were reviewed and approved on* **4/30/2015**. *The protocol is approved for a period of* 3 years with annual continuation.

Title of Application: Synergistic Mechanisms Between Traumatic Brain Injury and Migraine

ACC Number: 15-066

Initial Approval Period: 4/30/2015 to 4/21/2016

Condition of Initiation: ***Prior to the initiation of any work with animals, PI must obtain approval from DOD ACURO and provide copy of approval to OACIB.***

Current Funding: ***Portions of this protocol are supported by the funding sources indicated in the table below.***
Number of funding sources: 1

Funding Agency	**Funding Title**			**Portion of Proposal Matched**
DOD- Department of Defense	*Synergistic Mechanisms Between Traumatic Brain Injury and Migraine*			*All matched*
Funding Number	**Current Status**	**UIC PAF NO.**	**Performance Site**	**Funding PI**
W81XWH-14-PRMRP-DA	*Pending*	*2015003338*	*UIC*	*Amynah Pradhan*

This institution has Animal Welfare Assurance Number A3460.01 on file with the Office of Laboratory Animal Welfare (OLAW), NIH. **This letter may only be provided as proof of IACUC approval for those specific funding sources listed above in which all portions of the funding proposal are matched to this ACC protocol.**

In addition, all investigators are responsible for ensuring compliance with all federal and institutional policies and regulations related to use of animals under this protocol and the funding sources listed on this protocol. Please use OLAW's "*What Investigators Need to Know about the*

APPENDIX D: ANIMAL PROTOCOL 16-022

UIC UNIVERSITY OF ILLINOIS AT CHICAGO

Office of Animal Care and Institutional
Biosafety Committee (M/C 672)
Office of the Vice Chancellor for Research
206 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612

May 10, 2016

Amynah Pradhan
Psychiatry
M/C 912

Dear Dr. Pradhan:

The modifications requested in modification indicated below pertaining to your approved protocol indicated below have been reviewed and approved in accordance with the Animal Care Policies of the University of Illinois at Chicago on ***05/09/2016***.

Title of Application: The Development of Delta Opioid Receptor Agonists for The Treatment of Psychiatric and neurologic disorders

ACC Number: **16-022**

Modification Number: *2*

Nature of Modification: ***Addition of new personnel: Sarah Louis Tangsgaard Christensen***

Condition of Initiation: ***New personnel added must complete facility orientation/zoonotic training and enter the UIC Occupational Health Program for Individuals with Animal Contact prior to initiation of any work with animals. For rodents, contact Dr. Jeanette Purcell (996-7051) for BRL, BBC, MBRB, COD, SES, and all satellites), Dr. Cynthia Adams for the mouse barrier in COMRB (996-9236), or Ms. Kelly Pavlik for BSB (996-7810). For large animal areas of the BRL and COMRB, contact Dr. Kelly Garcia (996-8619). For primates, contact Dr. Lisa Halliday (996-9453). Facility access will not be granted until this condition is completed.***

Protocol Approved: *2/29/2016*

Current Approval Period: *2/29/2016 to 2/16/2017. Protocol is eligible for 2 additional years of renewal prior to expiration and resubmission.*

Current Funding: *Portions of this protocol are supported by the funding sources indicated in the table below.*
Number of funding sources: 2

Funding Agency	Funding Title	Portion of Funding Matched

Phone (312) 996-1972 · Fax (312) 996-9088

VITA

NAME: Laura Segura Moye

EDUCATION: B.Sc., Neuroscience, Agnes Scott College, Decatur, GA, 2014

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Promoting Success in STEM Graduate Education (PASSAGE) Scholar, University of Illinois at Chicago, Chicago, IL, June-August 2014

PUBLICATIONS: LS Moye, AF Tipton, I Dripps, Z Sheets, A Crombie, JD Violin, AA Pradhan. (2018) Delta opioid receptor agonists are effective for multiple types of headache disorders. *Neuropharmacology*.

H Jeong, LS Moye, B Southey, A Hernandez, I Dripps, EV Romanova, S Roubakhin, J Sweedler, A Pradhan, S Rodriguez-Zas. (2018) Gene network dysregulation in the trigeminal ganglia and nucleus accumbens of a model of chronic migraine-associated hyperalgesia. *Frontiers in Systems Neuroscience*.

LS Moye, ML Novack, AF Tipton, H Krishnan, SC Pandey, and AA Pradhan. (2018). The development of a mouse model of mTBI-induced post-traumatic migraine, and identification of the delta opioid receptor as a novel therapeutic target. *Cephalalgia :* an international journal of headache, 333102418777507.

MB Aissa, AF Tipton, Z Bertels, R Gandhi, LS Moye, ML Novack, BM Bennett, Y Wang, V Litosh, SH Lee, IN Gaisina, G Thatcher, AA Pradhan. (2017) Soluble guanylate cyclase is a critical regulator of migraine-associated pain. *Cephalalgia*, doi: 10.1177/0333102417737778

LS Moye and AAA Pradhan. (2017) Animal model of chronic migraine-associated pain. *Current Protocols in Neuroscience, 80,* 9.60.1-9.60.9. doi: 10.1002/cpns.33

LS Moye and AA Pradhan. (2016) From blast to bench: a translational mini-review of post-traumatic headache. *Journal of Neuroscience Research*.

A Vicente-Sánchez, L Segura, AA Pradhan. (2016) The Delta Opioid Receptor Tool Box. *Neuroscience.* http://dx.doi.org/10.1016/j.neuroscience.2016.06.028

AA Pradhan, J Perroy, W Walwyn, M Smith, A Vicente-Sanchez, L Segura, A Bana, B Kieffer, C Evans. (2016) Agonist-selective recruitment of arrestin isoforms differentially modify delta opioid receptor function. *Journal of Neuroscience.* 36(12):3541–3551.

N Bartolotti, L Segura, O Lazarov. (2015) Diminished CRE-induced plasticity is linked to memory deficits in familial Alzheimer's Disease mice. *Journal of Alzheimer's Disease* 50(2):477-489.

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