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MIGRAINE (R COWAN, SECTION EDITOR)

Update on Animal Models of Migraine

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Abstract Migraine is a severe and debilitating disorder of the brain that involves a constellation of neurological symptoms alongside head pain. Its pathophysiology is only beginning to be understood, and is thought to involve activation and sensitization of trigeminovascular nociceptive pathways that innervate the cranial vasculature, and activation of brain stem nuclei. Much of our understanding of migraine pathophysiology stems from research conducted in animal models over the last 30 years, and the development of unique assays in animals that try to model specific aspects of migraine pathophysiology related to particular symptoms. This review will highlight some of the latest findings from these established animal models, as well as discuss the latest in the development of novel approaches in animals to study migraine

Keywords Migraine · Pathophysiology · Cranial vasculature · Trigeminovascular nociceptive pathways · Behavioral model · Animal models

Introduction

Migraine is a chronic and disabling disorder of the brain that affects at least 15 % of the US population [1]. It is predominantly thought of as a head pain disorder; however, a closer inspection of the migraine classification demonstrates a greater level of complexity [2]. The overall symptomatology of migraine points to a more general disorder of sensory processing, which includes hypersensitivity to light, sound, touch, smell, and food, as well as altered cognition, emotion, and general homeostasis. These symptoms can present before, during, and even continue after the head pain symptoms of migraine, which may indicate a more generalized migrainous brain state, rather than a simple pain disorder. Our understanding of migraine pathophysiology over the last 25 – 30 years is based largely on animal models that study the nociceptive pathways of the trigeminovascular system and their ascending projections to brainstem and diencephalic nuclei, as well as the control these structures have on nociceptive and other sensory processing pathways that result in migraine symptoms [3–5]. These animal models use electrophysiology, video imaging, laser Doppler flowmetry, immunohistochemistry, biochemistry, and behavioral assessments to determine the pathophysiological basis for migraine symptoms (Table 1). While these models pertain to be animal models of migraine, it is more accurate to say they model certain aspects of migraine pathophysiology, rather than migraine per se. The majority focus on the pain aspects of migraine, while ignoring for a long time the other associated neurological symptoms, but in more recent years discoveries about the neural basis of these other symptoms have been made, using in part, some of these existing animal models. This review will focus on the latest advances and adaptations in animal models and how they have helped us to understand more about the pathophysiology of migraine, and its specific symptoms. Firstly, we will discuss the anesthetized models of migraine, which have helped dissect neuronal pathways, and the neural basis of migraine and symptoms. Secondly, we will discuss conscious, behavioral models of migraine, where perhaps there has been the greatest advancement over the last few years, which use measures of sensory hypersensitivity, light aversion, and other assessments

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Table 1 Experimental tools involved in the study of migraine in animals

Technique	
Electrophysiology	A recording electrode is placed in discrete nuclei in the brain or spinal cord (trigeminocervical complex) and real-time changes in electrical activity (flow of ions) are measured, such as action potentials with single unit recording, or changes in local field potentials by measuring the net activity of a population of neurons, in response to external stimuli
Intravital video microscopy	A video microscope is placed over a cranial window to visualize a blood vessel, most commonly the dural middle meningeal artery, and using acquisition hardware and software, changes in the size of the vessel can be determine in response to dural stimulation or chemical mediators
Laser Doppler flowmetry	Used in hemodynamics to measure changes blood flow. Can be used on any vascular bed, but commonly used to measure flow in dural vasculature, trigeminocervical complex, and cerebral cortex. A low power laser light from a fiber optic probe penetrates the tissue and is scattered with a Doppler shift by red blood cells and returned to detector to give a measure of flow.
Micro-iontophoresis/ Microinjection	Used to apply pharmacological agents to discrete areas of the brain. Iontophoresis uses an electric field to charge pharmacological agents in aqueous solution to eject drug and can be combined with a recording electrode to determine the pharmacology of a neuron. Injection uses pressure to apply a known volume into an area of the brain to determine effects elsewhere.
Histology/ Immunohistochemistry	Used to study the anatomy of brain cells and specific nuclei in the brain. Can be combined with immunohistochemistry, detection of antigens with specific antibodies to highlight proteins, to determine cellular activation and the pharmacological profile in areas of the brain.
Biochemistry	Used to study chemical processes at the molecular level, such as the structure, function, and interaction of biochemical molecules, including proteins, lipids, and carbohydrates. Similar to immunohistochemistry, it uses detection of target antigens with specific antibodies to highlight these biochemical
Evoked behavioral testing	Measures allodynia and hyperalgesia with mechanical and thermal methodology and devices. The animal tested has 'control' of the duration of the stimuli (withdrawal)
Spontaneous behavioral testing	Measures spontaneous, non-evoked behaviors that may normally be presented in rodents but may be affected by the study paradigm (noxious stimulation). Comprehends grooming patterns, facial grimace, blinking, exploratory behaviors, rearing, gnawing, freezing, and locomotion.
Conditioned placed preference	Measures the motivational effects to objects or experiences. The mice are conditioned to associate the pain/discomfort to a compartment that has cues such as specific textures/patterns where they received a noxious stimulus (aversive effect) and to associate a control stimulus (saline) to another, different compartment.
Elevated plus shape maze	This apparatus is based on the mouse's aversion to open spaces. Mice avoid open areas and prefer to be confined to enclosed spaces.

that we will explain further as correlates of migraine symptoms. Finally, we will briefly discuss the new genetic models of migraine, based on known gene mutations in the human condition, which incorporate the experimental methods described above.

Anesthetized Models of Migraine

Animal Models of Migraine Headache: Intracranial Dural Stimulation

While the pathophysiology of migraine is only beginning to be understood, the headache component of migraine is thought to involve activation and sensitization of the trigeminovascular system. This includes the rich plexus of nociceptive primary nerve fibers that originate in the trigeminal ganglion and innervate the peripheral cranial vasculature, including the nociceptive-specific dura mater and its meninges, the central projection to the medullary dorsal horn, and ascending projections up to the higher brainstem and diencephalic nuclei [6, 7, 3]. Activation of these structures in conscious patients, particularly the dura mater, with mechanical, chemical, or electrical stimulation results in severe headache pain, lateralized to the side of stimulation, very similar to the pain in migraine, as well as other symptoms associated with migraine, including nausea and photophobia [8–10]. Of further interest is that stimulation of sites away from these blood vessels is much less nociceptive, with correspondingly lower symptoms of headache. Therefore, animal models have been developed, particularly in anesthetized animals, to model specifically these phenomena using intracranial stimulation of the

dural vasculature, with neuronal and vascular changes recorded along the peripheral and central migraine pain pathway, to determine the neural and vascular basis of migraine-like symptoms, but particularly head pain.

The two major methods for intracranial dural stimulation involve either dural electrical stimulation [11, 12] or application of chemical mediators including inflammatory soup (IS) [13], nitric oxide donors [14], or calcitonin gene-related peptide (CGRP) [15]. These methods aim to stimulate the trigeminal nociceptive nerve innervation of the dural vasculature and, therefore, manipulate an area adjacent to or on the dural blood vessels, including meningeal, sagittal, or transverse sinuses. In preclinical studies, intracranial stimulation of the superior sagittal sinus or middle meningeal artery, described and reviewed in detail previously [16–19], has been demonstrated to cause similar activation in the periphery and brain as that found during a migraine attack [20–23]. This includes dural vascular and neuronal changes in the brainstem and diencephalic nuclei, reviewed in detail elsewhere [5, 3, 24], that are likely to contribute to the headache, but are also involved in modulation of nociceptive inputs during migraine, as well as mediating autonomic, endocrine, and affective symptoms that accompany migraine headache.

Some of the most recent findings in our understanding of the anatomy of migraine pain pathways has used these animal models to dissect out thalamocortical projections involved in craniovascular nociceptive processing, which explain how patients can localize their pain to specific cranial regions, which are also implicated in other symptoms. Two recent studies demonstrated that dural nociceptive ventroposteromedial thalamic neurons project to mainly primary (S1) and secondary (S2) somatosensory cortices, as well as the insula. These data suggest that the processing of craniovascular sensory and discriminatory information, particularly in the ophthalmic (V1) trigeminal division, is somatotopically organized to cortical regions [25•, 26•]. This may account for the ability of migraineurs to localize their intracranial pain to specific head regions, as well as characterize the intensity and quality of their pain. Furthermore, dural nociceptive posterior (PO), lateral posterior (LP), and lateral dorsal (LD) thalamic neurons project to S1 and S2, but also to motor, parietal association, retrosplenial, auditory, visual, and olfactory cortices [26•]. These data demonstrate that the processing of craniovascular nociceptive information in PO, LP, and LD thalamic neurons relay directly to cortical areas, which suggests a role in cognitive and motor deficits during migraine, as well as allodynia, photophobia, phonophobia, and osmophobia.

Perhaps the biggest criticism of this approach is that it is not particularly translational, despite reflecting many of the clinical features of migraine. A further criticism is that headache is not the first symptom during a migraine attack and, therefore, it does not readily explain what causes activation of these nociceptive pathways during migraine, or indeed the mechanism for these premonitory symptoms. However, it does directly activate the nociceptive pathways thought to be involved in migraine headache, and it helps us to understand how these pathways may be involved in the generation of other associated neurological symptoms of migraine. Also, this approach causes similar release of neuropeptides into the extracranial vasculature as demonstrated during migraine, which includes CGRP and pituitary adenylate cyclase-activating peptide (PACAP) [27–30].

Animal Models of Associated Migraine Symptoms

Studies that use intracranial dural stimulation have also recently been able to dissect the neural basis for associated neurological migraine symptoms. Using the dural inflammatory soup, it has been shown that peripheral and central sensitization of trigeminovascular neurons causes hypersensitive responses to mechanical stimulation of the intracranial dural and extracranial cutaneous facial receptive fields [31, 13]. Peripheral and central trigeminovascular sensitization likely explains the exacerbation of pain due to physical activity, or the avoidance of physical activity (intracranial hypersensitivity), that is part of migraine classification [2]. Sensitization of central trigeminovascular neurons likely explains the referral of pain to extracranial regions, and the development of symptoms of facial cutaneous hyperalgesia and cutaneous allodynia [32]. Recent studies have also been able to dissect the neural basis for referral of symptoms of somatosensory hypersensitivity to extracephalic areas, such as arms and legs, which often occurs as migraine becomes more severe and frequent [33]. In animal models using a dural inflammatory soup, this extracephalic hypersensitivity is mediated by sensitization of trigeminothalamic neurons, a response that is supported in migraine patients who experience expansion of hypersensitive symptoms to extracephalic regions [21]. A final symptom of hypersensitivity in migraine that has been modeled using intracranial activation is photophobia, either hypersensitivity to light, or light exacerbating migraine pain. A recent study mapped dural nociceptive intracranial neurons to a subset of posterior thalamic nuclei that were modulated by light intensity, whose axons projected extensively to the somatosensory, visual, and associative cortices. The cell bodies and dendrites of these posterior thalamic neurons were apposed by axons originating in retinal ganglion cells [34••]. The implication is that the convergence of photic signals onto dural nociceptive trigeminothalamic neurons that project to the somatosensory cortex exacerbates nociceptive processing, and the exacerbation of migraine headache due to light, while similar neurons that ultimately project to the visual cortex cause a hypersensitivity to light during migraine [35].

Animal Models of Migraine: Brainstem Dysfunction/Modulation

A criticism for using intracranial dural stimulation is that it does not explain what triggers activation of these nociceptive pathways during migraine, which seems to occur without a direct stimulus. If migraine is to be considered a brain state, and migraine symptoms are caused by changes in activation in specific brain nuclei, it should be possible to model aspects of migraine by manipulation of these nuclei. Models of intracranial nociceptive activation have been adapted to allow one to understand how other brain nuclei may be involved in the descending modulation of trigeminovascular nociceptive processing and, therefore, potentially implicate these regions in triggering or modulating nociceptive activation of migraine pain pathways resulting in head pain and other symptoms. Several brainstem and hypothalamic nuclei, including the ventrolateral periaqueductal grey (vlPAG), rostral ventromedial medulla (RVM), nucleus raphe magnus (NRM), superior salivatory nucleus (SuS), and posterior and A11 hypothalamic nuclei have been shown to modulate intracranial trigeminovascular nociceptive processing, via various neurotransmitter pathways, reviewed in [3]. Most recently, dysfunctional endocannabinoid mechanisms have been implicated in modulating trigeminovascular intracranial nociceptive neurons [36], which may show that this system is involved in triggering migraine, or at least in modulating migraine pain.

In several recent studies from our laboratory, we were able to demonstrate that stimulation of the pontine SuS independently produces short term neuronal activation in the trigeminocervical complex (TCC), as well as autonomic facial symptoms, that are modulated by specific migraine and TAC therapeutics [37, 38••]. The SuS is the origin of cells of the parasympathetic autonomic vasodilator pathway [39], activation of which likely contributes to autonomic symptoms during migraine [40]. It receives a reflex connection from the trigeminal nucleus, as well as direct projections from the paraventricular hypothalamic nucleus (PVN). Furthermore, trigeminovascular nociceptive processing is modulated by direct manipulation of the PVN through GABAergic, PACAPergic, and triptanergic approaches, and the strength of these responses are limited by stress triggers [41•]. Through these data, hypothalamic nuclei, known to be involved in the regulation of body homeostasis, are implicated in producing nociceptive activation of trigeminovascular neurons, and thus potentially triggering migraine. Likewise, the SuS may represent a migraine gateway through its connections with the TCC, hypothalamic, limbic, and cortical neurons, implicating it in prolonging migraine duration through a perpetual feedback mechanism, but also in triggering many migraine symptoms through connections with hypothalamic nuclei involved in migraine triggers, such as stress, feeding, and sleep disturbance.

However, several major criticisms distract from the findings. Firstly, while manipulation of these nuclei alters the way evoked nociceptive responses are processed over a short time, these responses are transient, lasting less than 30 min, or represent permanent changes, whereas the hypothesized changes during migraine would last much longer and also be reversible. Secondly, it is not clear whether this manipulation actually switches on previously quiescent nociceptive neurons, or just causes a hypersensitive response to evoked stimulation. There is no real measure in these anesthetized animals that spontaneous throbbing head pain is caused, as would be expected in migraine. It is hoped that future adaptations, which incorporate conscious behavioral observations, will be able to demonstrate prolonged, reversible changes that represent the neural substrate of throbbing head pain

Animal Models of Aura: Cortical Spreading Depression

Approximately 30 % of migraine patients also experience transient neurological deficits, the migraine aura [42], and cortical spreading depression (CSD) is thought to be its underlying mechanism [43, 44]. In animals models, CSD is induced by placement of KCl on the cerebral cortex, or electrical or mechanical stimulation of the cortex [45]. It is measured by an initial depolarization followed by a prolonged hyperpolarization (or quiescence) of neurons and glia, and is usually accompanied by changes in perfusion, with hyperaemia followed by sustained oligaemia (reduced blood flow) [45], similar to the cortical blood flow changes demonstrated during migraine aura [46, 47].

The most recent advances in studying mechanisms of CSD in migraine have concentrated on whether it has a role in triggering trigeminovascular activation, and as a consequence, triggering migraine. Imaging studies in rodents have demonstrated that CSD causes vasodilation of meningeal blood vessels, both concurrent with CSD, but also independent of this response, that is accompanied by neuronal activation in the trigeminocervical complex [48]. Electrophysiological studies demonstrated that mechanical-, chemical-, or electrical-induced CSD causes activation of meningeal nociceptors and central trigeminovascular neurons approximately 50 % of the time [49, 50]. The implication is that CSD induces dural activation that subsequently activates the peripheral and central aspects of the trigeminovascular nociceptive pain pathway. However, CSD can induce inhibition as much as facilitation of trigeminovascular neurons [51•] depending on the site of initiation, and does not necessarily require a peripheral input [52]. With these contradictions, and the fact that clinically migraine more often occurs without aura, the full role of CSD in migraine is not completely clear. However, the most recent adaptations and studies of animal models of migraine aura have led to a better understanding of the neurophysiology of these responses, and the generation of

new hypotheses and approaches to understand migraine pathophysiology.

Animal Models of Migraine: Pharmacological Provocation Studies

Migraine provocation studies using several pharmacological agents have proven very successful at inducing headache in migraineurs that is classified as migraine [2]. The most notable of these is nitroglycerin (NTG), a nitric oxide donor [53], although other molecules have shown similar effects, including CGRP, PACAP, and prostaglandins E2 (PGE2) and I2 (PGI2), reviewed in detail previously [54]. It was natural to transfer these molecules to animal models as they represent a genuine translational opportunity to determine their vascular and neuronal effects on migraine pain pathways [18, 19, 55]. These molecules are potent vasodilators of the cranial vasculature in humans and rodents, although these vascular changes do not seem to coincide with migraine headache. It now seems most likely that migraine is not accompanied by an obvious vasodilation [56, 57], and any that does occur is an epiphenomenon [58]. In animal models, NTG activates neurons along the migraine pain pathway [18, 19, 55]. CGRP has also been studied and appears to have effects when applied locally [59], although systemic effects remain unclear [60, 61].

However, there has been much criticism of the use of chemical migraine triggers, particularly NTG, in animal models [19, 55]. It is certainly the case that much higher doses of NTG and CGRP have been necessary to induce neuronal changes than is used clinically. Furthermore, it could be argued that since these chemical mediators do not trigger migraine in healthy controls, to study their effects in animals that presumably do not have migraine is irrelevant. However, some believe migraine is a disorder of thresholds, in that the level of activation along migraine pain pathways necessary to trigger an attack in migraineurs is much lower than healthy subjects, rather than simply activation itself triggering an attack. In other words, activation may occur regardless of the patient phenotype, but it is how the brain perceives this activation, which results in migraine symptoms. Therefore, measuring responses in animal models is relevant as migraine pain pathways are activated when dosed sufficiently; what is perhaps more important is how these mediators are used in studies and how the data is interpreted with respect to studying many symptoms of migraine.

The original provocation animal studies looked simply at short term neuronal changes, but migraine is a multifactorial disorder that affects many sensory modalities at different time points, and all aspects need to be taken into account. More recent studies have looked across the migraine phenotype more thoughtfully, with conscious, behavioral studies (discussed below), leading the way. But even in anesthetized studies there has been very recent progress. The fact that NTG and CGRP trigger a delayed, rather than an immediate migraine attack is important, and several electrophysiological studies have now demonstrated that the effects of NTG are at least biphasic, with an immediate increase, and then a delayed and more prolonged activation [62] for up to 3 h [63]. Furthermore, it has been argued that the dural inflammatory soup assay lacks translation as there is little evidence of release of the inflammatory mediators at the dural level during a migraine attack, and the assay cannot be replicated in patients. However, using similar measures of intra- and extracranial hypersensitivity, it has been further demonstrated that NTG causes a delayed sensitization of central trigeminovascular neurons, accompanied by hypersensitive responses to intra- and extracranial noxious and innocuous stimulation [63], similar to migraine patients' response to NTG. Also, this sensitization and hypersensitive response to evoked stimuli are aborted by acute migraine treatments [64]. These data demonstrate that combining several of these animal models perhaps reflects more the migrainous phenotype and makes them more translational, rather than looking at the independent neuronal changes these chemical mediators produce.

Behavioral Models of Migraine

Many of the assays described above have allowed us to understand better the neural basis for migraine symptoms, yet they suffer from having an anesthetized preparation, which means rather than pain being measured, only nociceptive pathways are described, and pain is somewhat assumed. Pain is a subjective experience; we identify pain based on behavior [65] and only a conscious individual will report painful and non-painful symptomatology [66••]. Therefore, conscious behavioral assays of migraine are extremely important since they attempt to correlate as close as possible the human experience of this multi-symptom disorder. These models allow the careful measurement of noxious stimuli translated into behavioral responses in addition to other non-painful symptoms present in migraine.

Behavioral Models of Trigeminovascular Nociception

To study headache in conscious animals it is essential to have a measure of pain referred to the craniofacial region. It is thought that activation and sensitization of central trigeminovascular neurons is the neural substrate for headache in migraine, and also importantly, the neural substrate of facial cutaneous allodynia. Facial cutaneous allodynia is a symptom described in 60 – 80 % of migraineurs, particularly those with more chronic forms of the disorder [32, 67], where normally innocuous stimuli are perceived as painful. Therefore,

measurements of allodynia in the craniofacial region serve as an excellent marker of a migraineous phenotype in behavioral animal models in addition to helping us explore the use of novel therapeutics. Cutaneous allodynia is determined by measuring sensory nociceptive thresholds, usually in response to applying calibrated or electronic von Frey monofilaments to the head, usually in the peri-orbital region, where headache occurs in patients. Response of rats or mice is measured as a retraction of their head or washing stroke if the monofilament is considered noxious [66••, 68, 69]. Similar to studies in anesthetized animals, it is assumed that activation of the trigeminovascular system should do this; therefore, assays used in anesthetized animals have been carefully transferred to conscious animals. Using dural inflammatory soup, injected through an implanted cannula to activate the trigeminovascular nociceptive innervation of the dural vasculature [13] can significantly reduce measurements of sensory nociceptive thresholds in the peri-orbital (ophthalmic trigeminal) region [68, 69], implying cephalic cutaneous allodynia.

Migraine patients can also have cutaneous allodynia in extracephalic areas, such as arms and legs [70, 71, 33]. More severe and prolonged symptoms of cutaneous allodynia are associated with increased frequency of migraine, and these patients are less likely to respond to triptan therapy [72]. In rats, a single dose of dural inflammatory soup produced a reduction in sensory thresholds not only in the cephalic (peri-orbital), but also extracephalic (hind paw) regions that reached its maximum at 3 h and only returned to baseline after 5 – 6 h [68]. Administration of sumatriptan or naproxen either prior to or 30 min after inflammatory soup were able to prevent and reverse allodynia, whereas sumatriptan and naproxen given 1.5 or 2.5 h after dural imflammatory soup had no effects. These data translate to the clinical setting of reduced effect of triptans when cutaneous allodynia is established. Administration of a CGRP receptor antagonist after 30 min also abolished allodynia [68].

To recreate recurrent headaches, rats have also been given episodic infusions of dural inflammatory soup [69], which repeatedly activate and sensitize central trigeminovascular neurons. These repeated infusions transiently decrease sensory thresholds in the peri-orbital region each day, whereas after eight infusions during several weeks, the decrease in sensory thresholds is sustained without additional infusion for a further 3 weeks. This assay is thought to represent the transformation from episodic to chronic migraine, and can be used to study these mechanisms. These rats also presented more sustained symptoms of peri-orbital cutaneous allodynia after NTG [69].

Migraine is not just restricted to the ophthalmic (V1) dermatome, and the latest IHS classification has included facial migraine, where, in a subset of patients that present with typical migraine, headache is localized in the face [2]. In some cases, migraine pain can be felt in the maxillary (V2) or mandibular (V3) divisions of the trigeminal nerve [73–75]. A similar study used inflammatory soup infusions on the dura mater, and measured sensory thresholds in the peri-orbital region and in the orofacial region (masseter area and whisker pad), in both male and female rats [76]. The orofacial sensitivity assay consisted of training rats to reach a bottle with sucrose solution. This reaching allowed the experimenter to access the peri-orbital, peri-masseter, and whisker pad areas for threshold testing with von Frey stimulation. It is well known that migraine is more prevalent in females [1], and in this model female rats needed less dural inflammatory soup to reduce their nociceptive thresholds in the periorbital and peri-masseter areas. In addition, female rats presented a reduction in locomotor activity when compared to male rats [76] that may imply reduction of physical activity due to the pain.

Similar to the conscious models that use dural inflammatory soup, NTG as a trigger of migraine has also been studied. In mice, a high dose of NTG (10 mg/kg, ip) induced mechanical and thermal extracephalic allodynia (hind paw) that was reversed by sumatriptan [77]. A criticism of this approach is that craniofacial sensory threshold analysis was not performed, but neural activation was represented at the trigeminal nucleus caudalis by increased Fos expression. Chronic, intermittent NTG has also been used to model the progression to more chronic migraine. In mice, it results in acute hind paw mechanical hyperalgesia with each injection and becomes progressive and sustained [78]. Sumatriptan was able to reduce the acute hyperalgesia, without effecting basal hyperalgesia, whereas topiramate was able to reduce both. In a further paper, the same group expanded their assessment of nociceptive behaviors to include also a conditioned place aversion paradigm to NTG injections [79••]. This assay brings behavioral testing to another level since it measures the motivational effects to objects or experiences [80] and, in this case, to NTG. The assay consists in an apparatus with two Plexiglas boxes divided into two compartments that are separated by a guillotine door. Each compartment has a different floor texture and wall pattern. The mice are conditioned to associate the pain/discomfort to the compartment (texture/pattern) where they received the NTG treatment (aversive effect) and to associate a control stimulus (saline) in the other compartment. Then, time spent in each compartment is measured. In this study, the NTG-induced acute and chronic mechanical and thermal hyperalgesia in mice was reduced by a δ-opioid receptor agonist, SNC80, which reversed the NTG-conditioned place aversion. This study demonstrated that not only are δ-opioid receptor agonists a potential therapy for migraine, but also that they may alleviate the negative emotional estate of migraine.

Behavioral Models of Spontaneous Nociceptive Responses in the Craniofacial Region

The pain experience is subjective and complex in itself. In primary headaches the presence of spontaneous pain is inherent to the disorder and allodynic symptoms may be additional and not present in all patients. Classical pain studies in animal models quantify evoked responses to noxious stimuli (mechanical, thermal) applied by the experimenter [81]. In this paradigm, the animal has control of the duration of the stimuli (withdrawal) and spontaneous, non-evoked, responses are not measured. However, spontaneous pain is thought to be a much better predictor as a pain rating [82] and is closer to the pain experience that a human may present. Therefore, new animal models that assess spontaneous behavior have been developed and are great tools to characterize the headache experience further [66••]. These models include a freely moving animal and video recording.

Pain in the craniofacial area of rodents induces changes in their normal grooming patterns. In a novel mouse model of orofacial pain induced by complete Freund's adjuvant injection into the masseter muscle, three different patterns of non-evoked behaviors in freely moving rodents directed to the area of injection were described [83•]. The grooming patterns consisted of repetitive washing strokes performed using their forepaws, repetitive rubbing of their cheek and chin area against the bottom of the cage, and scratching of the affected area with their hind paw. These behaviors correlated to Fos expression in the trigeminal nucleus caudalis and were arrested by morphine [83•]. These data demonstrate that these nociceptive behaviors were mediated by trigeminal nerve activation. Similar facial grooming behaviors were also reported in another study related more specifically to head pain, which also coded for changes in body grooming, exploratory behaviors, resting, freezing, and other non-specific behaviors intrinsic to the animals analyzed, after meningeal nociceptive activation induced with the dural inflammatory soup [84•]. Meningeal nociception increased resting and freezing behavior, as well as reducing exploratory behavior, which could be described as a correlate of the avoidance of physical activity described in migraineurs [2]. Furthermore, the facial grooming behaviors were unilateral (ipsilateral to the inflammatory soup infusion), similar to the unilateral pain in migraine, and these nociceptive behaviors were significantly reduced with zolmitriptan, where freezing was completely abolished [84•]. These studies provide mechanisms to code spontaneous behaviors specific to pain in the craniofacial area that can be used to study the pathophysiology of headache disorders, and correlate these behaviors to the neural approaches in anesthetized animals.

A mouse grimace scale (MGS) has also been established, which encodes changes in facial expressions of mice when they experience pain of any modality [85], and this scale has also been used in a genetic migraine mouse model, the CACN A1A knock-in mouse (see below for description) [86]. They found that the MGS baseline scores were increased in this migraine model, implying more spontaneous pain that was reversed by rizatriptan treatment [85]. In another study using these mice, nine standard nociceptive sensitivity assays were assessed [87], in addition to measures of spontaneous behaviors that were considered representative of trigeminally mediated head pain, such as grooming patterns directed to the head, eye–blinking, and whole body shuddering. There were no differences in the standard sensitivity tests compared to wild-type. However, lateralized head grooming directed to the oculotemporal area, eye–blinking, and shuddering behaviors, in addition to measures of photophobia (see next section) were all significantly higher in the migraine mice, and head grooming and eye-blinking were reduced with rizatriptan [88••]. In the same study, they took it a step further to determine if stress can exacerbate or trigger these nociceptive behaviors. Mice were tested in different enclosures: their home cage with mates, a new enclosure, but alone, and in restraining cylinders. Stress levels were higher in the latter two enclosures, and this resulted in higher eye-blinking and shuddering in mutant mice, particularly more severe migraine genotypes, compared to wild-type mice. The implication is that these behaviors are migraine-specific and the authors stated that their findings may be the first behavioral evidence of spontaneous headache since they illustrate several pain behaviors that could be considered representative of headache, and certainly demonstrate a nociceptive phenotype in these mice.

Behavioral Models of Associated Neurological Symptoms

As described above, photophobia is an associated symptom of migraine that gives clear evidence of a wider sensory hypersensitivity to external stimuli, and its neural basis is thought to stem from the convergence of photic signals on trigeminal [89] and trigeminothalamic neurons that project to the somatosensory cortex [35]. Photophobia has also been measured and determined in conscious mice, using two slightly different methodologies. Firstly, using the light-dark box exploratory test of anxiety, mice with elevated expression of the hRAMP1 subunit of the CGRP receptor presented a strong aversion to light, preferring the dark compartment [90, 91]. The response was exacerbated after administration of CGRP, and mice also showed diminished locomotion and rearing activity. Again, as mentioned above, these behaviors are indicative of migraineurs seeking out a dark room and reduced activity. The second assay uses the elevated plus-shaped maze with two closed arms "safe" and two open arms to measure symptoms of photophobia, more commonly used to measure anxiety, based on the mouse's aversion to open spaces [88••]. Mice were assigned to one of three conditions: room lights on, room lights off, or bright open arms where the room lights are off,

but the closed arms are illuminated with fluorescent lights. In this study, the CACNA1A migraine mice demonstrated light aversion, choosing to remain longer in the open space, compared to wild-types, in order to avoid bright light.

Nausea and vomiting is another symptom in the migraine constellation. Rodents are incapable of vomiting and, therefore, behavioral models are necessary to study these often debilitating symptoms. Paradigms with conditioned taste aversion may serve as a good substitute in rodents, but they are still untested for their outcomes related to migraine. However, conscious rats have shown a loss of appetite (anorexia) with the dural inflammatory soup assay, which also induces activation of anorectic peptides in the brainstem and dienchephalic nuclei. This might imply that sensitization of the trigeminovascular system and its resultant pain may induce loss of appetite by activating centers in the brain that control feeding and appetite [92].

Genetic Models of Migraine

Familial Hemiplegic Migraine

During the last few decades, several gene mutations have become associated with specific severe and rare forms of migraine using a classic linkage analysis approach, which has helped support the hypothesis that migraine may be an inherited disorder. As described above briefly, the behavioral characterization of genetic models of migraine adds a translational step, which is a particularly significant advantage that transgenic mouse technologies offer, in helping to understand migraine pathophysiology and to test novel therapeutic targets. Here, we outline a few of those described and their significant data. The first of these are related to familial hemiplegic migraine (FHM), a rare form of migraine with aura where patients have severe hemiplegic aura. Three monogenic gene mutations have been determined so far, termed FHM1-3, caused by mutations to the CACNA1A, ATP1A2, and SCNA1A genes, respectively [93, 94]. Two mouse models of FHM1, R192Q [86] and S218L [95], and one for FHM2, W887R [96], have been generated with the human mutated genes inserted into the genome that form genetic mouse models of migraine. These animal models have been used in conjunction with the assays described above to help gain insight into the phenotypic consequences of these mutations in migraine pathophysiology. Mice with these mutations each independently show reduced induction threshold and increased propagation velocity of CSD. Furthermore, the R192Q mice showed reduced CGRP-immunoreactivity in trigeminal ganglia and the superficial laminae of the trigeminocervical complex [97]. There is also a reduced neuronal response to nociceptive activation of the trigeminovascular system compared to wild-type animals [98], indicating they respond differentially to common nociceptive signals relevant to migraine. Additionally, they have an increased susceptibility to present with nociceptive headache-related spontaneous pain behaviors and photophobia [85, 88••]

Casein Kinase 1δ (CK1δ)

A mutation in the gene encoding casein kinase 1δ (T44A) has been described in patients with familial advanced sleep phase (FASP) syndrome, characterized by early sleep times and early morning waking [99]. In six individuals of one family, FASP is also associated with migraine with and without aura [100••], indicating that the CK1δ gene may also be functionally related to migraine pathophysiology. Mice carrying the T44A human gene mutation also showed an increased susceptibility to CSD induction, and hypersensitive behavioral responses, as well as increased neuronal activation in the trigeminocervical complex, when exposed to NTG [100••]. These data indicate that this gene may have a pathophysiological influence on CSD, as well as trigeminovascular nociceptive activation, and more generally, may contribute to the pathogenesis of migraine with and without aura.

These genetic models of migraine, accompanied by assays that study physiological pathways involved in migraine offer a mechanism to understand the phenotype of the human condition in these rarer forms or associations in migraine, which may be extrapolated to more common and generalized forms of migraine to unlock pathways and loci otherwise not explored. The one caveat to the huge advances in basic research that these genetic models may provide is that these gene mutations are considered rare compared to the high prevalence of migraine in the general population, and despite considerable efforts, it has not be possible to determine similar mutations in more common migraine. Furthermore, how far we can generalize findings from these animal models at the extreme end of the migraine spectrum, or with co-morbidities with other disorders to the pathophysiology of common forms of migraine is still to be determined, and thus one must be cautious. As case in point are patients with FHM who do not share the same hypersensitivity to NTG or CGRP as a migraine trigger compared to patients with more common types of migraine [54]. This demonstrates potentially separate pathways of pathophysiology for different migraine phenotypes. But, with all that said, these genetic models of migraine do represent opportunities to understand the pathophysiology of specific symptoms, rather than generalizing to all migraine. Recently, a number of genes have been discovered for more common forms of migraine using genome-wide association studies (GWAS), and in time, development of animal models with these mutations may shed light on the role

these genes have on migraine pathophysiology, opening up better opportunities for drug development.

Conclusion

A disorder is a disturbance of the normal homeostasis, and to study a disorder, a model (system) that recreates this disorder is needed. Animal models for the study of migraine include anesthetized (unconscious) and conscious paradigms that attempt to recreate as close as possible the pathophysiology of migraine from physiological, behavioral, and genetic levels. Thanks to the animal models of headache, our knowledge has advanced tremendously in the last decade. In addition, these models are being used to refine current treatment approaches and to develop new therapeutic targets.

Compliance with Ethics Guidelines

Conflict of Interest Dr. Marcela Romero-Reyes and Dr. Simon Akerman each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Lipton RB, Bigal ME, Diamond M, Freitag F, Reed ML, Stewart WF. Migraine prevalence, disease burden, and the need for preventive therapy. Neurology. 2007;68(5):343–9.
2. Headache Classification Committee of the International Headache Society. The International Classification of Headache Disorders, 3rd edition (beta version). Cephalalgia. 2013;33(9):629–808.
3. Akerman S, Holland PR, Goadsby PJ. Diencephalic and brainstem mechanisms in migraine. Nat Rev Neurosci. 2011;12(10):570–84.
4. Pietrobon D, Moskowitz MA. Pathophysiology of migraine. Annu Rev Physiol. 2013;75:365–91.
5. Noseda R, Burstein R. Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, CSD, sensitization and modulation of pain. Pain. 2013.
6. Bernstein C, Burstein R. Sensitization of the trigeminovascular pathway: perspective and implications to migraine pathophysiology. J Clin Neurol. 2012;8(2):89–99.
7. Goadsby PJ, Lipton RB, Ferrari MD. Migraine–current understanding and treatment. N Engl J Med. 2002;346(4):257–70.
8. Penfield W, McNaughton F. Dural headache and innervation of the dura mater. Arch Neurol Psychiatry. 1940;44:43–75.
9. Ray BS, Wolff HG. Experimental studies on headache. Pain sensitive structures of the head and their significance in headache. Arch Surg. 1940;41:813–56.
10. McNaughton FL, Feindel W. Innervation of intracranial structures: a reappraisal. In: Rose FC, editor. Physiological aspects of clinical neurology. Oxford: Blackwell Scientific; 1977. p. 279–93.
11. Lambert GA, Goadsby PJ, Zagami AS, Duckworth JW. Comparative effects of stimulation of the trigeminal ganglion and the superior sagittal sinus on cerebral blood flow and evoked potentials in the cat. Brain Res. 1988;453(1–2):143–9.
12. Hoskin KL, Zagami AS, Goadsby PJ. Stimulation of the middle meningeal artery leads to Fos expression in the trigeminocervical nucleus: a comparative study of monkey and cat. J Anat. 1999;194(Pt4):579–88.
13. Strassman AM, Raymond SA, Burstein R. Sensitization of meningeal sensory neurons and the origin of headaches. Nature. 1996;384(6609):560–4.
14. Strecker T, Dux M, Messlinger K. Increase in meningeal blood flow by nitric oxide - interaction with calcitonin gene-related peptide receptor and prostaglandin synthesis inhibition. Cephalalgia. 2002;22(3):233–41.
15. Kurosawa M, Messlinger K, Pawlak M, Schmidt RF. Increase of meningeal blood flow after electrical stimulation of rat dura mater encephali: mediation by calcitonin gene-related peptide. Br J Pharmacol. 1995;114(7):1397–402.
16. Akerman S, Holland PR, Hoffmann J. Pearls and pitfalls in experimental in vivo models of migraine: Dural trigeminovascular nociception. Cephalalgia. 2013;33(8):577–92.
17. Andreou AP, Summ O, Charbit AR, Romero-Reyes M, Goadsby PJ. Animal models of headache: from bedside to bench and back to bedside. Expert Rev Neurother. 2010;10(3):389–411. doi:10.1586/ern.10.16.
18. Bergerot A, Holland PR, Akerman S, Bartsch T, Ahn AH, Maassenvandenbrink A, et al. Animal models of migraine: looking at the component parts of a complex disorder. Eur J Neurosci. 2006;24(6):1517–34.
19. Jansen-Olesen I, Tfelt-Hansen P, Olesen J. Animal migraine models for drug development: status and future perspectives. CNS Drugs. 2013;27(12):1049–68. doi:10.1007/s40263-013-0121-7.
20. Afridi SK, Giffin NJ, Kaube H, Friston KJ, Ward NS, Frackowiak RS, et al. A positron emission tomographic study in spontaneous migraine. Arch Neurol. 2005;62(8):1270–5.
21. Burstein R, Jakubowski M, Garcia-Nicas E, Kainz V, Bajwa Z, Hargreaves R, et al. Thalamic sensitization transforms localized pain into widespread allodynia. Ann Neurol. 2010;68(1):81–91.
22. Denuelle M, Fabre N, Payoux P, Chollet F, Geraud G. Hypothalamic activation in spontaneous migraine attacks. Headache. 2007;47(10):1418–26.
23. Weiller C, May A, Limmroth V, Juptner M, Kaube H, Schayck RV, et al. Brain stem activation in spontaneous human migraine attacks. Nat Med. 1995;1(7):658–60.
24. Goadsby PJ, Charbit AR, Andreou AP, Akerman S, Holland PR. Neurobiology of migraine. Neuroscience. 2009;161(2):327–41.
25.• Lambert GA, Hoskin KL, Michalicek J, Panahi SE, Truong L, Zagami AS. Stimulation of dural vessels excites the SI somatosensory cortex of the cat via a relay in the thalamus. Cephalalgia. 2013. doi:10.1177/0333102413508239. *Alongside 26, the first studies to how patients can localize their pain to specific cranial regions, through activation of specific cortical regions.*
26.• Noseda R, Jakubowski M, Kainz V, Borsook D, Burstein R. Cortical projections of functionally identified thalamic trigeminovascular neurons: implications for migraine headache and its associated symptoms. J Neurosci. 2011;31(40):14204–17.

Alongside 25, the first studies to how patients can localize their pain to specific cranial regions, through activation of specific cortical regions.

27. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann Neurol. 1990;28(2):183–7.
28. Tuka B, Helyes Z, Markovics A, Bagoly T, Szolcsanyi J, Szabo N, et al. Alterations in PACAP-38-like immunoreactivity in the plasma during ictal and interictal periods of migraine patients. Cephalalgia. 2013;33(13):1085–95.
29. Zagami AS, Edvinsson L, Hoskin KL, Goadsby PJ. Stimulation of the superior sagittal sinus causes exracranial release of PACAP. Cephalalgia. 1995;14 Suppl 14:109.
30. Zagami AS, Goadsby PJ, Edvinsson L. Stimulation of the superior sagittal sinus in the cat causes release of vasoactive peptides. Neuropeptides. 1990;16(2):69–75.
31. Burstein R, Yamamura H, Malick A, Strassman AM. Chemical stimulation of the intracranial dura induces enhanced responses to facial stimulation in brain stem trigeminal neurons. J-Neurophysiol. 1998;79(2):964–82.
32. Burstein R, Yarnitsky D, Goor-Aryeh I, Ransil BJ, Bajwa ZH. An association between migraine and cutaneous allodynia. Ann Neurol. 2000;47(5):614–24.
33. Bigal ME, Ashina S, Burstein R, Reed ML, Buse D, Serrano D, et al. Prevalence and characteristics of allodynia in headache sufferers: a population study. Neurology. 2008;70(17):1525–33.
34.•• Noseda R, Kainz V, Jakubowski M, Gooley JJ, Saper CB, Digre K, et al. A neural mechanism for exacerbation of headache by light. Nat Neurosci. 2010;13(2):239–45. *First studies to demonstrate the neural basis by photophobia in migraine, mediated by dural nociceptive afferents*.
35. Noseda R, Burstein R. Advances in understanding the mechanisms of migraine-type photophobia. Curr Opin Neurol. 2011;24(3):197–202.
36. Akerman S, Holland PR, Lasalandra MP, Goadsby PJ. Endocannabinoids in the brainstem modulate dural trigeminovascular nociceptive traffic via CB1 and "triptan" receptors: implications in migraine. J Neurosci. 2013;33(37):14869–77. doi:10.1523/JNEUROSCI.0943-13.2013.
37. Akerman S, Holland PR, Lasalandra MP, Goadsby PJ. Oxygen inhibits neuronal activation in the trigeminocervical complex after stimulation of trigeminal autonomic reflex, but not during direct dural activation of trigeminal afferents. Headache. 2009;49(8):1131–43.
38.•• Akerman S, Holland PR, Summ O, Lasalandra MP, Goadsby PJ. A translational in vivo model of trigeminal autonomic cephalalgias: therapeutic characterization. Brain. 2012;135(Pt 12):3664–75. *Characterisation of an animal model of trigeminal autonomic cephalagias, with prominent trigeminovascular and autonomic features, and describes a novel locus for treatments through the parasympathetic outflow to the cranial vasculature*.
39. Spencer SE, Sawyer WB, Wada H, Platt KB, Loewy AD. CNS projections to the pterygopalatine parasympathetic preganglionic neurons in the rat: a retrograde transneuronal viral cell body labeling study. Brain Res. 1990;534(1–2):149–69.
40. Lai T-H, Fuh J-L, Wang S-J. Cranial autonomic symptoms in migraine: characteristics and comparison with cluster headache. J Neurol Neurosurg Psychiatry. 2009;80:1116–9.
41.• Robert C, Bourgeais L, Arreto CD, Condes-Lara M, Noseda R, Jay T, et al. Paraventricular hypothalamic regulation of trigeminovascular mechanisms involved in headaches. J Neurosci. 2013;33(20):8827–40. *Demonstrates paraventricular mediated modulation of nociceptive trigeminovascular neurons, with implications of hypothalamic nuclei involved in the triggering of migraine*.
42. Rasmussen BK, Olesen J. Migraine with aura and migraine without aura: an epidemiological study. Cephalalgia. 1992;12:221–8.
43. Lashley KS. Patterns of cerebral integration indicated by the scotomas of migraine. Arch Neurol Psychiatr. 1941;46:331–9.
44. Lauritzen M. Pathophysiology of the migraine aura. The spreading depression theory. Brain. 1994;117(Pt 1):199–210.
45. Eikermann-Haerter K, Moskowitz MA. Animal models of migraine headache and aura. Curr Opin Neurol. 2008;21(3):294–300.
46. Hadjikhani N, Sanchez Del Rio M, Wu O, Schwartz D, Bakker D, Fischl B, et al. Mechanisms of migraine aura revealed by functional MRI in human visual cortex. Proc Natl Acad Sci U S A. 2001;98(8):4687–92. doi:10.1073/pnas.071582498.
47. Olesen J, Larsen B, Lauritzen M. Focal hyperemia followed by spreading oligemia and impaired activation of rCBF in classic migraine. Ann Neurol. 1981;9(4):344–52.
48. Bolay H, Reuter U, Dunn AK, Huang Z, Boas DA, Moskowitz MA. Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. Nat Med. 2002;8(2):136–42.
49. Zhang X, Levy D, Kainz V, Noseda R, Jakubowski M, Burstein R. Activation of central trigeminovascular neurons by cortical spreading depression. Ann Neurol. 2011;69(5):855–65.
50. Zhang X, Levy D, Noseda R, Kainz V, Jakubowski M, Burstein R. Activation of meningeal nociceptors by cortical spreading depression: implications for migraine with aura. J Neurosci. 2010;30(26):8807–14.
51.• Noseda R, Constandil L, Bourgeais L, Chalus M, Villanueva L. hanges of meningeal excitability mediated by corticotrigeminal networks: a link for the endogenous modulation of migraine pain. J Neurosci. 2010;30(43):14420–9. *First study to demonstrate that CSD triggered in different cortical regions may inhibit or excite trigeminovascular noxious and innocuous responses*.
52. Lambert GA, Truong L, Zagami AS. Effect of cortical spreading depression on basal and evoked traffic in the trigeminovascular sensory system. Cephalalgia. 2011.
53. Iversen HK, Olesen J, Tfelt-hansen P. Intravenous Nitroglycerin As an Experimental-Model of Vascular Headache - Basic Characteristics. Pain. 1989;38(1):17–24.
54. Schytz HW, Schoonman GG, Ashina M. What have we learnt from triggering migraine? Curr Opin Neurol. 2010;23(3):259–65.
55. Olesen J, Jansen-Olesen I. Towards a reliable animal model of migraine. Cephalalgia. 2012;32(7):578–80. doi:10.1177/0333102412441719.
56. Amin FM, Asghar MS, Hougaard A, Hansen AE, Larsen VA, de Koning PJ, et al. Magnetic resonance angiography of intracranial and extracranial arteries in patients with spontaneous migraine without aura: a cross-sectional study. Lancet Neurol. 2013;12(5):454–61.
57. Schoonman GG, van der Grond J, Kortmann C, van der Geest RJ, Terwindt GM, Ferrari MD. Migraine headache is not associated with cerebral or meningeal vasodilatation–a 3T magnetic resonance angiography study. Brain. 2008;131(Pt 8):2192–200.
58. Asghar MS, Hansen AE, Amin FM, van der Geest RJ, Koning P, Larsson HB, et al. Evidence for a vascular factor in migraine. Ann Neurol. 2011;69(4):635–45.
59. Storer RJ, Akerman S, Goadsby PJ. Calcitonin gene-related peptide (CGRP) modulates nociceptive trigeminovascular transmission in the cat. Br J Pharmacol. 2004;142(7):1171–81.
60. Cumberbatch MJ, Williamson DJ, Mason GS, Hill RG, Hargreaves RJ. Dural vasodilation causes a sensitization of rat caudal trigeminal neurones in vivo that is blocked by a 5-HT1B/1D agonist. Br J Pharmacol. 1999;126(6):1478–86.
61. Levy D, Burstein R, Strassman AM. Calcitonin gene-related peptide does not excite or sensitize meningeal nociceptors: implications for the pathophysiology of migraine. Ann Neurol. 2005;58(5):698–705.
62. Koulchitsky S, Fischer MJ, De Col R, Schlechtweg PM, Messlinger K. Biphasic response to nitric oxide of spinal

trigeminal neurons with meningeal input in rat–possible implications for the pathophysiology of headaches. J Neurophysiol. 2004;92(3):1320–8.
63. Akerman S, Hoffmann J, Goadsby PJ. A Translational Approach to Studying Triptan-Induced Reversal of Established Central Sensitization of Trigeminovascular Neurons. Cephalalgia. 2013;33(8 (S1)):211.
64. Akerman S, Goadsby PJ. Acute Anti-Migraine Treatments Abort Established Central Sensitization of Trigeminovascular Neurons: Validation of a Novel Translational Approach. Headache. 2014;54(S1):2–3.
65. Mogil JS, Davis KD, Derbyshire SW. The necessity of animal models in pain research. Pain. 2010;151(1):12–7.
66.•• Romero-Reyes M, Ye Y. Pearls and pitfalls in experimental in vivo models of headache: Conscious behavioral research. Cephalalgia. 2013;33(8):566–76. doi:10.1177/0333102412472557. *A detailed review of the methodology of behavioral animal models of headache.*
67. Lipton RB, Bigal ME, Ashina S, Burstein R, Silberstein S, Reed ML, et al. Cutaneous allodynia in the migraine population. Ann Neurol. 2008;63(2):148–58.
68. Edelmayer RM, Vanderah TW, Majuta L, Zhang ET, Fioravanti B, De Felice M, et al. Medullary pain facilitating neurons mediate allodynia in headache-related pain. Ann Neurol. 2009;65(2):184–93.
69. Oshinsky ML, Gomonchareonsiri S. Episodic dural stimulation in awake rats: a model for recurrent headache. Headache. 2007;47(7):1026–36. doi:10.1111/j.1526-4610.2007.00871.x.
70. Tfelt-Hansen P, Lous I, Olesen J. Prevalence and significance of muscle tenderness during common migraine attacks. Headache. 1981;21(2):49–54.
71. Oshinsky ML. Insights from experimental studies into allodynia and its treatment. Curr Pain Headache Rep. 2006;10(3):225–30.
72. Burstein R, Collins B, Jakubowski M. Defeating migraine pain with triptans: a race against the development of cutaneous allodynia. Ann Neurol. 2004;55(1):19–26.
73. Daudia AT, Jones NS. Facial migraine in a rhinological setting. Clin Otolaryngol Allied Sci. 2002;27(6):521–5.
74. Obermann M, Mueller D, Yoon M-S, Pageler L, Diener H, Katsarava Z. Migraine With Isolated Facial Pain: A Diagnostic Challenge. Cephalalgia. 2007;27(11):1278–82. doi:10.1111/j.1468-2982.2007.01413.x.
75. Silberstein. Headache and other head pain. 7th ed. New York: Oxford University Press; 2001.
76. Stucky NL, Gregory E, Winter MK, He YY, Hamilton ES, McCarson KE, et al. Sex differences in behavior and expression of CGRP-related genes in a rodent model of chronic migraine. Headache. 2011;51(5):674–92.
77. Bates E, Nikai T, Brennan K, Fu YH, Charles A, Basbaum A et al. Sumatriptan alleviates nitroglycerin-induced mechanical and thermal allodynia in mice. Cephalalgia. 2009.
78. Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A. Characterization of a novel model of chronic migraine. Pain. 2014;155(2):269–74. doi:10.1016/j.pain.2013.10.004.
79.•• Pradhan AA, Smith ML, Zyuzin J, Charles A. delta-Opioid receptor agonists inhibit migraine-related hyperalgesia, aversive state and cortical spreading depression in mice. Br J Pharmacol. 2014;171(9):2375–84. doi:10.1111/bph.12591. *Use of conditioned place aversion in a model of chronic migraine with the use of NTG.*
80. Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol. 2007;12(3–4):227–462. doi:10.1111/j.1369-1600.2007.00070.x.
81. Mogil JS. Animal models of pain: progress and challenges. Nat Rev Neurosci. 2009;10(4):283–94.
82. Backonja MM, Stacey B. Neuropathic pain symptoms relative to overall pain rating. J Pain. 2004;5(9):491–7.
83.• Romero-Reyes M, Akerman S, Nguyen E, Vijjeswarapu A, Hom B, Dong HW, et al. Spontaneous Behavioral Responses in the Orofacial Region: A Model of Trigeminal Pain in Mouse. Headache. 2013;53(1):137–51. *Characteriation of spontaneous (non-evoked) grooming nociceptive behaviors in the orofacial region.*
84.• Melo-Carrillo A, Lopez-Avila A. A chronic animal model of migraine, induced by repeated meningeal nociception, characterized by a behavioral and pharmacological approach. Cephalalgia. 2013;33(13):1096–105. doi:10.1177/0333102413486320. *Chronic model of migraine who presents spontaneos (non-evoked) behaviors as unilateral grooming in response to inflammatory soup infusion.*
85. Langford DJ, Bailey AL, Chanda ML, Clarke SE, Drummond TE, Echols S, et al. Coding of facial expressions of pain in the laboratory mouse. Nat Methods. 2010;7(6):447–9.
86. van den Maagdenberg AM, Pietrobon D, Pizzorusso T, Kaja S, Broos LA, Cesetti T, et al. A Cacna1a knockin migraine mouse model with increased susceptibility to cortical spreading depression. Neuron. 2004;41(5):701–10.
87. Mogil JS, Ritchie J, Sotocinal SG, Smith SB, Croteau S, Levitin DJ, et al. Screening for pain phenotypes: analysis of three congenic mouse strains on a battery of nine nociceptive assays. Pain. 2006;126(1–3):24–34. doi:10.1016/j.pain.2006.06.004.
88.•• Chanda ML, Tuttle AH, Baran I, Atlin C, Guindi D, Hathaway G, et al. Behavioral evidence for photophobia and stress-related ipsilateral head pain in transgenic Cacna1a mutant mice. Pain. 2013;154(8):1254–62. doi:10.1016/j.pain.2013.03.038. *Multiple behavioral assessments including photophobia supporting Cacna1a mice as a reliable model to study migraine pathophysiology and to screen novel therapeutics.*
89. Okamoto K, Tashiro A, Chang Z, Bereiter DA. Bright light activates a trigeminal nociceptive pathway. Pain. 149(2):235-42.
90. Recober A, Kuburas A, Zhang Z, Wemmie JA, Anderson MG, Russo AF. Role of calcitonin gene-related peptide in light-aversive behavior: implications for migraine. J Neurosci. 2009;29(27):8798–804.
91. Recober A, Kaiser EA, Kuburas A, Russo AF. Induction of multiple photophobic behaviors in a transgenic mouse sensitized to CGRP. Neuropharmacology. 2009.
92. Malick A, Jakubowski M, Elmquist JK, Saper CB, Burstein R. A neurohistochemical blueprint for pain-induced loss of appetite. Proc Natl Acad Sci U S A. 2001;98(17):9930–5.
93. Barrett CF, van den Maagdenberg AM, Frants RR, Ferrari MD. Familial hemiplegic migraine. Adv Genet. 2008;63:57–83. doi:10.1016/S0065-2660(08)01003-1.
94. Pietrobon D. Familial hemiplegic migraine. Neurother: J Am Soc Exp NeuroTher. 2007;4(2):274–84. doi:10.1016/j.nurt.2007.01.008.
95. van den Maagdenberg AM, Pizzorusso T, Kaja S, Terpolilli N, Shapovalova M, Hoebeek FE, et al. High cortical spreading depression susceptibility and migraine-associated symptoms in Ca(v)2.1 S218L mice. Ann Neurol. 2010;67(1):85–98.
96. Leo L, Gherardini L, Barone V, De Fusco M, Pietrobon D, Pizzorusso T, et al. Increased susceptibility to cortical spreading depression in the mouse model of familial hemiplegic migraine type 2. PLoS Genet. 2011;7(6):e1002129. doi:10.1371/journal.pgen.1002129.
97. Mathew R, Andreou AP, Chami L, Bergerot A, van den Maagdenberg AM, Ferrari MD, et al. Immunohistochemical characterization of calcitonin gene-related peptide in the trigeminal system

of the familial hemiplegic migraine 1 knock-in mouse. Cephalalgia. 2011;31(13):1368–80. doi:10.1177/0333102411418847.

98. Park J, Moon H, Akerman S, Holland PR, Lasalandra MP, Andreou AP, et al. Differential trigeminovascular nociceptive responses in the thalamus in the familial hemiplegic migraine 1 knock-in mouse: A Fos protein study. Neurobiol Dis. 2014;64:1–7.
99. Xu Y, Padiath QS, Shapiro RE, Jones CR, Wu SC, Saigoh N, et al. Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. Nature. 2005;434(7033):640–4. doi:10.1038/nature03453.
100.•• Brennan KC, Bates EA, Shapiro RE, Zyuzin J, Hallows WC, Huang Y, et al. Casein kinase idelta mutations in familial migraine and advanced sleep phase. Sci Transl Med. 2013;5(183):183ra56. *1-11. Demonstration of genetic link to migraine that is not related to familial hemiplegic migraine, but a sleep disorder, and the first characterisation of a mutant mouse carrying the human gene mutation and its migrainous phenotype.*