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Spontaneous Trigeminal Allodynia in Rats: A Model of Primary Headache

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Abstract

Animal models are essential for studying the pathophysiology of headache disorders and as a screening tool for new therapies. Most animal models modify a normal animal in an attempt to mimic migraine symptoms. They require manipulation to activate the trigeminal nerve or dural nociceptors. At best, they are models of secondary headache. No existing model can address the fundamental question: How is a primary headache spontaneously initiated? In the process of obtaining baseline periorbital von Frey thresholds in a wild-type Sprague-Dawley rat, we discovered a rat with spontaneous episodic trigeminal allodynia (manifested by episodically changing periorbital pain threshold). Subsequent mating showed that the trait is inherited. Animals with spontaneous trigeminal allodynia allow us to study the pathophysiology of primary recurrent headache disorders. To validate this as a model for migraine, we tested the effects of clinically

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proven acute and preventive migraine treatments on spontaneous changes in rat periorbital sensitivity. Sumatriptan, ketorolac, and dihydroergotamine temporarily reversed the low periorbital pain thresholds. Thirty days of chronic valproic acid treatment prevented spontaneous changes in trigeminal allodynia. After discontinuation, the rats returned to their baseline of spontaneous episodic threshold changes. We also tested the effects of known chemical human migraine triggers. On days when the rats did not have allodynia and showed normal periorbital von Frey thresholds, glycerol trinitrate and calcitonin gene related peptide induced significant decreases in the periorbital pain threshold. This model can be used as a predictive model for drug development and for studies of putative biomarkers for headache diagnosis and treatment.

Keywords

animal model; migraine; headache trigger; chronic daily headache; primary headache; allodynia

Recurrent headache is an extremely prevalent neurologic disorder, and migraine is one of the most disabling disorders.¹ Animal models are essential to study basic headache mechanisms and improve treatment. Most headache models modify an animal to mimic migraine symptoms.²⁻⁷ This usually requires significant manipulation of the animal, ie, craniotomy with electrical or chemical stimulation of the dura.⁸⁻¹³ Many of these models are probably models of secondary headache.¹⁴ Migraine is more than a pain disorder.¹ Migraineurs have interictal hypersensitivity and changes in sensory processing¹⁵⁻¹⁹ as well as increased sound sensitivity even between migraine attacks.^{20,21} The best migraine animal model should closely mimic the natural disorder.

Could rodents have spontaneous headaches? We previously described a chemical induction model of recurrent headache that results in referred pain in the rat's periorbital region. This is similar to the referred pain that migraineurs experience.^{22,23} In our experiments, to control for the effects of the craniotomy and to track the rat's recovery, we repeatedly tested the periorbital region with von Frey hairs prior to surgery for the implantation of the cannula. To our surprise, we found a male rat with spontaneous fluctuations in its periorbital pain thresholds (PPT). Thresholds were nearly normal (von Frey threshold ~10 g) 1 day, but very low (<2 g) on another. We then mated the male rat with a normal female. In the next generation, ~50% of the offspring had low PPTs. We have now produced 12 generations; between 50% and 75% of the offspring have the trait.

This study summarizes the testing we performed on rats with spontaneously changing trigeminal pain thresholds. We used sensory stimuli and pharmacologic treatments clinically relevant to humans with migraine, ie, von Frey trigeminal pressure thresholds, sound sensitivity, chemical headache triggers, and acute and preventive migraine treatments.

We will refer to these rats as spontaneous trigeminal allodynia (STA) rats in lieu of a more technical description of them as rats with the inherited trait of spontaneously changing trigeminal von Frey thresholds. Our results show that these rats are a novel model of spontaneous headache and can be used as a model of primary headache.²⁴

METHODS

Rats (Sprague Dawley from Charles River, In-house colony, $n = 107$, 200-600 grams) were group housed in a temperature-controlled environment under a 12-hour light/dark cycle and allowed access to food and water *ad libitum*.

Ethics Statement

All procedures performed on the animals were approved by the Thomas Jefferson University Institutional Animal Care and Use Committee, animal welfare assurance number A3085-01. All efforts were made to minimize the number of animals used and their suffering.

Tactile Sensory Testing

Rats were trained and acclimated to a plastic tube restraint and entered uncoaxed.²² This restrainer allowed the rats to undergo sensory testing.

Periorbital, hind-paw, and jaw-pressure thresholds were determined by applying von Frey monofilaments (Stoelting Co., Wood Dale, IL, USA). Each monofilament is identified by manufacturer-assigned force values (26, 15, 10, 8, 6, 4, 2, 1.4, 1, 0.6, 0.4, 0.07 g). The von Frey hairs are calibrated nylon monofilaments that generate a reproducible buckling stress. For trigeminal testing, the filaments were tested on both the left and right sides of the face, over the rostral portion of the eye for periorbital testing and on the skin over the masseter muscle for jaw testing. We did not touch the vibrissae during the testing. For the hind-paw testing, the filaments were applied to the mid-plantar region of the left and right hind paws, avoiding the less sensitive foot pads. For the hind-paw testing, the maximum value tested was 26 g; the rats that did not respond to this stimulus were assigned this value. Left and right threshold data were recorded separately. The von Frey stimuli were presented in sequential order, either ascending or descending, as necessary, to determine the threshold of response.²⁵ After a positive response, a weaker stimulus was presented, and after a negative response, a stronger stimulus was presented. Results are presented either as the threshold in grams $\pm$ standard error of the mean (SEM), or as a percent change from baseline on the side that had the lowest value. The threshold is defined as a positive response to 2 of 3, or in some cases 3 of 5 trials of a single von Frey monofilament. The value of the von Frey filament that elicited head withdrawal in 2 of 3 repetitions of the stimulus was designated as that day's threshold. Several behaviors were considered a positive head-withdrawal response. The rat would vigorously stroke its face with the ipsilateral forepaw and quickly recoil its head away from the stimulus or vocalize. For the periorbital von Frey testing, rats that did not respond to the 10-g stimulus were assigned 10 g as their threshold, except for those on chronic valproic acid treatment, where we extended our testing to a 15-g stimulus.

Acoustic Startle Threshold Test

The SR-LAB Startle Response System (San Diego Instruments, Inc., San Diego, CA, USA) was used to measure the startle reflex.²⁶ The hardware consisted of an isolation cabinet (15 $\times$ 16 $\times$ 23 in) that minimizes the effects of extraneous noise and vibrations. The isolation cabinet was equipped with an internal light and fan as well as a complete sound generation system to produce white noise stimuli up to 120 dB and separately adjustable background

noise level. A cylindrical startle chamber made of transparent acrylic was situated in the center of the isolation cabinet and was used to restrain minimally the animal during testing. The system was calibrated using a digital sound level meter (Radio Shack model 33-2055, dB SPL) that was placed in the cylindrical startle chamber.

During the startle threshold test, the animal was placed in the cylindrical startle chamber located in the isolation cabinet. The internal light was turned off so that all auditory testing took place in the dark. The rat was then exposed to a startle session that contained trials of white noise bursts ranging from 90 dB to 120 dB (in triplicates) in 3 dB increments with a background noise level of 65 dB. A total of 33 stimuli were presented. The intensity of the stimuli were organized in a pseudo-random order. Each 100 ms noise burst had a 0 ms rise/fall time. The inter-stimulus intervals were randomly generated and ranged from 5 to 30 s.²⁷

The data for each session were relayed to a PC and analyzed using the SR-LAB Startle Response System software (San Diego Instruments, Inc.). The waveforms generated by the rat's movement during the startle response were reviewed for each trial to determine if the movement recorded represented a typical startle-response waveform in a treatment-blinded manner. Each sound intensity was presented 3 times; the acoustic startle threshold (ASR) was the minimum intensity required to elicit a startle response in 2 of 3 trials of that intensity.

Headache Triggers

Glyceryl Trinitrate (GTN): To test the effects of GTN on sensory thresholds on the face of the rat, GTN (0.1 mg/kg, intraperitoneal [i.p.], Sigma) was injected following a baseline sensory test and periorbital thresholds were monitored at 0.33, 1.5, 2.5, and 5 hours after injection.

Calcitonin Gene-Related Peptide (CGRP)

CGRP (4 µg/kg, Sigma) was injected i.v. through the tail vein while the rat was restrained. Successful injections were verified through an immediate flushing due to the vasodilatory actions of CGRP. Tactile sensory thresholds were recorded prior to and following the injection of CGRP for up to 4.5 hours.

Acute Drug Treatments

STA rats and litter mates without the trait were injected with sumatriptan (1 mg/kg i.p., Sigma), ketorolac (0.4 mg/kg i.p., Sigma), or dihydroergotamine mesylate (DHE, 1 mg/kg, Bedford Laboratories) on days when their thresholds in spontaneous allodynia rats were 4 g or below for STA rats. Testing days for each of these drugs were separated by at least 1 week. Tactile sensory thresholds were recorded prior to and following injections for 4.5–24 hours.

Preventive Drug Treatment (Valproic Acid)

Pressure thresholds for STA rats (n = 7) were recorded 5 days per week in the morning for 1 month. Following this, valproic acid (200 mg/kg i.p., Sigma) was injected (i.p.) daily for 1 month (7 days per week). Thresholds were recorded 5 days per week during drug treatment

and for 1 additional month following cessation of treatment. Sensory testing occurred at the same time each day using von Frey monofilaments up to 15 g. Testing was done once per day in the morning, 5 times per week. These dosages are based on previous published studies of rat headache models and our experience with other rat models of trigeminal hypersensitivity.^{11,28,29}

Statistical Analyses

Data were collected and organized in Microsoft Excel. A *t*-test was used to compare acoustic startle thresholds. A repeated measures ANOVA was used to compare von Frey thresholds using SPSS v.12 (IBM Co., Chicago, IL, USA).

RESULTS

Von Frey Threshold Testing

Testing was done once per day in the morning 5 times per week for 3-4 weeks. The value of the von Frey filament that elicited head withdrawal in 2 of 3 repetitions of the stimulus was designated as that day's threshold. Figure 1A shows the response of 10 normal rats to daily von Frey sensory testing. Their thresholds were stable and varied no more than ± 1 von Frey step between days (range of thresholds = 8-10 g). The results of tracking 10 STA for 4 weeks, 5 times per week is shown in Figure 1B. Figure 1C shows the results of tracking the 10 siblings of spontaneous allodynia rats that do not show the trait of spontaneous trigeminal allodynia.

The pattern of the change in periorbital von Frey thresholds in the STA rats is individual dependent. Figures 2A-J show rats that displayed thresholds 4 g or below during the 4 weeks of testing. The rat in Figure 2A episodically changed between 10 and 4 g during the first 3 weeks of the testing period, and during the last week its threshold decreased to 1.7 g. The rat in Figure 2B oscillated between 8 and 1.4 g during weeks 2-3 of sensory testing. Figure 2G shows a rat that had normal thresholds between 10 and 8 g during the first week, but had very low thresholds during weeks 2-4. Together, these graphs demonstrate that 2-4 weeks of sensory testing are required to determine if the rats have episodic trigeminal allodynia.

We also quantified the head-withdrawal thresholds of the STA rats in the V2 trigeminal region (masseter muscle area) and in the hind paw. We consider the allodynia trait to be present if the rat had a low threshold for at least 2 of the 10 days of testing in the masseter region. In the trigeminal region, the masseter muscle-withdrawal thresholds were 2 g or below in 23 of 34 rats tested (mean: 1.37 ± 1.93 g). The hind-paw withdrawal threshold was tracked in rats with low PPT across 3 generations ($n = 12$). None had low hind-paw thresholds; they did not respond to the 26-g monofilament, the maximum filament tested (Fig. 3). Rats that did not respond to the maximum value tested were just assigned 26 g as their threshold in order to generate the graph.

Inherited Low Trigeminal Threshold Trait

The trait of changing trigeminal von Frey thresholds is inherited in both male and female offspring of a single parent with the trait. We tracked the rats at least 3 times per week for 1

month to determine if they exhibited changes in their von Frey thresholds below the levels in normal rats (8-10 g). The rats were required to display at least 3 episodes of thresholds of 4 g or below during the 1 month of testing to be designated as having spontaneous trigeminal allodynia. The pedigree chart in Figure 4 shows the results of 3 generations of matings. The first mating was between a male rat with the low threshold trait and a wild-type normal female that did not carry the trait. Eight of the 15 offspring showed the low trigeminal threshold trait. The mating of male and female siblings with the trait yielded 4 offspring, of which 3 of the 4 showed the trait. The next mating of siblings with the trait resulted in 12 offspring where 8 of the 12 exhibited the spontaneous allodynia phenotype. The penetration of the trait in the next generation is between 50 and 75% in the male and female offspring.

Sound Sensitivity

We quantified increased sound sensitivity (phonophobia) in the spontaneous allodynia rats by measuring the intensity of sound required to induce the acoustic startle reflex (ASR). We exposed the rats to a pseudo-random order of 100 ms long noise bursts ranging from 90-120 dB. Each sound intensity was repeated 3 times; the threshold was defined as the lowest intensity that elicited an ASR in 2 of the 3 repetitions. The ASR threshold of normal rats is 112.5 ± 2.9 dB. The rats with the spontaneous allodynia trait had an ASR threshold of 101.6 ± 2.2 dB. This corresponds to a difference of 10.9 dB between the 2 groups (*t*-test, $P < .011$). Since the dB scale is a logarithmic scale, this represents a more than 10-fold increase in sound sensitivity (Fig. 5).

Chemical Triggers of Headache

A migraine attack in humans can be induced by chemical triggers, such as GTN and CGRP.³⁰⁻³⁴ We evaluated these triggers in both normal control rats and STA rats. STA rats were studied on a day when their threshold was in the normal range (8 g or greater). In response to GTN (0.1 mg/kg), the normal rats and the STA rats had a decrease in their threshold for up to 1.5 hours (Fig. 5A). By 2.5 hours, the thresholds of the control rats had returned to baseline, but the STA rats had significantly lower thresholds for more than 5 hours (ANOVA, $P < .01$).

The control rats showed no significant response to i.v. injection of CGRP (4 μ g/kg). The decrease in PPT in the STA rats continued for up to 5 hours following the CGRP injection (significant decrease in comparison to the normal rats) (ANOVA, $P < .01$). The PPT of all of the rats returned to normal levels 24 hours following CGRP injection (Fig. 6B).

Pharmacologic Headache Treatments

We tested the effects of the migraine acute treatments sumatriptan, ketorolac, and DHE on PPT in STA rats. The STA rats were tested on days when their threshold was 4 g or lower. Sumatriptan (1 mg/kg) increased the threshold 2.5 times the baseline level of the spontaneous allodynia rats within 30 minutes, and the effect lasted more than 5 hours (Fig. 7A). Ketorolac (0.4 mg/kg) had a slower onset than sumatriptan, with no effect during the first 30 minutes (Fig. 7B). During the 1.5, 2.5, and 5 hours testing periods, a significant increase in pressure was required to elicit nociceptive-like behaviors (ANOVA, $P < .001$). At the 24-hour time point, all of the STA rats had returned to their baseline low thresholds.

DHE (0.1 mg/kg) significantly increased the periorbital thresholds up to 3.89 ± 0.94 times the baseline level (ANOVA, $P < .001$), beginning at 30 minutes and lasting for the duration of testing, 2.5 hours (Fig. 7C).

We tested the effect of the preventive migraine medication, valproic acid (200 mg/kg, i.p.), by tracking the PPT. STA rats were tested 5 times per week for 4 weeks ($n = 7$), for a total of 20 sensory tests before drug treatment. The rats were then treated with daily injections of valproic acid for 4 weeks. Their PPT was then tracked for 4 weeks post treatment. Before treatment, the PPT episodically changed and were often 4 g or below. After 3 days of valproic acid treatment, 5 of the 7 rats had PPT of 10-15 g (Fig. 8A). During the fourth week of treatment and after the injections were stopped, the PPT decreased, and all rats returned to episodically changing thresholds. After only 14 days of treatment, 2 of the 5 rats that initially responded to the valproic acid injections began to have lower PPT again, despite continued treatment for the rest of the month (Fig. 8A). Comparing the pretesting to the valproic acid treatment phase, there was a significant increase in the withdrawal PPT ($P < .001$, ANOVA). There was no significant difference between the pre- and post-treatment phase ($P = .124$, ANOVA). Two of the 7 rats had no effect from the valproic acid treatment (Fig. 8B). These rats had the lowest thresholds, consistently below 2 g in the pretesting phase.

DISCUSSION

We demonstrate that spontaneous trigeminal allodynia rats are similar to persons with migraine in 5 ways. First, STA rats have episodically changing trigeminal von Frey thresholds. This may reflect general trigeminal hypersensitivity, since low withdrawal thresholds were also found in the masseter muscle region of the jaw but not in the hind paws. Clinical studies have noted that allodynia is a common symptom of migraine. In fact, it is present in up to 80% of migraineurs.²³ Although allodynia may be present in other headache and trigeminal pain disorders, it is more common in patients with migraine and chronic migraine compared to tension type headache or headaches that are due to a secondary cause such as meningitis or head injury.³⁵ Second, the STA trait is inherited. If both of the parents have the trait, the offspring are more likely to have spontaneous trigeminal allodynia than if 1 parent has the trait. This has been demonstrated in families with migraine.^{36,37} The third way that STA rats are similar to persons with migraine is that STA rats have increased sound sensitivity in comparison to normal control rats (+10.9 dB); this is similar to the differences in sound sensitivity between migraineurs and non-migraineurs (+12-14 dB).^{20,21} The increased sensitivity to chemical headache triggers, including GTN and CGRP, is the fourth way that STA rats are similar to migraineurs.^{33,38-41} The fifth way that STA rats are similar to migraineurs is that the rats' PPT is normalized by clinically proven acute and preventive pharmacologic migraine treatments. Sumatriptan, ketorolac, and DHE transiently returned the PPT of the STA rats to normal levels. The migraine preventive treatment valproic acid restored the PPT in 5 of 7 STA rats to normal levels during treatment, 2 of 7 STA rats did not respond to valproic acid treatment. This reflects the effect of valproic acid in humans with migraine where clinical trials demonstrate that it is effective in 50% of human migraineurs. Since STA rats with episodically changing

trigeminal von Frey thresholds share many of these phenotypes and sensitivities of migraineurs, we believe that they are a good model for studying migraine in rats.

Animal Model of Primary Headache

Our spontaneous trigeminal allodynia model does not require a craniotomy or other invasive manipulation. It has the potential to unravel the pathophysiology of migraine. It will facilitate the use of quantitative trait loci (QTL) mapping or high throughput techniques for genetic analysis to discover new genes that are associated with the allodynia and sound sensitivity traits.^{42,43}

Behavioral tests coupled with traditional physiological measures, such as electrophysiology, microdialysis, and molecular studies, will allow researchers to study the associated symptoms of migraine and other recurrent headache disorders. Using these techniques, this animal model has the potential to facilitate the discovery of the molecular mechanisms that underlie this disorder and allow for screening for novel prophylactic and abortive therapeutics.¹⁴

Although there are many similarities between the STA rats and patients with migraine, there are also other neurological disorders where the patients have allodynia. Examples of this are multiple sclerosis,⁴⁴ Parkinson's disease,⁴⁵ fibromyalgia,⁴⁶ post-stroke pain,⁴⁷ and syringomyelia.⁴⁸ Although even in these conditions the intensity of pain felt by the patient and the symptoms of allodynia can increase and decrease over time, we believe that STA rats are a better model for migraine than these other neurological conditions because the allodynia is inherited, restricted to the trigeminal region, is associated with increased sound sensitivity, and it is responsive to acute migraine treatments such as sumatriptan.

Inherited Allodynia Trait

Several clinical studies suggest that genetics plays an important role in migraine.^{36,37} Migraine runs in families, and there is a high rate of migraine in twins reared together or apart. Heritability estimates ranged from 30 to 65% across studies.^{49,50} Twin projects suggest heritability rates from 34 to 57%.⁵¹ Higher concordance rates for migraine between monozygotic twins compared to dizygotic twins were found, even when the twins were raised apart. Taken together, the results of twin and family studies suggest ~50% heritability of migraine with polygenic inheritance.³⁷

Spontaneous trigeminal allodynia is an inheritable trait in rats with a heritability estimate within our colony of 50-75%. There is a diversity of phenotypes in the STA rats. Some rats have thresholds well below normal for more than 15 days per month; they are similar to chronic migraineurs. Others have a more episodic pattern, with periods of normal thresholds and periods of low thresholds, similar to episodic migraine. Multiple genes that confer the low trigeminal threshold trait and the frequency of the expression of the trait might exist. This may also explain why 2 of the 7 rats did not respond to valproic acid treatment (Fig. 8B), since in clinical trials not all patients respond to valproic acid.^{37,52}

Phonophobia

The ASR is the response to a sudden unexpected stimulus, such as a loud noise. In humans and rats, the reaction includes a sudden movement away from the stimulus, flexion of the muscles in the arms and legs, and blinking. It may also include blood pressure and respiratory changes. Children and adults with migraine may have an exaggerated ASR.^{53,54} The threshold for eliciting acoustic startle is a measure of phonophobia.

The difference in sound aversiveness between migraineurs and non-migraineurs is 12-14 dB between the frequencies of 1-8 kHz.^{20,21} The difference in the sound sensitivity in the spontaneous allodynia rats compared with normal rats is 11 dB, which is similar to the differences between migraineurs and non-migraineurs. Our rats can be used to study the physiological mechanism of headache-related phonophobia.

Headache Triggers

In migraineurs, CGRP and nitric oxide donors such as GTN induce attacks that are indistinguishable from spontaneous migraine attacks. The exact mechanism for trigger-induced migraine is uncertain.³⁰ The headache induced by GTN in non-migraineurs is short and does not have the features of migraine.^{33,34,55,56} CGRP infusion does not induce headache in non-migraineurs.^{57,58} The effects of these migraine triggers are mimicked in the STA rats. GTN induced a decrease in head-withdrawal thresholds for more than 5 hours compared with up to 1.5 hours in normal rats (Fig. 6A). CGRP decreased head-withdrawal thresholds only in spontaneous allodynia rats (Fig. 6B). CGRP may excite a migraine-specific neuronal pathway.³⁰ STA rats may have a mechanism for headache induction similar to human migraineurs and can be used to study the effects of the triggers to determine their mechanism of action in migraineurs.

Pharmacologic Treatments

One validation of a migraine animal model is the effect of clinically proven acute and preventive medications.¹⁴ Acute treatment (sumatriptan, ketorolac, and DHE) transiently increased von Frey thresholds (Fig. 7A-C). Preventive daily treatment with valproic acid significantly stabilized the von Frey thresholds within the normal range for 5 of the 7 rats (Fig. 8A, $P < .001$, ANOVA). Two of the rats did not respond to valproic acid; their periorbital thresholds were low throughout the valproic acid treatment. These data suggest that a larger dose of valproate may be needed or that the phenotype of the colony is diverse, and multiple genes are important for the trait, which may reflect the range of the response to drugs. There was no significant difference between the pretreatment and the washout phase, all of the rats reverted to episodically changing thresholds following 1 month of treatment ($P = .124$, ANOVA). Our paradigm reflects the current system of clinical trials of migraine medications, wherein there is a run-in period to assess the baseline headache frequency followed by an extended treatment phase. Our model of testing may be predictive of putative preventive drug effectiveness.

CONCLUSION AND FUTURE DIRECTIONS

Despite more than 40 years of basic science research in pain using various animal models, there has been only limited success in finding novel and safe treatments. Many of the treatment candidates that were effective in animal models were not efficacious in humans.⁵⁹ Nearly all of these studies used animal models of externally induced pain. A better model of recurrent headache might be our spontaneous allodynia rat. Idiopathic pain/allodynia may be mediated by different mechanisms than pain induced by chemical or electrical stimulation. Rats with spontaneous trigeminal allodynia provide a unique system with which to study the pathophysiology of spontaneous episodic trigeminal pain disorders.

We believe this is the first study that demonstrates a rodent model of spontaneous allodynia/headache that is phenotypically similar to migraine. The development of new therapeutic targets and new hypotheses for migraine pathophysiology require new animal models that reflect the phenotype of migraine in humans. We believe that these rats can be used for pharmacologic investigations to identify new treatments. There is also the potential to discover a new gene or biomarker for migraine in humans.

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References

1. Lipton RB, Newman LC. Epidemiology, impact, and comorbidities of migraine headaches in the United States. *Neurology*. 2003; 60(Suppl. 2):S2–S8.
2. Bergerot A, Holland PR, Akerman S, et al. Animal models of migraine: Looking at the component parts of a complex disorder. *Eur J Neurosci*. 2006; 24:1517–1534. [PubMed: 17004916]
3. Buzzi MG, Bonamini M, Moskowitz MA. Neurogenic model of migraine. *Cephalalgia*. 1995; 15:277–280. [PubMed: 7585923]
4. Ghelardini C, Galeotti N, Grazioli I, Uslenghi C. Indomethacin, alone and combined with prochlorperazine and caffeine, but not sumatriptan, abolishes peripheral and central sensitization in vivo models of migraine. *J Pain*. 2004; 5:413–419. [PubMed: 15501422]
5. Greco R, Gasperi V, Sandrini G, et al. Alterations of the endocannabinoid system in an animal model of migraine: Evaluation in cerebral areas of rat. *Cephalalgia*. 2010; 30:296–302. [PubMed: 19515121]
6. Phebus, LA.; Johnson, KW. Dural inflammation model of migraine pain. In: Crawley, JN., editor. *Curr Protoc Neurosci*. Vol. Chapter 9. 2001.
7. Shimazawa M, Hara H. An experimental model of migraine with aura: Cortical hypoperfusion following spreading depression in the awake and freely moving rat. *Clin Exp Pharmacol Physiol*. 1996; 23:890–892. [PubMed: 8911731]
8. Demirpence S, Kurul SH, Kiray M, Tugyan K, Yilmaz O, Köse G. The effect of sumatriptan on nitric oxide synthase enzyme production after iatrogenic inflammation in the brain stem of adolescent rats: A randomized, controlled, experimental study. *Curr Ther Res Clin Exp*. 2009; 70:129–135. [PubMed: 24683224]
9. Eikermann-Haerter K, Moskowitz MA. Animal models of migraine headache and aura. *Curr Opin Neurol*. 2008; 21:294–300. [PubMed: 18451713]
10. Malick A, Burstein R. Peripheral and central sensitization during migraine. *Funct Neurol*. 2000; 15(Suppl. 3):28–35. [PubMed: 11200798]

11. Oshinsky ML, Luo J. Neurochemistry of trigeminal activation in an animal model of migraine. *Headache*. 2006; 46:S39–S44. [PubMed: 16927963]
12. Reuter U, Sanchez Del Rio M, Moskowitz MA. Experimental models of migraine. *Funct Neurol*. 2000; 15:9–18. [PubMed: 11200808]
13. Wieseler J, Ellis A, Sprunger D, et al. A novel method for modeling facial allodynia associated with migraine in awake and freely moving rats. *J Neurosci Methods*. 2010; 185:236–245. [PubMed: 19837113]
14. 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; 10:389–411. [PubMed: 20187862]
15. Bramanti P, Grugno R, Vitetta A, Marino S, Di Bella P, Nappi G. Ictal and interictal hypoactivation of the occipital cortex in migraine with aura. A neuroimaging and electrophysiological study. *Funct Neurol*. 2005; 20:169–171. [PubMed: 16483455]
16. De Tommaso M, Guido M, Libro G, et al. Interictal lack of habituation of mismatch negativity in migraine. *Cephalalgia*. 2004; 24:663–668. [PubMed: 15265055]
17. Sand T, Zhitniy N, Nilsen KB, Helde G, Hagen K, Stovner LJ. Thermal pain thresholds are decreased in the migraine preattack phase. *Eur J Neurol*. 2008; 15:1199–1205. [PubMed: 18795945]
18. Schoenen J. Neurophysiological features of the migrainous brain. *Neurol Sci*. 2006; 27(Suppl. 2):S77–S81. [PubMed: 16688634]
19. Borsook D, Hargreaves R. Brain imaging in migraine research. *Headache*. 2010; 50:1523–1527. [PubMed: 20958298]
20. Ashkenazi A, Mushtaq A, Yang I, Oshinsky ML. Ictal and interictal phonophobia in migraine – A quantitative controlled study. *Cephalalgia*. 2009; 29:1042–1048. [PubMed: 19735532]
21. Ashkenazi A, Yang I, Mushtaq A, Oshinsky ML. Is phonophobia associated with cutaneous allodynia in migraine? *J Neurol Neurosurg Psychiatry*. 2010; 81:1256–1260. [PubMed: 20562466]
22. Oshinsky ML, Gommonchareonsiri S. Episodic dural stimulation in awake rats: A model for recurrent headache. *Headache (US)*. 2007; 47:1026–1036.
23. Burstein R, Cutrer MF, Yarnitsky D. The development of cutaneous allodynia during a migraine attack clinical evidence for the sequential recruitment of spinal and supraspinal nociceptive neurons in migraine. *Brain*. 2000; 123:1703–1709. [PubMed: 10908199]
24. Ramadan NM, Olesen J. Classification of headache disorders. *Semin Neurol*. 2006; 26:157–162. [PubMed: 16628525]
25. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J Neurosci Methods*. 1994; 53:55–63. [PubMed: 7990513]
26. Blaszczyk JW, Tajchert K, LapoLapo I, Sadowski B. Acoustic startle and open-field behavior in mice bred for magnitude of swim analgesia. *Physiol Behav*. 2000; 70:471–476. [PubMed: 11111000]
27. Berg WK, Balaban MT. Startle elicitation: Stimulus parameters, recording techniques, and quantification. *Startle Modification: Implications for Neuroscience, Cognitive Science, and Clinical Science*. 1999:21–50.
28. 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; 58:1531–8249.
29. Oshinsky ML. Insights from experimental studies into allodynia and its treatment. *Curr Pain Headache Rep*. 2006; 10:225–230. [PubMed: 18778578]
30. Levy D, Strassman AM, Burstein R. A critical view on the role of migraine triggers in the genesis of migraine pain. *Headache*. 2009; 49:953–957. [PubMed: 19545256]
31. Afridi SK, Matharu MS, Lee L, et al. A PET study exploring the laterality of brainstem activation in migraine using glyceryl trinitrate. *Brain*. 2005; 128:932–939. [PubMed: 15705611]
32. Burstein R, Jakubowski M. Unitary hypothesis for multiple triggers of the pain and strain of migraine. *J Comp Neurol (United States)*. 2005; 493:9–14.

33. Christiansen I, Daugaard D, Thomsen LL, Olesen J. Glyceryl trinitrate induced headache in migraineurs – Relation to attack frequency. *Eur J Neurol*. 2000; 7:405–411. [PubMed: 10971600]
34. Bigal ME, Ashina S, Burstein R, et al. Prevalence and characteristics of allodynia in headache sufferers: A population study. *Neurology*. 2008; 70:1525–1533. [PubMed: 18427069]
35. Christiansen I, Thomsen LL, Daugaard D, Ulrich V, Olesen J. Glyceryl trinitrate induces attacks of migraine without aura in sufferers of migraine with aura. *Cephalalgia*. 1999; 19:660–667. [PubMed: 10524660]
36. Hershey AD. Genetics of migraine headache in children. *Curr Pain Headache Rep*. 2007; 11:390–395. [PubMed: 17894930]
37. Gardner KL. Genetics of migraine: An update. *Headache*. 2006; 46(Suppl. 1):S19–S24. [PubMed: 16927960]
38. Vincent V. GTN in migraine. *Cephalalgia*. 1999; 19:626.
39. Villalón CM, Olesen J. The role of CGRP in the pathophysiology of migraine and efficacy of CGRP receptor antagonists as acute antimigraine drugs. *Pharmacol Ther*. 2009; 124:309–323. [PubMed: 19796656]
40. Edvinsson L, Ho TW. CGRP receptor antagonism and migraine. *Neurother*. 2010; 7:164–175.
41. Maxwell CR, Spangenberg RJ, Hoek JB, Silberstein SD, Oshinsky ML. Acetate causes alcohol hangover headache in rats. *PLoS ONE*. 2010; 5:e15963. [PubMed: 21209842]
42. Yi N, Xu S, George V, Allison DB. Mapping multiple quantitative trait loci for ordinal traits. *Behav Genet*. 2004; 34:3–15. [PubMed: 14739693]
43. DiPetrillo K, Wang X, Stylianou IM, Paigen B. Bio-informatics toolbox for narrowing rodent quantitative trait loci. *Trends Genet (England)*. 2005; 21:683–692.
44. Svendsen KB, Jensen TS, Hansen HJ, Bach FW. Sensory function and quality of life in patients with multiple sclerosis and pain. *Pain*. 2005; 114:473–481. [PubMed: 15777872]
45. Brefel-Courbon C, Payoux P, Thalamas C, et al. Effect of levodopa on pain threshold in Parkinson's disease: A clinical and positron emission tomography study. *Mov Disord*. 2005; 20:1557–1563. [PubMed: 16078219]
46. Vargas A, Vargas A, Hernandez-Paz R, et al. Sphygmomanometry-evoked allodynia-a simple bedside test indicative of fibromyalgia: A multi-center developmental study. *J Clin Rheumatol*. 2006; 12:272–274. [PubMed: 17149055]
47. McGeoch PD, Williams LE, Song T, Lee RR, Huang M, Ramachandran VS. Post-stroke tactile allodynia and its modulation by vestibular stimulation: A MEG case study. *Acta Neurol Scand*. 2008; 119:404–409. [PubMed: 18853944]
48. Ducreux D, Attal N, Parker F, Bouhassira D. Mechanisms of central neuropathic pain: A combined psychophysical and fMRI study in syringomyelia. *Brain*. 2006; 129:963–976. [PubMed: 16434417]
49. Honkasalo ML, Kaprio J, Winter T, Heikkilä K, Sillanpää M, Kaskenvuo M. Migraine and concomitant symptoms among 8167 adult twin pairs. *Headache*. 1995; 35:70–78. [PubMed: 7737864]
50. Russell MB, Olesen J. Increased familial risk and evidence of genetic factor in migraine. *Br Med J*. 1995; 311:541–544. [PubMed: 7663209]
51. Mulder EJ, Van Baal C, Gaist D, et al. Genetic and environmental influences on migraine: A twin study across six countries. *Twin Res*. 2003; 6:422–431. [PubMed: 14624726]
52. Freitag FG. Divalproex in the treatment of migraine. *Psychopharmacol Bull*. 2003; 37(Suppl. 2): 98–115. [PubMed: 15021865]
53. Kroner-Herwig B, Ruhmland M, Zintel W, Siniatchkin M. Are migraineurs hypersensitive? A test of the stimulus processing disorder hypothesis. *Eur J Pain*. 2005; 9:661–671. [PubMed: 16246819]
54. Duncko R, Cui L, Hille J, Grillon C, Merikangas KR. Startle reactivity in children at risk for migraine. *Clin Neurophysiol*. 2008; 119:2733–2737. [PubMed: 18951839]
55. Olesen J, Jansen-Olesen I. Nitric oxide mechanisms in migraine. *Pathol Biol*. 2000; 48:648–657. [PubMed: 11072643]

56. Lassen LH, Christiansen I, Iversen HK, Jansen-Olesen I, Olesen J. The effect of nitric oxide synthase inhibition on histamine induced headache and arterial dilatation in migraineurs. *Cephalalgia*. 2003; 23:877–886. [PubMed: 14616929]
57. Lassen LH, Haderslev PA, Jacobsen VB, Iversen HK, Sperling B, Olesen J. CGRP may play a causative role in migraine. *Cephalalgia*. 2002; 22:54–61. [PubMed: 11993614]
58. Offenhauser N, Zinck T, Hoffmann J, et al. CGRP release and c-fos expression within trigeminal nucleus caudalis of the rat following glyceryltrinitrate infusion. *Cephalalgia*. 2005; 25:225–236. [PubMed: 15689199]
59. Mogil JS. Animal models of pain: Progress and challenges. *Nat Rev Neurosci*. 2009; 10:283–294. [PubMed: 19259101]

Abbreviations

ASR	acoustic startle reflex
CGRP	calcitonin gene-related peptide
DHE	dihydroergotamine
GTN	glyceryl trinitrate
IS	inflammatory soup
i.v.	intravenous
NO	nitric oxide
NSAID	nonsteroidal anti-inflammatory drug
PPT	periorbital pain threshold
QTL	quantitative trait loci
SEM	standard error of the mean
TNC	trigeminal nucleus caudalis

Fig 1.

Trigeminal threshold tracking: These overlay graphs show von Frey periorbital pressure thresholds in normal rats, spontaneous allodynia rats and siblings of the spontaneous allodynia rats that do not have the low trigeminal von Frey threshold trait. (A) Von Frey thresholds in normal rats ($n = 10$) during 4 weeks of testing, 5 times per week for a total of 20 periorbital von Frey threshold tests. It is difficult to differentiate between the individual rats because there are 10 graphs plotted, and all of the rats responded to either 10 or 8 g. (B) Results of tracking spontaneous allodynia rats that display spontaneous periorbital allodynia ($n = 10$). Periorbital von Frey thresholds are displayed for 4 weeks of testing, 5 times per week. (C) Results of tracking the siblings of spontaneous allodynia rats that do not show the trait of spontaneous trigeminal allodynia.

Fig 2.

Trigeminal threshold tracking: A-J show the pattern of periorbital von Frey threshold changes in 10 individual spontaneous allodynia rats. Tests were done 5 times per week for 4 weeks. Thresholds were obtained in the morning, within 3 hours after the lights were turned on.

Fig 3.

Hindpaw testing. Hindpaw von Frey threshold testing in 12 individual spontaneous allodynia rats. Tests were done 3 times per week for 2 weeks for a total of 6 threshold tests. There was no response in any of the rats to the maximum von Frey value tested, 26 g. Rats that did not respond to this von Frey filament were assigned this value as their threshold for illustration purposes. Although there is only 1 line on the graph, the graph summarizes the data for 12 rats. All of the data points of the rats lay atop the points for the other rats because there was no variation in their thresholds during the 2 weeks of testing.

Fig 4.

Pedigree chart: Pedigree of the spontaneous allodynia trait resulting from 3 pairings, representing 3 generations. Rats who carry the spontaneous allodynia trait (male: black squares, females: black circles), and male and female rats without the trait are identified (white squares and white circles). The first mating listed in this pedigree is from the F7 generation.

Fig 5.

Phonophobia testing: The average (mean $\pm$ SEM) of the acoustic startle threshold (ASR) in normal rats compared to spontaneous allodynia rats. The threshold of normal rats before their infusions was 112.5 ± 2.9 dB. The spontaneous allodynia rats have an ASR threshold of 101.6 ± 2.2 dB. This corresponds to a difference of 10.9 dB between the 2 groups (*t*-test, $P < .011$).

Fig 6.

Headache trigger testing: Comparison of the effect of headache triggers on the von Frey head-withdrawal threshold between normal and spontaneous allodynia rats. The sensory testing was done before the injection of the chemical trigger and at several time points afterward. (A) GTN (0.1 mg/kg i.p.) decreased the threshold in both normal and spontaneous allodynia rats. After 1.5 hours, the normal rats recovered; the spontaneous allodynia rats maintained low thresholds for more than 5 hours. (* $P < .01$). (B) CGRP (4 µg/kg i.v.) decreased the threshold only in spontaneous allodynia rats. The duration of the effect was approximately 5 hours (* $P < .01$).

Fig 7.

Acute headache treatment testing: Comparison of the effect of acute headache treatments vs saline treatment on the von Frey head-withdrawal threshold in spontaneous allodynia rats. The sensory testing was done before the injection of the drug and up to 24 hours afterward. (A) Sumatriptan (1 mg/kg, i.p.) decreased the sensitivity of the spontaneous allodynia rats to periorbital testing for at least 5 hours (* $P < .001$). After 24 hours, the rats had returned to their low thresholds. (B) Ketorolac (0.4 mg/kg i.p.) decreased sensitivity of the spontaneous allodynia rats to periorbital testing beginning at 1.5 and lasted for at least 5 hours. After 24 hours, the rats had returned to their low thresholds. (C). DHE (0.1 mg/kg, i.p.) immediately decreased the sensitivity of the spontaneous allodynia rats to periorbital testing ($P < .001$). We tracked the rats for 2.5 hours after the injection; the effect was significant for the duration of the testing (* $P < .001$). After 24 hours, the next day, the rats had returned to their low thresholds.

Fig 8.

Preventive testing – Valproic acid (200 mg/kg, i.p.): Periorbital thresholds were tracked in spontaneous allodynia rats 5 times per week for 1 month ($n = 7$) before treatment, during treatment, and afterward, for a total of 20 sensory tests. (A) Before treatment, the thresholds were episodically changing and were often 4 g or below. After 3 days of valproic acid treatment, 5 of the 7 rats had thresholds of 10-15 g ($P < .001$, ANOVA). During the fourth week of treatment and after the injections were stopped, the periorbital von Frey thresholds decreased and all rats returned to episodically changing thresholds ($P = .124$, ANOVA). (B) Two of the 7 rats had no effect from the valproic acid treatment (Fig. 8B). These rats had the lowest thresholds, consistently below 2 g, in the pretesting phase.