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Hypothalamic and basal ganglia projections to the posterior thalamus: Possible role in modulation of migraine headache and photophobia

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Abstract

Migraine attacks are typically described as unilateral, throbbing pain that is usually accompanied by nausea, vomiting, and exaggerated sensitivities to light, noise and smell. The headache phase of a migraine attack is mediated by activation of the trigeminovascular pathway; a nociceptive pathway that originates in the meninges and carries pain signals through meningeal nociceptors to the spinal trigeminal nucleus and from there to the cortex through relay neurons in the thalamus. Recent studies in our lab have identified a population of trigeminovascular neurons in the posterior (Po) and lateral posterior (LP) thalamic nuclei that may be involved in the perception of whole-body allodynia (abnormal skin sensitivity) and photophobia (abnormal sensitivity to light) during migraine. The purpose of the current study was to identify sub-cortical areas that are in position to directly regulate the activity of these thalamic trigeminovascular neurons. Such process begins with anatomical mapping of neuronal projections to the posterior thalamus of the rat by performing discrete injections of the retrograde tracer Fluorogold into the Po/LP region. Such injections yielded retrogradely labeled neurons in the nucleus of the diagonal band of Broca, the dopaminergic cells group A11/A13, the ventromedial and ventral tuberomammillary nuclei of the hypothalamus. We also found that some of these neurons contain acetylcholine, dopamine, cholecystokinin and histamine, respectively. Accordingly, we speculate that these forebrain/hypothalamic projections to Po and LP may play a role in those migraine attacks triggered by disrupted sleep, skipping meals and emotional reactions.

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INTRODUCTION

It is generally believed that the headache phase of migraine is mediated by a neural circuit known as the trigeminovascular pathway, which includes a peripheral component of nociceptors innervating the meningeal tissue, and a central component including trigeminovascular neurons located in the spinal trigeminal nucleus (Sp5C), thalamus and cortex. The trigeminovascular pathway is thought to be activated during migraine through a complex neurovascular interplay that involves pro-inflammatory neuropeptides released by activated meningeal nociceptors and proinflammatory molecules released by meningeal mast cells, platelets, and dilated meningeal arteries (Goadsby et al., 2009, Olesen et al., 2009). However, despite much progress in the understanding of migraine pathophysiology, the anatomical and functional relationship between activation of the trigeminovascular pathway and the accompanying neurological symptoms is largely unknown.

In the past few years, it has been shown that the development of whole-body allodynia (abnormal skin sensitivity all over the body) during migraine is mediated by a group of trigeminovascular neurons in the posterior-most region of the thalamus that process nociceptive information from the entire body. To verify the clinical relevance of these findings, migraine patients exhibiting whole-body allodynia were scanned using fMRI imaging techniques and found abnormal neuronal activation in the pulvinar, a nucleus located in the most posterior region of the human thalamus (Burstein et al., 2010).

Along with that study, we have also shown that the spontaneous activity of some of these thalamic trigeminovascular neurons increased dramatically under ambient illumination and returned to baseline in dark conditions. Using a combination of anatomical and electrophysiological techniques, we then found that those neurons located in the posterior (Po) and lateral posterior (LP) thalamic nuclei integrate photic signals from intrinsically photosensitive retinal ganglion cells with nociceptive signals from the meninges. Again, we explored the clinical relevance of these findings by obtaining testimonies of blind migraine patients with different retinal pathologies that ended up in complete or partial blindness. These findings gave rise to a groundbreaking understanding of the mechanism that underlies migraine-type photophobia, defined as exacerbation of headache by light (Nosedá et al., 2010).

Based on these studies (Burstein et al., 2010, Nosedá et al., 2010), we sought to unravel other sources of input that Po/LP region receive in order to identify additional factors that may play a role in the modulation of migraine headache and its exacerbation by exposure to light. Specifically, we focused on the possibility that sub-cortical areas involved in regulation of sleep, food intake, and mood contain neurons whose axons project directly to this thalamic region. The premise behind this anatomical study is that the connectivity of the brain is pivotal in defining a better framework at which we think about migraine pathophysiology.

METHODS

Animals

A total of 25 male Sprague-Dawley rats weighting 250–350 g were used in this study. Experiments were conducted in accordance with NIH guidelines and approved by the Institutional Animal Care and Use Committee at Harvard Medical School and Beth Israel Deaconess Medical Center.

Iontophoresis of the retrograde tracer fluorogold into Po/LP

Rats were initially anesthetized with a single dose of Brevital sodium (45 mg/kg i.p.) to allow endotracheal intubation. Each rat was then mounted on a stereotaxic frame, and the general anesthesia was switched to vaporized isoflurane (2% in O₂; 100 ml/min) delivered through an automatic ventilation system. Body temperature, breathing and heart rates, blood oxygen saturation, and end-tidal CO₂ levels were continuously monitored and maintained within a physiological range. A craniotomy was performed at the right parietal bone to allow the access of a glass micropipette (40 µm diameter tip) into the posterior thalamus for iontophoretic administration of the retrograde tracer fluorogold (2% in dH₂O; Fluorochrome, LLC). Fluorogold (FG) was injected for 10–15 min by delivering pulses of positive current (5 µA) at 10 s on/off intervals, as described elsewhere (Nosedá et al., 2010). After the injection, micropipettes were pulled out of the brain, wounds were sutured and disinfected, and each animal was allowed to recover in their cages for 72 h.

Immunohistochemistry and Immunofluorescence

Rats were re-anesthetized with an overdose of pentobarbital sodium (100 mg/kg) and perfused with 200 ml of warm (37°C) heparinized saline, followed by 400 mL of a fixative solution containing phosphate-buffered solution (PBS; 0.2 M; pH 7.3), 4% paraformaldehyde, and 0.05% picric acid. The brain was removed and soaked in the later solution for 2 h, then cryoprotected in 30% sucrose phosphate buffer for 48 h. Tissue was frozen and cut into serial coronal sections (60 µm thick) using a cryostat (Leica). For immunohistochemistry, free-floating sections were processed in the following sequence: (a) quenching with 3:1 methanol/PBS solution containing 1% H₂O₂ for 1 h; (b) pre-incubation with PBS solution containing 2% fetal bovine serum (FBS) and 1% Triton X-100 for 1 h; (c) incubation with the primary antibody—rabbit antifuorogold (1:5,000; Millipore) for 48 h at 4°C; (d) rinsing with PBS and incubation with the secondary antibody—biotinylated goat anti-rabbit (1:500; Jackson ImmunoResearch) for 2 h; (e) rinsing, amplification and labeling using avidin-biotin complex kit (ABC, Vector Labs) and DAB-nickel kit (Vector Labs). For immunofluorescence, free-floating sections were pre-incubated with PBS solution containing 2% FBS and 1% Triton X-100 for 1 h; then incubated for 48 h at 4°C with one of the following primary antibodies: (i) goat anti-Choline Acetyltransferase, ChAT (1:1,000; Millipore); (ii) guinea pig anti-Histidine Decarboxylase, HDC (1:1,000; ARP, Inc); (iii) mouse anti-Tyrosine Hydroxylase, TH (1:5,000; Immunostar); (iv) rabbit anti-Cholecystokinin Octapeptide, CCK-8 (1:5,000; Immunostar); and finally, rinsed and incubated for 1–2 h at room temperature with the corresponding fluorescent secondary antibody (Alexa Fluor 594; Invitrogen) against the Ig's of the animal in which the primary antibody was raised (dilution range 1:200 – 1:500). Consecutive immunostained sections

were mounted on glass slides, coverslipped with Cytoseal or fluorescent mounting media, and analyzed under the microscope.

Digital Imaging and Tissue Analysis

Digital imaging of retrograde labeling and immunoreactive cell bodies for the different neuronal biomarkers was performed using bright field and epifluorescence scanning microscopy that compiled 1–1.5 μm -thick scans using z-stacking software (Leica). Injection sites in Po/LP region and retrogradely-labeled neurons in the forebrain and hypothalamus were mapped using a microscope (Olympus) equipped with camera lucida and both bright- and darkfield. Immunofluorescent labeling of FG was visualized by excitation/emission at 360/408 nm (blue). The blue color emitted by FG was colored green for presentation purposes. For the neuronal labeling of the biomarkers with Alexa Fluor 594, the signal was detected by excitation/emission at 590/617 nm (red). Photomicrographs of co-labeling were achieved by superimposition of the red and green channels. Location of neuronal cell bodies and delimitation of anatomical structures were based on an atlas of the rat brain (Paxinos and Watson, 2008).

RESULTS

Injection sites into thalamic Po/LP area

In order to identify forebrain/hypothalamic nuclei projecting to the Po/LP area that contains thalamic trigeminovascular neurons, 19 out of 25 iontophoretic injections (one/rat) of FG were selected for further analysis on the basis of their restricted locations and sizes. The anatomical analysis of all 19 selected FG injection sites shows that the tracer covers the dorsal most half of Po, the dorsal and ventral aspects of LP (laterorostral part, LPLR; and mediorostral part, LPMR), the caudal most aspect of the laterodorsal thalamic nucleus (ventrolateral part, LDVL), and a small area of the dorsomedial aspect of the ventral posteromedial nucleus of the thalamus (VPM) from – 3.0 to –4.0 mm behind *bregma* (Fig. 1). Camera lucida reconstructions of all 19 injection sites are depicted in Figure 1A. A representative injection site covering the dorsal most aspect of Po and the whole depth of LP is shown in Figure 1B. The retrograde labeling obtained from this injection was used for mapping the location of neuronal cell bodies in the forebrain and hypothalamic regions of interest.

Retrograde labeling from thalamic Po/LP area and co-labeling with neuronal markers of Acetylcholine, Dopamine, Cholecystikinin and Histamine

As shown in Figure 2, the location of basal forebrain neurons projecting to the Po/LP area is mainly restricted to the horizontal limb of the diagonal band of Broca (HDB), and for a lesser extent, to the vertical limb of the diagonal band of Broca (VDB), covering an area from 1.5 to – 1.0 mm in relation to *bregma* (Fig. 2A). Based on the labeling obtained from the injection site presented in Figure 1, a total number of 97 neurons (93 in HDB, 4 in VDB) were counted in these 2 nuclei. Taking into account all the injected brains processed for immunofluorescence, 48% of these projecting neurons were immunoreactive to ChAT (Fig. 2B).

The largest amount of neurons projecting to the Po/LP area was found in three different nuclei of the hypothalamus. First, the A11/A13 dopaminergic cells groups of the dorsal hypothalamus contained a total of 148 retrogradely-labeled neurons from Po/LP, covering an area from -2.0 to -4.5 mm in relation to *bregma* (Fig. 3A). A large proportion of them (77%) were co-labeled with TH (Fig. 3B). Second, the ventromedial hypothalamic nucleus (VMH) contained the densest population of neurons from -1.8 to -3.4 mm that projects to the posterior thalamic area (192 neurons; Fig. 4A). Scattered neurons were also observed in the ventrolateral nucleus of the posterior hypothalamic area (VLH; not shown). As shown in Figure 4B, the VMH presents a dense pattern of terminal labeling immunopositive for CCK, which is intermingled with most neurons labeled with FG that are restricted to the VMH (Fig. 4C). Finally, the ventral tuberomammillary nucleus of the hypothalamus (VTM) accounted for 35 neurons between -3.7 and -4.7 mm *bregma* (Fig. 5A–B). From all injections, nearly 41% of these projecting neurons were co-labeled with HDC (Fig. 5C).

Although we choose to focus on sub-cortical structures that may be relevant to migraine pathophysiology, we also found retrograde labeling from well-known projecting areas such as the cortex, reticular thalamic nucleus, spinal cord, spinal trigeminal nuclei and medial lemniscus. Other projections to the Po/LP area were found in the zona incerta, superior colliculus, periaqueductal gray and locus coeruleus. A schematic representation summarizing the projections described in this study, as well as the trigeminovascular pathway and retinal projections to the posterior thalamus thought to be involved in migraine pain and photophobia is shown in Figure 6.

DISCUSSION

Using retrograde tracing we demonstrated direct projection to Po/LP thalamic area from neurons located in the forebrain nucleus of the diagonal band of Broca, dopaminergic cells group of the hypothalamus, ventromedial and ventral tuberomammillary nucleus of the hypothalamus. Such direct connections may play a role in the onset of early morning migraines or those precipitated by insufficient sleep, skipping meals, stress and anxiety. These projections can also contribute to prodromes such as food craving and nausea.

Migraine attacks often begin between 4 and 6 AM (Gori et al., 2005, Aguggia et al., 2011, Gori et al., 2012). Mechanistically, it has been difficult to determine whether it is because of the headache that patients wake up or because of the sleep disruption that the migraine is triggered. In principle, any brain area that regulates sleep pattern and communicate with trigeminovascular neurons may be considered as candidate. In this study, we traced such connections from two areas that regulate, at least in part, the sleep-wake cycle – the DBB and the VTM. The DBB contains cholinergic neurons whose activity has been associated with sleep homeostasis (Monti and Jantos, 2004, Kalinchuk et al., 2006). Remarkably, evidence supporting the involvement of acetylcholine in migraine has emerged in clinical studies showing that centrally acting cholinesterase inhibitors are effective in preventing migraine (Nicolodi et al., 2002), and that anti-cholinergic drugs trigger migraine-like headaches and worsen the headache during ongoing attacks in migraineurs (Ikeda et al., 2009). Although the drugs used in these studies may act in the peripheral vasculature, central effects on cholinergic DBB neurons are also possible. Actually, effective triggering

of migraine-like headaches can be achieved by nitric oxide donors' infusion, which has been shown to alter DBB cholinergic neuronal activity (Kostin et al., 2008).

On the other hand, the VTM is the sole provider of histamine within the CNS (Haas and Panula, 2003). Because histaminergic neurons project heavily to the cortex, and begin to fire at the end of the sleep period (Takahashi et al., 2006), histamine release has been implicated in the awakening process and wakefulness (Schwartz et al., 1991, Lin et al., 1996, Steininger et al., 1999). Also, a recent study provided evidence for a functional link between DBB and VTM, in which the awakening-related firing of VTM neurons releases histamine onto cholinergic DBB neurons that in turn induce cortical activation and facilitate awakening (Zant et al., 2012). Since histamine can trigger a migraine (Krabbe and Olesen, 1980, Lassen et al., 1995), and since many migraine begin in the early hours of the morning, the results obtained in this study prompted us to propose 3 different scenarios for migraines triggered by firing of VTM neurons: (a) VTM firing leads to massive release of histamine in the cortex which then diffuses to cerebral blood vessels and induce the release of nitric oxide which in turn activates meningeal nociceptors; (b) the massive release of histamine triggers a morning migraine by activating meningeal nociceptors directly; and (c) firing of VTM neurons activates dura- and dura/light-sensitive Po/LP neurons through the projections described in this study.

The hallmark of migraine headache is its unique host of associated symptoms including nausea and vomiting. Interestingly, intravenous administration of dopamine agonists induces the premonitory symptoms commonly seen in migraineurs several hours before the onset of an attack such as nausea, vomiting, yawning and hyperosmia, (Cerbo et al., 1997, Giffin et al., 2003). During the headache phase of migraine, the role of dopamine is less clear. There are, however, some reports showing that infusion of commonly used D2 receptor blockers may have some effectiveness in alleviating migraine headache and its most common associated symptoms (Tek et al., 1990, Bigal et al., 2002, Colman et al., 2004, Marmura, 2012). Although dopamine may be involved in migraine (Sicuteri, 1977, Peroutka, 1997, Charbit et al., 2010), conflicting data using dopaminergic drugs have hampered the proposal of a unitary hypothesis about its role in migraine pathophysiology. Nevertheless, based on the dopaminergic pathway described in this study, abnormal firing of A11/A13 neurons during premonitory symptoms may alter the excitability of dura- and dura/light-sensitive neurons in Po/LP, and thus, contribute to the development of migraine headache and the associated nausea and vomiting.

Migraine can be triggered by skipping a meal and be relieved by eating. We observed labeling in both VMH and VLH hypothalamic areas, whose neurons regulate food intake through glucose-sensing properties (Qu et al., 1996, Sakurai et al., 1998, Elmquist et al., 1999, Kang et al., 2004). Extensive review of the literature suggests that activation of VMH neurons signal satiety and is associated with cessation of feeding, whereas activation of VLH signal hunger and thus is associated with food intake (Gao and Horvath, 2007). Accordingly, it is reasonable to speculate that GABAergic VMH neurons that are activated by food intake (Karnani and Burdakov, 2011) may inhibit thalamic trigeminovascular processing through the projections described in this study, and thus prevent or alleviate ongoing attacks. Also, migraine triggered by skipping a meal may be mediated through

activation of orexinergic VLH neurons that signal hunger and promote food intake, and that the prodrome of food craving (which begins 4–12 hrs before onset of headache) is mediated by abnormal activity of hypothalamic neurons that regulate feeding behavior in general. These speculations are further supported by recent surge of publications on the potential role the hypothalamus may play in the pathophysiology of migraine (Malick et al., 2001, Denuelle et al., 2007, Akerman et al., 2011, Nosedá and Burstein, 2013).

Because migraine is associated with a unique set of neurological symptoms, and is well known to be influenced by sleep, feeding and emotions, the projections from the basal ganglia and hypothalamus to Po/LP may be relevant for these associations. However, given that the posterior thalamic area is involved in the processing of diverse sensory modalities, we cannot rule out that the pathways described in this study can also influence sensory processing that is not related to migraine headache. Therefore, specific interactions between these pathways and thalamic trigeminovascular neurons should be considered for future investigations seeking to understand the initiation of migraine and the development of its associated symptoms.

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REFERENCES

- Aguggia M, Cavallini M, Divito N, Ferrero M, Lentini A, Montano V, Tinebra MC, Saracco MG, Valfre W. Sleep and primary headaches. *Neurol Sci.* 2011; 32(Suppl 1):S51–S54. [PubMed: 21533713]
- Akerman S, Holland PR, Goadsby PJ. Diencephalic and brainstem mechanisms in migraine. *Nat Rev Neurosci.* 2011; 12:570–584. [PubMed: 21931334]
- Bigal ME, Bordini CA, Speciali JG. Intravenous chlorpromazine in the emergency department treatment of migraines: a randomized controlled trial. *The Journal of emergency medicine.* 2002; 23:141–148. [PubMed: 12359281]
- Burstein R, Jakubowski M, Garcia-Nicas E, Kainz V, Bajwa Z, Hargreaves R, Becerra L, Borsook D. Thalamic sensitization transforms localized pain into widespread allodynia. *Ann Neurol.* 2010; 68:81–91. [PubMed: 20582997]
- Cerbo R, Barbanti P, Buzzi MG, Fabbrini G, Brusa L, Roberti C, Zanette E, Lenzi GL. Dopamine hypersensitivity in migraine: role of the apomorphine test. *Clinical neuropharmacology.* 1997; 20:36–41. [PubMed: 9037571]
- Charbit AR, Akerman S, Goadsby PJ. Dopamine: what's new in migraine? *Curr Opin Neurol.* 2010; 23:275–281. [PubMed: 20164768]
- Colman I, Brown MD, Innes GD, Grafstein E, Roberts TE, Rowe BH. Parenteral metoclopramide for acute migraine: meta-analysis of randomised controlled trials. *BMJ.* 2004; 329:1369–1373. [PubMed: 15550401]
- Denuelle M, Fabre N, Payoux P, Chollet F, Geraud G. Hypothalamic activation in spontaneous migraine attacks. *Headache.* 2007; 47:1418–1426. [PubMed: 18052951]
- Elmqvist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. *Neuron.* 1999; 22:221–232. [PubMed: 10069329]
- Gao Q, Horvath TL. Neurobiology of feeding and energy expenditure. *Annu Rev Neurosci.* 2007; 30:367–398. [PubMed: 17506645]
- Giffin NJ, Ruggiero L, Lipton RB, Silberstein SD, Tvedskov JF, Olesen J, Altman J, Goadsby PJ, Macrae A. Premonitory symptoms in migraine: an electronic diary study. *Neurology.* 2003; 60:935–940. [PubMed: 12654956]

- Goadsby PJ, Charbit AR, Andreou AP, Akerman S, Holland PR. Neurobiology of migraine. *Neuroscience*. 2009; 161:327–341. [PubMed: 19303917]
- Gori S, Lucchesi C, Morelli N, Maestri M, Bonanni E, Murri L. Sleep-related migraine occurrence increases with aging. *Acta neurologica Belgica*. 2012; 112:183–187. [PubMed: 22450708]
- Gori S, Morelli N, Maestri M, Fabbrini M, Bonanni E, Murri L. Sleep quality, chronotypes and preferential timing of attacks in migraine without aura. *J Headache Pain*. 2005; 6:258–260. [PubMed: 16362680]
- Haas H, Panula P. The role of histamine and the tuberomammillary nucleus in the nervous system. *Nat Rev Neurosci*. 2003; 4:121–130. [PubMed: 12563283]
- Ikedo K, Kawase Y, Takazawa T, Iwamoto K, Ito H, Murata K, Sato R, Nagaoka T, Sekine T, Nagata R, Nakamura Y, Hirayama T, Ishikawa Y, Miura K, Yoshii Y, Iwasaki Y. Scopolamine-induced migraine like headache. *Intern Med*. 2009; 48:681–685. [PubMed: 19420813]
- Kalinchuk AV, Lu Y, Stenberg D, Rosenberg PA, Porkka-Heiskanen T. Nitric oxide production in the basal forebrain is required for recovery sleep. *J Neurochem*. 2006; 99:483–498. [PubMed: 17029601]
- Kang L, Routh VH, Kuzhikandathil EV, Gaspers LD, Levin BE. Physiological and molecular characteristics of rat hypothalamic ventromedial nucleus glucosensing neurons. *Diabetes*. 2004; 53:549–559. [PubMed: 14988237]
- Karnani M, Burdakov D. Multiple hypothalamic circuits sense and regulate glucose levels. *American journal of physiology Regulatory, integrative and comparative physiology*. 2011; 300:R47–R55.
- Kostin A, Stenberg D, Kalinchuk AV, Porkka-Heiskanen T. Nitric oxide modulates the discharge rate of basal forebrain neurons. *Psychopharmacology (Berl)*. 2008; 201:147–160. [PubMed: 18661122]
- Krabbe AA, Olesen J. Headache provocation by continuous intravenous infusion of histamine. *Clinical results and receptor mechanisms*. *Pain*. 1980; 8:253–259. [PubMed: 7402688]
- Lassen LH, Thomsen LL, Olesen J. Histamine induces migraine via the H1- receptor. Support for the NO hypothesis of migraine. *Neuroreport*. 1995; 6:1475–1479. [PubMed: 7579128]
- Lin JS, Hou Y, Sakai K, Jouvett M. Histaminergic descending inputs to the mesopontine tegmentum and their role in the control of cortical activation and wakefulness in the cat. *J Neurosci*. 1996; 16:1523–1537. [PubMed: 8778302]
- 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; 98:9930–9935. [PubMed: 11504950]
- Marmura MJ. Use of dopamine antagonists in treatment of migraine. *Current treatment options in neurology*. 2012; 14:27–35. [PubMed: 22012659]
- Monti JM, Jantos H. Microinjection of the nitric oxide synthase inhibitor LNAME into the lateral basal forebrain alters the sleep/wake cycle of the rat. *Progress in neuro-psychopharmacology & biological psychiatry*. 2004; 28:239–247. [PubMed: 14751418]
- Nicolodi M, Galeotti N, Ghelardini C, Bartolini A, Sicuteri F. Central cholinergic challenging of migraine by testing second-generation anticholinesterase drugs. *Headache*. 2002; 42:596–602. [PubMed: 12482211]
- Nosedo R, Burstein R. Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neurological symptoms, CSD, sensitization and modulation of pain. *Pain*. 2013 in press.
- Nosedo R, Kainz V, Jakubowski M, Gooley JJ, Saper CB, Digre K, Burstein R. A neural mechanism for exacerbation of headache by light. *Nat Neurosci*. 2010; 13:239–245. [PubMed: 20062053]
- Olesen J, Burstein R, Ashina M, Tfelt-Hansen P. Origin of pain in migraine: evidence for peripheral sensitisation. *Lancet Neurol*. 2009; 8:679–690. [PubMed: 19539239]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*. Academic Press; 2008.
- Peroutka SJ. Dopamine and migraine. *Neurology*. 1997; 49:650–656. [PubMed: 9305317]
- Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, Mathes WF, Przypek R, Kanarek R, Maratos-Flier E. A role for melanin-concentrating hormone in the central regulation of feeding behaviour. *Nature*. 1996; 380:243–247. [PubMed: 8637571]
- Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, Williams SC, Richardson JA, Kozlowski GP, Wilson S, Arch JR, Buckingham RE, Haynes AC, Carr SA, Annan RS, McNulty

- DE, Liu WS, Terrett JA, Elshourbagy NA, Bergsma DJ, Yanagisawa M. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*. 1998; 92:573–585. [PubMed: 9491897]
- Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M. Histaminergic transmission in the mammalian brain. *Physiological reviews*. 1991; 71:1–51. [PubMed: 1846044]
- Sicuteri F. Dopamine, the second putative protagonist in headache. *Headache*. 1977; 17:129–131. [PubMed: 893082]
- Steininger TL, Alam MN, Gong H, Szymusiak R, McGinty D. Sleep-waking discharge of neurons in the posterior lateral hypothalamus of the albino rat. *Brain Res*. 1999; 840:138–147. [PubMed: 10517961]
- Takahashi K, Lin JS, Sakai K. Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. *J Neurosci*. 2006; 26:10292–10298. [PubMed: 17021184]
- Tek DS, McClellan DS, Olshaker JS, Allen CL, Arthur DC. A prospective, double-blind study of metoclopramide hydrochloride for the control of migraine in the emergency department. *Annals of emergency medicine*. 1990; 19:1083–1087. [PubMed: 2221512]
- Zant JC, Rozov S, Wigren HK, Panula P, Porkka-Heiskanen T. Histamine release in the basal forebrain mediates cortical activation through cholinergic neurons. *J Neurosci*. 2012; 32:13244–13254. [PubMed: 22993440]

Abbreviations

Hb	habenula
MD	mediodorsal thalamic
PC	paracentral thalamic
CL	centrolateral thalamic
VM	ventromedial thalamic
LPMR	lateral posterior thalamic, mediorostral part
LPLR	lateral posterior thalamic, laterorostral part
LDVL	laterodorsal thalamic, ventrolateral part
Po	posterior thalamic
VPM	ventral posteromedial thalamic
VPL	ventral posterolateral thalamic
AV	anteroventral thalamic
VL	ventrolateral thalamic
CM	central medial thalamic
ZI	zona incerta
DLG	dorsal lateral geniculate
VLG	ventral lateral geniculate
Rt	reticular thalamic
VPPC	ventral posterior thalamic, parvicellular
fr	fasciculus retroflexus

PF	parafascicular thalamic
MS	medial septum
VDB	vertical limb of the diagonal band of broca
HDB	horizontal limb of the diagonal band of broca
CPu	caudate putamen
ec	external capsule
aca	anterior commissure
3V	third ventricle
LV	lateral ventricle
A11/13	dopaminergic cells group of the hypothalamus
Pa	paraventricular hypothalamic
f	fornix
mt	mammillothalamic tract
ic	internal capsule
VMH	ventromedial hypothalamus
opt	optic tract
cp	cerebral peduncle
Arc	arcuate hypothalamic
DM	dorsomedial hypothalamic
PH	posterior hypothalamus
VTM	ventral tuberomammillary
pm	principal mammillary tract
MRe	mammillary recess 3V.

HIGHLIGHTS

- Forebrain sends cholinergic projections to thalamic trigeminovascular area.
- A11/13 nuclei send dopaminergic projections to thalamic trigeminovascular area.
- Ventromedial hypothalamus sends projections to thalamic trigeminovascular area.
- Histaminergic projections innervate thalamic area of trigeminovascular processing.

Figure 1.

Injection sites of the retrograde tracer fluorogold into Po/LP thalamic area. (A) Coronal view illustrating the location and extent of all FG injection sites into the thalamus using camera lucida reconstruction. Numbers indicate distance from *bregma*. (B) Bright-field image showing a representative injection site of FG into Po/LP area. Note that the center of the injection is located in the dorsal most part of Po, and spreads to the LP nucleus. Scale bars = A–B: 500 μ m.

Figure 2.

Retrograde labeling in the forebrain VDB and HDB nuclei from thalamic Po/LP. (A) Camera lucida reconstruction of brain slices containing all the retrogradely labeled neurons in VDB/HDB from the injection shown in Figure 1B. Numbers indicate distance from *bregma*. (B) Fluorescent images from top to bottom: fluorogold-labeled neurons (green) in the HDB; Choline Acetyltransferase (ChAT) staining (red) in the HDB; Merged images indicating double labeled neurons (yellow) with arrowheads. Scale bars = A: 1 mm, B: 100 μm.

Figure 3.

Retrograde labeling in the hypothalamic A11/A13 dopaminergic cells groups from thalamic Po/LP. (A) Camera lucida reconstruction of brain slices containing all the retrogradely labeled neurons in A11/A13 from the injection shown in Figure 1B. Numbers indicate distance from *bregma*. (B) Fluorescent images from top to bottom: fluorogold-labeled neurons (green) in the A11 nucleus; Tyrosine Hydroxylase (TH) staining (red) in A11; Merged images indicating double labeled neurons (yellow) with arrowheads. Scale bars = A: 1mm, B: 100µm.

Figure 4.

Retrograde labeling in the VMH nucleus from thalamic Po/LP. (A) Camera lucida reconstruction of brain slices containing all the retrogradely labeled neurons in VMH from the injection shown in Figure 1B. Numbers indicate distance from *bregma*. (B) Low-power magnification image showing the axonal pattern of Cholecystikinin immunoreactivity (white) in the hypothalamus. (C) Fluorescent image showing retrogradely labeled neurons with FG (green) intermingled with CCK positive fibers (red). Scale bars = A: 1mm, B–C: 100 μ m.

Figure 5.

Retrograde labeling in the VTM nucleus of the hypothalamus from thalamic Po/LP. (A) Camera lucida reconstruction of brain slices containing all the retrogradely labeled neurons in VTM from the injection shown in Figure 1B. Numbers indicate distance from *bregma*. (B) Bright-field image showing FG labeled neurons in VTM processed with immunohistochemistry. (C) Fluorescent images from top to bottom: fluorogold-labeled neurons (green) in the VTM; Histidine Decarboxylase (HDC) staining (red) in the VTM; FG + HDC

Merged images indicating double labeled neurons (yellow) with arrowheads. Scale bars = A: 1mm, B: 50 μ m, C: 100 μ m.

Figure 6.

Schematic illustration of the proposed pathways involved in the modulation of migraine headache and photophobia at thalamic level. The peripheral (meningeal nociceptors) and central (trigemino-thalamic) components of the trigeminovascular pathway are shown in blue. The axons of retinal ganglion cells that carry photic information to the posterior thalamus are shown in red. Green arrows represent the projections described in this study. Arrow thickness represents the relative contribution of each pathway to the modulation of thalamic activity based on the density of retrogradely-labeled projecting neurons obtained in this study and Nosedá et al., 2010.