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REVIEW

Modelling headache and migraine and its pharmacological manipulation

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Similarities between laboratory animals and humans in anatomy and physiology of the cephalic nociceptive pathways have allowed scientists to create successful models that have significantly contributed to our understanding of headache. They have also been instrumental in the development of novel anti-migraine drugs different from classical pain killers. Nevertheless, modelling the mechanisms underlying primary headache disorders like migraine has been challenging due to limitations in testing the postulated hypotheses in humans. Recent developments in imaging techniques have begun to fill this translational gap. The unambiguous demonstration of cortical spreading depolarization (CSD) during migraine aura in patients has reawakened interest in studying CSD in animals as a noxious brain event that can activate the trigeminovascular system. CSD-based models, including transgenics and optogenetics, may more realistically simulate pain generation in migraine, which is thought to originate within the brain. The realization that behavioural correlates of headache and migrainous symptoms like photophobia can be assessed quantitatively in laboratory animals, has created an opportunity to directly study the headache in intact animals without the confounding effects of anaesthetics. Headache and migraine-like episodes induced by administration of glyceryltrinitrate and CGRP to humans and parallel behavioural and biological changes observed in rodents create interesting possibilities for translational research. Not unexpectedly, species differences and model-specific observations have also led to controversies as well as disappointments in clinical trials, which, in return, has helped us improve the models and advance our understanding of headache. Here, we review commonly used headache and migraine models with an emphasis on recent developments.

LINKED ARTICLES
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Abbreviations
CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CGRP, calcitonin gene-related peptide; CSD, cortical spreading depolarization; FHM, familial hemiplegic migraine; GTN, glyceryltrinitrate; HMGB1, high mobility group protein-B1; IS, inflammatory soup; MGS, Mouse Grimace Scale; MMA, middle meningeal artery; NK_1, neurokinin-1; NSAID, non-steroidal anti-inflammatory drug; PAC_1, PACAP receptor type 1; PACAP, pituitary adenylate cyclase-activating polypeptide; PPE, plasma protein extravasation; TCC, trigeminocervical complex; TG, trigeminal ganglion; TNC, trigeminal nucleus caudalis; TRPA1, transient receptor potential channel-A1; TVS, trigeminovascular system; VIP, vasoactive intestinal polypeptide; VPAC, VIP receptor

Introduction

About half of the adult population suffers from headache at least once annually according to the World Health Organization. The majority of these headaches are caused by primary headache disorders and have no serious underlying medical problem. Migraine is one of the most common primary headache disorders, affecting about 1/6 of the population

(Headache Classification Committee of the International Headache Society, 2013). It is usually characterized by a unilateral, throbbing headache aggravated by exercise and accompanied by nausea, vomiting, sensitivity to light and sound (Headache Classification Committee of the International Headache Society, 2013). In approximately 20–30% of patients, a transient neurological dysfunction called aura precedes the headache by 20–60 min. With a complex and yet incompletely understood pathophysiology, migraine headache has been subject of extensive clinical and experimental research. Advances in the last decades have led to the development of effective migraine-specific novel drugs with the help of various experimental headache models. Although no model is able to simulate all aspects of this complex paradigm, they have been instrumental in the scientific progress achieved. The aim of this report is to review the currently available headache/migraine models by focusing particularly on recent developments.

Pathophysiology of migraine headache

Pain-sensitive intracranial structures, such as dura mater, venous sinuses, meningeal and pial arteries are richly innervated by afferent sensory branches of the trigeminocervical nerves (Penfield, 1932; Ray and Wolff, 1940; Mayberg *et al.*, 1984; Figure 1). This structure composed of blood vessels, trigeminal afferents and their central projections is called the trigeminovascular system (TVS; Andreou *et al.*, 2010; Edvinsson, 2011; Noseda and Burstein, 2013). Cell bodies of trigeminal afferents lie in the trigeminal ganglion (TG). Mainly Aδ and C-type nociceptive fibres are responsible for conduction of pain signals to the brain. Central projections of the trigeminocervical neurons terminate on the second-order nociceptive neurons within the trigeminocervical complex (TCC; Kaube *et al.*, 1993; Burstein *et al.*, 1998; Strassman and Levy, 2006; Andreou *et al.*, 2010). TCC extends from the rostral pons to upper cervical spinal cord levels. Nociceptive neurons in TCC cross to the other side, ascend rostrally and terminate on the third-order neurons in thalamic nuclei, mainly within the ventralposteromedial (also posterior and parafascicular) nucleus (Akerman *et al.*, 2011; Noseda and Burstein, 2013). Thalamic neurons project to the primary somatosensory cortex, insular cortex, limbic structures and hypothalamus for conscious perception of the headache as well as to modify pain-related behaviours and autonomic responses (Noseda and Burstein, 2013; Pietrobon and Moskowitz, 2013).

During migraine headache, the TVS becomes sensitized and remains overactive for an extended period of time. Activated perivascular nociceptive afferents release vasoactive mediators (mainly calcitonin gene-related peptide, substance P, neurokinin A and pituitary adenylate cyclase-activating polypeptide), trigger mast cell degranulation and cause sterile neurogenic inflammation in dura mater (Moskowitz, 1984; Markowitz *et al.*, 1987; Levy *et al.*, 2007). Additionally, a mono-synaptic reflex arc between the TCC and superior salivatory nucleus activates the parasympathetic nerve endings around dural blood vessels (which release ACh, NO and vasoactive intestinal polypeptide) and cause vasodilatation (Noseda and Burstein, 2013; Pietrobon and Moskowitz, 2013). The neurogenic inflammation probably sustains the nociceptive activity and sensitizes afferents (Burstein, 2001; Dalkara *et al.*, 2006; Levy, 2012). Sensitization of perivascular afferents to mechanical stimuli is thought to account for the characteristic pulsatile, throbbing migraine headache although a recent work has challenged this view by showing that subjective report of the rhythm of throbbing sensation is distinct from the pulse rate but coincided with oscillations of the EEG α-power (Mo *et al.*, 2013). However, sensitization of higher order central neurons leads to cutaneous allodynia and muscle tenderness, whereas convergence of retinal pathways with nociceptive pathways in the thalamus is believed to underly photophobia (Burstein *et al.*, 2000; Dodick and Silberstein, 2006).

Although it is well established that the TVS mediates the migraine headache, the exact nature of the triggering stimuli responsible for this activation is currently unclear. The migraine generator theory proposes that migraine is a primary disorder caused by episodic activation of the TCC (Weiller *et al.*, 1995; Akerman *et al.*, 2011). Another theory proposes that the TCC activation is secondary to a noxious intracranial event such as cortical spreading depolarization (CSD), the putative cause of aura (Moskowitz *et al.*, 1993; Pietrobon and Moskowitz, 2013; Figure 1). To improve our understanding of migraine pathophysiology and develop better therapeutics, we still need good experimental headache models, despite recent advances in imaging techniques for studying headache and CSD in patients. Striking similarities, despite expected differences, in the anatomy, physiology and pharmacology of headache between rodents, guinea pigs, cats and primates have encouraged the use of these animals to model headache and predict therapeutic potential of anti-migraine agents (Amenta *et al.*, 1980; Edvinsson and Uddman, 1981; Keller *et al.*, 1985; Uddman *et al.*, 1989).

Electrophysiological recordings in TG and TCC

Neuronal activity in the TVS can be directly assessed with electrophysiological recordings from the TG or TCC (trigeminal nucleus caudalis and C1–2; Strassman *et al.*, 1996; Messlinger and Ellrich, 2001; Strassman and Levy, 2006; Zhang *et al.*, 2010; 2011a; Akerman *et al.*, 2013; Noseda and Burstein, 2013; Figure 1). This technique allows detection of the corresponding dural and cutaneous receptive fields for each unit and, real-time monitoring of the nociceptive activity. TCC can be activated by electrical, chemical (e.g. 100 mM KCl, capsaicin, inflammatory molecules) or mechanical (e.g. calibrated von Frey monofilaments) stimulation of the ipsilateral dura, superior sagital sinus or TG and by stimulation of the facial skin (with brush, pressure or pinch). Studies on experimental animals have revealed that the majority of the dural nociceptors mediating headache are polymodal Aδ and C fibres, sharing several common features with peripheral nociceptors (Strassman *et al.*, 1996; Strassman and Levy, 2006). They also provided insight into some features of migraine headache such as its throbbing character and aggravation on coughing or bending by demonstrating that dural

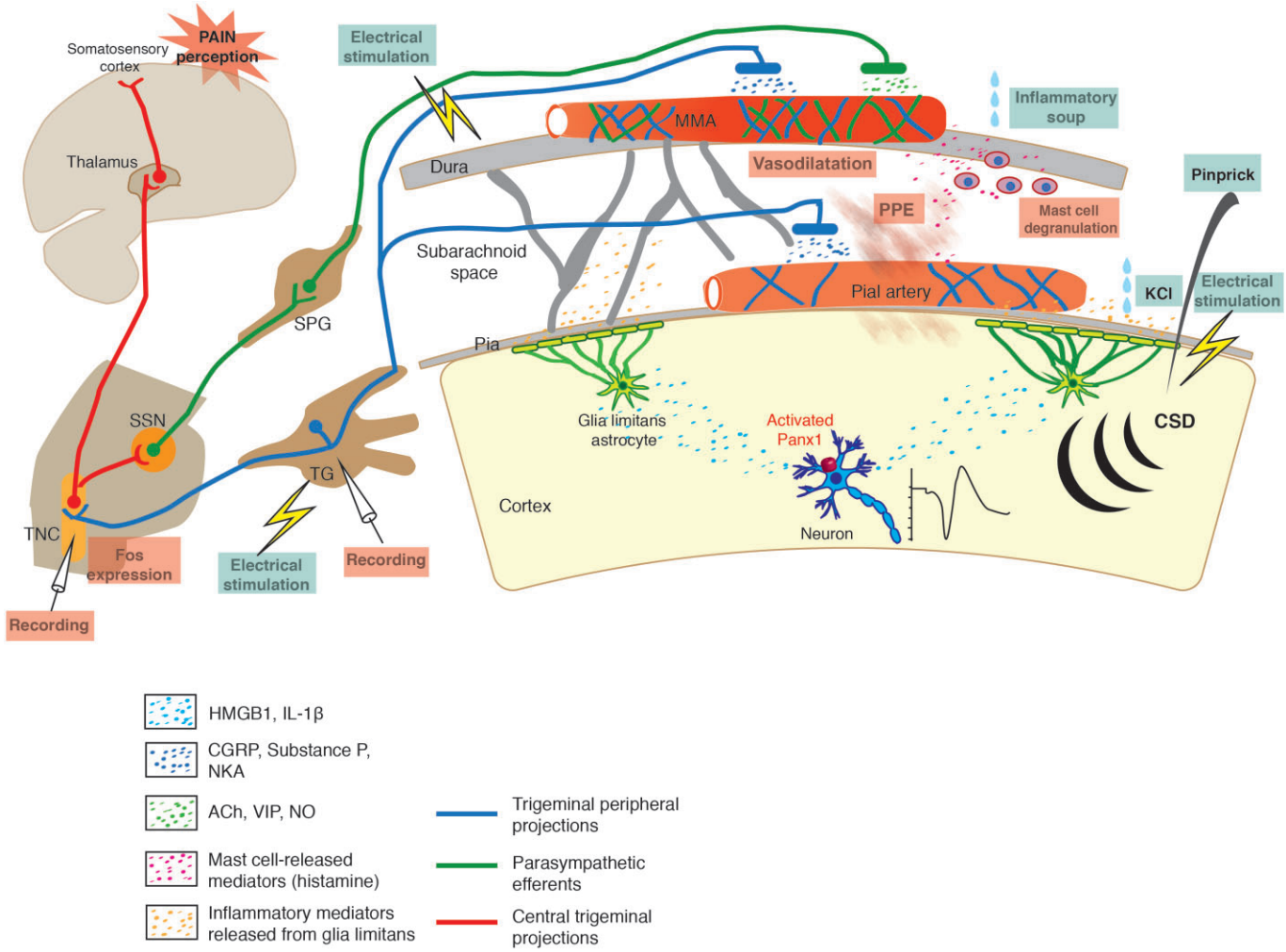

Figure 1

Schematic representation of the TVS mediating headache and some of the experimental methods used to activate it. Peripheral processes of the TG neurons terminate over the pial and dural vessels, whereas central processes terminate in TNC. Neurons in TNC project to thalamus and send collaterals to parasympathetic superior salivatory nucleus (SSN), which innervates dural vessels by way of spehenopalatine ganglion (SPG). Activation of TVS lead to release of several peptides and mediators from nerve endings around vessels, which induce dural vasodilatation, PPE and mast cell degranulation. TVS can be activated by electrical stimulation of the dura or TG, dural application of the inflammatory mediators (soup) as well as by CSD (evoked by pinprick, electrical stimulation or KCl). CSD stimulates trigeminal fibres around pial vessels by initiating a parenchymal inflammatory response triggered by the opening of neuronal pannexin-1 (Panx1) channels and release of HMGB1 and IL-1β. Activated nociceptors can be recorded from TG or TNC or detected by Fos immunolabelling in TNC. Dural vessel dilatation, PPE and mast cell degranulation can also be used to monitor TVS activation.

nociceptors become rapidly sensitized to mechanical stimuli once activated, whereas sensitization of the second-order neurons in the TCC was shown to mediate cephalic allodynia and muscle tenderness (Burstein *et al.*, 1998; Noseda and Burstein, 2013). Unambiguous identification of the nociceptive neurons in TG or TCC with the help of the above stimulation methods have revealed how they are activated by intrinsic noxious brain events such as CSD, providing strong evidence that CSD, the putative cause of migraine aura could also trigger headache (Zhang *et al.*, 2010; 2011a). These studies also revealed that neuronal activation started with a mean delay of 14 and 25 min after CSD in the majority of the TG and TNC units, respectively, in accordance with the time gap between migraine aura and headache in patients. Moreover, electrophysiological recordings from the TCC combined with anterograde and retrograde tracing methods can be used to study modulation of the CSD-induced dural nociceptor activation by corticotrigeminal descending pathways (Noseda *et al.*, 2010a) and to investigate the characteristics of third-order neurons in the thalamus, which has provided insights into migraine-associated symptoms such as photophobia (Noseda *et al.*, 2010b). Neuronal activity in the ventroposteromedial nucleus in response to TVS stimulation is reportedly modulated by triptans (Shields and Goadsby, 2006) as well as drugs used in migraine prophylaxis such as propranolol (Shields and Goadsby, 2005), topiramate (Andreou and Goadsby, 2011) and valproate (Sokolov *et al.*, 2013).

Increased metabolism in TCC

Increased neuronal activity in the TNC and upper cervical dorsal horn and related nociceptive structures results in increased blood flow. A laser Doppler probe positioned over these structures can demonstrate the activity-dependent blood flow changes, serving as an indirect marker of TCC activation (Goadsby and Zagami, 1991; Goadsby *et al.*, 1997; Goadsby and Classey, 2000; Andreou *et al.*, 2010). Similar results have also been obtained when the metabolic activity was measured by autoradiographic detection of glucose utilization (Goadsby and Zagami, 1991; Goadsby *et al.*, 1997). PET and blood oxygen level-dependent functional MRI have been instrumental in showing the areas activated during induced or spontaneous migraine attacks and have allowed the investigation of migraine-associated phenomena such as allodynia in humans (Burstein *et al.*, 2010; Denuelle *et al.*, 2011; May, 2013).

Increased c-fos expression in TCC

The expression of Fos protein, the product of the *c-fos* gene is widely used as a marker of TCC activation (Morgan and Curran, 1989). Also, there is increasing evidence showing that phosphorylation of the extracellular signal-regulated kinase can be used as a marker of TG activation (Sixt *et al.*, 2009; Iwashita *et al.*, 2013). Activation of the *c-fos* gene begins within 5 min following stimulus and continues until the stimulus ceases. Fos protein becomes immunohistochemically detectable in neuronal nuclei approximately 30 min after the stimulus (Morgan and Curran, 1989). It has a half-life of 2 h after the end of stimulus (Svendsen and Lykkegaard, 2001). In addition to Fos immunoreactivity, *c-fos* mRNA can also be studied (Uhl *et al.*, 1991; Nozaki *et al.*, 1992; Ashmawi *et al.*, 2003; Benjamin *et al.*, 2004). Activation of the TCC by mechanical, chemical or electrical stimulation of the dura, superior sagital sinus or TG as well as by CSD results in immunoreactivity in nociception-related superficial laminae (I and II) of the C1–2 dorsal horns and TNC, especially in the ventrolateral segment of the region receiving inputs from the ophthalmic nerve (Uhl *et al.*, 1991; Nozaki *et al.*, 1992; Kaube *et al.*, 1993; Moskowitz, 1993; Clayton *et al.*, 1997; Mitsikostas *et al.*, 1998; Mitsikostas and Sanchez del Rio, 2001) (Figure 2). Lamina V and other laminae may also be positively labelled for Fos but to a lesser extent. Depending on the stimulus used, the laminae involved may vary and *c-fos* expression in other brain regions such as periaqueductal grey matter, central nucleus of amygdala and hypothalamic nuclei is observed (Tassorelli and Joseph, 1995; Keay and Bandler, 1998; Mitsikostas and Sanchez del Rio, 2001). *c-fos* expression in TNC has been shown to be modulated by 5-$HT_{1B/1D/1F/2B}$, neurokinin-1 (NK_1), $GABA_A$, NMDA, AMPA, metabotropic glutamate and μ-opioid receptors (for receptor nomenclature see Alexander *et al.*, 2013a) and to be inhibited by triptans, calcitonin gene-related peptide (CGRP) antagonists and ergot alkaloids (Mitsikostas and Sanchez del Rio, 2001; Sixt *et al.*, 2009).

The number of Fos-positive nuclei within the superficial laminae can be used for quantification. Although it is a technically simple method to study neuronal activation, Fos is a rather difficult marker to evaluate. Its expression may be induced or inhibited by many confounding stimuli such as skin incisions, meningeal irritation, ear bars used to fix the head of the animal and hypotension; therefore, the changes detected may not be always specific to the stimulus used. A good experimental design controlling the confounding variables, adequate anaesthesia and maintenance of physiological conditions are essential. Measures such as topical application of lidocaine to skin incisions may be taken to increase specificity (Akerman *et al.*, 2013). Additionally, to be able to unambiguously detect expression changes in Fos, repeated or intense stimulation may be required (Bullitt, 1989; Lima and Avelino, 1994). When evaluating Fos immunoreactivity, cell counting by at least two investigators blinded to the experimental groups is advisable.

Middle meningeal artery (MMA) dilatation

MMA dilatation can be induced by electrical stimulation of the MMA, dura mater or TG and, also by CSD (Williamson *et al.*, 1997a,b; Bolay *et al.*, 2002; Li *et al.*, 2009; Karatas *et al.*, 2013; Figure 1). The intravital microscopy is generally used to study dural blood vessel diameter changes through a closed cranial window (Williamson *et al.*, 1997a). Although rats are commonly used for this model, guinea pigs and mice have also been used with some modifications (Williamson *et al.*, 2001; Gupta *et al.*, 2006). The skull of the animal is thinned with a micro drill so that dural and pial blood vessels under the parietal bone can be visualized. The window is covered with mineral oil preheated to 37°C. The image is displayed on a monitor and the diameters of the selected blood vessels are measured to determine their response to stimuli. In the past, video dimension analysers were used for this purpose, but today, many image-processing software can perform such actions. Usually, MMA, the largest artery supplying the dura mater is selected for analysis. It can easily be identified in rats with help of its convoluted course (Figure 2). In mice, the artery looks relatively straight and shows variable location, yet is still identifiable under a stereomicroscope as the artery with the largest diameter among the dural arteries, which are more superficially located and thinner compared with the underlying pial arteries.

The MMA dilatation triggered by TVS activation is mediated by parasympathetic nerves and the peptides released from nociceptive afferents. The arterial smooth muscle cells express CGRP receptor, vasoactive intestinal polypeptide (VIP) receptor 1 and 2 ($VPAC_1$ and $VPAC_2$) and pituitary adenylate cyclase-activating polypeptide (PACAP) receptor type 1 (PAC_1), which can be stimulated with CGRP and PACAP released from trigeminal nociceptors and by VIP released from parasympathetic neurons (Oliver *et al.*, 2002; Boni *et al.*, 2009; Baun *et al.*, 2011; Syed *et al.*, 2012). These GPCRs are all vasodilatory (Edvinsson *et al.*, 2002; Fizanne *et al.*, 2004; Boni *et al.*, 2009; Syed *et al.*, 2012). The electrical stimulation-induced dilatation of dural blood vessels can be blocked with triptans by inhibiting CGRP release via presynaptic 5-$HT_{1B/D}$ receptors on nociceptive afferents and with

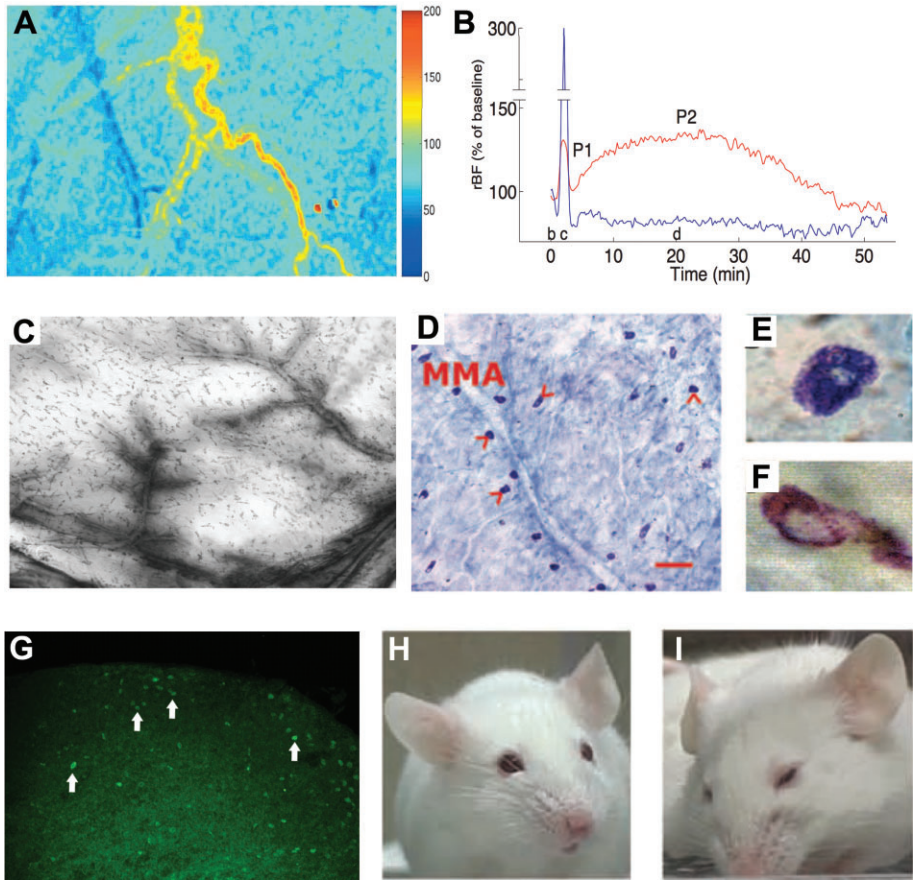

Figure 2

Activation of the TVS can be investigated with various techniques. (A) MMA blood flow: laser speckle images of the cortex, cortical blood vessels and MMA captured in a rat under anaesthesia illustrate the blood flow changes as two-dimensional relative flow maps. Cold colours indicate a decrease, whereas hot colours depict an increase in blood flow compared with baseline as shown in the scale bar on the right. Twenty minutes after induction of a single CSD with a pinprick, the cortex is hypoperfused (blue), whereas blood flow in the MMA is increased (yellow-red). (B) The MMA flow increase (red line) peaks around 20 min after CSD and lasts about 1 h during which the cortical blood (blue line) remains oligemic. (C) Plasma protein extravasation: leakage of i.v. administered HRP in a rat whole-mount dura preparation illustrates plasma protein extravasation from dural vessels after CSD. (D–F) Mast cell degranulation: incubation of dura with methylene blue reveals mast cells (arrowheads) along the course of MMA in a mouse; scale bar = 100 μm (D). Degranulated mast cells (F) can easily be distinguished from resting cells (E) with loss of blue cytoplasmic granules. (G) c-fos expression: Fos immunoreactivity appears in TNC 1 h after KCl-induced CSDs. Majority of the labelled cells (arrows) are located in the superficial layers (laminae I and II). (H, I) Pain-related behaviour: (H) normal mouse facial appearance, (I) facial expression of a mouse suffering from pain. A, B, C were reproduced from Bolay *et al.*, 2002; D, E, F, from Karatas *et al.*, 2013; and H, I; from Langford *et al.*, 2010 with permission.

CGRP receptor antagonists (Kurosawa *et al.*, 1995; Messlinger *et al.*, 1997; Petersen *et al.*, 2004). PACAP-mediated vasodilatation can be blocked by $VPAC_1$ or PAC_1 receptor antagonists in experimental animals (Fizanne *et al.*, 2004; Syed *et al.*, 2012) and hence, targeting these receptors may have a therapeutic potential in migraine similar to CGRP antagonists and triptans. CGRP or PACAP infusion triggers migraine-like episodes in susceptible individuals, which can also be inhibited by triptans (Lassen *et al.*, 2002; Schytz *et al.*, 2009; Hansen *et al.*, 2010). Interestingly, VIP does not cause attacks in migraineurs (Rahmann *et al.*, 2008). Mast cells may play an important role in sustained activation of TVS as they express CGRP and $VPAC_1$ receptors (Levy *et al.*, 2007; Seeliger *et al.*, 2010) and both CGRP and PACAP have been shown to degranulate dural mast cells (Levy *et al.*, 2007; Levy, 2009; Baun *et al.*, 2012). The dural stimulation induces NO release from the arterial endothelium, which also contributes to vasodilatation by facilitating CGRP release from nociceptors (Messlinger *et al.*, 2000).

The time profile of the dural vascular response depends on the nature of the stimulus activating the TVS. Electrical stimulation of MMA or TG causes a rapid dilatation within 30 s that ends a few minutes after stimulation (Shepherd *et al.*, 1997; Williamson *et al.*, 1997a; Li *et al.*, 2009). In contrast, a delayed and sustained dilation in MMA, beginning in 5–10 min and lasting an hour is observed after CSD (Bolay *et al.*, 2002; Karatas *et al.*, 2013). Perhaps this time-limited dilatation of the MMA could account for failure of some MR angiography studies to detect MMA dilatation during nitroglycerin-induced or spontaneous migraine attacks

(Schoonman *et al.*, 2008; Amin *et al.*, 2013). Indeed, MMA dilatation was revealed when patients were scanned as soon as the delayed headache attack started about 5.5 h after CGRP infusion (Asghar *et al.*, 2011). Controversies notwithstanding, these studies convincingly showed that MMA dilatation in humans was not always associated with headache (see also previous paragraph) despite the fact that the MMA dilatation, as a surrogate marker of dural neurogenic inflammation, has been instrumental in the development of triptans and CGRP antagonists using experimental models (see the 'Neurogenic inflammation' section below for details). This discrepancy is not surprising considering that several other successful drug screening tests (e.g. tests for antidepressant action) show quantitative and mechanistic differences compared with the disease mechanisms.

It should be noted that the diameter of the MMA is sensitive to hypocapnia or hypercapnia and the temperature of the animal; therefore, these physiological parameters should be monitored during the experiment (Petersen *et al.*, 2005; Akerman *et al.*, 2013). Unlike pial arteries, however, the MMA diameter does not significantly change in response to alterations in systemic arterial BP (Petersen *et al.*, 2005). In mice, a methodological modification is necessary as the dura mater is very thin and more sensitive to manipulation compared with the other species. Dural blood vessels maximally dilate at the end of drilling process. Endothelin was used to preconstrict the arteries before activating TVS in the mouse (Gupta *et al.*, 2006). As endothelin could exert confounding effects, a better alternative is to wait overnight after the surgery before starting MMA recordings, allowing time for the arteries to recover (Karatas *et al.*, 2013).

An open cranial window can also be used after removing the skull in rats. This technique allows topical application of various chemicals and direct contact with dura for electrical stimulation; however, it may cause unwanted irritation of trigeminal afferents. Continuous application of artificial CSF at 37°C is necessary to prevent drying of dura and to mimic the physiological environment. The open window is nearly impossible in mice, in our experience, since dural blood vessels are thrombosed soon after the skull is removed.

MMA blood flow

In addition to measurement of the MMA diameter, the blood flow velocity may also be recorded as an indirect but more sensitive measure of the vessel calibre as the small diameter changes cannot be reliably assessed in video images, whereas the flow increases with the 4th power of the diameter. For laser Doppler flowmetry, a Doppler probe is placed over the MMA to record the blood flow changes. Precise positioning of the probe over the MMA is essential as displacements and small probe movements may corrupt the data. The laser speckle flowmetry is another technique introduced for studying dural and cortical blood flow by Dunn and his colleagues (Dunn *et al.*, 2001). This technique allows construction of two-dimensional blood flow maps of an area of interest, and the temporal changes in the blood flow of a selected artery or tissue can be determined compared with baseline. Simultaneous evaluation of dural, pial and tissue blood flow changes is a major advantage. The target area is illuminated with a laser diode and raw speckle images are captured by a CCD camera. A special software then converts these images into speckle contrast and correlation time value images, the latter of which are inversely proportional to the blood flow velocity (Dunn *et al.*, 2001). Laser speckle technique has successfully been used to test the activation of TVS in mice and rats (Bolay *et al.*, 2002; Karatas *et al.*, 2013; Figure 2). The same concerns described above for video imaging of the MMA through a closed cranial window also apply to the laser speckle monitoring.

Neurogenic inflammation

Sterile inflammation in the dura mater, initiated by peptides released from the trigeminal afferents is another measurable feature of the TVS activation. In addition to vasodilatation of dural vessels reviewed above, the neurogenic inflammation is characterized by increased vascular permeability, the resultant plasma protein extravasation (PPE) and mast cell degranulation (Figures 1 and 2). Rodents, including mice and guinea pigs, are suitable models for studying PPE (Markowitz *et al.*, 1987; 1988; Yu *et al.*, 1996). A radioisotope ^{125}I linked with BSA is used to measure PPE (Markowitz *et al.*, 1987), which is regarded as the most sensitive method (Bergerot *et al.*, 2006). Five minutes after [^{125}I]-BSA injection i.v., a stimulus is applied to activate the TVS. Dura is then bilaterally removed and the extravasated [^{125}I]-BSA counts are compared between the stimulated and unstimulated sides. Additionally, detection of dural Evans blue leakage by fluorescence (Johnson and Phebus, 1998) or confocal microscopy (Schuh-Hofer *et al.*, 2003) has been described. Evans blue (a dye binding to albumin) allows real-time monitoring of dural extravasation with intravital microscopy and quantification of the leakage by spectrophotometry at the end of the experiment (Johnson and Phebus, 1998). HRP administered i.v. has also been used to examine dural plasma extravasation *ex vivo* (Bolay *et al.*, 2002; Figure 2).

The dural vasodilatation model was successfully used to predict the efficacy of antimigraine drugs like 5-HT$_{1B/1D}$ agonist triptans and dihydroergotamine (Kurosawa *et al.*, 1995; Messlinger *et al.*, 1997; Petersen *et al.*, 2004). NK$_1$ receptor antagonists on the other hand failed to inhibit dural vasodilatation and were also found to be clinically ineffective for migraine (Goldstein *et al.*, 1997; Williamson *et al.*, 1997b). Unlike dural vasodilatation, dural PPE is suppressed by clinically ineffective (neurokinin A and substance P inhibitors and endothelin blockers) as well as clinically effective (triptans and CGRP antagonists) agents (Moskowitz, 1992; 1993; O'Shaughnessy and Connor, 1994; Shepheard *et al.*, 1995; May *et al.*, 1996; Goldstein *et al.*, 1997; Williamson *et al.*, 1997c; Yu *et al.*, 1997). These inconsistencies are further aggravated by species differences: for example, 5-HT$_{1D}$ receptor agonists more potently inhibit PPE in rats and guinea pigs (Shepherd *et al.*, 1997), whereas 5-HT$_{1B}$ agonists are more potent in mice (Yu *et al.*, 1996). Although neuromediators and their receptor subtypes involved in neurogenic inflammation vary between species, CGRP is reportedly a common mediator in most mammalian species (Geppetti *et al.*, 2005). The above drawbacks led to concerns that the dural neurogenenic inflammation model could be more akin to

meningitis than migraine. However, the rat experimental meningitis consistently causes gadolinium enhancement in meninges in MR, whereas dural neurogenic inflammation induced by TG stimulation does not, suggesting that the intensity of the inflammatory response is considerably different between the two models although some common mechanisms are shared (Hoffmann *et al.*, 2002; Wiesmann *et al.*, 2002).

To simulate neurogenic inflammation, the dura mater can be chemically stimulated by topical application of inflammatory substances. These can be applied through plastic cannulae that are fixed to the cranium without touching the dura (Wei *et al.*, 2011). A few microliters of a mixture called 'inflammatory soup' (IS), composed of histamine, 5-HT, bradykinin and PGE_2 can be used to activate the TVS (Strassman *et al.*, 1996; Burstein *et al.*, 1998; Figure 1). Capsaicin, IL-6, IL-1β, TNF-α, transient receptor potential channel-A1 (TRPA1) agonists and PGI_2 are other inflammatory stimulants used (Mitsikostas *et al.*, 1998; Zhang *et al.*, 2007; 2011b; 2012; Materazzi *et al.*, 2013).

TRPA1 channels (Alexander *et al.*, 2013b) have recently emerged as a novel target based on the findings from experimental migraine models, underscoring the value of the animal models in identification of new pharmacological targets. These excitatory non-selective cation channels are expressed in a population of trigeminal afferent neurons innervating the dura (Edelmayer *et al.*, 2012; Huang *et al.*, 2012). They can be activated by various exogenous and endogenous stimuli such as cold temperatures, volatile irritants (e.g. formaldehyde, chlorine), cigarette smoke, isothiocyanates and PGs (Jordt *et al.*, 2004; Bautista *et al.*, 2005; 2006; McNamara *et al.*, 2007; Andre *et al.*, 2008; Bessac and Jordt, 2008; Materazzi *et al.*, 2008; del Camino *et al.*, 2010). Based on the observations that inhalation of TRPA1-activating irritants can trigger headaches in susceptible migraineurs, TRPA1 channels have been proposed to mediate irritant and odour hypersensitivity in migraine (Ziem and McTamney, 1997; Kelman, 2007; Lima *et al.*, 2011). Indeed, TRPA1 agonists promote CGRP release from trigeminal nerve endings and increase meningeal blood flow (Kunkler *et al.*, 2011; Nassini *et al.*, 2012). TRPA1 activators like mustard oil and umbellulone, when topically applied to the dura in rats, cause cutaneous allodynia and headache-related behaviour such as decreased exploratory rearing activity in novel environments (Edelmayer *et al.*, 2012). Selective TRPA1 antagonists like HC-030031 (McNamara *et al.*, 2007) can inhibit CGRP release, meningeal vasodilatation and headache-related behaviour (Kunkler *et al.*, 2011; Edelmayer *et al.*, 2012).

Inflammatory substances can also be applied in awake animals for behavioural experiments and also for chronic dural stimulation with some modifications (Oshinsky and Gomonchareonsiri, 2007; Edelmayer *et al.*, 2012; Yan *et al.*, 2012; Melo-Carrillo and Lopez-Avila, 2013). For this, an indwelling supradural catheter is used to administer inflammatory mediators to awake and freely moving rats (Wieseler *et al.*, 2012). Topical dural exposure to IS for 5 min results in short-term hyperactivity followed by long-term (approximately 2 h) hyper-responsiveness and increased receptive fields of the central trigeminal neurons (Jakubowski *et al.*, 2007). This is inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) with their direct action on central and peripheral neurons (Jakubowski *et al.*, 2005; 2007; Levy *et al.*, 2008). Unlike COX 1/2 inhibitors, triptans are unable to reverse central sensitization once it is formed in experimental animals. Similarly, triptans are not satisfactorily effective in patients who have already developed allodynia, unlike i.v. NSAIDs (Burstein and Jakubowski, 2004; Levy *et al.*, 2004; Jakubowski *et al.*, 2005).

Inflammatory mediators can alternatively be injected into cisterna magna through a catheter after making an incision extending from occipital protuberance to cervical area in rodents. It is suggested that the animals are held in reverse Trendelenburg position for 2 h following injection for effective distribution of the stimulants within subarachnoid space (Mitsikostas *et al.*, 1998). Inflammatory substances activate meningeal sensory afferents and increase the jugular CGRP level within minutes (Hoffmann *et al.*, 2009). This activation is blocked by either chronic administration of topiramate or acute i.v. triptan injection, simulating prophylactic and acute treatment of migraine.

Mast cell degranulation

Activation of the TVS results in degranulation of mast cells in the ipsilateral dura by way of the peptides released from trigeminal nerve endings (Dimitriadou *et al.*, 1991; Keller *et al.*, 1991; Levy *et al.*, 2007; Figures 1 and 2). Mast cells release histamine and several other inflammatory mediators in their granules and add to the meningeal inflammatory changes, sensitization of afferent fibres and sustained nociceptive activity (Zhang and Levy, 2008; Messlinger *et al.*, 2012). Dura mater is heavily populated by mast cells in close proximity to meningeal nociceptors around vessels (Dimlich *et al.*, 1991; Rozniecki *et al.*, 1999; Artico and Cavallotti, 2001). Compound 48/80, a mast cell secretagogue agent, results in degranulation of dural mast cells, activation of meningeal nociceptors in electrophysiological recordings and increased *c-fos* expression in TCC (Shefler and Sagi-Eisenberg, 2001; Levy *et al.*, 2007). Of note, a mast cell independent, direct excitatory action of compound 48/80 on enteric neurons and visceral afferents has also been reported (Schemann *et al.*, 2012). The mast cell degranulation is also used as a TVS activation marker in headache models (Markowitz *et al.*, 1989; Levy *et al.*, 2007). For this, the dura is removed from mice and rats transcardially perfused with formaldehyde, then it is incubated with methylene blue. The degranulated mast cells can easily be detected by the absence of their typical blue, scattered cytoplasmic granules (Markowitz *et al.*, 1989; Dimitriadou *et al.*, 1991; Figure 2).

Neuropeptide changes in blood

Peptides released from activated trigeminal afferents can be measured in blood (Hoffmann *et al.*, 2012). Jugular venous blood is preferred over the peripheral blood to avoid dilution of peptides in extracerebral circulation. Indeed, CGRP was found to increase during migraine attacks in the external jugular blood but did not change in the antecubital blood (Goadsby *et al.*, 1990). Unlike migraine attacks, trigeminal

nerve stimulation in humans results in increased substance P levels, in addition to CGRP, in jugular venous blood (Goadsby *et al.*, 1988). CGRP and substance P were also shown to increase in the external jugular blood of experimental animals after trigeminal activation (Goadsby *et al.*, 1988; Goadsby and Edvinsson, 1993). CGRP is rapidly degraded in plasma and has a short half-life of approximately 10 min (Deng and Li, 2005). Accordingly, the blood collected must be immediately centrifuged (Akerman *et al.*, 2013). PACAP has recently been shown to be elevated ictally in patients' cubital vein blood compared with interictal periods (Tuka *et al.*, 2013). Similarly, in experimental rat models, increased levels of PACAP were demonstrated in blood following electrical TG stimulation or nitroglycerin injection (Tuka *et al.*, 2012). Interestingly, neuropeptide Y, VIP as well as substance P levels in jugular blood were found to be unaltered during migraine attacks (Goadsby *et al.*, 1990).

NO degradation products and pro-inflammatory cytokines have been found to be elevated in the internal jugular venous blood during migraine attacks (Sarchielli *et al.*, 2000; 2004; 2006a,b). The internal jugular blood in humans carries less than 3% blood of extracerebral origin (Hodde and Sercombe, 1996), hence, mainly reflects the changes in brain parenchyma, rather than meninges, which drain into the external jugular vein. Therefore, it remains to be established whether or not these alterations in the internal jugular blood are caused by a parenchymal inflammatory response triggering the migraine headache as recently proposed (Karatas *et al.*, 2013).

Pain-related behaviour

The very end point of nociceptive activation is the pain perception. Therefore, well-established models of pain-related behaviour in animals are badly needed in headache research. Various experimental approaches reported illustrate that both headache and associated migrainous symptoms such as allodynia, light and sound hypersensitivity can be modelled in mice and rats, which have recently been reviewed in detail (Romero-Reyes and Ye, 2013b). Spontaneous pain-related behaviours in rodents can be evaluated by video-taping a freely moving animal, and either still images or video fragments of these records can then be assessed off-line (Bennett, 1993; Akcali *et al.*, 2010; Langford *et al.*, 2010; Romero-Reyes *et al.*, 2013a).

In a mouse model of trigeminal pain, masseter muscle was unilaterally injected with Freund's adjuvant and facial grooming patterns such as forepaw rubbing and hind paw scratching directed to the injection area were analysed (Romero-Reyes *et al.*, 2013a). Pain-related behaviours can also be elicited in rats with superior sagital sinus stimulation (Zhang *et al.*, 2013a). With some modifications of the technique for use in conscious animals, the researchers detected a stimulation frequency-dependent alteration of grooming and head-flick activities, which were reversed by morphine and rizatriptan (Dong *et al.*, 2011). Another approach called 'Mouse Grimace Scale' (MGS) was developed based on changes in facial expression like orbital tightening and nose bulging in response to painful stimuli (Langford *et al.*, 2010) and has been successfully used to demonstrate the CSD-induced headache in wild type as well as CACNA1A transgenic mice that are prone to generate spontaneous CSDs (Langford *et al.*, 2010; Karatas *et al.*, 2013; Figure 2). More recently, a 'rat grimace scale' has been developed. This is largely similar with MGS apart from some differences in nose and cheek appearance (Sotocinal *et al.*, 2011). Conditioned place preference in rats, an indicator of the motivated behaviour to seek relief, can also be used to evaluate potential headache therapeutics against various headache triggers such as dural inflammatory mediators (De Felice *et al.*, 2013).

Periorbital cutaneous hypersensitivity and allodynia emerging during neurogenic dural inflammation (e.g. induced by topical application of inflammatory mediators) can be assessed *in vivo* by measuring head withdrawal latency in response to pressure applied by von Frey filament or to heat (Yamamura *et al.*, 1999; Levy *et al.*, 2007; Oshinsky and Gomonchareonsiri, 2007; Edelmayer *et al.*, 2009; Bates *et al.*, 2010; Maxwell *et al.*, 2010; Elliott *et al.*, 2012). An automatic system has been developed that increases the amount of force applied by the filament until head withdrawal occurs (Cunha *et al.*, 2004). Head withdrawal can be accompanied by grooming behaviour directed to the stimulated area (Vos *et al.*, 1998; Edelmayer *et al.*, 2009). For these cutaneous hypersensitivity tests, the animal may need to be put in a restrainer to access the craniofacial region, creating a stressful situation for the animal. However, allodynia can also be evaluated in freely moving animals (Wieseler *et al.*, 2010; 2012; Fioravanti *et al.*, 2011) as well as in the periphery. Withdrawal latency of the hind paw after heat application or mechanical nociceptive thresholds with von Frey filaments confirmed development of thermal and mechanical allodynia 30–60 min after i.p. glyceryltrinitrate (GTN) application in mice (Bates *et al.*, 2010) or dural application of mustard oil and environmental irritant umbellulone in rats (Edelmayer *et al.*, 2012).

Photophobia can experimentally be studied by light-aversion behaviour in a chamber with two compartments, one is dark and closed and the other is light and open (Crawley and Goodwin, 1980). GTN, injected i.p., has been shown to trigger light-aversive behaviour in mice, 0–30 and 90–120 min after injection (Markovics *et al.*, 2012). An interesting study in cats showed that superior sagittal sinus stimulation resulted in activation of cells in nucleus tractus solitarius, which is involved in regulating vomiting, and this connection is interrupted with triptans by their action on 5-$HT_{1B/1D}$ receptors (Hoskin *et al.*, 2004).

CSD-mediated trigeminal activation

It is generally agreed that aura symptoms preceding headache by 20–60 min in migraineurs are mediated by CSD, a wave of neuronal and glial depolarization that spreads along the cortex at a speed about 2–6 mm·min^{-1} followed by depression of neuronal activity (Somjen, 2001). Although CSD is used as a model of migraine aura, recent experimental data suggest that CSD can also activate TVS (Bolay *et al.*, 2002; Zhang *et al.*, 2010; 2011a; Karatas *et al.*, 2013). It has been shown that CSD leads to a delayed and sustained increase in MMA blood flow along with plasma protein extravasation in dura (Bolay *et al.*, 2002). MMA dilatation and PPE were found to be mediated by trigeminal and parasympathetic nerves supply-

ing the dura, and they were inhibited when CSDs were suppressed by an NMDA antagonist MK-801. It would be interesting to see whether or not a noxious brain event like CSD could more realistically model TVS activation than direct stimulation of the dura and TG (Dalkara *et al.*, 2006), especially in transgenic or optogenetically engineered mice, in whom CSDs spontaneously emerge or could be evoked rather non-invasively by blue light respectively (Eikermann-Haerter *et al.*, 2009; Charles and Baca, 2013)

CSD can be triggered in mammalian brains by electrical, mechanical or chemical stimuli. Agyrencephalic brains as in rodents are more susceptible to CSD generation compared with primate brains with sulci and gyri. Whatever the triggering stimulus is, the main requirement for CSD is collective depolarization of a critical mass of cortical tissue (Matsuura and Bures, 1971). Then, a depolarization wave starts to spread to the neighbouring areas. A basic mechanical method to trigger CSD is to swiftly prick the cortex by a needle, briefly known as the 'pinprick' method. Being relatively easy and highly reproducible, this technique also has the advantage of studying the effect of single CSDs that can be triggered at the exact time of interest. However, it is not suitable for triggering repeated CSDs, and it does not allow determination of a CSD threshold. Electrical stimulation of the cortex is another method for CSD induction; a series of square pulses is applied at varying current intensities to determine a threshold current value (Ayata *et al.*, 2006; Ayata, 2013). Topical application over the dura or into the cortex of concentrated KCl (usually 0.5–1 M) is frequently used for initiating repeated CSDs. A threshold concentration can easily be detected (Reuter *et al.*, 1998; Ayata *et al.*, 2006). Other chemical stimuli that can be used to trigger CSD include glutamate, NMDA, sodium channel activators like aconitine and Na^+/K^+ ATPase inhibitor ouabain (Balestrino *et al.*, 1999; Ayata, 2013). These chemicals can be applied topically over the dura or intraparenchymally by pressure injection or microdialysis (Obrenovitch *et al.*, 2009).

CSD may be recorded by intracortical microelectrodes or by extracellular electrodes placed over the dura or cranium. A characteristic-negative slow potential shift can easily be recognized, and amplitude and duration of the wave can be measured. A simultaneous electrocorticogram recording can show suppression of the EEG activity for 15–30 min. When two or more electrodes are used, propagation speed of the depolarization wave can also be calculated. Although the electrophysiological recording is the standard method to detect CSDs, one can also monitor CSD-associated cortical blood flow changes by laser Doppler or laser speckle imaging techniques. In rats, cats and primates, a biphasic blood flow response consisting of an initial hyperaemia (corresponding to the depolarization phase) followed by a long-lasting oligaemia is recorded (Lauritzen *et al.*, 1982; Wahl *et al.*, 1987; Lacombe *et al.*, 1992; Yokota *et al.*, 2002). In mice, a triphasic blood flow response characterized by a brief hypoperfusion preceding the hyperperfusion and long-lasting hypoperfusion, is observed (Ayata *et al.*, 2004; Brennan *et al.*, 2007). An alternative method to detect CSD is to monitor the intrinsic optical signals in the brain tissue as light absorption characteristics of the tissue change due to shifts in haemoglobin concentration and water content (Ba *et al.*, 2002; Brennan *et al.*, 2007). A method combining voltage-sensitive dyes with the laser speckle technique was also proposed to study the relationship between depolarization and associated blood flow changes (Obrenovitch *et al.*, 2009).

Monitoring physiological parameters of the animal is essential as the arterial hypotension and hypoxia can modify characteristics of CSD and accompanying blood flow changes (Sukhotinsky *et al.*, 2008). CSD susceptibility may also vary depending on the body temperature, blood pH and pCO_2 (Ayata, 2013; Hoffmann *et al.*, 2013). The choice of anaesthetic is also critical, not only because anaesthesia may induce systemic hypotension but also because of the direct inhibitory effects of anaesthetics on cortical excitability. Urethane and α-chloralose have been found to have a much lower potential for suppressing CSD susceptibility when compared with ketamine, halothane, isoflurane and N_2O (Saito *et al.*, 1995; Kudo *et al.*, 2008). Propofol also suppresses CSD (Sonn and Mayevsky, 2006; Dhir *et al.*, 2012).

Evoking CSD in awake rodents induces anxiety, pain-related behaviour and allodynia (Akcali *et al.*, 2010; Fioravanti *et al.*, 2011; Karatas *et al.*, 2013); however, it also poses some technical difficulties. A plastic tubing can be placed and fixed over a burr hole covering it without piercing the dura. Stimulating chemicals can be applied through the tubing by a microinjector (Akcali *et al.*, 2010). In a recent study on freely moving mice, CSDs were triggered by a dry KCl pellet placed over the dura through a burr hole (Karatas *et al.*, 2013).

CSD can also be employed to study the mechanisms of action of migraine prophylaxis and for development of prophylactic drugs. Indeed, more than 1 month (but not acute) administration of topiramate, valproate, propranolol, amitriptyline and methysergide have all been found to dose-dependently suppress CSD frequency and increase CSD induction threshold in parallel with the time required for effective prophylaxis in their clinical use (Ayata *et al.*, 2006). This delayed action, which is also typical for several other CNS drugs like antidepressants, suggests drug-induced changes in gene expression and synaptic reorganization, reducing the susceptibility to CSD. However, these interesting possibilities still await clarification. Spreading depolarizations can also be triggered in the hippocampus *in vivo* by pressure microinjections of KCl, which also induce c-fos expression in TNC (Kunkler and Kraig, 2003). Hippocampal spreading depolarizations can bring insight to the mechanisms of memory impairment observed during migraine attacks. Moreover, hippocampal slices have been instrumental in studying neural mechanisms triggering spreading depolarization and to identify agents that can suppress susceptibility to spreading depolarization such as insulin-like growth factor-1 (Somjen, 2001; Grinberg *et al.*, 2012).

Recently, CSD induced by pinprick or epidural KCl has been shown to open neuronal pannexin-1 channels and release high mobility group protein-B1 (HMGB1) and IL-1β, which then activate the NF-κB pathway in astrocytes (Karatas *et al.*, 2013). NF-κB, by inducing expression of the inflammatory enzymes, triggered release of inflammatory mediators into subarachnoid space via glia limitans, and hence, activated the perivascular trigeminal afferents. In this model, TVS activation was monitored by MMA dilatation, dural mast cell degranulation and headache-related behaviours in mice. Trigeminal activation was blocked by knocking down or

inhibiting pannexin-1 channels and HMGB1 as well as by NF-κB activation inhibitors and naproxen. These findings suggest that pannexin-1 channels may serve to detect neuronal stress and alarm the organism with headache by activating the parenchymal inflammatory pathways. Unlike transient release of potassium, NO and other vasoactive mediators to extracellular space during CSD, the above summarized inflammatory response better explains the delayed appearance of headache 20–60 min after aura (CSD). Activation of an inflammatory mechanism after CSD is also supported by PET findings in rats with 11C-PK11195, a ligand for peripheral-type benzodiazepine receptors showing microglial activation (Cui *et al.*, 2009).

Mutant mouse models

It is now accepted that the susceptibility to migraine and CSD is, at least in part, genetically determined (van den Maagdenberg *et al.*, 2007). Mutations affecting ion channels or transporters may modify the balance between the excitatory and inhibitory activity in cortex, making it more susceptible to spontaneous or induced CSD generation (Pietrobon and Moskowitz, 2013). Patients with familial hemiplegic migraine (FHM) and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) bear mutations leading to recurrent transient neurological symptoms and headache (Thomsen *et al.*, 2002; Chabriat *et al.*, 2009). The mutations identified in those patients have created an opportunity for building genetic models of CSD and migraine. However, it should be noted that mice carrying FHM mutations may also exhibit features more reminiscent of atypical rather than common forms of migraine (Schytz *et al.*, 2010).

Vascular NOTCH3 knock-out or the NOTCH3 R90C knock-in mice have been developed for modelling CADASIL (Krebs *et al.*, 2003; Ruchoux *et al.*, 2003). Electrical and KCl stimulation showed decreased CSD thresholds in these mice, more prominently in knock-outs (Eikermann-Haerter *et al.*, 2011). NOTCH3-associated vasculopathy may lead to brief ischaemic episodes in the brain and induce CSDs. However, the exact mechanism underlying the increased CSD susceptibility in CADASIL is unknown, and an impaired neurovascular coupling leading to disturbed energy homeostasis in cortex is also possible (Eikermann-Haerter *et al.*, 2011).

Mouse knock-in FHM1 models bearing two different mutations (R192Q and S218L) in CACNA1A gene encoding the P/Q-type voltage-gated Ca^{2+} channel have been developed (van den Maagdenberg *et al.*, 2004; 2010). Mutations in the presynaptic voltage-gated Ca channels result in their opening at more negative potentials, hence, in enhanced glutamate release (van den Maagdenberg *et al.*, 2004; 2010; Tottene *et al.*, 2009). In these mice, CSD threshold is decreased and propagation velocity is increased (van den Maagdenberg *et al.*, 2004; 2010; Tottene *et al.*, 2009). CSDs may lead to transient neurological deficits. The CSD susceptibility in FHM1 models is dependent on the allele dosage and type of mutation. Homozygous R192Q mice have no major phenotypic abnormalities; however, homozygous S218L mice exhibit persistent ataxia, seizures and spontaneous hemiparesis attacks, in line with the more severe clinical phenotype in S218L FHM1 (Kors *et al.*, 2001; van den Maagdenberg *et al.*, 2004; 2010). Female knock-in mice are more susceptible to CSD as seen in migraine (Eikermann-Haerter *et al.*, 2009). FHM1 mutations, besides promoting CSD formation, also increase the tendency for inflammation in the TVS (Franceschini *et al.*, 2013). Trigeminal ganglia from FHM1 mice display an enhanced neuroinflammatory reaction on exposure to lipopolysaccharide compared with wild-type mice. Behavioural studies also demonstrated that FHM1 mutations exibited behaviours that could be attributed to photophobia and spontaneous unilateral headache in mice (Chanda *et al.*, 2013).

ATP1A2, associated with FHM2, is responsible for maintaining the resting membrane potential and, is found to be essential for clearance of released potassium and glutamate from synaptic clefts by astrocyte endfeet (Ikeda *et al.*, 2003). Homozygous T2763C mutations are lethal in neonatal stage but heterozygous knock-in mutants are available. These mice have a lower CSD threshold and higher CSD velocity (Pietrobon, 2007; Leo *et al.*, 2011). Haploinsufficiency of ATP1A2 leads to increased extracellular potassium and glutamate, promoting depolarization and therefore making the cortex more susceptible to CSD (Montagna, 2004; Pietrobon, 2007; Gritz and Radcliffe, 2013). These mice also show increased fear and anxiety behaviour (Gritz and Radcliffe, 2013).

Based on identification of a missense mutation in the casein kinase Iδ gene in two families with migraine, a mouse model carrying the T44A mutation has also been investigated. The T44A mutation causes reduced enzyme activity and results in lower CSD threshold, enhanced vascular response to CSD and a hyperalgesic phenotype after GTN administration (Brennan *et al.*, 2013).

GTN infusion

An i.v. infusion of GTN, a NO donor, was shown to trigger headache attacks with a 4–6 h delay in migraine patients (Olesen *et al.*, 1994; Iversen, 2001). These attacks can be aborted by triptans (Juhasz *et al.*, 2005) and can be prevented by prophylactic use of propranolol and valproate (Tvedskov *et al.*, 2004a,b). In rodent models, GTN can be applied either by i.v. infusion, s.c., i.p. or topically over the dura. Alternatively, infusion of sodium nitroprusside, another NO donor, can also be used (Galeotti and Ghelardini, 2013). GTN-induced changes vary with dose (e.g. Fos expression in TNC), and high doses lead to hypotension especially in anaesthetized animals (Tassorelli and Joseph, 1995; Ramachandran *et al.*, 2012). It should be noted that GTN may be absorbed onto plastic or glass surfaces, causing the concentration to be lower than expected in injection solutions (Bergerot *et al.*, 2006).

In contrast to the delayed onset of migraine-like headache after GTN, GTN induces dural and pial vasodilatation within minutes, making vasodilatation an unlikely mechanism for the attack (Akerman *et al.*, 2002a; Strecker *et al.*, 2002; Gozalov *et al.*, 2007). However, GTN application also induces inflammatory changes in dura and mast cell degranulation (Reuter *et al.*, 2001; Kim *et al.*, 2008; Galeotti and Ghelardini, 2013). In cats (Lambert *et al.*, 2000) and rodents, GTN infusion results in a delayed increase in mechanosensitivity of

meningeal nociceptors (Zhang *et al.*, 2013b) and in firing rate of individual TNC neurons (Koulchitsky *et al.*, 2009). GTN application in rodents increases *c-fos* expression in TNC, periaqueductal grey matter, central nucleus of amygdala and hypothalamic nuclei (Tassorelli and Joseph, 1995; Greco *et al.*, 2005). GTN also elicits pain-related behaviours in animal models. An i.p. application of 10 $mg \cdot kg^{-1}$ GTN in rats triggers hyperalgesic behaviour within 4 h as demonstrated by formalin and tail-flick tests (Greco *et al.*, 2008). GTN also dose-dependently causes development of mechanical and thermal hind paw allodynia in mice, starting 30–60 min after its i.p. administration (Bates *et al.*, 2010). Light-aversive behaviour in mice associated with an increase in meningeal blood flow was also observed with GTN and this response is thought to be mediated by PACAP (Markovics *et al.*, 2012).

In human studies, GTN is usually infused for approximately 20–30 min at a dose of 0.125–0.5 $\mu g \cdot kg^{-1}$ min^{-1} (Thomsen *et al.*, 1994; Christiansen *et al.*, 2000; Bednarczyk *et al.*, 2002; Tvedskov *et al.*, 2004b). Equivalent doses of drugs are 12 times that of humans in mice and six times in rats, based on body-surface area (US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research, 2005). Therefore, an approximate dose of 0.75–6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ can be considered the human equivalent in rodent models. However, most of the studies in rats have employed i.p. application doses such as 10 $mg \cdot kg^{-1}$, which is a few orders higher compared with doses used in humans (Reuter *et al.*, 2001; Gozalov *et al.*, 2007; Varga *et al.*, 2007; Greco *et al.*, 2008; Markovics *et al.*, 2012; Olesen and Jansen-Olesen, 2012). Such high doses can induce systemic hypotension especially in anaesthetized rats (Needleman, 1970), which can independently induce c-fos expression (Tassorelli and Joseph, 1995). Therefore, GTN dose should be carefully controlled in experimental models. A low-dose GTN model (4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ infusion for 20 min) in awake animals was introduced to overcome the hypotensive effect, and an up-regulation of c-fos expression in TNC was still observed in the delayed phase (Ramachandran *et al.*, 2012).

CGRP administration

Given the important role played by CGRP in migraine pathophysiology and effective treatment of migraine attacks with CGRP antagonists, exogenous application of CGRP has also been used to trigger migraine attacks (Williamson *et al.*, 1997a; Cumberbatch *et al.*, 1999; Akerman *et al.*, 2002b; Gupta *et al.*, 2010). CGRP, i.v., induces a delayed migraine-like episode in susceptible migraine patients and headache in non-migraineurs (Lassen *et al.*, 2002). CGRP causes MMA dilatation in the rat accompanied by central sensitization of the TVS (Williamson *et al.*, 1997a; Cumberbatch *et al.*, 1999). 5-$HT_{1B/1D}$ agonists abolish this sensitization (Williamson *et al.*, 1997a; Cumberbatch *et al.*, 1999). Recordings from the meningeal nociceptors in TG showed that CGRP did not directly modify the activity or sensitivity of peripheral nociceptors (Levy *et al.*, 2005). Instead, it promoted mast cell degranulation (Levy *et al.*, 2005; Levy, 2009). However, CGRP has also central actions; i.c.v. administered CGRP triggers light-aversive behaviour in mice, which can be reduced by rizatriptan (Kaiser *et al.*, 2012). This response is more prominent in transgenic mice sensitized to CGRP action by overexpressing RAMP1 protein, a regulatory subunit of the CGRP receptor involved in its trafficking and CGRP binding (Recober *et al.*, 2010).

Chronic migraine models

Migraine becomes a chronic disorder in 3% of patients (Bigal and Lipton, 2008). Therefore, models involving chronic stimulation of trigeminal system have been developed to simulate the chronic nature of the pain. In a rodent model, repeated episodes of IS were applied to dura in awake rats for 4 weeks (Oshinsky and Gomonchareonsiri, 2007). This application increased the trigeminal sensitivity up to 3 weeks as tested by periorbital von Frey ligament thresholds (Burstein *et al.*, 1998). In another study, dural IS was applied for 7 subsequent days and behavioural changes of animals were tested before and after. IS resulted in decreased exploratory and increased nociceptive behaviour and these behavioural changes were inhibited by NSAIDs as well as zolmitriptan (Melo-Carrillo and Lopez-Avila, 2013). However, chronic intermittent administration of GTN results in a progressive and sustained basal hyperalgesia along with acute hyperalgesia with each GTN dose (Pradhan *et al.*, 2013). Acute hyperalgesia in this model is reversed by triptans while both acute and chronic hyperalgesia is diminished by prophylactic administration of topiramate. The medication overuse is a major risk factor for transformation from episodic to chronic migraine (Diener and Limmroth, 2004; D'Amico *et al.*, 2005; Headache Classification *et al.*, 2006). Several animal models of medication overuse headache have been developed. Continuous infusion or repeated administration of sumatriptan or naratriptan to rats elicits time-dependent sustained allodynia that can be reversed by CGRP antagonists or neuronal NOS inhibitors (De Felice *et al.*, 2010a,b). These rats also show increased CGRP release to the circulation following administration of the NO donor, sodium nitroprusside (De Felice *et al.*, 2010b) and an increased expression of NOS in trigeminal dural afferents (De Felice *et al.*, 2010a), which may underlie the triptan-induced latent sensitization.

Conclusion

Headache models, like the models in other fields of science are merely an approximation of the reality based on the limited knowledge available. Accordingly, while they may provide significant insights into one aspect of headache, they may disappoint in other directions. Unlike cerebral ischaemia, for instance, in which the initial event starting the pathophysiological cascade is well known and common to other species, modelling becomes more complicated for disorders of unknown aetiology such as migraine. However, the limitations of the present animal models should not be considered discouraging as we always learn from our failures, and thus, can improve our models for deeper insight and better predictability.

At the present stage, models used for investigating the nociceptive mechanisms mediating the headache, which are

in general similar to pain mechanisms in the periphery are thought to be satisfactory to a large extent. Nevertheless, the intrinsic brain events that trigger migraine headache are still an enigma. Despite much debate on the initiating mechanisms, however, there is agreement that they start within the brain. Therefore, current efforts should focus on models that activate the TVS by way of intrinsic brain events like CSD. Although CSD is the biological substrate of migraine with aura, as a model, it may also provide clues as to why and how TVS can be activated by episodic disorders that disturb the brain homeostasis in headache patients in general. Induction of CSD by non-invasive methods like optogenetics in awake animals holds promise in this regard. Optogenetics could also be instrumental for non-invasive stimulation of CNS cells and pathways as well as specific receptors selectively in brain areas of interest, providing unprecedented advantage over the conventional methods (Airan *et al.*, 2009; Daou *et al.*, 2013). Neurogenic inflammation induced by intrinsic brain events might more realistically simulate the meningeal events in migraine compared with the direct stimulation of meninges with chemical or electrical methods. Progress in monitoring pain-related behaviours in unanaesthetized animals along with human models and imaging studies (Wager *et al.*, 2013) may also bring about exciting opportunities to investigate headache mechanisms and screen pharmacological agents.

Several technical aspects of the present models also need improvements. For example, paying attention to the use of human-equivalent doses and detection of the drug concentrations achieved at the site of action should be mandatory in every model used, as the observed changes, activated systems and receptors significantly vary with dose. Biodistribution and pharmocokinetics of the agents used should also be considered as effects in non-targeted tissues such as changes in BP and hormones may substantially modify CNS responses. Increasing collaboration between laboratories experienced on different techniques may allow improvements in the current models by bringing complementary expertise together. Proteomic and genomic studies may reveal species differences with regard to mediators involved in models and help predicting discrepancies.

Finally, improvements in the present models in parallel with the new discoveries in humans may increase the much desired translational potential of headache models. Ever expanding use of genetically engineered animals and innovative methods may lead to the creation of unprecedented novel models. In summary, the next decade holds great promise for the successful use of animal models in concert with human studies to understand headache and migraine, and develop new anti-migraine therapies

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Conflict of interest

The authors declare no conflict of interest.

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