



King's Research Portal

DOI:
[10.1111/ejn.14423](https://doi.org/10.1111/ejn.14423)

Document Version
Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Hoffmann, J., Storer, R. J., Park, J.-W., & Goadsby, P. J. (2019). N-Methyl-d-aspartate receptor open-channel blockers memantine and magnesium modulate nociceptive trigeminovascular neurotransmission in rats. *European Journal of Neuroscience*, 50(5), 2847-2859. <https://doi.org/10.1111/ejn.14423>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

DR. ROBIN JAMES STORER (Orcid ID : 0000-0003-0157-2919)

DR. PETER GOADSBY (Orcid ID : 0000-0003-3260-5904)

Article type : Research Report

***N*-Methyl-D-aspartate receptor open-channel blockers memantine and magnesium modulate nociceptive trigeminovascular neurotransmission in rats**

Jan Hoffmann,^{1,2*} Robin James Storer,^{1,3*} Jeong-Wook Park,^{1,†} and Peter J. Goadsby^{1,2}

¹Department of Neurology, University of California, San Francisco, San Francisco CA USA

²Headache Group, Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, UK

³Office of Research Affairs, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand

*J.H. and R.J.S. contributed equally to this work as first authors.

Currently

[†]Department of Neurology, Catholic University, Seoul, South Korea

Correspondence:

Professor P. J. Goadsby, NIHR-Wellcome Trust King's Clinical Research Facility

King's College Hospital, London SE5 9PJ, UK

Email: peter.goadsby@kcl.ac.uk

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ejn.14423

This article is protected by copyright. All rights reserved.

Keywords: migraine, headache, microiontophoresis, glutamate, naratriptan

Abstract

Experimental and clinical studies suggest that the low-affinity *N*-methyl-D-aspartate (NMDA) receptor open-channel blockers Mg^{2+} and memantine are effective in reducing trigeminal nociceptive activation. The aim of the present study was to investigate the apparent effectiveness of these channel blockers using a model of trigeminal activation *in vivo*. Rats were anesthetized before electrically stimulating the dura mater adjacent the middle meningeal artery. Neurons responding to stimulation were recorded extracellularly using electrophysiological methods while L-glutamate or NMDA and Mg^{2+} , memantine, or sodium controls were applied locally using microiontophoresis. Microiontophoretic application of Mg^{2+} or memantine into the trigeminocervical complex inhibited mechanically and electrically-stimulated craniovascular afferent, L-glutamate, or NMDA-evoked neuronal activity at the second order trigeminal synapse of craniovascular afferents. By contrast, intravenous administration of $MgSO_4$ (100 mg/kg) or memantine (10 mg/kg) did not significantly affect electrically-stimulated afferent-evoked activity within the trigeminocervical complex. The Mg^{2+} and memantine concentrations achieved after systemic administration may not effectively inhibit activation of the trigeminocervical complex, perhaps providing an explanation for the relatively poor efficacy of these NMDA receptor open-channel blockers for headache treatment in clinical studies. Nevertheless, the present results suggest blocking of NMDA-receptor open channels inhibits nociceptive activation of the trigeminocervical complex. Further exploration of such channel blockers as a therapeutic strategy for primary head pain is warranted.

Introduction

Migraine is the most common cause of disability globally (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017). It is a brain disorder (Akerman *et al.*, 2011; Goadsby *et al.*, 2017) characterized by impaired habituation to sensory information (Ambrosini *et al.*, 2003). Clinical and experimental evidence suggests that glutamate receptors are involved in the pathophysiology of migraine (Nicolodi & Sicutieri, 1995; Ramadan *et al.*, 2003; Vikelis & Mitsikostas, 2007), which thus offers potential targets for therapeutic approaches to migraine and primary head pain (Andreou & Goadsby, 2009).

The NMDA receptor is widely distributed in the central nervous system. It generally comprises a tetramer, formed by two GluN1 subunits, which are present in all functional NMDA receptors, and two GluN2 subunits of various types A–D to form a channel pore.

GluN1 subunits express a glycine-binding site and GluN2 subunits express a glutamate-binding site (Kew & Kemp, 2005; Parsons *et al.*, 2007). NMDA-receptor activation requires simultaneous binding of glycine and glutamate, together with removal of the endogenous channel-pore blocker Mg^{2+} in a voltage-dependent manner (Kleckner & Dingledine, 1988).

The NMDA-receptor channel is normally relatively permeable to Ca^{2+} , and at resting potentials Mg^{2+} , memantine, or ketamine, can block the channel, impeding Ca^{2+} influx (Alexander *et al.*, 2011). During local depolarization of neurons, the NMDA receptor channel changes conformation allowing Mg^{2+} to leave the channel pore, and Ca^{2+} influx. Memantine and ketamine also dissociate from the channel pore in a voltage-dependent manner, but require a greater change in voltage to do so (Parsons *et al.*, 1999).

A role for Mg^{2+} deficiency in headache was proposed in the 1980s (Altura, 1985). Clinical studies have found that Mg^{2+} levels are reduced in the saliva, serum (Sarchielli *et al.*, 1992), and cerebrospinal fluid (Jain *et al.*, 1985) of migraineurs, during and between migraine attacks. These findings are further supported by a study using nuclear magnetic resonance

spectroscopy that found low levels of Mg^{2+} in the brains of migraineurs (Ramadan *et al.*, 1989). At least three other studies have found reduced Mg^{2+} levels in the serum of migraineurs (Mauskop *et al.*, 1995; Soriani *et al.*, 1995; Mauskop *et al.*, 1996). Clinical trials of migraine treatment with magnesium showed promising results initially (Facchinetti *et al.*, 1991; Mauskop *et al.*, 1995). However, randomized, double-blind, placebo-controlled trials have mostly failed to realize this initial promise (Peikert *et al.*, 1996; Pfaffenrath *et al.*, 1996; Bigal *et al.*, 2002).

The potential efficacy of memantine for the treatment of migraine has been tested clinically including in open-label pilot studies, a case series, and most recently a randomized placebo-controlled trial, which taken together suggest effectiveness (Cammarata & Krusz, 2005; Kew & Kemp, 2005; Charles *et al.*, 2007; Bigal *et al.*, 2008; Spengos *et al.*, 2008; Noruzzadeh *et al.*, 2016). Memantine is generally well tolerated because of its relatively fast channel off-rate and partial untrapping (Parsons *et al.*, 2007). It does not substantially accumulate in the channel to interfere with subsequent normal synaptic transmission (Lipton, 2007). By contrast, other NMDA receptor antagonists such as ketamine, with its higher affinity for the channel, while perhaps effective in migraine treatment (Nicolodi & Sicutieri, 1995; Kaube *et al.*, 2000; Afridi *et al.*, 2013) has unacceptable dose-limiting clinical side effects (Hocking & Cousins, 2003; Visser & Schug, 2006; Chizh, 2007; Blonk *et al.*, 2010).

In experimental studies, both Mg^{2+} and memantine inhibited cortical spreading depression (CSD), considered a pathophysiological analog of migraine aura (Mody *et al.*, 1987; van der Hel *et al.*, 1998; Peeters *et al.*, 2007). Consistent with this inhibition, Mg^{2+} deficiency increases the sensitivity of NMDA receptors to glutamate-induced CSD (Van Harreveld & Fifkova, 1973). NMDA receptor antagonists including the high-affinity channel blocker MK-801 block trigeminal nociceptive transmission (Storer & Goadsby, 1999). The obligate NR1 subunit of NMDA receptors exists in the trigeminal nucleus caudalis (Bereiter & Bereiter,

2000; Otahara *et al.*, 2003) so NMDA channel agonists and blockers may act to modulate nociceptive neurotransmission at the second order synapse in this region. We therefore investigated the efficacy of Mg²⁺ and memantine to modulate trigeminovascular nociception in our rat model of trigeminovascular nociceptive activation, comparing direct application onto receptors in the trigeminal nucleus caudalis via microiontophoresis with systemic administration.

Materials and Methods

All protocols involving animals were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of California, San Francisco following the U.S. National Academy of Sciences Guide for the Care and Use of Laboratory Animals and in compliance with the U.S. Health Research Extension Act as specified by the U.S. National Institutes of Health. The molecular target nomenclature used conforms with the Guide to Receptors and Channels (Alexander *et al.*, 2011). Male Sprague Dawley rats ($n = 30$) were used in the present study to reduce the effect of fluctuations in estrogen that can be avoided if male rats are used. If female rats are used we would need to ensure that they were at the same stage of their estrous cycle, which is impractical. This is because migraine (and therefore trigeminal excitability and activation) can be influenced by estrogen levels (Saleeon *et al.*, 2016), and in particular, to changes in these levels. In women, it is not the absolute level that triggers attacks, it is the sudden fall of estrogen levels (which triggers menstruation) that causes attacks (Chai *et al.* 2014). The rats were housed in a specific pathogen free facility in cages containing groups of rats on nesting material with at least one piece of environmental enrichment per cage (e.g., nestlet, shelter, or additional nesting material) and monitored for welfare considerations as specified by the Laboratory Animal Resource Center (LARC) of the University of California, San Francisco, under a 12 h light–dark cycle with free access to

standard laboratory food and sterilized water and acclimatized for at least a week before use.

Surgical preparation of rats for electrophysiology

A single dose of pentobarbital (60 mg/kg i.p., Nembutal, Lundbeck, Deerfield, IL) was used to induce anesthesia in previously untreated (drug naïve) male Sprague Dawley rats (300–350 g, Charles River Laboratories, Hollister, CA, USA) and propofol (20–25 mg/kg per hour intravenously (i.v.); Propoflo, Abbott, Abbott Park, IL, USA) was used to maintain anesthesia throughout the experiments using systemic administration of test substances ($n = 25$ rats). For the longer term microiontophoretic experiments ($n = 5$ rats) anesthesia was maintained with intravenous infusions of α -chloralose dissolved in 40% 2-hydroxypropyl- β -cyclodextrin (initial dose 60–70 mg/kg, maintenance dose 10–20 mg/kg per hour) (Storer *et al.*, 1997). Left and right femoral veins were cannulated for administration of anesthetics and drugs.

Physiological monitoring

The left femoral artery was cannulated for continuous monitoring of arterial blood pressure using a transducer (DTX Plus DT-XX; Becton Dickinson, Sandy, UT, USA) connected to an amplifier (PM-1000; CWE, Ardmore, PA, USA). After a tracheostomy, the trachea was cannulated and the rats were ventilated (model 7025; Ugo Basile, Comerio, VA, Italy) with oxygen-enriched air (40% v:v, 1.0 L/min, 2.5–3.5 mL per stroke, 80–100 strokes per minute), and end-tidal CO₂ was monitored (CapStar-100; CWE) and maintained between 3.5% and 4.5%. The rats were placed on a heating blanket with a DC-power supply designed to emit only extremely low levels of electromagnetic-radiofrequency (EMF–RF) noise interference and thermostatically regulated by continuous feedback from a thermistor circuit (Homeothermic blanket system; Harvard Apparatus, Holliston, MA, USA) to maintain core body temperature at 37 ± 0.5 °C. The rat's heads were fixed in a stereotaxic frame (Kopf

Instruments, Tujunga, CA, USA). Data regarding the variables described above, blood pressure, tidal CO₂, and core temperature, was fed to a data acquisition system (Power 1401, CED, Cambridge, UK) and laptop computer where they were continuously displayed and monitored to assist maintenance between physiological limits, and stored on hard disk.

Preparation for neuronal activity recording

A dental burr was used under constant saline irrigation to create a craniectomy over the parietal cortex to expose the middle meningeal artery, its branches, and adjacent supratentorial dura mater (MMA). To avoid desiccation of the tissues, a thin layer of mineral oil was applied over the craniectomy. A bipolar electrode (NE200; Rhodes Medical Instruments, Summerland, CA, USA), connected to a stimulus isolation unit (SIU5A, Grass Instruments, West Warwick, RI, USA), was placed straddling the MMA with its poles touching the dura mater on either side. After separating the neck muscles, a C1 partial hemilaminectomy was performed to allow access to the cervical trigeminocervical complex (TCC). The spinal dura mater covering the area of the laminectomy was removed, and a tungsten electrode with a nominal impedance of 1.0 M Ω (TM31A10; World Precision Instruments, Sarasota, FL) was introduced into the TCC for extracellular recording. For microiontophoretic experiments, a carbon-fiber recording electrode with an impedance of 1.0 \pm 0.3 M Ω when measured at 1 kHz in 0.9% saline (Impedance Check Module; FHC, Bowdoinham, ME, USA) tip size 19.5 \pm 4.1 μ m, mean \pm SD, incorporated into a multibarreled microiontophoretic pipette assembly (Carbostar-7S; Kation Scientific, Minneapolis, MN, USA), was used to record extracellular activity. To localize recording sites electrodes were advanced or retracted in 5 μ m steps using a piezoelectric motor-driven microelectrode positioner attached to a micromanipulator (model 1760-61; Kopf Instruments) while testing for receptive fields and poststimulus activity. Wide dynamic range (WDR)

neurons in the cervical trigeminocervical complex with convergent input from the MMA and cutaneous receptive fields were identified by noxious pinch and innocuous brush of the skin innervated by the first (ophthalmic) branch of the trigeminal nerve (V) and their response to electrical stimulation of the perivascular trigeminal afferents from the MMA and surrounding dura.

Stimulation and recording of activity in the trigeminocervical complex

Perivascular trigeminal afferents innervating the MMA and surrounding dura were stimulated using trains of 20 (experiments using systemic administration of test substances) or 25 (microiontophoretic experiments) electrical square-wave pulses (8–22 V for 0.1–0.2 ms at 0.5 Hz; S88 Stimulator, Grass Instruments, West Warwick, RI, USA) delivered through a bipolar NE200 stimulating electrode as described above.

Signals from the recording electrodes were passed into a head-stage amplifier (NL100AK, Neurolog, Digitimer, Welwyn Garden City, Hertfordshire, UK) then passed to an AC preamplifier (NL104, Digitimer), set to a gain of 1000× or 2000× employing a low frequency cut-off filter at 10 Hz to remove DC components, before further band-pass filtering from 300 Hz to 10 kHz using a filter module (NL125/126, Digitimer) without using the active 60 Hz notch filter to remove line frequency interference that was instead removed by passing the signal to a Hum Bug noise subtraction system (Quest Scientific, Vancouver, BC, Canada), which cancels 60 Hz line noise and its related harmonics up to 4 kHz without distortion of critical components or frequency characteristics of the desired signal that may be removed by notch filtering, before further amplification using an AC–DC amplifier (NL106, Digitimer) with a gain range of about 50–100×. From the AC–DC amplifier the signal was passed to a data acquisition system (Power 1401 with a Spike 2 expansion box; CED) and to a gated amplitude discriminator (NL201 Spike Trigger, Digitimer) the output of which was fed to the

Accepted Article

data acquisition system to allow frequency logging, and to an oscilloscope and audio amplifier (NL120, Digitimer) to assist with spike discrimination by monitoring the direct output from the NL106 amplifier or spikes gated by the NL201. A storage oscilloscope triggered by the stimulus of the perivascular MMA afferents further assisted real-time spike discrimination by displaying activity from up to 100 ms post stimulation. All spike data where they were continuously displayed on a PC using Spike software (version 5; CED), and stored on hard disk as poststimulus histograms and as continuously monitored activity.

Test substance administration

Multibarreled microiontophoretic application

On the day of the experiments, test substances for microiontophoresis were dissolved in water for injection (USP) and filtered to 0.02 μm . Memantine hydrochloride (Tocris Bioscience) 100 mM, pH 5.0; MgCl (Fisher Scientific) 100 mM, pH 5.0; monosodium L-glutamate (200 mM, pH 8.0) and *N*-methyl-D-aspartate (NMDA 100 mM, pH 8.5) were both from Sigma-Aldrich; pH of solutions was adjusted by adding solid sodium bicarbonate. Sodium chloride at neutral pH was used in a current control barrel (100 mM) and a current balance barrel (200 mM). After filling, microiontophoretic pipette barrels passing useful (>10 nA) iontophoretic currents at ± 135 V compliance usually had impedances of 5–20 $\text{M}\Omega$ tested at 10 nA peak-to-peak at 1 kHz in saline (Impedance Check Module; FHC) and resistances of 20–200 $\text{M}\Omega$ in situ (6400A; Dagan, Minneapolis, MN, USA).

A microiontophoresis current generator (6400A; Dagan) provided the currents for ejecting substances from the micropipette barrels and output current levels were passed to the data acquisition system (Power 1401 with a Spike 2 expansion box; CED) where it was collected and displayed simultaneously with free running spike activity using Spike 2 software (version 5; CED, Cambridge, UK). L-Glutamate and NMDA were ejected as anions and retained with

small positive currents (approximately 3–5 nA) to restrain passive diffusion from barrels between ejection periods. If a neuron was responsive to L-glutamate and NMDA, the tip of the microiontophoretic pipette assembly was assumed to be close to a neuronal soma that has accessible glutamate receptors (and therefore second order synapse), and the ejection current required to produce a stable baseline response across at least 5 epochs of L-glutamate application or NMDA application, as demanded for cell testing, was established empirically for each neuron such that the firing activity of neurons was at a maximum of 30–200 Hz during pulses of application to avoid excitotoxicity. This current ranged from –10 to –60 nA. The current pulses were typically applied for 10 s and alternated with a similar or longer period of retention, so that inhibition, or facilitation, of neuronal firing by the test substances, could be determined and distinguished from random firing and noise without overexciting the neurons. Responses were quantified in continuous rate histograms using 1 s bins or using 0–50 ms poststimulus histograms. Ejection currents for memantine, magnesium, and Na⁺ control (with a Cl⁻ balancing current) were positive, ejecting these substances as cations in the range of from 5 to 80 nA, and small retaining currents of approximately –3 to –5 nA were used. Controls were based on passing currents of the same magnitude and direction through the barrel containing 100 mM NaCl. Active current balancing was always provided through the barrel containing 200 mM NaCl.

Poststimulus histograms

Baseline histograms of activity after stimulating perivascular trigeminal afferents innervating the MMA and surrounding dura were obtained by calculating the mean of three consecutive poststimulus histograms, each consisting of 20 stimuli (25 in microiontophoretic experiments) before any test substance or vehicle administration. The poststimulus histograms were collected immediately before, during, and after (to assess recovery for up to

1 h) microiontophoretically delivered substance interventions, which at the “during” phase had produced a steady state of inhibition at their highest dose. Nerve firing activity from the WDR neurons in the cervical trigeminocervical complex with convergent input from the perivascular MMA afferents and cutaneous receptive fields innervated by the ophthalmic division of trigeminal nerve were similarly collected while mechanically stimulating cutaneous receptive fields as described earlier. In practice about 5–6 epochs of excitatory amino acid ejections equivalent to about 5 min of test substance ejection at the highest dose were made before poststimulus histogram collections during interventions and neuronal activity had recovered to baseline levels within 15 min of ceasing the microiontophoretic ejection currents and applying the described small retaining currents.

Microiontophoretic study design

For microiontophoretic experiments data were collected from up to several neurons, identified as described, in each previously untreated rat ($n = 5$ rats). We aimed to collect data from at least 5 and up to 7 neurons for each iontophoretic current used and for each substance tested. Glutamate and NMDA were applied in approximately 10 s pulses as described and then simultaneously, the iontophoretic currents for Mg^{2+} , memantine, and Na^+ (as proxies for doses based on Faraday’s Law, and as current controls in the case of Na^+) were applied in a cumulative-dose fashion of at least 3 and up to 4 doses until a steady-state dose–response effect was seen at each dose (for at least 5 pulses of the excitatory amino acid) before allowing up to 1 h for recovery to pretest levels. The activity from 5 epochs of glutamate or NMDA application was averaged for each data point. The order of application of Mg^{2+} , memantine, and Na^+ was alternated to avoid ordering effects. The resource equation method (http://www.3rs-reduction.co.uk/html/6__power_and_sample_size.html) indicates an E of between 12 and 25 for these conditions, which should be adequate to detect substantial

effects with a low probability of a Type II error.

Recording site localization

At the end of the microiontophoretic protocols, the ends of electrode tracks were marked with electrothermolytic lesions (anodal DC current of 25 μ A for 20–30 s; Model DC-LM5 lesion maker, Grass Instruments). The traditional Chicago Sky Blue marking method was not used, allowing an additional barrel to be used for test substances providing direct within cell comparisons of responses to test substances and controls. At the end of experiments rats were humanely killed with an overdose of sodium pentobarbital (Nembutal, Hospira, Lake Forest, IL; 80 mg/kg i.p.). The lesioned spinal cord tissue was excised by dissection, fixed in neutral buffered 10% formalin (4% formaldehyde), cryoprotected with 30% sucrose (w/v) in phosphate buffered saline at 4 °C until saturated, embedded in optimal cutting temperature medium for frozen tissue specimens (OCT, Sakura Finetek, Torrance, CA) on a cryostat freezing chuck, sectioned in a coronal plane perpendicular to the imaginary horizontal plane joining Bregma and lambda at 40 μ m intervals on a cryostat (CM3050 S; Leica Microsystems, Nussloch, Germany) at –16 to –25 °C, and serial sections mounted on gelatin-coated glass slides. The lesion marks were visualized using a Zeiss Axioplan Universal microscope (Carl Zeiss Microscopy). Images were processed using AxioVision LE software (version 4.6, Carl Zeiss MicroImaging) and recording positions relative to the lesion marks and cord surface determined using digital caliper software and from digital positioning output from the microelectrode positioner and stereotaxic measurements from the midline of the cord and the obex. In this manner, the recording positions were reconstructed and projected onto a schematic representation of the cord at C2 (Molander & Grant (1995).

Systemic test substance administration and study design

After acquiring three baseline poststimulus histograms (15, 10 and 5 min before intravenous infusion of test substances or vehicle control), memantine (10 mg/kg in water for injection USP) or magnesium sulphate (Fisher Scientific, Pittsburg, PA; 100 mg/kg in water for injection USP) were administered intravenously. The volume administered was 1 mL. As a positive control, naratriptan (Sigma-Aldrich, St. Louis, MO; 5 mg/kg in water for injection USP)(Cumberbatch *et al.*, 1998), or the vehicle control were administered. Poststimulus histograms were acquired before and at 5, 10, 15, 20, 25, 30 and 45 min after administering test substances or controls. We allocated 6 previously untreated rats to each of MgSO₄, memantine, and the vehicle control treatments, and 7 rats for the positive naratriptan control treatment in an unselected manner. The resource equation method indicates an adequate E of 21 for these conditions, albeit to detect substantial effects.

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics for Windows (version 19.0, IBM Corporation, Armonk, NY). Data are expressed as mean values with standard errors of the mean (SEM) unless otherwise specified. Treatment effects within one group were analyzed using an ANOVA with repeated measures. If the assumption of sphericity was violated Greenhouse–Geisser corrections were applied. Bonferroni or Dunnett post hoc tests were applied for multiple comparisons. Two-sided unpaired Student *t* tests were used to compare poststimulus histograms with pretreatment baseline values. $P < 0.05$ was considered significant in tests of inference. The results are expressed as percentages of baseline.

Results

Microiontophoretic administration of low-affinity NMDA-receptor channel blockers significantly inhibits NMDA-, glutamate-, craniovascular afferent-, and receptive field-evoked trigeminal neuron firing

All neurons tested in microiontophoretic experiments were classified as wide-dynamic range neurons and had facial cutaneous receptive fields, primarily restricted to the territory of the first (ophthalmic or V₁) division of the trigeminal nerve ($n = 11$ neurons), including the cornea, although some overlap with the second (maxillary or V₂) trigeminal division was sometimes observed. Microiontophoretic application of magnesium and memantine at the second-order synapse of trigeminocervical neurons in the trigeminovascular system linked to MMA-stimulation and convergent V₁ input from receptive fields (Fig. 1) inhibited the neuronal response to L-glutamate (Fig. 2A) and NMDA (Fig. 2B) applied microiontophoretically in the same region, in a reversible, dose-dependent manner. The inhibition was significant even at low currents after NMDA-evoked firing compared with current-matched sodium chloride controls. By contrast, although the inhibition of L-glutamate-evoked firing was significant, the inhibition was usually less than the inhibition of NMDA-evoked firing and not complete, even at the highest currents used. Neuronal firing was inhibited more profoundly by memantine than by magnesium (Fig. 2A–C) and activity inhibited by memantine recovered more slowly (Fig. 2C). Current-matched sodium controls (with an equivalent chloride balance current) did not affect firing significantly, although an increased tendency was noted at the highest current (80 nA) used.

Magnesium and memantine delivered microiontophoretically to the second-order synapse of trigeminocervical neurons in the trigeminovascular system (Fig. 1) also inhibited the neuronal response to V₁ cutaneous receptive field mechanostimulation (Fig. 3A) and electrical stimulation of afferents from the MMA and perivascular dura (Fig. 3B). Current-matched

sodium chloride controls did not significantly inhibit the responses evoked by mechanostimulation of cutaneous receptive fields or electrical stimulation of afferents from the MMA and perivascular dura. Representative poststimulus histograms show reversible inhibition by memantine and Mg^{2+} , but not sodium chloride, of neuronal firing evoked by electrical stimulation of afferents from the MMA and perivascular dura (Fig. 3C).

Intravenous systemic administration of moderate concentrations of low-affinity NMDA-receptor channel blockers does not inhibit stimulus-evoked firing significantly

Systemic administration of magnesium at 100 mg/kg intravenously did not significantly inhibit stimulus-evoked neuronal firing in the trigeminocervical complex ($F_{2.5, 12.37} = 1.72$, $P = 0.217$, $n = 6$; ANOVA followed by a Bonferroni post hoc test). Similarly, memantine administered at 10 mg/kg intravenously failed to inhibit neuronal firing in the trigeminocervical complex significantly ($F_{2.2, 11.2} = 0.70$, $P = 0.531$, $n = 6$). By contrast, naratriptan at 5 mg/kg intravenously, which was used as a positive control, was strongly inhibitory ($F_{3.3, 19.9} = 33.43$, $P < 0.001$, $n = 7$), with inhibition reaching a maximum 30 minutes after intravenous administration ($61 \pm 6\%$, $t_6 = 11.00$, $P < 0.001$). Vehicle control administered intravenously had no significant effect on the neuronal firing ($F_{2.5, 12.4} = 0.98$, $P = 0.419$, $n = 6$) (Fig. 5). As can be seen from Figures 4B and 4C the effect of memantine appears to have a bimodal distribution, with some animals responding to about 30% inhibition at 5 min with others showing no effect (nonresponders) or even excitation. By 30 min the effect of Mg^{2+} is reducing, while the effect of naratriptan increases, the effect of memantine is about the same, perhaps slightly increased in the animals that responded. Compared with vehicle controls, magnesium and memantine exerted strong, but short lasting, reductions in arterial blood pressure. The maximum reduction was $49 \pm 11\%$ ($t_5 = 4.54$, $P = 0.006$) after administering magnesium and $31 \pm 5\%$ ($t_5 = 6.31$, $P = 0.001$) after memantine.

Blood pressure returned to pretreatment levels in both groups within 5 min. Naratriptan induced a weaker, but longer lasting reduction in blood pressure ($26 \pm 5\%$, $t_6 = 5.35$, $P = 0.002$) that did not return to pretreatment levels within 45 min.

Discussion

In the present study, we demonstrate that the microiontophoretic delivery of the low-affinity NMDA-receptor channel blockers magnesium and memantine into the trigeminocervical complex inhibit L-glutamate- and NMDA-evoked responses from trigeminocervical complex neurons at the second order trigeminal synapse linked to afferents from the MMA and its perivascular dura and cutaneous receptive fields in a rat model of trigeminal nociception.

Moreover, microiontophoretic delivery of magnesium and memantine also inhibited nociceptive responses from the same trigeminocervical complex neurons following electrical stimulation of afferents from the MMA and its perivascular dura and mechanostimulation of cutaneous afferents. However, when magnesium and memantine were delivered systemically at moderate concentrations these nociceptive responses were not significantly inhibited.

Higher concentrations of magnesium and memantine may be necessary to obtain significant inhibition.

Glutamate, well known as the most important and abundant excitatory neurotransmitter in the CNS, is likely to be fundamental to the pathophysiology of migraine (Ramadan *et al.*, 2003; Vikelis & Mitsikostas, 2007). NMDA receptor channel blockers, which effectively impede the influx of Ca^{2+} and thereby channel function, differentiate among each other in their affinity for the receptor, their blocking and unblocking kinetics, and consequently in their voltage-dependency. A lower receptor affinity leads to faster blocking and unblocking kinetics, and a higher voltage-dependency. Substances with these lower NMDA receptor affinity properties, such as magnesium and memantine are characterized by fewer and less

severe side effects than substances with slower receptor kinetics, and are therefore widely used for several clinical conditions. Substances with slow receptor kinetics such as ketamine, phencyclidine, and dizolcipine (MK-801) induce clinically unacceptable side effects, such that their use is limited to certain conditions and routes of administration impede their use on an outpatient basis (ketamine) or even at all (dizolcipine) (Chizh, 2007). Magnesium and memantine show only very limited side effects compared with current preventive treatment options. Magnesium represents one of the very few preventives that can be used during most trimesters of pregnancy.

That several NMDA receptor channel blockers suppress CSD in the rat (Lauritzen & Hansen, 1992; van der Hel *et al.*, 1998; Peeters *et al.*, 2007) supports the hypothesis that glutamate is fundamental to the pathophysiology of migraine. Consistent with these findings, magnesium deficiency increases the sensitivity of NMDA receptors to glutamate-induced CSD (Van Harreveld & Fifkova, 1973). Memantine significantly reduces nociceptive behavioral responses and c-Fos expression in the trigeminocervical complex induced by the injection of formalin into the periorbital area of rats (Park *et al.*, 2012). Experiments from our laboratory in cats have demonstrated that intravenous administration of the NMDA-receptor channel blocker dizolcipine is able to inhibit neuronal firing (Storer & Goadsby, 1999) and c-Fos expression in the trigeminocervical complex evoked by electrical stimulation of craniovascular and perivascular dural afferents (Classey *et al.*, 2001). These findings strongly suggest a glutamatergic mechanism for trigeminal nociception and the potential of NMDA receptor channels as therapeutic targets for migraine.

Clinical studies of migraineurs support the evidence obtained from laboratory research. By analogy with the findings from *in vivo* experiments showing that NMDA receptor channel blockers are able to inhibit CSD, intranasally administered ketamine is able to attenuate migraine aura (Kaube *et al.*, 2000; Afridi *et al.*, 2013). Results from a small clinical trial

suggest that subcutaneously administered ketamine is useful for the treatment of migraine pain (Nicolodi & Sicutieri, 1995).

Low magnesium levels have been found in serum, saliva, and CSF of migraineurs (Jain *et al.*, 1985; Sarchielli *et al.*, 1992). The noninvasive measurement of brain magnesium by NMR spectroscopy showed reduced levels in migraineurs (Ramadan *et al.*, 1989). Measurements of ionized magnesium in serum also showed reduced levels in migraineurs (Mauskop *et al.*, 1995; 1996). Subsequently, clinical studies were conducted to elucidate the potential efficacy of the low-affinity NMDA-receptor channel blockers magnesium and memantine for migraine treatment. Many of these studies had several shortcomings including small sample sizes, retrospective design, or lack of separation between different types of primary headaches. Nevertheless, these studies showed promising results (Mauskop *et al.*, 1995; 1996; Charles *et al.*, 2007; Bigal *et al.*, 2008), including a positive randomized placebo-controlled trial (Noruzzadeh *et al.*, 2016). However, the promising results for magnesium could not always be satisfactorily reproduced in the randomized controlled trials (Peikert *et al.*, 1996; Pfaffenrath *et al.*, 1996; Bigal *et al.*, 2002). To our knowledge, randomized controlled trials analyzing the efficacy of memantine for migraine treatment have not yet been reported.

Local administration of magnesium and memantine into the trigeminocervical complex inhibits trigeminal nociceptive responses in the trigeminocervical complex, perhaps through a sufficiently high concentration at the NMDA receptors to block transmission of nociception there. However, using intravenous administration, which provides a systemic dose, more closely resembling administration in humans, we failed to find significantly reduce neuronal responses in the trigeminocervical complex to electrical craniovascular and meningeal stimulation at moderate, and perhaps clinically achievable concentrations. This finding may provide an explanation for the contradictory results of randomized controlled trials with

magnesium. A lower concentration at the sites of action within the CNS after systemic administration might account for the lack of clinically significant effects. However, the doses used in this study are high compared with those used in humans, where the usual magnesium dosage used in the clinical trials for the prophylactic treatment of migraine was 1000 mg per day (Mauskop *et al.*, 1995; 1996), and that for memantine is 10–20 mg per day for treatment of Alzheimer's disease. Even considering the difficulty in comparing dosages used in humans to those used in animals during experiments *in vivo*, a further increase in dose was not practical in the experiments performed, because the intravenous doses used for both magnesium and memantine already induced significant decreases in blood pressure that might affect firing rate of trigeminal nociceptive neurons or even result in cerebral hypoperfusion, albeit temporarily. A change of structure that leads to different unblocking kinetics for the channel blocker might provide a solution to this problem, by providing blocking sufficiently high enough to block nociception, but not so strong as to produce unwanted side effects impeding its clinical use.

The present study is limited in that we did not specifically identify neurons in the trigeminocervical complex with dural input in the immunohistochemical study. Identification of neurons with dural input would require further experiments such as might be conducted using retrograde tracers such as DiI applied to the perivascular dura (Fioretti *et al.*, 2011). The retrograde tracing might be combined with further confirmatory localization such as *in situ* hybridization and further immunochemical controls, such as the use of other primary antibodies directed against a different epitope, and more precise confocal laser scanning microscopy.

Although the concept of dose in microiontophoretic experiments essentially follows Faraday's Law, for various reasons it is difficult to control the dose of substance delivered to the neuron and each experimental circumstance may differ slightly (Stone, 1985). It is possible that magnesium and memantine were acting nonspecifically at high local concentrations and that including AMPA as a control might have improved interpretation. Aside from technical limitations on the number of agonists and antagonists that can be used in the experiments, the specificity of magnesium as a channel blocker is well known. Herero *et al.* (1994) had already determined that memantine is relatively specific for NMDA receptors so we wanted to determine the overall effect of memantine and magnesium on glutamatergic transmission to estimate the contribution of NMDA receptor channels on glutamatergic transmission overall. Both substances are not particularly potent with memantine being about 6 times weaker than ketamine (Herero *et al.*, 1994). We were able to see effects at relatively low currents being significant at 5–20 nA in the case of memantine. That NMDA and glutamate evoked activity was similarly inhibited, although glutamate to a lesser extent suggests that NMDA receptors make a substantial contribution to the overall glutamatergic transmission, which may explain the relative lack of clinical effectiveness of AMPA receptor antagonists such as BGG492 (Gomez-Mancilla *et al.*, 2014). In the microiontophoretic experiments we recorded from 11 neurons in 5 rats. An advantage of microiontophoresis is of being able to record more than one unit in a single rat reducing the number of animals required. Moreover, the multibarreled technique and fast diffusion of substances from the area of local application allows for the testing of multiple substances on a single neuron.

For technical reasons involving blood pressure and solubility we did not use the higher doses of memantine of up to 32 mg/kg that have been used by others (Herrero *et al.*, 1994). The means that we may have missed seeing a significant effect of memantine that might have been seen at a higher concentration. The effect of memantine appears to be distributed bimodally, with some animals responding while others are nonresponders. For the animals that did respond, the effect of memantine was greater than for Mg^{2+} , at least at 30 min, which is what is expected and consistent with the iontophoretic data. It would be useful to review the data presented in the clinical studies (Cammarata & Krusz, 2005; Kew & Kemp, 2005; Charles *et al.*, 2007; Bigal *et al.*, 2008; Spengos *et al.*, 2008; Noruzzadeh *et al.*, 2016) to see if there are groups of responders and nonresponders.

Conclusion

To our knowledge, this is the first study that compares the effectiveness of the low affinity NMDA-receptor channel blockers memantine and magnesium on central trigeminal nociceptive transmission and demonstrates poor effectiveness in their more clinically relevant systemic administration providing a potential explanation for their uncertain effect in the treatment of migraine and primary head pain. Nevertheless, the present results suggest blocking NMDA-receptor channels is an effective way to inhibit nociceptive activation in the trigeminocervical complex. Further exploration of such channel blockers as a therapeutic strategy for primary head pain is warranted.

Funding

This work was supported by the Sandler Foundation; the Migraine Trust; the Korean travelling fellowship grant (J.W.P.), the German Research Foundation (Deutsche Forschungsgemeinschaft; DFG grant number HO4369/1-1 to J.H.), The Wellcome Trust

(104033) and EUROHEADPAIN European Union FP7 (602633).

Acknowledgements

The authors thank Drs. Simon Akerman and Weera Supornsilpchai for their valuable discussions and comments on the manuscript and Michele Lasalandra for advice and technical assistance with the histology to locate recording sites shown in Figure 1.

Competing interests

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership or other equity interest; and expert testimony or patent-licensing arrangements) or nonfinancial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. Unrelated to the submitted work, J.H. is consulting and/or serving on advisory boards for Allergan, Autonomic Technologies Inc. (ATI), Chordate Medical AB, Eli Lilly, Hormosan Pharma, Novartis and Teva Pharmaceuticals. He received honoraria for speaking from Allergan, Chordate Medical AB, Novartis and Teva. Unrelated to this work, P.J.G. reports grants and personal fees from Amgen and Eli-Lilly and Company, and personal fees from Alder Biopharmaceuticals, Allergan, Autonomic Technologies Inc., Dr Reddy's Laboratories, Electrocore LLC, eNeura, Novartis, Scion, Teva Pharmaceuticals, and Trigemina Inc., and personal fees from MedicoLegal work, Journal Watch, Up-to-Date, Oxford University Press, Massachusetts Medical Society, and Wolters Kluwer; and a patent Magnetic stimulation for headache assigned to eNeura without fee. R.J.S., and J.W.P. declare that they have no potential conflicts of interest to disclose.

Data Accessibility

Data files from the experiments conducted sufficient to fully reproduce the results of the article have been uploaded to Figshare a permanent data archiving public repository with the DOI: EJN0000000000. A demonstration version of Spike2 for Windows to view the original electrophysiology files with *.smr, *.S2R, and *.srf extensions is available for download along with supporting documentation and help files at <http://ced.co.uk/upgrades/spike2demo>. SigmaPlot *.JNB files can be viewed with software available for download from <https://systatsoftware.com/downloads/download-sigmaplot/>.

Contributions

J.H. (jan.hoffmann@kcl.ac.uk) conceived, designed and conducted the systemic administration study, acquired, analyzed, and interpreted the data, contributed equally with R.J.S. to drafting the initial manuscript, critically revised and edited the manuscript, and approved the final version, and agrees to be accountable for all aspects of the work.

R.J.S. (james.s@chula.ac.th) conceived and designed the study, and conducted the microiontophoresis experiments, supervised the immunohistology and acquired, analyzed, and interpreted the data, drafted the initial manuscript, critically revised and edited the manuscript, and approved the final version, and agrees to be accountable for all aspects of the work.

J.W.P. (pjw516@catholic.ac.kr) assisted with conducting the systemic administration study, acquiring the data and analysis, contributed to preparing the manuscript, approved the final version, and agrees to be accountable for all aspects of the work.

P.J.G. acquired the funding and provided resources, helped design, and oversaw the conduct of the study, contributed to interpreting the data, and drafting and critically revising the manuscript, approved the final version, and agrees to be accountable for all aspects of the work.

Abbreviations:

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; MMA, middle meningeal artery; TCC, trigeminocervical complex; CGRP, calcitonin gene-related peptide.

Figure 1. Sites of neurons ($n = 11$) recorded in the trigeminal nucleus caudalis/cervicalis (trigeminocervical complex) at the level of C1, reconstructed from marks made at the end of electrode tracts using electrothermolytic lesions. Figure 1 shows the recording sites for the microiontophoresis experiments on the same neurons using different stimuli, as shown in Figure 2 (excitatory amino acid (microelectrophoretic delivery) stimulated activity) and Figure 3 (V_1 cutaneous receptive field mechanostimulation (Figure 3A) and stimulation of afferents from the middle meningeal artery or its branches and periarterial dura mater (MMA) (Figure 3B) with microiontophoretic delivery of memantine and Mg^{2+} at various currents in Figure 2 and at maximal currents in Figure 3. The timing of MMA stimulus is seen in Figure 2C. The cord representation is based on a figure by Molander and Grant (Molander & Grant, 1995) with permission.

Figure 2. Scatter plots showing that microiontophoretic application of magnesium and memantine (5–80 nA) inhibited the response of trigeminovascular neurons to L-glutamate ($n = 9$ neurons for memantine, and 7 neurons for Mg^{2+} , panel A) and NMDA ($n = 5$ neurons for memantine, and 7 neurons for Mg^{2+} , panel B) in a reversible, dose-dependent manner, with inhibition of NMDA-evoked activity by memantine reaching significance even at the lowest current used (5 nA, $t = 6.23$, $P < 0.001$) compared with current-matched sodium controls. Memantine and Mg^{2+} were applied to the same neurons in alternating order with NMDA and L-glutamate in alternating order to avoid ordering effects. However, because of technical difficulties including channel blocking and electrode drift, not all neurons provided data for all substances. The inhibition of L-glutamate-evoked activity was significant, but usually partial that of NMDA, even at the highest currents used (20 nA memantine, $t = 6.83$, $P < 0.01$; 80 nA Mg^{2+} , $t = 3.73$, $P < 0.05$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, #### $P < 0.001$. The bars indicate means. Asterisks (*) indicate comparisons with sodium current controls (Dunnett's t). Hash marks (#) indicate comparisons with recovery of activity from inhibition. R indicates recovery from inhibition during which time memantine, Mg^{2+} , and Na^+ were retained in the barrels of the microiontophoretic electrode. (C) Representative inhibition of NMDA-evoked firing shown as free-running activity in a histogram with 1 s bins. Neuronal firing was more profoundly inhibited by memantine and activity recovered more slowly than with magnesium. MMA (bars) represent electrical stimulation of afferents from the middle meningeal artery or its branches and periarterial dura mater. Sodium current matched controls (with an equivalent chloride balance current) did not significantly affect firing, although sodium's tendency to inhibit was noted at the highest current used.

Figure 3. Memantine and magnesium reversibly inhibited the response of trigeminovascular neurons to (A) V₁ cutaneous receptive field mechanostimulation ($n = 5$ neurons, $F_{2,10} = 27.5$, $P < 0.001$) and (B) stimulation of afferents from the middle meningeal artery or its branches and periarterial dura mater (MMA), whereas no significant inhibition, shown as percentage change, was observed with current-matched sodium (chloride) controls ($n = 6$ neurons for memantine, $t_9 = 5.15$, $P < 0.001$; 5 neurons for Mg^{2+} , $t_9 = 2.93$, $P < 0.05$; 5 for Na^+), $*P < 0.05$, $***P < 0.001$. The bars indicate means. (C) Representative poststimulus histograms showing reversible inhibition by memantine and Mg^{2+} , but not sodium chloride, of neuronal firing evoked by electrical stimulation of afferents from the MMA and perivascular dura. The stimulus artifact is seen on the far left of each histogram. Sites of neurons ($n = 11$) recorded in the trigeminal nucleus caudalis/cervicalis are seen in Figure 1.

Figure 4. Effect of intravenous administration of magnesium (100 mg/kg), memantine (10 mg/kg), and naratriptan (5 mg/kg) on inhibition of neuronal firing in the trigeminocervical complex evoked by electrical stimulation of afferents from the MMA and perivascular dura. (A) Time course in minutes with baseline firing measured at 5, 10, and 15 min before systemic administration of test substances and controls at time 0 min. The group means are displayed as lines and shaded regions represent standard errors above and below the means. (B) Difference plot (from 0) of individual data at 5 min. (C) Difference plot (from 0) of individual data at 30 min. Bars show median values. Drawn using the interactive line graph tool available at <http://statistika.mfub.bg.ac.rs/interactive-linegraph/> as described by Weissgerber *et al.* (2016).

References

- Afridi, S., Giffin, N.J., Kaube, H. & Goadsby, P.J. (2013) A randomized controlled trial of intranasal ketamine in migraine with prolonged aura. *Neurology*, **80**, 642–647.
- Akerman, S., Holland, P. & Goadsby, P.J. (2011) Diencephalic and brainstem mechanisms in migraine. *Nature Rev. Neurosci.*, **12**, 570–584.
- Alexander, S.P., Mathie, A. & Peters, J.A. (2011) Guide to Receptors and Channels (GRAC), 5th edition. *Br J Pharmacol*, **164 Suppl 1**, S1–S324.
- Altura, B.M. (1985) Calcium antagonist properties of magnesium: implications for antimigraine actions. *Magnesium*, **4**, 169–175.
- Ambrosini, A., de Noordhout, A.M., Sandor, P.S. & Schoenen, J. (2003) Electrophysiological studies in migraine: a comprehensive review of their interest and limitations. *Cephalalgia*, **23**, 13–31.
- Andreou, A.P. & Goadsby, P.J. (2009) Therapeutic potential of novel glutamate receptor antagonists in migraine. *Exp. Rev. Investig. Drugs*, **18**, 789–804.
- Bigal, M., Rapoport, A., Sheftell, F., Tepper, D. & Tepper, S. (2008) Memantine in the preventive treatment of refractory migraine. *Headache*, **48**, 1337–1342.
- Bigal, M.E., Bordini, C.A., Tepper, S.J. & Speciali, J.G. (2002) Intravenous magnesium sulphate in the acute treatment of migraine without aura and migraine with aura. A randomized, double-blind, placebo-controlled study. *Cephalalgia*, **22**, 345–353.
- Blonk, M.I., Koder, B.G., van den Bemt, P.M. & Huygen, F.J. (2010) Use of oral ketamine in chronic pain management: a review. *Eur. J. Pain*, **14**, 466–472.
- Bereiter, D.A. & Bereiter, D.F. (2000) Morphine and NMDA receptor antagonism reduce c-fos expression in spinal trigeminal nucleus produced by acute injury to the TMJ region. *Pain*, 2000 **85**, 65–77.
- Cammarata, D. & Krusz, J. (2005) Memantine: Novel mechanism for migraine and headache

prophylaxis. *Headache*, **45**, 820.

Chai, N.C., Peterlin, B.L., Calhoun, A.H. (2014) Migraine and estrogen. *Curr. Opin. Neurol.*, 2014 **27**, 315–324.

Charles, A., Flippen, C., Romero Reyes, M. & Brennan, K.C. (2007) Memantine for prevention of migraine: a retrospective study of 60 cases. *J. Headache Pain*, **8**, 248–250.

Chizh, B.A. (2007) Low dose ketamine: a therapeutic and research tool to explore *N*-methyl-D-aspartate (NMDA) receptor-mediated plasticity in pain pathways. *J. Psychopharmacol.*, **21**, 259–271.

Classey, J.D., Knight, Y.E. & Goadsby, P.J. (2001) The NMDA receptor antagonist MK-801 reduces Fos-like immunoreactivity within the trigeminocervical complex following superior sagittal sinus stimulation in the cat. *Brain Res.*, **907**, 117–124.

Cumberbatch, M.J., Hill, R.G., Hargreaves, R.J. (1998) Differential effects of the 5HT_{1B/1D} receptor agonist naratriptan on trigeminal versus spinal nociceptive responses. *Cephalalgia*, **18**, 659-663.

GBD 2016 Disease and Injury Incidence and Prevalence Collaborators. (2017) Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet*, **390**, 1211–1259.

Facchinetti, F., Sances, G., Borella, P., Genazzani, A.R. & Nappi, G. (1991) Magnesium prophylaxis of menstrual migraine: effects on intracellular magnesium. *Headache*, **31**, 298–301.

Fioretti, B., Catacuzzeno, L., Sforza, L., Gerke-Duncan, M.B., van den Maagdenberg, A.M.J.M., Franciolini, F., Connor, M. & Pietrobon, D. (2011) Trigeminal ganglion neuron subtype-specific alterations of CaV2.1 calcium current and excitability in a Cacna1a mouse model of migraine. *J. Physiol.* **589(Pt 23)**, 5879–5895.

- Goadsby, P.J., Holland, P.R., Martins-Oliveira, M., Hoffmann, J., Schankin, C. & Akerman, S. (2017) Pathophysiology of Migraine: A disorder of sensory processing. *Physiol. Rev.*, **97**, 553–622.
- Gomez-Mancilla, B., Brand, R., Jurgens, T. P., et al. (2014) Randomized, multicenter trial to assess the efficacy, safety and tolerability of a single dose of a novel AMPA receptor antagonist BGG492 for the treatment of acute migraine attacks. *Cephalalgia*, **34**, 103–113.
- Herrero, J.F., Headley, P.M., Parsons, C.G. (1994) Memantine selectively depresses NMDA receptor-mediated responses of rat spinal neurons in vivo. *Neurosci. Letts.* **165**, 37–40.
- Hocking, G. & Cousins, M.J. (2003) Ketamine in chronic pain management: an evidence-based review. *Anesth Analg.* **97**, 1730–1739.
- Jain, A.C., Sethi, N.C. & Babbar, P.K. (1985) A clinical electroencephalographic and trace element study with special reference to zinc copper and magnesium in serum and cerebrospinal fluid in cases of migraine. *J. Neurol.*, **232**, 161.
- Kaube, H., Herzog, J., Kaufer, T., Dichgans, M. & Diener, H.C. (2000) Aura in some patients with familial hemiplegic migraine can be stopped by intranasal ketamine. *Neurology*, **55**, 139–141.
- Kew, J.N. & Kemp, J.A. (2005) Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology (Berl)*, **179**, 4–29.
- Kleckner, N.W. & Dingledine, R. (1988) Requirement for glycine in activation of NMDA-receptors expressed in *Xenopus* oocytes. *Science*, **241**, 835-837.
- Lauritzen, M. & Hansen, A.J. (1992) The effect of glutamate receptor blockade on anoxic depolarization and cortical spreading depression. *J. Cereb. Blood Flow Metab.*, **12**, 223–229.
- Lipton, S.A. (2007) Pathologically-activated therapeutics for neuroprotection: mechanism of NMDA receptor block by memantine and S-nitrosylation. *Curr. Drug Targets*, **8**, 621-632.
- Mauskop, A., Altura, B.T., Cracco, R.Q. & Altura, B.M. (1995) Intravenous magnesium

sulphate relieves migraine attacks in patients with low serum ionized magnesium levels: a pilot study. *Clin. Sci. (Lond.)*, **89**, 633–666.

Mauskop, A., Altura, B.T., Cracco, R.Q. & Altura, B.M. (1996) Intravenous magnesium sulfate rapidly alleviates headaches of various types. *Headache*, **36**, 154–160.

Melone, M., Burette, A. & Weinberg, R.J. (2005) Light microscopic identification and immunocytochemical characterization of glutamatergic synapses in brain sections. *J. Comp. Neurol.*, **492**, 495–509.

Mody, I., Lambert, J.D. & Heinemann, U. (1987) Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. *J. Neurophysiol.*, **57**, 869–888.

Molander, C. & Grant, G. (1995) Spinal cord cytoarchitecture. In Paxinos, G. (ed) *The Nervous System*. Academic Press, San Diego, pp. 40–42.

Nicolodi, M. & Sicutieri, F. (1995) Exploration of NMDA receptors in migraine: therapeutic and theoretic implications. *Int J Pharmacol Res*, **15**, 181-189.

Noruzzadeh, R., Modabbernia, A., Aghamollaii, V., Ghaffarpour, M., Harirchian, M.H., Salahi, S., Nikbakht, N., Noruzi, N. & Tafakhori, A. (2016) Memantine for prophylactic treatment of migraine without aura: a randomized double-blind placebo-controlled study. *Headache*, **56**, 95–103.

Otahara, N., Ikeda, T., Sakoda, S., Shiba, R. & Nishimori T. (2003) Involvement of NMDA receptors in Zif/268 expression in the trigeminal nucleus caudalis following formalin injection into the rat whisker pad. *Brain Res. Bull.*, 2003, **62**, 63–70.

Park, J.W., Suh, G.I., Shin, H.E. & Park, G.E. (2012) Influence of memantine on nociceptive responses of the trigeminocervical complex after formalin injection. *Cephalalgia*, **32**, 308–316.

Parsons, C.G., Danysz, W. & Quack, G. (1999) Memantine is a clinically well tolerated N-

methyl-D-aspartate (NMDA) receptor antagonist—a review of preclinical data.

Neuropharmacology, **38**, 735–767.

Parsons, C.G., Stoffler, A. & Danysz, W. (2007) Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system - too little activation is bad, too much is even worse. *Neuropharmacology*, **53**, 699–723.

Peeters, M., Gunthorpe, M.J., Strijbos, P.J., Goldsmith, P., Upton, N. & James, M.F. (2007) Effects of pan- and subtype-selective N-Methyl-D-aspartate receptor antagonists on cortical spreading depression in the rat: therapeutic potential for migraine. *J. Pharmacol. Exp. Ther.*, **321**, 564–572.

Peikert, A., Wilimzig, C. & Kohne-Volland, R. (1996) Prophylaxis of migraine with oral magnesium: results from a prospective, multi-center, placebo-controlled and double-blind randomized study. *Cephalalgia*, **16**, 257–263.

Pfaffenrath, V., Wessely, P., Mener, C., Isler, H.R., Evers, S., Grotemeyer, K.H., Taneri, Z., Soyka, D., Gobel, H. & Fischer, M. (1996) Magnesium in the prophylaxis of migraine- a double-blind placebo-controlled study. *Cephalalgia*, **16**, 436–440.

Ramadan, N.M., Halvorson, H., Vande-Linde, A., Levine, S.R., Helpert, J.A. & Welch, K.M.A. (1989) Low brain magnesium in migraine. *Headache*, **29**, 416–419.

Ramadan, N.M., Van den Hende, F. & Granier, L.A. (2003) A double blind, placebo-controlled, crossover study to investigate the effect of subcutaneous sumatriptan or placebo on nitroglycerin-induced migraine symptoms, calcitonin gene-related peptide and glutamate levels. *Neurology*, **60**, A93.

Saleon W, Jansri U, Srikiatkachorn A, Bongsebandhu-phubhakdi S. (2016) Estrous cycle induces peripheral sensitization in trigeminal ganglion neurons: an animal model of menstrual migraine. *J. Med. Assoc. Thai.*, **99**, 206–212.

Sarchielli, P., Coata, G., Firenze, C., Morucci, P., Abbritti, G. & Gallai, V. (1992) Serum and

salivary magnesium levels in migraine and tension-type headache. Results in a group of adult patients. *Cephalalgia*, **12**, 21–27.

Soriani, S., Arnaldi, C., De Carlo, L., Arcudi, D., Mazzotta, D., Battistella, P.A., Sartori, S. & Abbasciano, V. (1995) Serum and red blood cell magnesium levels in juvenile migraine patients. *Headache*, **35**, 14–16.

Spengos, K., Theleritis, C. & Paparrigopoulos, T. (2008) Memantine and NMDA antagonism for chronic migraine: a potentially novel therapeutic approach? *Headache*, **48**, 284–286.

Stone, T.W. Microiontophoresis and pressure ejection. In Smith, A.D. (general ed), *International Brain Research Organization handbook series. Methods in the neurosciences*; volume 8. John Wiley & Sons, Chichester.

Storer, R.J., Butler, P., Hoskin, K.L. & Goadsby, P.J. (1997) A simple method, using 2-hydroxypropyl- β -cyclodextrin, of administering α -chloralose at room temperature. *J. Neurosci. Meth.*, **77**, 49–53.

Storer, R.J. & Goadsby, P.J. (1999) Trigeminovascular nociceptive transmission involves *N*-methyl-D-aspartate and non-*N*-methyl-D-aspartate glutamate receptors. *Neuroscience*, **90**, 1371–1376.

van der Hel, W.S., van den Bergh, W.M., Nicolay, K., Tulleken, K.A. & Dijkhuizen, R.M. (1998) Suppression of cortical spreading depressions after magnesium treatment in the rat. *Neuroreport*, **9**, 2179–2182.

Van Harreveld, A. & Fifkova, E. (1973) Mechanisms involved in spreading depression. *J. Neurobiol.*, **4**, 375–387.

Vikelis, M. & Mitsikostas, D.D. (2007) The role of glutamate and its receptors in migraine. *CNS Neurol Disord Drug Targets*, **6**, 251–257.

Visser, E. & Schug, S.A. (2006) The role of ketamine in pain management. *Biomed. Pharmacother.*, **60**, 341–348.

Waite, P.M.E. (2004) Trigeminal sensory system. In Paxinos, G. (ed) *The rat nervous system*. Elsevier Academic Press, San Diego, pp. 817–851.

Weissgerber, T.L., Garovic, V.D., Savic, M.D., Winham, S.J. & Milic, N.M. From static to interactive: transforming data visualization to improve transparency. *PLoS Biol.*, **14**(6), e1002484.



















