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Published in:
Neuroscience

DOI (link to publication from Publisher):
[10.1016/j.neuroscience.2016.08.007](https://doi.org/10.1016/j.neuroscience.2016.08.007)

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Publication date:
2016

Document Version
Accepted author manuscript, peer reviewed version

[Link to publication from Aalborg University](#)

Citation for published version (APA):
O'Brien, M., & Cairns, B. E. (2016). Monosodium glutamate alters the response properties of rat trigeminovascular neurons through activation of peripheral NMDA receptors. *Neuroscience*, 334, 236-244. <https://doi.org/10.1016/j.neuroscience.2016.08.007>

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Monosodium Glutamate Alters the Response Properties of Rat Trigemino-vascular
Neurons through Activation of Peripheral NMDA Receptors

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Abstract

Ingestion of monosodium glutamate (MSG) has been shown to cause headaches in healthy
individuals and trigger migraine-like headaches in migraine sufferers. We combined
immunohistochemistry, *in vivo* electrophysiology, and laser Doppler recordings of dural
vasculature to investigate the effect of systemic administration of MSG on the
trigemino-vascular pathway. Immunohistochemical analysis confirmed the expression of NMDA
receptors on nerve fibres innervating dural blood vessels and excitatory amino acid transporter
2 on dural blood vessels. Systemic administration of MSG (50 mg/kg) evoked an increase in
ongoing discharge in 5/6 spinal trigeminal subnucleus caudalis (SpVc) neurons with dural input
recorded from male and female rats, respectively, as well as lowering their mechanical

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4 activation threshold. There were no sex-related differences in these effects of MSG. Neuronal
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7 discharge and mechanical sensitization were significantly attenuated by co-injection with the
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10 peripherally restricted NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid in both
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13 sexes. Systemic administration of MSG induced a 24.5% and 20.6% increase in dural flux in
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16 male and female rats, respectively. These results suggest that MSG-induced headache is
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19 mediated by the activation of peripheral NMDA receptors and subsequent dural vasodilation.
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22 Peripheral NMDA receptors are a potential target for the development of new drugs to treat
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27 headaches.

28 **Keywords**

29 Doppler flowmetry, Dura, Nerve fibers, Sensory Neuron, Trigeminal,
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Introduction

The pathophysiology of migraine has yet to be fully elucidated, however, the excitation of trigeminal afferent fibres which innervate the dura and converge at the spinal trigeminal subnucleus caudalis (SpVc), has been identified as a key mechanism in headache pain (Burstein et al., 2015). Historically, migraine has been considered a vascular headache, largely based on the observation of dilation of retinal and temporal blood vessels during a migraine attack (Goltman, 1936; Wolff et al., 1953). Recent studies are conflicting in their support of a vascular mechanism in migraine attacks (Friberg et al., 1991; Zwetsloot et al., 1993; Kruuse et al., 2003; Asghar et al., 2010). Dural blood vessels are heavily innervated by trigeminal afferent fibres, however, and several scenarios exist which could lead to excitation of afferents and also vasodilation during a migraine attack. In the first scenario, dilation of the highly innervated dural blood vessels can activate trigeminovascular afferent fibres, potentially leading to the sensitization of the trigeminovascular system (Levy and Burstein, 2011). In the second scenario, dural afferent fibres which have already been activated may induce vasodilation via neurogenic inflammation by releasing calcitonin gene related peptide (CGRP) and substance P (SP) in (Levy and Burstein, 2011).

Glutamate, an excitatory neurotransmitter, has been linked to migraine pathophysiology for several reasons. Glutamate levels in blood plasma, platelets, and cerebrospinal fluid (CSF) are elevated in migraineurs long after a migraine attack (Martinez et al., 1993; Cananzi et al., 1995; Eufemia et al., 1997), and several genetic variants affecting glutaminergic neurotransmission have been identified in migraine sufferers (Schürks, 2012; Burstein et al., 2015). Glutamate is also well known to be involved in the sensitization of trigeminal afferent fibres (Cairns et al.,

2007; Gazerani et al., 2010b; Laursen et al., 2014), as well as the transduction of nociceptive signaling (Klafke et al., 2012; Chan and MaassenVanDenBrink, 2014). Monosodium glutamate (MSG) is a naturally occurring form of glutamic acid, and is an International Headache Society recognized trigger for headache. MSG-related headache is classified as mild to moderate in non-migraineurs, but classified as episodic migraine in those who suffer from migraine (Headache Classification Committee of the International Headache Society, 2013). In recent studies, a single oral dose of 150mg/kg taken consecutively for five days resulted in headache and muscle tenderness when given to healthy young volunteers (Baad-Hansen et al., 2010; Shimada et al., 2013, 2015), which merit further studies as to the mechanism of MSG.

It is unknown how elevated levels of glutamate contribute to headache. Because glutamate does not cross the blood-brain-barrier (BBB) (Gasparini and Griffiths, 2013), ingestion of MSG likely induces headache through a peripheral mechanism. To investigate the potential mechanisms by which MSG may induce headache, we assessed (i) the expression the NMDA receptor and glutamate transporters in the dura, as well as (ii) the neuronal and (iii) vascular effects of systemic administration of MSG in rats. We also assessed whether co-administration of a peripherally restricted NMDA receptor antagonist, (2R)-amino-5-phosphonovaleric (APV), could attenuate effects of MSG administration on SpVc neurons.

Experimental procedures

Animals:

Male (305–480 g, n=30) and female (245–330 g, n=28) Sprague Dawley rats (Charles River, Canada) were used for these experiments. Animals were housed in groups of two or three and were subject to a 12-h light/dark cycle. Food and water were available ad libitum. All animal procedures were reviewed and approved by the University of British Columbia Animal Care Committee.

Surgical Procedure for Electrophysiological and Doppler Recordings:

Male and female Sprague-Dawley rats were anaesthetised with isoflurane (2–2.5% in oxygen 97–98%) and artificially ventilated via a trachea tube. Temperature was continually measured with a rectal thermometer and maintained at $37 \pm 0.5^\circ\text{C}$ using a heating pad (Fine Science Tools, Vancouver, Canada). Blood pressure was measured via carotid artery cannulation. The heart rate, blood pressure, and core body temperature were monitored throughout the experiments. The femoral vein was cannulated to administer drugs. Animals were placed in a Kopf stereotaxic frame, and the skull was exposed.

For electrophysiology experiments, the right parietal and the frontal bone were removed from the lambdoid suture to anterior of the orbit. An incision was also made in the skin and muscle over the neck to expose the brainstem, the dura was removed and a C1 laminectomy was performed. The exposed brainstem was bathed in mineral oil.

A separate group of animals were used for dural blood flow experiments. The closed window method was used to measure dural blood flux with a Doppler flowmeter (Akerman et al., 2013).

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4 Briefly, the parietal bone was thinned over the middle meningeal artery (MMA) using a small
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7 drill, creating a “closed window”. The Doppler probe was held in place over the window by a
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10 cotton-tipped applicator lowered in position by a micro manipulator. Mineral oil was used to
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12 keep the cotton-tipped applicator and exposed bone moist.
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15 **Electrophysiological Recordings:**

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18 Extracellular action potentials of SpVc neurons with dural receptive fields were recorded with a
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21 parylene-coated tungsten microelectrode (0.010”, 2M Ω , A-M Systems Inc., Carlsborg, WA, USA)
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24 in male (n=21) and female (n=19) rats. The recording electrode was lowered into the exposed
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27 brainstem over the spinal subnucleus caudalis (SpVc), 1-2mm lateral and 1-2mm caudal to the
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30 obex and at depth ranging from 50-2800 μ m at a 33° angle. The V1 and V2 dermatomes of the
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33 skin were brushed while the electrode was lowered. To identify potential areas of the SpVc
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36 which may have dural input, mechanoreceptors innervating the dura were identified by their
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39 response to mechanical stimulation applied to the dura. . Mechanoreceptors innervating the
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42 dura were identified by their response to mechanical stimulation applied to the dura. An
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45 electronic von Frey hair (model 1601C, Life Science, USA) was used to assess the mechanical
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48 activation threshold for dural responsive SpVc neurons. In some experiments multiple neurons,
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51 which could be differentiated by their action potential characteristic, shared a dural receptive
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54 field; in those cases, the acquired data from both neurons were used and spike sorting was
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57 applied with Spike 2 software (Cambridge Electronics, UK). The neurons were characterized by
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60 their receptive fields, their response to innocuous (brush) and noxious (pinch) stimuli applied to
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63 the skin, and also the latency to action potential discharge following electrical stimulation of
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66 the dura (0.5-5 mA, 0.5 ms, 0.5 Hz). In addition, the response of neurons to direct mechanical

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4 stimulation of the temporalis muscle with a blunt probe was determined by temporarily pulling
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6 the skin away from the muscle.
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10 Following the identification of a SpVc neuron with a dural receptive field, the baseline
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12 mechanical threshold (MT) was measured. Briefly, the von Frey hair was pressed against the
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14 dural receptive field with increasing force until it elicited an action potential discharge; the
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16 smallest force which elicited an action potential was recorded as the MT. The mean baseline
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18 MT was calculated from five evoked responses separated by one minute intervals. A five-
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20 minute baseline was recorded to assess ongoing discharge in the neuron, after which 50mg
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22 MSG was administered alone or in combination with APV (5mg/kg or 50mg/kg). Post-injection
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24 discharge was monitored for 5 minutes. Cumulative discharge was calculated by subtracting
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26 the number of action potentials during the pre-injection period from those during the post-
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28 injection period. The criterion for neuronal activation was a positive difference between the
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30 total number of action potentials fired following MSG administration and action potentials fired
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32 during the baseline period. The MT was then assessed for a period of 10 minutes. Mean MT
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34 values were calculated for the two 5-minute post-injection time periods. Animals were
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36 euthanized at the end of the experiments (pentobarbital 100 mg/kg).
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46 **Laser Doppler Recordings:**

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50 Five male and five female Sprague Dawley rats were used to measure relative changes in dural
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52 blood flow following MSG administration. Dural blood flux (measured in perfusion units, PU)
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54 was sampled at a rate of 1Hz. A 30-minute recovery period was allocated following the
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56 completion of surgery, before beginning the dural blood flux recording, because the surgical
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procedures might have altered meningeal blood flow. The laser Doppler fibre-optic probe was placed perpendicular to the dura over a branch of the MMA. The emitted laser light penetrates to a depth of 1mm. Thus, the signal recorded emanates primarily from blood flow through the MMA. The Doppler probe was calibrated in a polystyrene calibration solution prior to each use and mineral oil was applied once the probe was held in position over the closed window to minimize noise. After the recovery period, a five-minute baseline measurement of dural blood flux was recorded, followed by systemic injection of 50mg/kg MSG, which was flushed with 0.5ml of normal saline. Animals were euthanized at the end of experiments (pentobarbital 100 mg/kg).

Drugs:

L-glutamic acid sodium salt hydrate (Sigma Aldrich) was dissolved in water to a final concentration of 169.1mg/ml and administered at doses of 50mg/kg. This dose of MSG has been found to mechanically sensitize rat masseter muscle afferent fibres (Cairns et al., 2007). Fresh solutions of MSG were prepared weekly. DL-2-Amino-5-phosphonovaleric Acid (APV, Santa Cruz Biotechnology) was dissolved in 0.2M NaOH and phosphate buffered saline (PBS, Sigma Aldrich). Two concentrations of APV were prepared, 20mg/ml and 2mg/ml which were administered at doses of 50mg/kg and 5mg/kg, respectively. The experimenter was blinded to the dose of APV being administered.

Immunohistochemistry:

Male (n=4) and female (n=4) rats were anaesthetised and perfused with cold saline followed by paraformaldehyde (4%). The head of the rat was placed in 20% sucrose followed by 40%

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4 sucrose for 48 hours each to dehydrate the tissue. Whole mount tissue samples were stained
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7 with antibodies against protein gene product 9.5 (rabbit polyclonal 1:1500, Abcam) and von
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10 Willebrand Factor (sheep polyclonal 1:750, Cedarlane), to identify nerve fibres and blood
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13 vessels, respectively. All tissues were also incubated with antibodies against the NR2B subunit
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15 of the NMDA receptor (NMDAR; mouse monoclonal 1:400, Abcam). The expression of
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18 excitatory amino acid transporters 1-3 (EAAT1-3; sheep polyclonal 1:250, Santa Cruz) was also
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20 assessed. Tissue staining was done as described previously (Dong et al., 2007; Sung et al.,
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23 2008; Laursen et al., 2014). Tissues were visualized with Leica TCS SPE high resolution spectral
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26 confocal microscope (Leica DM 2500, Canada) equipped with a digital camera (Leica DFC310 FX,
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29 Canada) and the Leica application suite software (Version 2.3.6, build 5381). Images were taken
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31 in the x-y plane and z-stacking was used to view the tissue within 3-dimensions.

32 33 **Statistical Analysis:**

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36 An ANOVA sample size estimation suggested that groups of 6 animals were sufficient to detect
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39 an effect of MSG in electrophysiology experiments ($\alpha = 0.05$, power = 0.80). The median change
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42 in neuronal discharge in the MSG group and MSG+APV (5 or 50mg/kg) groups were compared
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45 using a 2-way ANOVA on ranks with sex and treatment as factors. The Holm-Sidak test was used
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48 post-hoc. The mean of the relative MT was calculated for the two post drug time points for
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51 each treatment group. Three-way repeated measures ANOVA was performed on the relative
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54 MT at the two different time points with sex and treatment as factors. The Fisher's least
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57 significant difference (LSD) test was used post-hoc.
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Results

Expression of Glutamate Receptors and Transporters in the Dura:

While glutamate has been proposed to play a role in neurogenic inflammation and excitation of trigeminal afferent fibres, the expression of glutamate receptors in the dura has not been assessed. Histological analysis revealed that NMDARs are expressed by nerve fibers innervating dural blood vessels (Figure 1). The receptor expression was localized to nerve endings rather than along axons distal to blood vessels. The expression of glutamate transporters EAAT1-3 was also assessed, however, only EAAT2 was identified on dural blood vessels (Figure 1). The expression of the NMDA receptor led to the use of the NMDA receptor antagonist, APV, in electrophysiology experiments.

Characteristics of SpVc neurons with dural input:

To determine the potential effect of MSG and APV (0, 5, 50 mg/kg) on the spontaneous and evoked activity of SpVc neurons with dural receptive fields, electrophysiological recordings from 36 neurons were undertaken (Figure 2). All neurons had receptive fields on the dura and most (34, 94%) also had additional receptive fields on the face. Receptive fields were predominantly located in the V1 (ophthalmic) dermatome, most commonly around the eye, but also could include both V1 and V2 (maxillary) dermatomes. The majority of neurons received input from both the skin (86%) and the temporalis muscle (89%; V3). Ninety-two percent of neurons were classified as wide-dynamic range (WDR), responding to both innocuous (brush) and noxious (pinch) stimuli, the remaining were classified as either nociceptive specific (NS, 5%) or low threshold (LT, 3%). Response to electrical stimulation of the dural receptive field was assessed in 25 neurons, 10 (40%) of these neurons responded with a mean latency of 11.8 ± 2.2

ms. The neurons recorded in this experiment have similar characteristics to wide dynamic range SpVc neurons reported in other studies (Akerman and Goadsby, 2015).

MSG activates and sensitizes trigeminovascular neurons:

The intravenous administration of 50mg/kg MSG induced an increase in the activity of SpVc neurons. An example of the responses from SpVc neurons to MSG is shown in Figure 3A and B.

In 5/6 male and 5/6 female rats, MSG evoked an increase in neuronal discharge (males: 0.44 ± 0.32 Hz; females: 0.89 ± 0.41 Hz) when compared with the baseline spontaneous activity (males: 0.09 ± 0.07 Hz; females: 0.75 ± 0.37 Hz) (Figure 3C). The median increase in the number of action potentials fired following MSG administration was 11 and 25 for male and female rats, respectively.

Mechanical sensitization of SpVc neurons was assessed by measuring mechanical thresholds prior to and after intravenous administration of 50mg/kg MSG. The changes induced in mechanical thresholds measured in male and female rats after injection of MSG are shown in Figure 4A. Two relative MTs were calculated, at 5-10 minutes and 10-15 minutes post MSG administration. In male rats, MSG induced a mean (\pm SE) $11.1 \pm 5.7\%$ decrease in MT, while in female rats the effect was smaller with a $0.4 \pm 12.5\%$ decrease in MT, 5-10 minutes after receiving MSG. The mechanical sensitization induced by MSG during the first five minute period returned to baseline values during the 10-15 minute period.

APV attenuates the activation and sensitization induced by MSG:

Co-administration of 5mg/kg, but not 50mg/kg APV, with MSG resulted in a statistically significant decrease in the median discharge following drug administration ($p = 0.015$, 2-way

ANOVA on RANKS, post-hoc Holm-Sidak). There was no statistically significant difference when male and female rats were compared (Figure 3D).

The combination of 50mg/kg APV with MSG induced a $21.2 \pm 15.0\%$ and $37.6 \pm 15.4\%$ increase in MT in male and female rats, respectively at 5-10 minutes post injection. At 10-15 minutes after the drug administration, the relative MT in the 50mg/kg APV + MSG treated animals had increased by $43.0 \pm 30.6\%$ and $67.8 \pm 12.1\%$ in male and female rats, respectively. The relative MTs of animals treated with MSG and 50mg/kg APV are statistically different from those who received MSG alone ($p=0.010$, 3-way rm ANOVA, post-hoc Fisher's least significant difference test). The relative mechanical thresholds from 10-15 minutes post drug administration are larger than those measured at 5-10 minutes post drug administration ($p=0.014$, 3-way repeated measures ANOVA). There was not a statistically significant difference in MTs between male and female rats. The effects of MSG administered alone or combined with APV on mechanical threshold are shown in Figure 4 B.

MSG induces dural vasodilation:

To determine whether MSG evoked activation and sensitization of SpVc neurons was associated with dural vasodilation, laser Doppler flowmetry was used to assess the dural flux in response to systemic administration of MSG in male ($n=5$) and female ($n=5$) rats. Figure 5 depicts an experimental trace of the MSG induced effect on dural flux and blood pressure. A mean maximal increase of $24.5 \pm 4.4\%$ and $20.6 \pm 2.8\%$ in the dural flux, relative to baseline, was measured in male and female rats, respectively. The duration of the MSG evoked surge in flux was approximately 170s in both sexes. Systemic blood pressure also increased but this began several seconds after the increase in dural flux.

Discussion

Consumption of MSG can induce a headache in normal healthy individuals and a migraine-like headache in migraineurs (Anon, 2013). MSG was first implicated in the 1960s to cause a number of adverse effects including muscle pain and weakness, cutaneous flushing, heart palpitations and headache (Ambos et al., 1968; Schaumburg et al., 1969). The symptoms were originally called 'Chinese restaurant syndrome' but later renamed 'MSG symptom complex'. Subsequent studies on 'MSG symptom complex' concluded that MSG consumption did not induce symptoms of pain or sensitivity, even in 'MSG sensitive patients', but many of these studies have been scrutinized for their poor methodology (Tarasoff and Kelly, 1993; Geha et al., 2000). More recent studies have found that oral or intravenous administration of MSG induces headache, muscle sensitivity and increased systolic blood pressure in healthy volunteers (Graham et al., 2000; Baad-Hansen et al., 2010; Shimada et al., 2013, 2015).

We propose that headache and pain associated with MSG consumption are mediated, in part, through activation of peripheral NMDARs. While the expression of NMDARs and transporters has been assessed in the TG (Larsen et al., 2014; Wong et al., 2014), little is known about their expression in the dura. Immunohistochemical analysis of the dura revealed the expression of the NMDAR on densely innervated dural blood vessels. The findings also revealed that EAAT2, but not EAAT1 or 3, were expressed in association with dural blood vessels. In the CNS, EAAT2 is expressed in astrocytes (Danbolt, 2001; Zhou and Danbolt, 2013), thus it is possible that EAAT2 is being expressed on glial cells near the surface of blood vessels in the dura. Inhibited clearance of glutamate by EAAT2 in the dura could affect sensory transmission by afferent fibres in migraine patients (Laursen et al., 2014). The effect of inhibiting EAAT1/2 in the TG on

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4 the excitability of temporalis or masseter muscle afferents in rats has recently be assessed
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6 (Laursen et al., 2014). Co-administration of EAAT1/2 inhibitor TFB-TBOA ((3S)-3-[[3-[[4-
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8 (trifluoromethyl)benzoyl]amino]phenyl]methoxy]-l-aspartic acid) and glutamate into the TG
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10 increased glutamate evoked neuronal discharge (Laursen et al., 2014). A genetic mutation in
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12 the gene that encodes EAAT2 has been linked to migraine (12), thus inhibited clearance of
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14 glutamate at the level of the dura may act to sensitize meningeal afferent fibres in migraineurs.
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16 These data suggest that elevated blood concentrations of glutamate could act on NMDARs
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18 expressed by dural vascular tissues.
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26 Excitation of trigeminal afferent fibres that innervate the dura and converge on the SpVc is
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28 thought to be a critical mechanism in migraine pathophysiology (Burstein et al., 2015). In this
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30 study, administration of 50mg/kg MSG induced both activation, as measured by an increase in
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32 neuronal discharge, and sensitization of SpVc neurons to mechanical stimulation of the dura.
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36 The excitation induced by MSG in the present study was attenuated when APV was co-
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38 administered. Interestingly, co-administration of APV with MSG not only reversed MSG-induced
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40 mechanical sensitization but actually appeared to increase the mechanical activation threshold
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42 of SpVc neurons with dural receptive fields. One interpretation of this result is that dural
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44 afferent mechanical sensitivity is dependent on the local release of glutamate, perhaps from
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46 the terminal endings of dural afferent fibres, as has been found for the temporalis muscle
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48 (Gazerani et al., 2010a). The co-administration of 50mg/kg APV with MSG also resulted in a
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50 decrease in neuronal discharge below that of the baseline values, which may reflect basal
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52 neuronal activation due to blood glutamate. As APV is thought to be peripherally restricted
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54 (Lodge et al., 1988; Whitten et al., 1990), these results suggest that the effects of MSG on SpVc
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neurons are mediated by peripheral NMDA receptors, however, because a relatively high dose was used it cannot be ruled out that a small amount of APV crossed the BBB (Lodge et al., 1988).

We also determined that MSG's ability to excite SpVc neurons was associated with increased blood perfusion through the medial meningeal artery. This effect likely reflects a combination of increased blood pressure and local vasodilation. Glutamate and NMDA applied to the pia, induced vasodilation of pial vessels in newborn pigs that was blocked by L-NNA (N^G -nitro-L-arginine), a potent nitric oxide synthetase inhibitor (Meng et al., 1995). This suggests that the glutamate induced dural vasodilation may be mediated by an increase of nitric oxide (NO), which is a potent vasodilator (Garthwaite, 1991; Meng et al., 1995). Intramuscular injections of glutamate in rats increase muscle blood flow for up to 30 minutes, which is attenuated with co-injections of kynurenic acid, APV, or a CGRP receptor antagonist (Cairns et al., 2003; Gazerani et al., 2010a), suggesting that the glutamate induced muscular vasodilation may be mediated by peripheral NMDA and non-NMDA receptors, through the release of CGRP (Jackson and Hargreaves, 1999; Gazerani et al., 2010a). The potent vasodilatory actions of CGRP have been implicated in migraine pathogenesis (Gazerani et al., 2010a; Burstein et al., 2015). Additional studies will be required to determine if MSG-induced dural vasodilation is mediated by activation of peripheral NMDARs.

In the present study, a sex-related difference in either neuronal discharge or mechanical sensitization induced by MSG administration was not identified. In previous electrophysiological experiments in rats, a sex-related difference in neuronal discharges evoked by glutamate was found. Intramuscular injections of glutamate into the masseter muscle provoked a larger

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4 increase in discharge of trigeminal afferent fibers in female than in male rats (Cairns et al.,
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7 2001). Similarly, women seem to respond more profoundly to MSG than men. Early
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9 experiments aimed at understanding mechanisms of 'MSG symptoms complex' found that
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11 women were more responsive to oral MSG than men and that men needed a higher dose to
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13 provoke a negative effect of MSG (Ambos et al., 1968; Tarasoff and Kelly, 1993). When injected
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15 subcutaneously into the forehead or intramuscularly into the masseter muscle, glutamate also
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17 evoked a higher pain rating score in women than men (Cairns et al., 2001; Svensson et al., 2003;
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19 Gazerani et al., 2006). The present study may have been underpowered to resolve a sexually
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Elevated levels of glutamate during and after migraine attacks (Martinez et al., 1993; Cananzi et al., 1995; Eufemia et al., 1997), genetic variants which alter glutamate homeostasis (Schürks, 2012), and the ability of MSG to induce a migraine-like attack in migraineurs rather than a mild-moderate headache (Anon, 2013), all suggest that glutamate may play a critical role in migraine headache pathophysiology. Previous findings also suggest that glutamate regulation may be an important mechanism in botulinum neurotoxin type A (BoNTA) migraine prophylaxis (Gazerani et al., 2010a). Pretreatment with BoNTA decreased interstitial glutamate concentrations in the temporalis muscle of rats and also prevented glutamate induced vasodilation and mechanical sensitivity (Gazerani et al., 2010a). These findings suggest that tissue glutamate concentrations are important for regulating the sensitivity of mechano-nociceptors, and that elevated concentrations of glutamate in the blood may be a cause rather than a consequence of migraine headache.

Conclusion

The present results suggest that MSG-induced headache could be mediated by the activation of peripheral NMDArs and subsequent dural vasodilation. The MSG induced sensitization of SpVc neurons with input from the dura and face correlate with clinical observations of allodynia and headache following the administration of MSG in humans. The findings merit further studies into the relationship between EAAT dysfunction, glutamate regulation, and the mechanisms underlying headache and migraine. The results also suggest peripheral NMDArs are a potential target for the development of new drugs to treat headaches.

Acknowledgement: The authors thank Drs Xudong Dong and Hayes Wong for their assistance with aspects of this study.

Funding: This research was supported by a 2012 Pfizer Canada Pain Research Award.

Conflict of interest: none to declare.

References

- Akerman S, Goadsby PJ (2015) Neuronal PAC1 receptors mediate delayed activation and sensitization of trigeminocervical neurons: Relevance to migraine. *Sci Transl Med* 7:308ra157.
- Akerman S, Holland P, Hoffmann J (2013) Pearls and pitfalls in experimental in vivo models of migraine: dural trigeminovascular nociception. *Cephalalgia* 33:577–592.
- Ambos M, Leavitt H, Marmorek L, Wolchina S (1968) Letter. *N Engl J Med* 279:105.
- Asghar M, Hansen A, Kapijimpanga T, van der Geest R, van der Koning P, Larsson H, Olesen J, Ashina M (2010) Dilation by CGRP of middle meningeal artery and reversal by sumatriptan in normal volunteers. *Neurology* 75:1520–1526.
- Baad-Hansen L, Cairns B, Ernberg M, Svensson P (2010) Effect of systemic monosodium glutamate (MSG) on headache and pericranial muscle sensitivity. *Cephalalgia* 30:68–76.
- Burstein R, Nosedá R, Borsook D (2015) Migraine: Multiple Processes, Complex Pathophysiology. *J Neurosci* 35:6619–6629.
- Cairns B, Dong X, Mann M, Svensson P, Sessle B, Arendt-Nielsen L, McErlane K (2007) Systemic administration of monosodium glutamate elevates intramuscular glutamate levels and sensitizes rat masseter muscle afferent fibers. *Pain* 132:33–41.
- Cairns B, Hu J, Arendt-Nielsen L, Sessle B, Svensson P (2001) Sex-related differences in human pain and rat afferent discharge evoked by injection of glutamate into the masseter muscle. *J Neurophysiol* 86:782–791.
- Cairns BE, Gambarota G, Dunning PS, Mulkern R V, Berde CB (2003) Activation of peripheral excitatory amino acid receptors decreases the duration of local anesthesia. *Anesthesiology* 98:521–529 Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12552214>.
- Cananzi A, D'Andrea G, Perini F, Zamberlan F, Welch K (1995) Platelet and plasma levels of glutamate and glutamine in migraine with and without aura. *Cephalalgia* 15:132–135.
- Chan K, MaassenVanDenBrink A (2014) Glutamate receptor antagonists in the management of migraine. *Drugs* 74:1165–1176.
- Danbolt N (2001) Glutamate uptake. *Prog Neurobiol* 65:1–105.
- Dong X-D, Mann MK, Kumar U, Svensson P, Arendt-Nielsen L, Hu JW, Sessle BJ, Cairns BE (2007) Sex-related differences in NMDA-evoked rat masseter muscle afferent discharge result

- from estrogen-mediated modulation of peripheral NMDA receptor activity. *Neuroscience* 146:822–832.
- Eufemia PD, Finocchiaro R, Lendvai D, Celli M, Viozzi L, Troiani P, Turri E (1997) Erythrocyte and plasma levels of glutamate and aspartate in children affected by migraine. *17*:652–657.
- Friberg L, Olesen J, Iversen HK, Sperling B (1991) Migraine pain associated with middle cerebral artery dilatation: reversal by sumatriptan. *Lancet (London, England)* 338:13–17.
- Garthwaite J (1991) Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci* 14:60–67.
- Gasparini CF, Griffiths LR (2013) The Biology of the Glutamatergic System and Potential Role in Migraine. *9*:1–8.
- Gazerani P, Au S, Dong X, Kumar U, Arendt-Nielsen L, Cairns BE (2010a) Botulinum neurotoxin type A (BoNTA) decreases the mechanical sensitivity of nociceptors and inhibits neurogenic vasodilation in a craniofacial muscle targeted for migraine prophylaxis. *Pain* 151:606–616.
- Gazerani P, Dong X, Wang M, Kumar U, Cairns BE (2010b) Sensitization of rat facial cutaneous mechanoreceptors by activation of peripheral N-methyl-d-aspartate receptors. *Brain Res* 1319:70–82.
- Gazerani P, Wang K, Cairns BE, Svensson P, Arendt-Nielsen L (2006) Effects of subcutaneous administration of glutamate on pain, sensitization and vasomotor responses in healthy men and women. *Pain* 124:338–348.
- Geha RS, Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC, Ditto AM, Harris KE, Shaughnessy MA, Yarnold PR, Corren J, Saxon A (2000) Review of Alleged Reaction to Monosodium Glutamate and Outcome of a Multicenter Double-Blind Placebo-Controlled Study. *J Nutr* 130:1058S – 62S.
- Goltman AM (1936) The mechanism of migraine. *J Allergy* 7:351–355.
- Graham TE, Sgro V, Friars D, Gibala MJ (2000) Glutamate ingestion: the plasma and muscle free amino acid pools of resting humans. *Am J Physiol Endocrinol Metab* 278:E83–E89.
- Headache Classification Committee of the International Headache Society (2013) The International Classification of Headache Disorders, 3rd Edition. *Cephalalgia* 33:629–808.
- Jackson DL, Hargreaves KM (1999) Activation of excitatory amino acid receptors in bovine dental pulp evokes the release of iCGRP. *J Dent Res* 78:54–60.

- 1
2
3
4 Klafke JZ, da Silva M a, Trevisan G, Rossato MF, da Silva CR, Guerra GP, Villarinho JG, Rigo FK,
5 Dalmolin GD, Gomez M V, Rubin M a, Ferreira J (2012) Involvement of the glutamatergic
6 system in the nociception induced intrathecally for a TRPA1 agonist in rats. *Neuroscience*
7 222:136–146.
8
9
10
11 Kruuse C, Thomsen LL, Birk S, Olesen J (2003) Migraine can be induced by sildenafil without
12 changes in middle cerebral artery diameter. *Brain* 126:241–247.
13
14
15 Larsen DB, Ingemann Kristensen G, Panchalingam V, Laursen JC, Nørgaard Poulsen J, Skallerup
16 Andersen M, Kandiah A, Gazerani P (2014) Investigating the expression of metabotropic
17 glutamate receptors in trigeminal ganglion neurons and satellite glial cells: implications for
18 craniofacial pain. *J Recept Signal Transduct Res* 34:261–269.
19
20
21 Laursen JC, Cairns BE, Dong XD, Kumar U, Somvanshi RK, Arendt-Nielsen L, Gazerani P (2014)
22 Glutamate dysregulation in the trigeminal ganglion: a novel mechanism for peripheral
23 sensitization of the craniofacial region. *Neuroscience* 256:23–35.
24
25
26 Levy D, Burstein R (2011) The vascular theory of migraine: leave it or love it? *Ann Neurol*
27 69:600–601.
28
29
30 Lodge D, Davies S, Jones M, Millar J, Manallack D, Ornstein P, Verberne A, Young N, Beart P
31 (1988) A comparison between the in vivo and in vitro activity of five potent and
32 competitive NMDA antagonists. *Br J Pharmacology* 95:957–965.
33
34
35 Martinez F, Castillo J, Rodriguez J, Leira, Noya M (1993) Neuroexcitatory amino acid levels in
36 plasma and cerebrospinal fluid during migraine attacks. *Cephalalgia* 13:89–93.
37
38
39 Meng W, Tobin JR, Busija DW (1995) Glutamate-induced cerebral vasodilation is mediated by
40 nitric oxide through N-methyl-D-aspartate receptors. *Stroke* 26:857–862.
41
42
43 Schaumburg H, Byck R, Gerstl R, Mashman J (1969) Monosodium l-glutamate: its pharmacology
44 and role in the Chinese restaurant syndrome. *Science* (80-) 163:826–828.
45
46
47 Schürks M (2012) Genetics of migraine in the age of genome-wide association studies. *J*
48 *Headache Pain* 13:1–9.
49
50
51 Shimada A, Baad-Hansen L, Castrillon E, Ghafouri B, Stensson N, Gerdle B, Ernberg M, Cairns B,
52 Svensson P, Svensson Odont P (2015) Differential effects of repetitive oral administration
53 of monosodium glutamate on interstitial glutamate concentration and muscle pain
54 sensitivity. *Nutrition* 31:315–323.
55
56
57 Shimada A, Cairns BE, Vad N, Ulriksen K, Pedersen AML, Svensson P, Baad-Hansen L (2013)
58 Headache and mechanical sensitization of human pericranial muscles after repeated intake
59 of monosodium glutamate (MSG). *J Headache Pain* 14:2.
60
61
62
63
64
65

- 1
2
3
4 Sung D, Dong X, Ernberg M, Kumar U, Cairns BE (2008) Serotonin (5-HT) excites rat masticatory
5 muscle afferent fibers through activation of peripheral 5-HT₃ receptors. *Pain* 134:41–50.
6
7
8 Svensson P, Cairns BE, Wang K, Hu JW, Graven-Nielsen T, Arendt-Nielsen L, Sessle BJ (2003)
9 Glutamate-evoked pain and mechanical allodynia in the human masseter muscle. *Pain*
10 101:221–227.
11
12
13 Tarasoff L, Kelly MF (1993) Monosodium L-glutamate: A double-blind study and review. *Food*
14 *Chem Toxicol* 31:1019–1035.
15
16
17 Whitten JP, Baron BM, Muench D, Miller F, White HS, McDonald IA (1990) (R)-4-oxo-5-
18 phosphononorvaline: a new competitive glutamate antagonist at the NMDA receptor
19 complex. *J Med Chem* 33:2961–2963.
20
21
22 Wolff H, Tunis M, Goodell H (1953) Studies on headache; evidence of damage and changes in
23 pain sensitivity in subjects with vascular headaches of the migraine type. *AMA Arch Intern*
24 *Med* 92:478–484.
25
26
27 Wong H, Kang I, Dong X-D, Christidis N, Ernberg M, Svensson P, Cairns BE (2014) NGF-induced
28 mechanical sensitization of the masseter muscle is mediated through peripheral NMDA
29 receptors. *Neuroscience* 269:232–244.
30
31
32 Zhou Y, Danbolt NC (2013) GABA and Glutamate Transporters in Brain. *Front Endocrinol*
33 (Lausanne) 4:165.
34
35
36 Zwetsloot CP, Caekebeke JF, Ferrari MD (1993) Lack of asymmetry of middle cerebral artery
37 blood velocity in unilateral migraine. *Stroke* 24:1335–1338.
38
39
40
41
42
43
44
45
46
47
48
49
50
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Figure Legends

Figure 1: Photomicrographs in A and B show expression of EAAT2 and NMDARs on dural blood vessels and within the dura. The small box on the composite shows a region of interest which has been magnified to the right for each fluorophore. In A, blood vessels are identified by the expression of von Willebrand Factor (vWF), and are innervated by nerve fibres (PGP 9.5), which express the NR2B subunit. In B, a number of nerve fibers are shown. Note that the nerve fiber in the region of interest is positively labelled for the NMDA receptor, and also weakly labeled for EAAT2. Calibration bar (small box): 25 μ m; Yellow arrows: blood vessels; White arrows: nerve fibers.

Figure 2: An electronic von Frey hair was used to determine the MTs of SpVc neurons with dural receptive fields, before and after administration of MSG alone or in combination with APV. A, experimental set-up for electrophysiological recordings of SpVc neurons. Blue areas on the exposed dura represent common dural receptive fields of the neurons recorded. B, method to record and calculate mechanical threshold (MT) from SpVc neurons. At one minute intervals throughout the recording, mechanical pressure was applied using the electronic von Frey instrument to the dural receptive field until an action potential (top trace) was fired. The lowest magnitude of mechanical pressure (bottom trace) required to generate an AP from this neuron was taken as its MT. C, facial receptive fields of the SpVc neurons recorded, darker blue represents the most common facial receptive field which includes the temporalis muscle and periorbital skin.

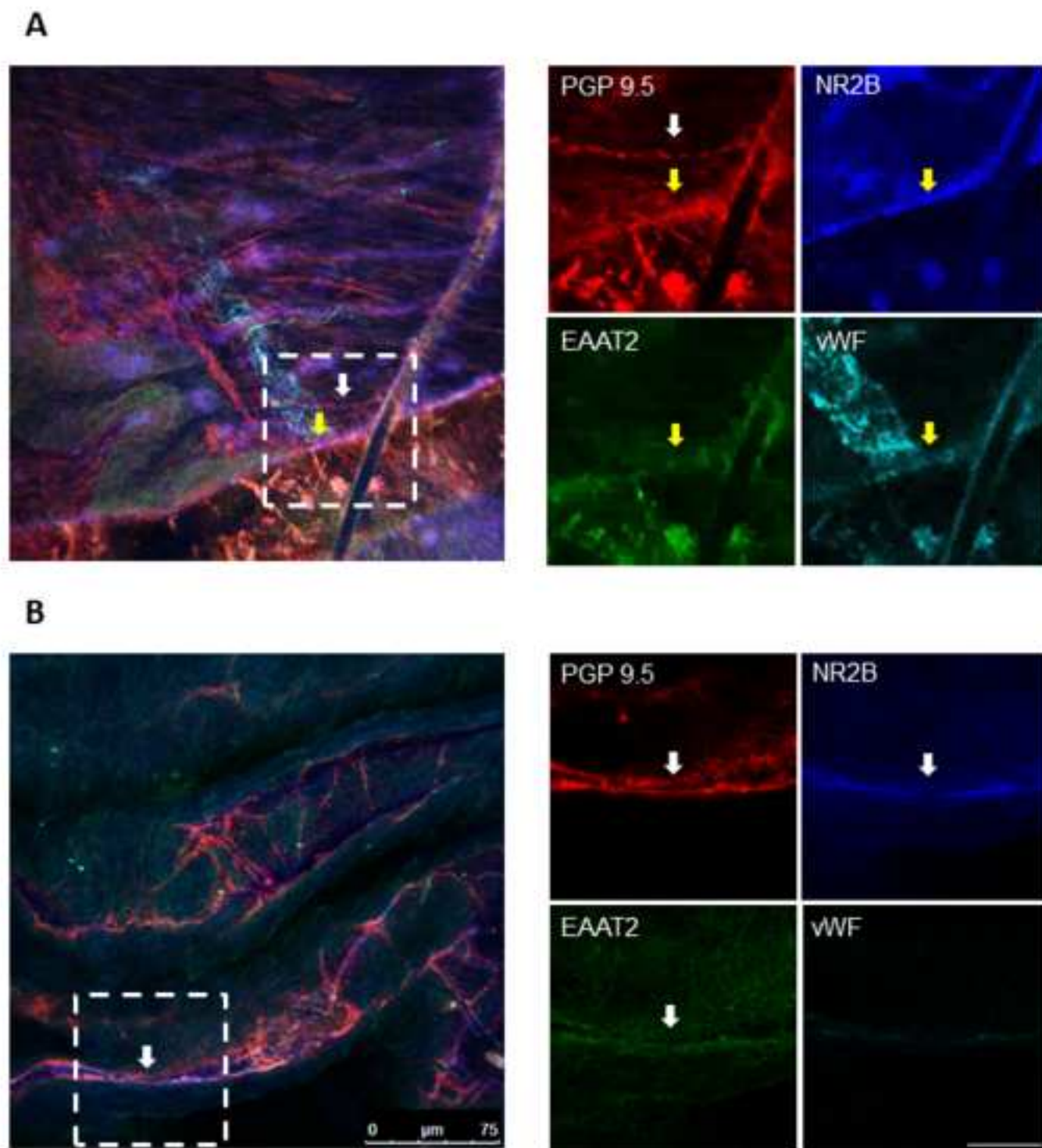
Figure 3: MSG induced an increase of neuronal discharge which was attenuate by co-administration of APV. In A and B, peri-stimulus histograms depict the increase in frequency (spikes/sec) of action potentials evoked from individual SpVc neurons following administration of 50mg/kg MSG (injected at 300s). The peristimulus histograms of A and B show a neuron that was quiet or had on-going activity prior to the administration of MSG, respectively. C, the total number of action potentials (neuronal discharge) recorded before and after administration of MSG (n=6 males, 6 females). D, the median cumulative discharge following administration of MSG + APV (0, 5, 50mg/kg, n=6). The asterisk indicates a statistically significant difference between the 5mg/kg APV treated groups (male + female) and 0mg/kg APV treated groups (male + female) ($P < 0.05$, post-hoc Holm-Sidak multiple comparison test). There was not a statistically significant difference based on sex ($P = 0.142$).

Figure 4: A. The relative mechanical threshold at 10 and 15 minutes post administration of MSG is shown (n=6 males, 6 females). The dotted line indicates no change in threshold. B. Co-administration of APV attenuated the MSG induced mechanical sensitization of SpVc neurons at 10 and 15 minutes post drug administration (n=6). Asterisks indicate thresholds significantly different between 50mg/kg APV + MSG and MSG treatment groups ($p < 0.05$, post-hoc Fisher's LSD test). There was not a statistically significant difference based on sex.

Figure 5: An experimental trace showing the response to 50mg/kg MSG administered intravenously in a male rat. Flux, measured in perfusion units (PU), and blood pressure,

measured in mmHg, both increase transiently following MSG administration (at 300s, dotted line).

Figure 1 (2 column):



Figure

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Figure 2 (1 column):

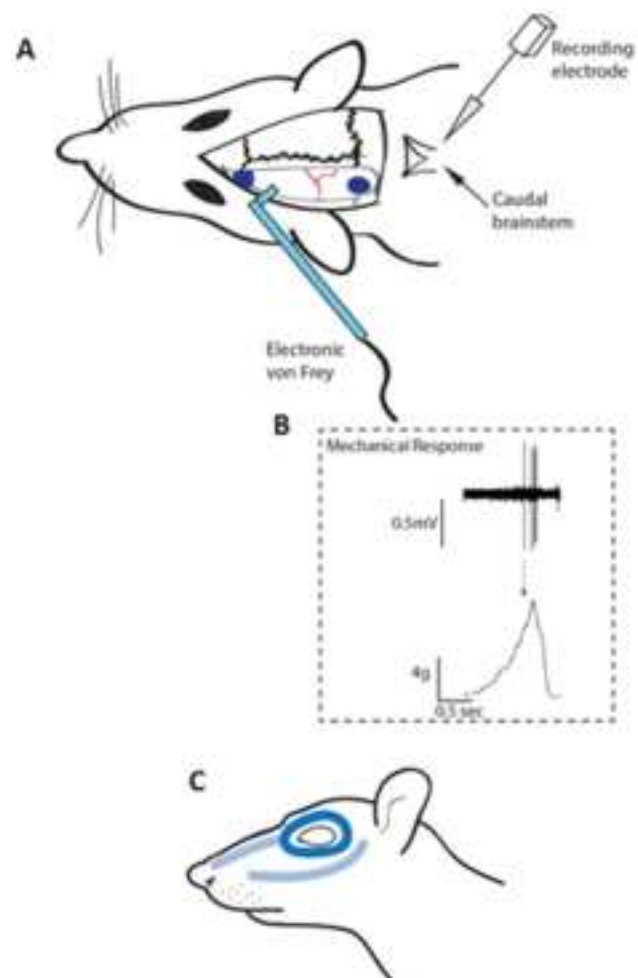


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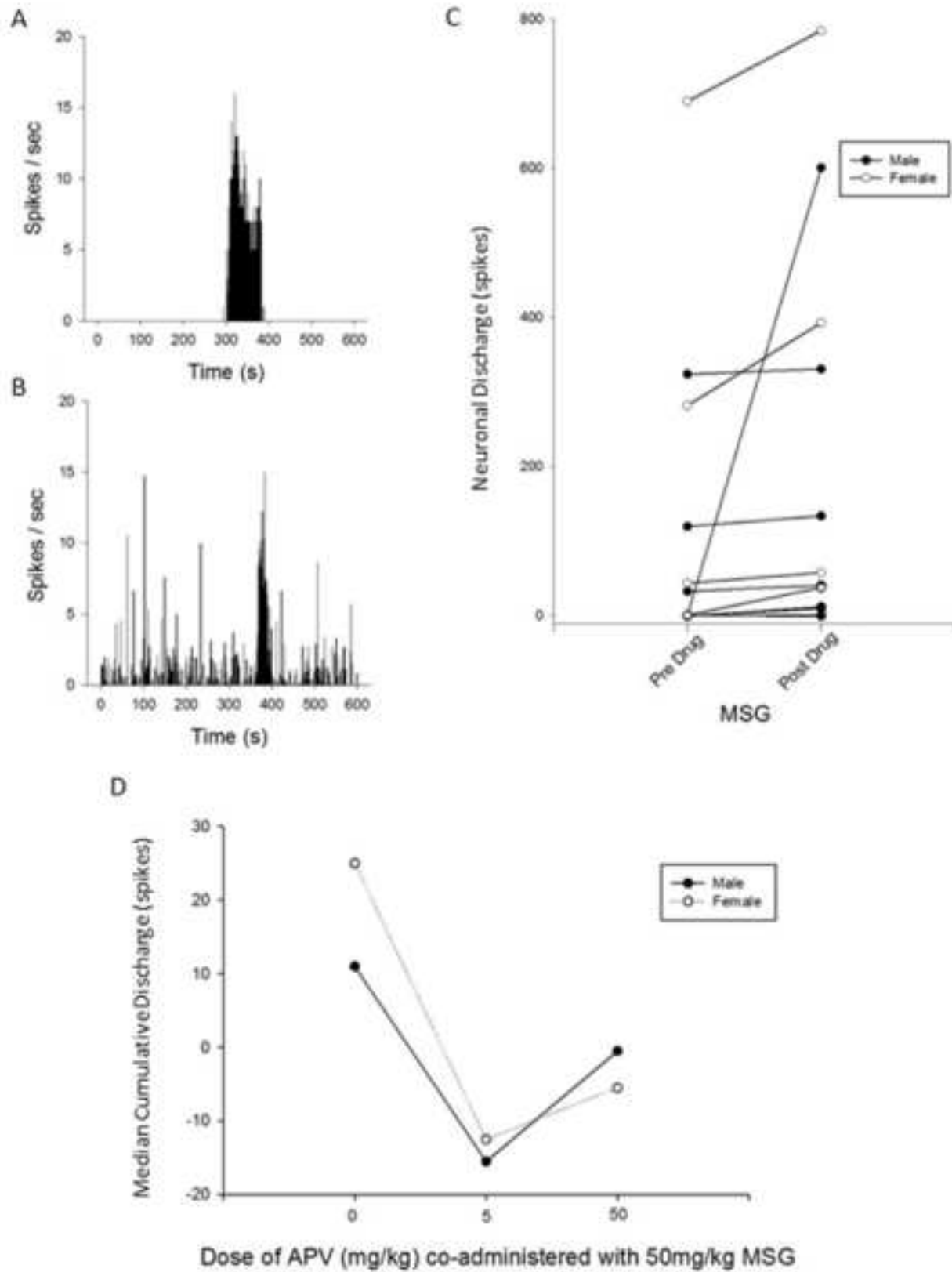
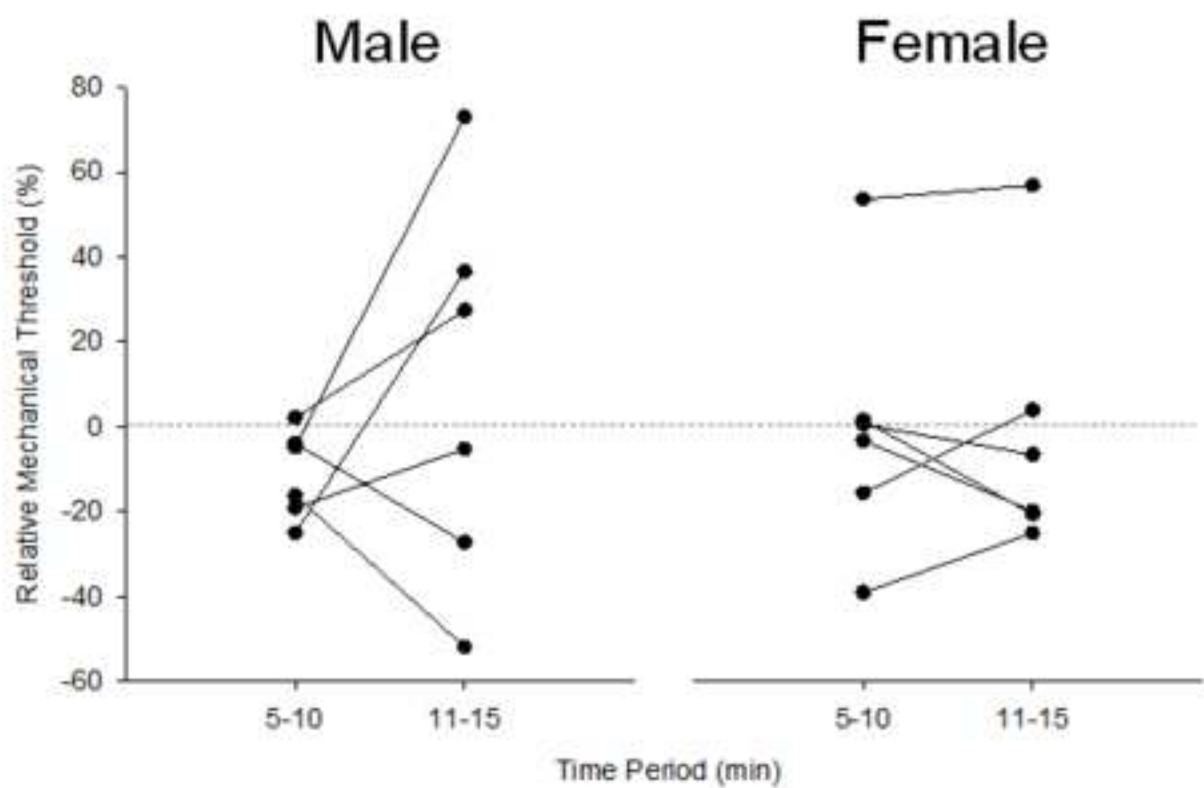


Figure 4 (2 column):

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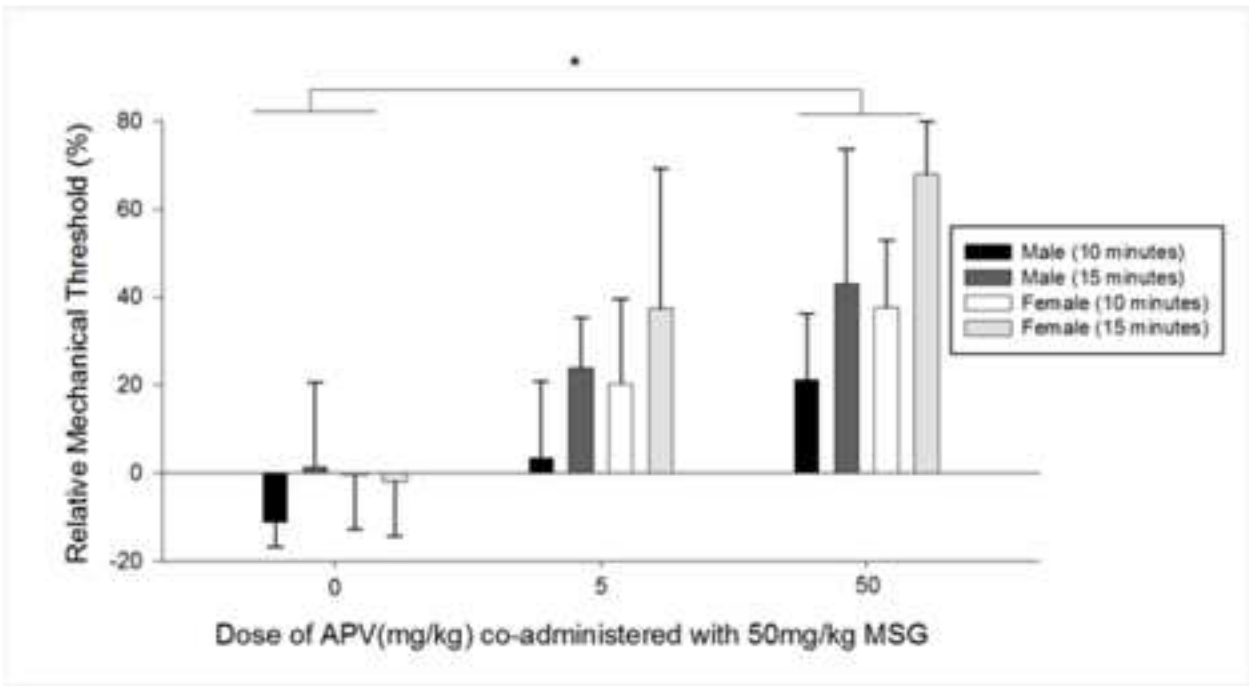


Figure 5 (1 column):

