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Harold G. Wolff Lecture Award Winner

Oxygen Inhibits Neuronal Activation in the Trigeminocervical Complex After Stimulation of Trigeminal Autonomic Reflex, But Not During Direct Dural Activation of Trigeminal Afferents

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Objective.—To understand the mechanism of action of oxygen treatment in cluster headache.

Background.—Trigeminal autonomic cephalalgias, including cluster headache, are characterized by unilateral head pain in association with ipsilateral cranial autonomic features. They are believed to involve activation of the trigeminovascular system and the parasympathetic outflow to the cranial vasculature from the superior salivatory nucleus (SuS) projections through the sphenopalatine ganglion, via the greater petrosal nerve of the VIIth (facial) cranial nerve. Cluster headache is remarkably responsive to treatment with oxygen, and yet our understanding of its mode of action is unknown.

Methods.—Combining models of trigeminovascular nociception and a novel approach that activates the trigeminal-autonomic reflex, using SuS/facial nerve stimulation, we explored the effect of oxygen on trigeminal nerve activation as well as on autonomic responses through blood flow observations of the lacrimal duct/sac.

Results.—Meningeal vasodilation and neuronal firing in the trigeminocervical complex (TCC), in response to dural electrical stimulation, was unaffected by treatment with 100% oxygen. Stimulation of the SuS via the facial nerve caused only marginal changes in dural blood vessel diameter, but did result in evoked firing in the TCC. Two populations of neurons were characterized, those responsive to 100% oxygen treatment, with a maximal inhibition of 33%, 20 minutes after the start of oxygen treatment ($t_{15} = 4.4$, $P < .0001$). A second population of neurons were not inhibited by oxygen and tended to have shorter latency. Oxygen also inhibited evoked blood flow changes in the lacrimal sac/duct caused by SuS stimulation.

Conclusions.—The data provide the first systematic, experimental evidence for a mechanism of action of oxygen in cluster headache. The data show oxygen has no direct effect on trigeminal afferents, acting specifically on the parasympathetic/facial nerve projections to the cranial vasculature to inhibit both evoked trigeminovascular activation and activation of the autonomic pathway during cluster headache attacks. Moreover, the studies begin to characterize a novel laboratory model for the most painful primary headache syndrome known – cluster headache.

Key words: oxygen, trigeminovascular, parasympathetic, cluster headache

Abbreviations: CGRP calcitonin gene-related peptide, MMA middle meningeal artery, NS nociceptive specific, PACAP pituitary adenylate cyclase-activating polypeptide, SUNCT short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing, SuS superior salivatory nucleus, TACs trigeminal autonomic cephalalgias, TCC trigeminocervical complex, VIP vasoactive intestinal peptide, WDR wide dynamic range

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Conflict of interest: P.J.G. has consulted with manufacturers of medical oxygen Air Products and Linde.

INTRODUCTION

The trigeminal autonomic cephalalgias (TACs),[1] including cluster headache, are primary headache disorders characterized by unilateral head pain that typically occurs in association with ipsilateral cranial autonomic features.[2] They are characterized by 3 major pathophysiological features: trigeminal distribution of pain, cranial autonomic features, and an episodic pattern of attacks. It is this last component that tends to define these disorders compared with migraine, although distinct patterns of the attack phenotype are well recognized.[3] Cluster headache attacks tend to have the longest duration with a lower attack frequency,[4] paroxysmal hemicrania an intermediate duration and attack frequency,[5] and short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing (SUNCT) having the shortest duration and up to 200 attacks a day.[6]

While our understanding of the pathophysiology of the TACs is limited, as their classification implies, they are believed to involve activation of the trigeminovascular system and its reflex connections to the cranial parasympathetic outflow (see Fig. 1). Activation of the trigeminovascular pathway by stimulation of the superior sagittal sinus in cats causes neuronal activation in the superior salivatory nucleus (SuS) within the pons,[7] which is the origin of cells for the cranial parasympathetic autonomic vasodilator pathway.[8] Activation of the VIIth cranial (facial) nerve and also stimulation of the sphenopalatine ganglion, which receives inputs from the SuS via the greater superficial petrosal nerve of the VIIth nerve, causes increases in cerebral blood flow,[9,10] as does direct stimulation of the SuS.[11] This parasympathetic outflow to the extracranial vessels uses vasoactive intestinal peptide (VIP) as its primary transmitter.[12] These data suggest activation of the trigeminovascular system, along with the autonomic reflex arc,[13] being integral to the physiology of TACs. The release of VIP during both facial nerve stimulation[14] and cluster headache[15] is consistent with this pathway being pivotal in the pathophysiology of these disorders.

The TACs are also characterized by their response to certain therapeutics that are not typically considered useful in migraine. Cluster headache, for example, has a robust response to high-flow oxygen as an abortive therapy.[16-18] It is not understood at all how oxygen works in cluster headache, and it has never been formally tested in migraine, although there has never been strong clinical support for its use in migraine. Remarkably in carefully studied cohorts of patients with paroxysmal hemicrania[5] or SUNCT[6] not a single patient reported good response to oxygen. In order to understand the mechanism of action of oxygen in cluster headache, and explore any potential efficacy in migraine, we used 100% oxygen treatment in several established models of trigeminovascular nociception that use stimulation of dural structures to activate trigeminal afferents.[19,20] We also wanted to explore the effects of oxygen in a more controlled model of trigeminal autonomic activation, specifically cluster headache symptoms, which we know to be responsive to oxygen. Given the evidence of the parasympathetic outflow to the extracranial blood vessels via the SuS, it was selected as a site of stimulation. We wanted to observe the response of SuS stimulation on dural arteries and neuronal firing within the trigeminocervical complex (TCC), and also to examine the cranial autonomic symptoms through changes in blood flow in the lacrimal duct of the ipsilateral eye, as tearing is a common symptom during these attacks.

METHODS

Animal Preparation.—All experiments were conducted under a protocol and ethical review by the Institutional Animal Care and Use Committee at the University of California, San Francisco. Male Sprague-Dawley rats (310-355 g) were anesthetized with sodium pentobarbitone (60 mg/kg i.p.) and maintained with propofol (Propoflo™) (25-30 mg/kg/hour i.v. infusion). During electrophysiological recording, animals undergoing dural stimulation were paralyzed with pancuronium bromide (Pavulon®, Organon) 0.4 mg initially and maintained with 0.2 mg every 35 minutes. The left femoral artery and left and right femoral vein were cannulated for blood pressure recording and intravenous infusion of anesthetic and test compounds, respectively. Temperature was maintained throughout using a homeothermic blanket system. The rats were placed in a stereotaxic frame and ventilated with medical air, 2.5 mL, 80-100 strokes per minute (Small Rodent Ventilator – Model

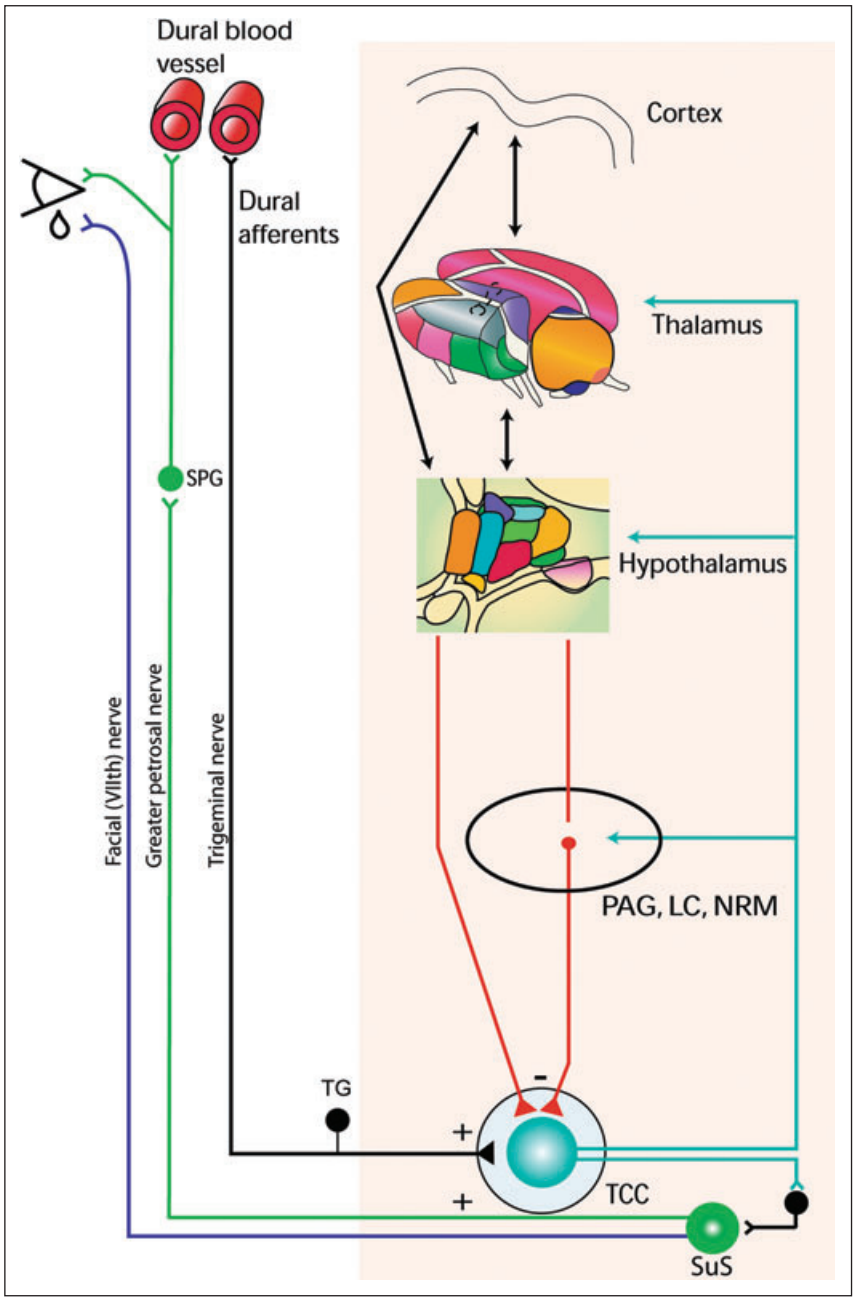

Fig 1.—Overview of the major anatomical and physiological pathways believed to be involved in trigeminal autonomic cephalalgias (TACs). Pain afferents from the trigeminovascular system traverse the ophthalmic division of the trigeminal nerve, taking signals from the cranial vessels and dura mater. These inputs synapse in the trigeminocervical complex (TCC) and project to higher brain structures such as the thalamus and cortex, resulting in pain. There is also activation of the parasympathetic reflex through the outflow from the superior salivatory nucleus (SuS) via the facial (VIIth cranial) nerve, predominantly involving the sphenopalatine ganglion (SPG), which acts to dilate blood vessels and activate trigeminal nerve endings. This autonomic activation leads to lacrimation, reddening of the eyes, and nasal congestion and a local third-order lesion because of carotid swelling. It is also thought that autonomic symptoms may be generated through the hypothalamus as well as controlling the cycling aspects of the TACs, and modulation of TCC firing may occur through the periaqueductal grey (PAG), locus coeruleus (LC), and nucleus raphe magnus (NRM).

683, Harvard Instruments, Kent, UK). End-tidal CO_2 was monitored (Capstar-100, CWE Inc., Ardmore, PA, USA) and kept between 3.5% and 4.5% and blood pressure was monitored continually. This allows one to monitor for changes to respiration and blood pressure because of long-term anesthetic maintenance. A sufficient depth of anesthesia was judged by the absence of paw withdrawal and corneal blink reflex, and during muscular paralysis by fluctuations of blood pressure.

Intravital Microscopy.—The skull was exposed and the right or left parietal bone thinned by drilling with a saline-cooled drill until the blood vessels of the dura mater were clearly visible through the intact skull. The cranial window was covered with mineral oil (37°C) and a branch of the middle meningeal artery (MMA) viewed using an intravital microscope (Microvision MV2100, Cambridge, UK) and the image displayed on a television monitor. Dural blood vessel diameter was continuously measured using a video dimension analyzer (Living Systems Instrumentation, Burlington, VT, USA) and displayed with blood pressure on an online data analysis system (CED spike2 v5 software). A bipolar stimulating electrode (NE 200, Clark Electromedical, UK) was placed on the surface of the cranial window approximately 200 μm from the vessel of interest, and stimulated at 5 Hz, 1 millisecond for 10 seconds (Grass Stimulator S88, Grass Instrumentation) with increasing voltage until maximal dilation was observed. Subsequent electrically induced responses in the same animal were then evoked using that voltage.[21,22] After 2 control responses to electrical stimulation, the animal was ventilated with 100% oxygen (1 L/minute) for 15 minutes and electrical stimulation repeated at 5, 15, and 30 minutes.

SuS Stimulation.—We developed a novel approach to activate trigeminal afferents through the trigeminal-autonomic reflex. An area of bone adjacent to midline, caudal to lambda and ipsilateral to the dural imaging site, was exposed to gain access to the SuS. A unipolar tungsten stimulating electrode (impedance 10-15 KΩ, tip diameter 3-4 μm, WPI Inc., Sarasota, FL, USA) was plunged into the stereotaxic co-ordinates for the SuS (AP 10.20-10.80 mm, DV 9.2-9.6 mm, and ML 2.1-2.4 mm).[23] A pulse generator (Neurolog NL301) divided by a pulse buffer (Neurolog NL510) activated by a constant current isolator (Neurolog NL800A) was used to stimulate the SuS (10-50 Hz, 150-μs duration and 10-50 μA for 10 seconds) and responses of dural blood vessel diameter recorded using intravital microscopy.

The parasympathetic outflow from the SuS is via the VIIth cranial (facial) nerve, which is predominantly through the sphenopalatine (pterygopalatine) ganglion. As a consequence of current spread in brain matter, where current may spread 50-500 μm with current of 5-50 μA applied to the area,[24] it is likely the facial nerve was also activated with the SuS. As a consequence, when we describe SuS stimulation, we assume an autonomic-facial reflex, as some component of facial nerve activation is unavoidable using this method, and could not be discounted. Electrode placement within the SuS was confirmed physiologically by observation of lacrimation as well as porphyrin discharge from the Harderian gland.[25,26] Precaution was also taken to limit the facial motor response; therefore, experiments were only conducted when no observable motor response was present. Lesion marks were made post experiment and the brain removed and post-fixed to confirm stimulation location (see Fig. 2).

Electrophysiological Recording in the TCC.—The muscles of the dorsal neck were separated, and a partial C_1 laminectomy carried out and the dura mater incised to expose the brainstem at the level of the caudal medulla. The electrode was slowly lowered into the brainstem at 5 μm increments with a piezoelectric motor controller (Exfo-Burleigh Instruments). Extracellular recordings were made from neurons in the TCC with cutaneous facial receptive fields with tungsten microelectrodes (WPI, impedance 0.5 MΩ, tip diameter 0.5 μm). The signal from the recording electrode attached to a high impedance headstage preamplifier (NL100AK; Neurolog, Digitimer, Herts, UK) was fed via an AC preamplifier (Neurolog NL104, gain ×1000) through filters (Neurolog NL125; bandwidth about 300 Hz to 20 kHz) and a 60 Hz noise eliminator (Humbug, Quest Scientific, North Vancouver, BC, Canada) to a second-stage amplifier (Neurolog NL106) providing variable gain

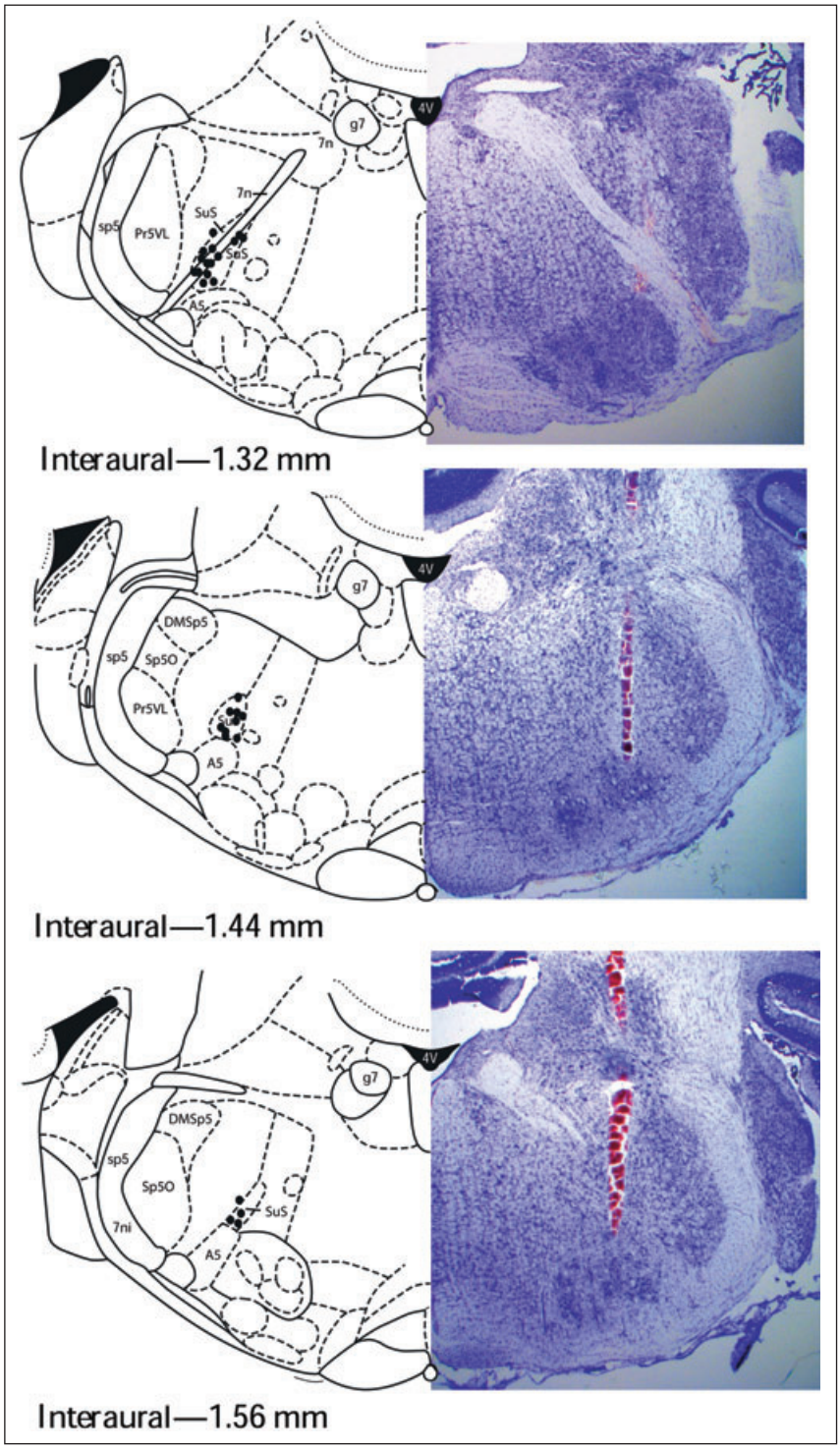

Fig 2.—Locations of lesion sites in the area of the superior salivatory nucleus (SuS) where stimulation took place. Stimulation sites in regions 1.32-1.56 mm behind the interaural point[34] highlight that the SuS was located as the stimulation site in each case and also the proximity of the facial nerve was <250 μm from the stimulation site and therefore likely to be simultaneously activated even if motor response was not observed. (4V, fourth ventricle; 7n, VIIth nerve; g7, genu of the facial nerve; Pr5VL, principle sensory trigeminal nucleus [ventrolateral part]; sp5, spinal trigeminal tract; Sp5O, spinal trigeminal nucleus oralis).

(×20 to ×90). This signal (total gain about ×20,000 to ×95,000) was fed to a gated amplitude discriminator (Neurolog NL201) and analogue-to-digital converter (Cambridge Electronic Design, Cambridge, UK), and to a microprocessor-based personal computer where the signal was processed and stored. Filtered and amplified electrical signals from action potentials were fed to a loudspeaker via a power amplifier (Neurolog NL120) for audio monitoring, and were displayed on analogue and digital-storage oscilloscopes (Goldstar, LG Precision, Seoul, Korea; Metrix Electronics, Chauvin Arnoux, Paris, France) to assist the isolation of single unit activity from adjacent cell activity and noise. Post- and peri-stimulus time histograms of neural activity were displayed and analyzed using Spike2 v5 (CED, Cambridge, UK).

Trigeminal afferents were activated using electrical stimulation of the dura mater adjacent to the MMA through an open cranial window, with a bipolar stimulating electrode (NE 200, Clark Electromedical, Kent, UK) using square-wave stimuli (0.6 Hz) of 100- to 200-μs duration, 8-20 V applied (S88 stimulator, Grass Instruments, MA, USA). We also used SuS stimulation to assess responses of neurons in the TCC through activation of the trigeminal-autonomic reflex. Methods are as above and the SuS was stimulated (0.5 Hz, 150-μs duration and 20-50 μA) and responses were recorded in the TCC.

Characterization of Neurons.—Neurons within the TCC were characterized for their cutaneous and deep receptive fields. The cutaneous receptive field, including cornea, was assessed in all 3 territories of the trigeminal innervations and identified as the recording electrode was advanced in the spinal cord. The receptive field was assessed for both non-noxious, with gentle brushing, and noxious responses, with pinching with forceps or applying heavy pressure that was painful when applied to humans. When a neuron sensitive to stimulation of the ophthalmic dermatome of the trigeminal nerve (first division) was identified it was tested for convergent input from either the dura mater or the SuS. According to the cutaneous receptive field properties, neurons were classified as low-threshold mechanoreceptors that responded only to innocuous stimulation, wide dynamic range (WDR) that responded to both noxious and non-noxious stimuli, or nociceptive specific (NS) that responded to only noxious input.

Recording of TCC Neurons Activated by Electrical Stimulation of the Dura Mater or SuS.—Trains of 20 stimuli were delivered at 5-minute intervals to assess the baseline response to electrical stimulation. Responses were analyzed using post-stimulus histograms with a sweep length of 100 milliseconds and a bin width of 1 millisecond that separated Aδ-fiber and C-fiber activated firing. Spontaneous activity (spikes per second, Hz) was recorded for 120-180 seconds preceding the electrical stimulation using peri-stimulus histogram.

Once it had been established that there was a TCC neuronal response to electrical-evoked stimulation and cutaneous and deep receptive field inputs from the ophthalmic division of the trigeminal nerve, the responses were tested before and after 100% oxygen (1 L/minute) using the following experimental protocol. After 3 baseline collections, animals were ventilated with 100% oxygen for 15 minutes and collection of neuronal responses to electrical stimulation was repeated at 5, 10, 15, 20, 25, 30, and 45 minutes.

Facial Blood Flow Responses.—To measure the effect of SuS stimulation on the blood flow within the eye and face around the tear ducts and lacrimal gland, as an indication of activation of the parasymapathetic outflow to the cranium, or autonomic reflex, we used laser Doppler probes (Moor Instruments, Devon, UK), and measured the blood flow in this area. Two baseline responses were performed in response to SuS stimulation (5 Hz, 500-μs duration and 40-70 μA for 10 seconds) and the animal was ventilated with 100% oxygen (1 L/minute) for 15 minutes and SuS stimulation repeated at 5-minute intervals for 30 minutes.

At the end of each experiment after euthanasia, a lesion was made in the TCC and SuS so that the exact area of recording or stimulation could be identified post experiment. The brain and spinal cord were removed and stored in 10% formalin for post-fixing for 24 hours and then cryopreserved in 30% sucrose. Thirty-micrometer sections were cut, slide mounted, and contra-stained with cresyl violet to highlight both the recording site in the TCC and the stimulation site of the SuS.

Data Analysis.—During intravital microscopy, the effects of electrical stimulation were calculated as a percentage increase from the prestimulation baseline diameter. The 2 baseline responses were averaged and used to test against drug effects. The typical vessel diameter measured ranges from 120 to 150 μm. The data collected after post-stimulus histograms after electrical stimulation of the dura mater for Aδ-fibers represent the number of cells fired over a 10-millisecond time period in the region 5-20 milliseconds post stimulation over the 20 collections. Similarly, data collected for C-fiber responses represent the number of all cells fired over 80 milliseconds (20-100 milliseconds post stimulation) from the 20 collections. During SuS stimulation, latency periods for Aδ-fibers were extended to 40 milliseconds because of the greater distance for the signal to travel and activation across multiple synapses. Spontaneous activity is measured in cell firings per second (Hz). Facial blood flow was measured as an average increase (in arbitrary units) over the 10 seconds from the baseline level.

All data are expressed as mean ± SEM. Statistical analysis was performed using an analysis of variance for repeated measures with Bonferroni *post hoc* correction for multiple comparisons used to measure the time cause of significant drug intervention, which included the 3 baselines. If Mauchly's test of sphericity was violated, we made appropriate corrections to degrees of freedom according to Greenhouse-Geisser. Student paired *t*-test for *post hoc* analysis was used to test for the time points of significance, using the average of the 2 or 3 baselines for comparison. A regression analysis was used to test the reliability of blood vessel diameter response to different frequencies and amplitudes.

RESULTS

Responses of Trigeminal Nerve Activation to 100% Oxygen.—Oyxgen (100%, 1 L/minute) did not affect neurogenic dural vasodilation as a result of electrical stimulation over 30 minutes ($F_{3,18} = 0.46$, $P = .71$, n = 7, see Fig. 3C). For electrophysiological responses, recordings were made from 7 neurons (5 WDR and 2 NS) responsive to dural stimulation and cutaneous receptive fields restricted to the first (ophthalmic) and second (maxillary) divisions of the trigeminal nerve, including the cornea. Neurons were found in the deep layers (laminae IV, V, and VI) of the dorsal horn of the C_1/trigeminal nucleus caudalis of the spinal cord (or TCC), at a range of depth 640-935 μm (see Fig. 3A and B for recording sites and receptive field areas). Units had a baseline firing latency after dural stimulation of 11 ± 0.8 milliseconds and 55 ± 7 milliseconds, respectively, for Aδ- and C-fiber inputs. The baseline spontaneous firing rate was 43.8 ± 4 Hz. Oxygen had no effect on trigeminal afferents responding in the Aδ-fiber ($F_{2.25,13.5} = 1.47$, $P = .27$, n = 7, see Fig. 3D) or C-fiber ($F_{1.8,10.7} = 0.47$, $P = .62$, n = 7) ranges. It did significantly alter the response of spontaneous activity ($F_{3.6,20.1} = 3.31$, $P < .05$, n = 7), with the 45 minutes time point significantly reduced ($t_6 = 2.81$, $P < .05$, n = 7). Responses to cutaneous stimulation, including innocuous brush of the trigeminal (V_1) dermatome, noxious pinch, and corneal responses, were not significantly altered.

Responses of SuS Activation on Dural Blood Vessel Diameter.—When all the data for electrical stimulation of the SuS/VIIth nerve were grouped across all amplitudes ($F_{1,78} = 0.21$, $P = .13$, for 10-50 μA, n = 83) or all frequencies ($F_{1,91} = 1.31$, $P = .3$, for 10-50 Hz, n = 96), there was no significant difference; therefore, data were pooled for the subsequent analysis (Fig. 4A and B). Using Student paired *t*-test, there was a small but significant 3.3 ± 0.8% ($t_{79} = 4.31$, $P < .05$, n = 80) increase in blood vessel diameter post stimulation.

Response of SuS Activation on Neuronal Firing in the TCC to 100% Oxygen.—Stimulation of SuS/VIIth nerve was also able to evoke neuronal firing in the TCC. Neurons were found in the deep layers (laminae IV, V, and VI) of the dorsal horn of the TCC at a range of depth 200-590 μm; see Figure 3A for recording sites and Figure 2 for stimulation sites proximal to the SuS. There were 2 populations of neuronal firing, dependent on response latency, with a shorter latency of baseline firing range of 3-20 milliseconds with an average response of 10.4 ± 0.5 milliseconds (see Fig. 5A) and a slightly longer latency of firing range of 9-40 milliseconds and an average of 20.3 ± 4 milliseconds (Fig. 5C). There was no evoked firing beyond 40 milliseconds. The baseline spontane-

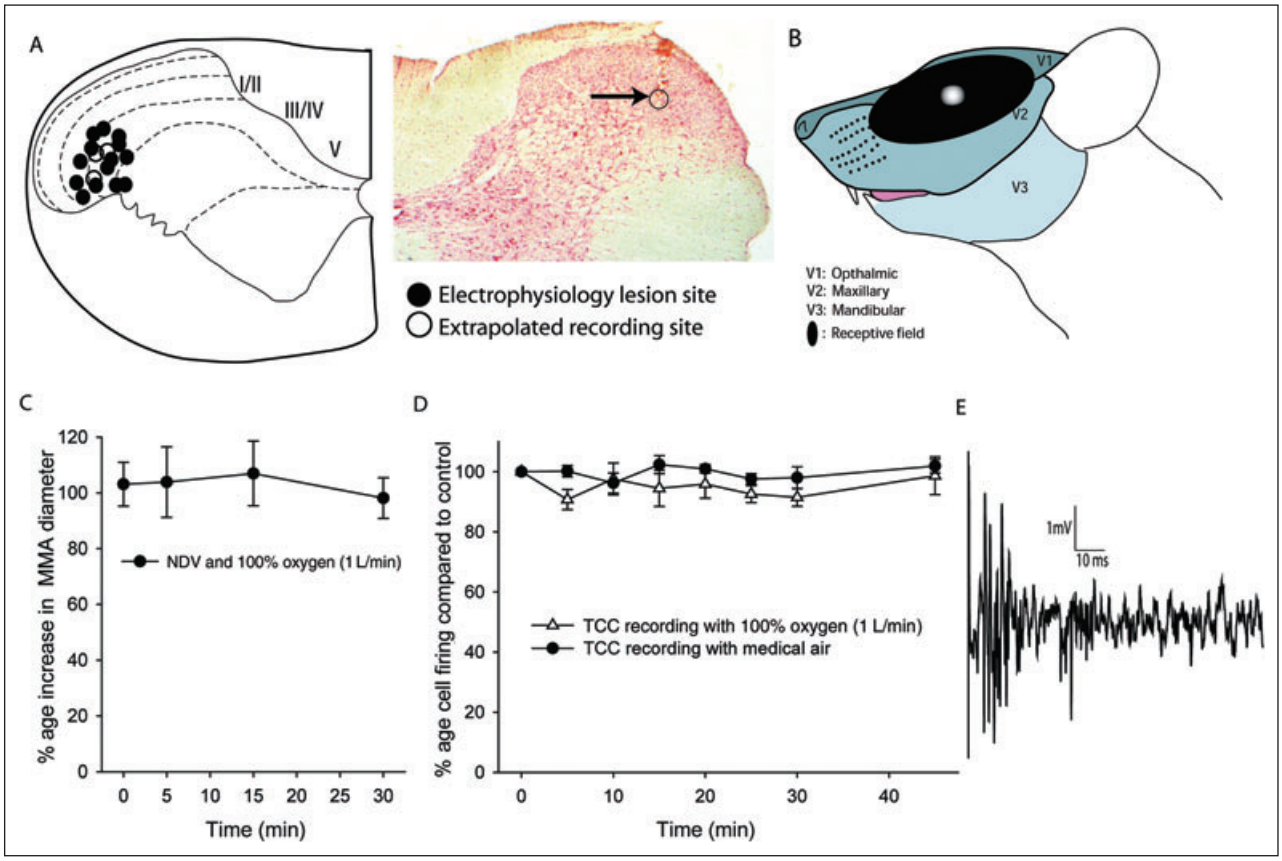

Fig 3.—The effects of oxygen on direct trigeminal nerve activation. (A) The location of recording sites in the trigeminal nucleus of neurons receiving convergent input from the dura mater and facial receptive field (closed circles are actual lesion sites, open circles reconstructed from lesions), and an example of a lesion site. (B) The receptive field of all neurons recorded was predominantly in the V1 ophthalmic dermatome and was characterized as wide dynamic range. Changes in (C) dural blood vessel diameter or (D) Aδ-fiber neuronal responses in the trigeminocervical complex (TCC) in response to stimulation of the dura mater, after treatment with 100% oxygen (1 L/minute). Neurogenic dural vasodilation and neuronal firing in the TCC were not inhibited by oxygen treatment. (E) Original tracing of a typical neuron responding to dural stimulation. MMA = middle meningeal artery.

ous firing rate was 31.3 ± 3 Hz. Oxygen had no effect on evoked responses in the TCC ($F_{7,63} = 1.13$, $P = .35$, n = 10) of cells firing with the shorter latency (Fig. 5B). The cells that were firing with a longer latency were significantly inhibited by 100% oxygen treatment across the 45 minutes ($F_{7,105} = 6.67$, $P < .000$, n = 16), with a maximum inhibition of 33% at 20 minutes ($t_{15} = 4.4$, $P < .0001$, Fig. 5D). Across all studies, dural and SuS-stimulated, oxygen was able to inhibit spontaneous activity ($F_{3.8,110.7} = 3.7$, $P < .05$, n = 30).

Response of SuS Activation on Blood Flow of the Cornea/Lacrimal Sac and in Response to 100% Oxygen.—Stimulation of the SuS/VIIth nerve resulted in increases in corneal/lacrimal sac blood flow, contiguous with the stimulation, that was reproducible over 30 minutes ($F_{2.45,12.24} = 0.33$, $P = .77$, n = 6, see Fig. 4C). Oxygen was able to reduce significantly increases in blood flow over the 30 minutes ($F_{6,48} = 3.25$, $P < .05$, n = 9) and maximally at 15 minutes by 20% ($t_8 = 4.16$, $P < .005$, Fig. 4D).

DISCUSSION

Treatment with 100% oxygen was able to inhibit neuronal firing in the TCC in response to stimulation of facial nerve/greater superficial petrosal nerve efferents that project from the SuS. Oxygen treatment had no effect on activation of trigeminal afferents in response to stimulation of dural structures, using both neuronal firing in the TCC and peripheral dural blood

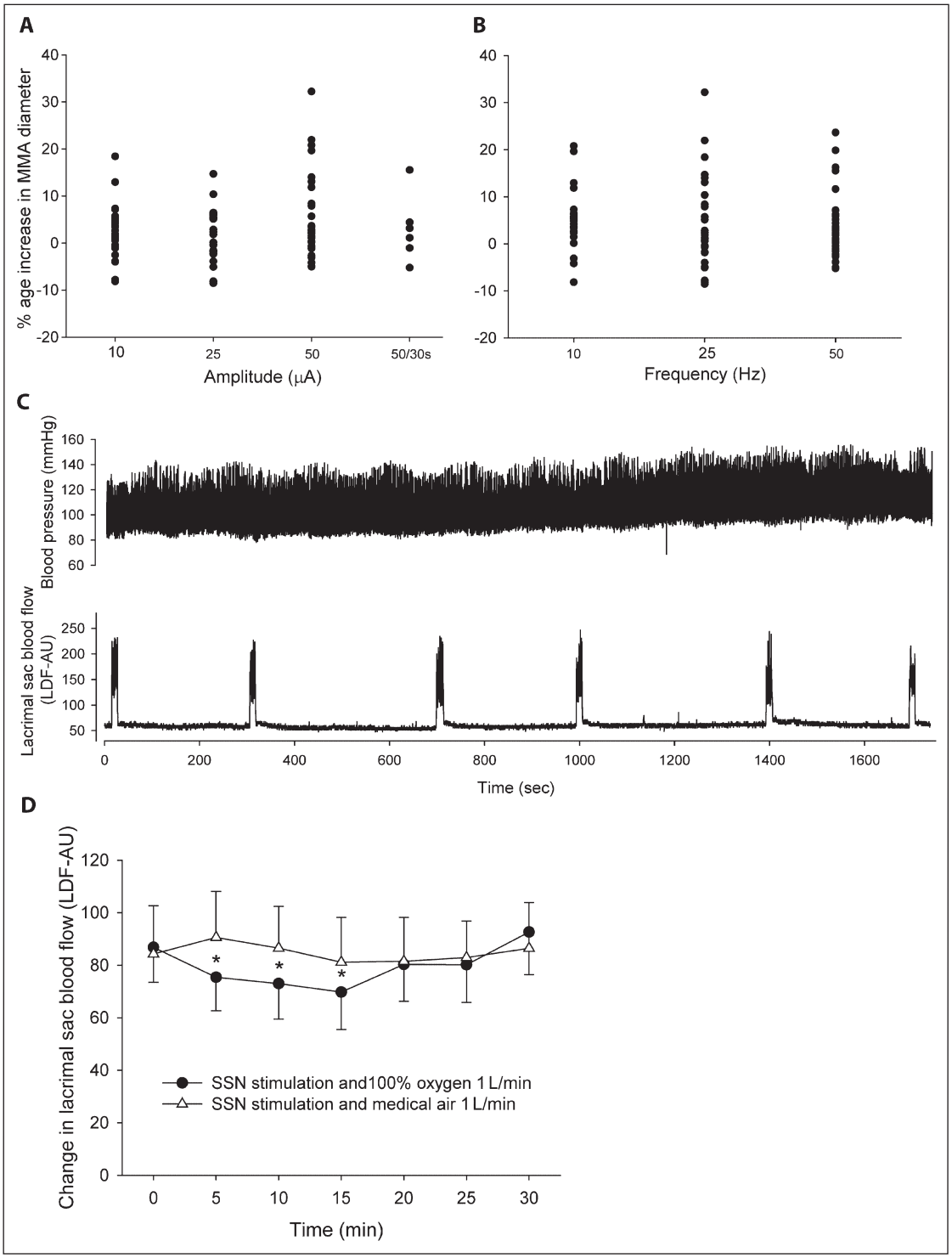

Fig 4.—Effects of oxygen on activation of the parasympathetic outflow to the cranial vasculature on dural blood vessel diameter and blood flow within the lacrimal duct/sac. Activation of the parasympathetic outflow to the cranial vasculature through stimulation of the superior salivatory nucleus and via the greater petrosal nerve of the VIIth nerve cause a minor but significant increase in dural blood vessel diameter, but there was no significant difference between different (A) currents and (B) frequencies applied. Activation of the trigeminal-autonomic arc also causes blood flow (laser Doppler flow-arbitrary unit, LDF-AU) increases within the lacrimal duct/sac and these responses were reproducible over 30 minutes (C). The blood flow increases (LDF-AU) were significantly inhibited by treatment with (D) 100% oxygen (1 L/minute). *$P < .05$. MMA = middle meningeal artery.

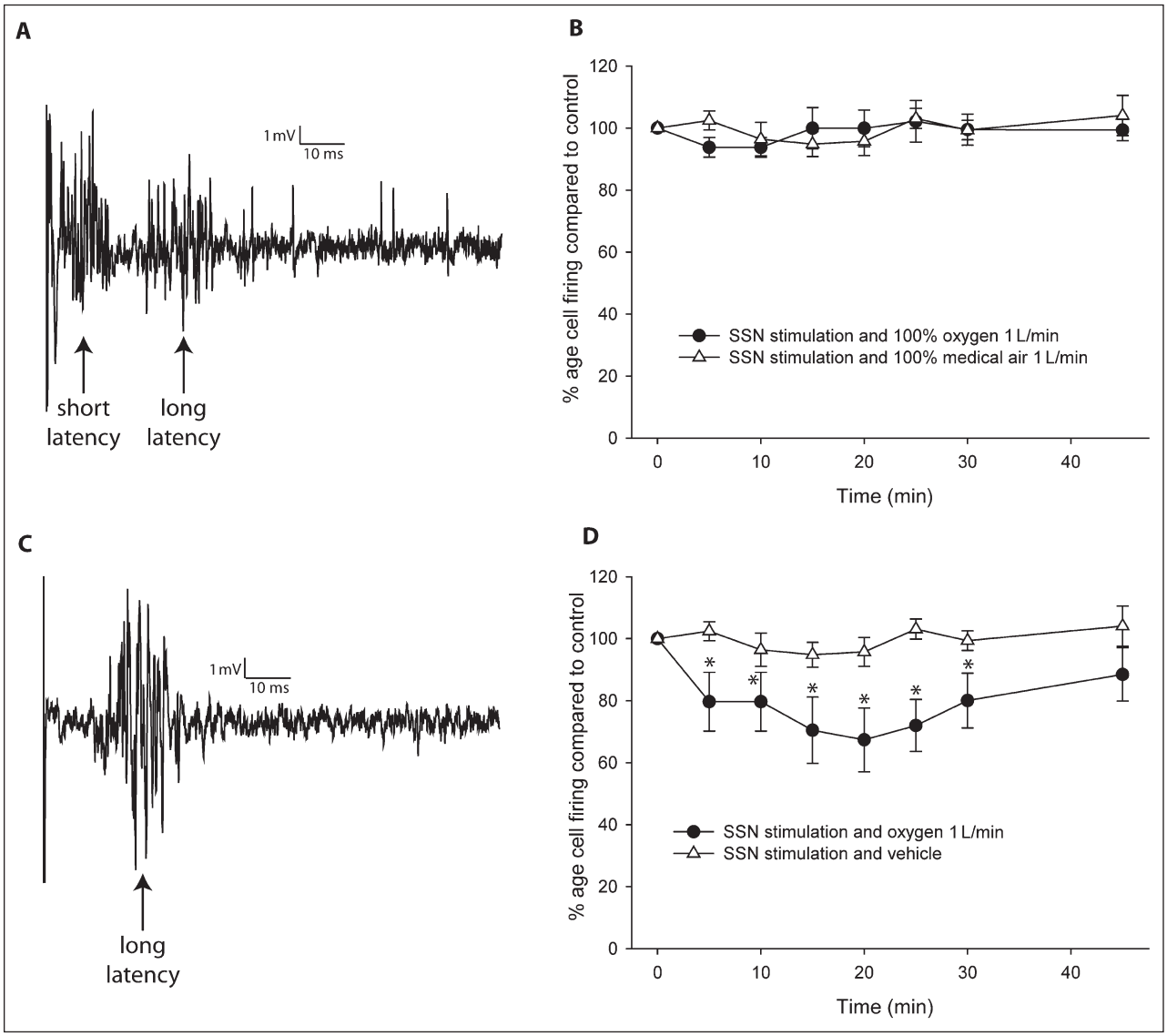

Fig 5.—Effects of oxygen on activation of the parasympathetic outflow to the cranial vasculature on evoked trigeminal nerve activation in the trigeminocervical complex. Activation of the parasympathetic outflow to the cranial vasculature through stimulation of the superior salivatory nucleus and via the greater petrosal nerve of the VIIth nerve causes activation of neurons with short latency (as highlighted by the first arrow), 3-20 milliseconds (A) that are unresponsive to treatment with (B) 100% oxygen (1 L/minute). Neuronal responses with longer latency (highlighted by the second arrow in A and the arrow in (C), 9-40 milliseconds were inhibited by oxygen treatment (D).

vessel dilation as the output response. This is the first study to suggest that oxygen may act specifically on the parasympathetic facial/greater superficial petrosal nerve pathway to exert its abortive effects in cluster headache, rather than directly on trigeminal afferents to the dural vasculature or within the trigeminal nucleus. The lack of a response of oxygen on durally evoked trigeminovascular activation, a model that has proved to have excellent reliability in predicting antimigraine action,[27] may also imply a lack of effect of oxygen in aborting migraine.

Activation of the parasympathetic outflow to the cranial vasculature, via stimulation of the SuS/facial nerve, resulted in evoked neuronal firing in the TCC and increases in blood flow of the lacrimal sac contiguous with the stimulation. There were 2 populations of neurons identified, those that responded to oxygen treatment and those that did not. SuS-evoked

neuronal firing that was responsive to oxygen treatment tended to be of slightly longer latencies, 9-40 milliseconds, with an average of 20 milliseconds. This is the first example of activation of neurons within the TCC that are a direct consequence of activation of the parasympathetic outflow, and this may explain the slightly longer latency as the responses are crossing multiple synapses and travelling a further 10 mm to the cranial vasculature. The population of neurons that did not respond to oxygen treatment had much shorter latencies, 3-20 milliseconds with an average of 10 milliseconds. It is possible that this population of neurons are a result of antidromic stimulation of the trigeminal system within the brain stem itself (Fig. 1).

Oxygen also had no significant effect on models of direct trigeminovascular nociception. It was unable to inhibit neurogenic dural vasodilation and neuronal firing in the TCC in response to dural stimulation. Looking at these data as a whole, it would indicate that the effects of oxygen are most likely only on the parasympathetic outflow from the SuS via the facial nerve/greater petrosal nerve with no direct effect on trigeminal afferents. This may explain why oxygen is only effective in cluster headache, and while, as yet, its direct effects on migraine are unknown these predict limited efficacy. This does not, however, explain why it is only specific to cluster headache compared to other TACs, and more work will be required to explore this question.

Another major feature of cluster headache is the prominence of cranial autonomic symptoms, such as lacrimation, reddening of the eye and nasal congestion. We tried to explore these parasympathetic effects by observing relative blood flow in the lacrimal sac as direct activation of this autonomic pathway and indicative of activation of the lacrimal gland. Activation of the SuS/facial nerve produced increases in blood flow within the lacrimal sac contemporaneously with the stimulation in a reproducible fashion (see Fig. 4). Oxygen was also able to attenuate these increases in blood flow. It is likely that this is again a direct consequence of an action of oxygen on the parasympathetic outflow from the SuS/facial nerve. This adds further support to the effects of oxygen in cluster headache relieving both the head pain and cranial autonomic symptoms.

Stimulation of the facial nerve[28] and the sphenopalatine ganglion[9] has been shown to cause increases in cerebral blood flow, and it is known that the dural vasculature receives VIPergic inputs from the SuS.[29,30] Therefore, these projections have a significant effect on the extracranial vasculature and this response is independent of direct activation of trigeminal afferents. It is interesting that SuS stimulation had little effect on the dural meningeal vessels, where VIP is believed to be the major vasodilator peptide release. VIP, pituitary adenylate cyclase-activating polypeptide (PACAP)-38 and -27, which act on the $VPAC_1$, $VPAC_2$, and PAC receptors are all able to induce dural blood vessel dilation when given exogenously.[31] Although it is worth noting that the level of vasodilation was significantly lower than that of calcitonin gene-related peptide (CGRP) in the same model[22] and 10-fold higher doses, at least, were used in those studies. It is possible that the exogenous doses of VIP, or even PACAP, were not sufficient to induce meningeal vasodilation in this study. Indeed, CGRP (1 μg/kg) causes maximal vasodilation of dural meningeal vessels,[32] whereas this same dose of VIP was unable to cause a significant change in blood vessel diameter.[31] It is known that VIP is released during cluster headache, perhaps as a reflection of cranial autonomic symptoms,[15] and indeed causes marked cephalic vasodilation in patients, but not migraine.[33] It is possible then that the neuronal effects of VIP in cluster headache are more important than the direct effects of VIP on blood vessels *per se*.

Understanding the pathophysiology of TACs, including cluster headache, is important in developing therapies that help relieve symptoms in these extremely debilitating conditions. While little is known about how current therapies work, they provide one of the few windows into improving our understanding of these conditions. The evidence from the effects of oxygen in the models of trigminovascular nociception, through direct activation of trigeminal afferents, would seem to imply that oxygen does not act directly on these nerves. However, by studying the parasympathetic outflow to the cranial vasculature and activation of the trigeminal-autonomic reflex, it would seem that stimulation of this pathway

results in significant evoked firing of neurons in the TCC and autonomic driven blood flow changes. It was shown that oxygen is able to attenuate these responses and it is likely that this is a direct action on the parasympathetic outflow itself rather than on trigeminal afferents. We believe that this is the first study to show that oxygen maybe acting directly on parasympathetic responses to exert its action in relieving cluster headache symptoms, and perhaps represents a future direction in designing therapeutics for this debilitating condition.

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