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Trigeminal interpolaris/caudalis transition neurons mediate reflex lacrimation evoked by bright light in the rat

Keiichiro Okamoto[1], **Akimasa Tashiro**[2], **Randall Thompson**[1], **Yasuhiro Nishida**[2], and **David A. Bereiter**[1]

[1]Department of Diagnostic and Biological Sciences, University of Minnesota School of Dentistry, Moos Tower 18-214, 515 Delaware St. SE, Minneapolis, MN 55455, USA

[2]Department of Physiology, National Defense Medical College, Namiki 3-2, Tokorozawa City, Saitama, 359-8513, Japan

Abstract

Abnormal sensitivity to bright light can cause discomfort or pain and evoke protective reflexes such as lacrimation. Although the trigeminal nerve likely is involved, the mechanism linking luminance to somatic sensory nerve activity remains uncertain. This study determined the effect of bright light on second-order ocular neurons at the ventral trigeminal interpolaris/caudalis transition (Vi/Vc) region, a major termination zone for trigeminal sensory fibers that innervate the eye. Most Vi/Vc neurons (80.9 %) identified by responses to mechanical stimulation of the ocular surface also encoded bright light intensity. Light-evoked neural activity displayed a long latency to activation (>10 s) and required transmission through the trigeminal root ganglion. Light-evoked neural activity was inhibited by intravitreal injection of phenylephrine or L-N^G-nitro-arginine methyl ester (L-NAME) suggesting a mechanism coupled to vascular events within the eye. Laser Doppler flowmetry revealed rapid light-evoked increases in ocular blood flow that occurred prior to the increase in Vi/Vc neural activity. Synaptic blockade of the Vi/Vc region by cobalt chloride prevented light-evoked increases in tear volume, whereas blockade at the more caudal spinomedullary junction (Vc/C1) had no effect. In summary, Vi/Vc neurons encoded bright light intensity and were inhibited by drugs that alter blood flow to the eye. These results support the hypothesis that light-responsive neurons at the Vi/Vc transition region are critical for ocular-specific functions such as reflex lacrimation, whereas neurons at the caudal Vc/C1 junction region likely serve other aspects of ocular nociception.

Keywords

trigeminal subnucleus caudalis; light sensitivity; lacrimation; ocular blood flow; nociception

Introduction

Abnormal sensitivity to light, commonly termed photophobia or photo-oculodynia (Lebensohn 1934; Fugate, 1957; Fine and Digre 1995; Murray et al., 2002), causes ocular sensations that can vary in intensity from mild discomfort to intolerable pain. Photophobia occurs in patients with such diverse conditions as headache (Goadsby et al. 2008; Noseda &

Corresponding author; Keiichiro Okamoto, DDS Ph.D., Dept. of Diagnostic and Biological Sciences, University of Minnesota School of Dentistry, Moos Tower 18-214 Minneapolis, MN 55455, USA, Phone; (612) 626-2768; fax (612) 626-2651. okamo007@umn.edu.

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Burstein 2011), eye injury or inflammation (Custer & Reistad, 2000; Cordero-Coma et al., 2007), blepharospam (Hallet et al., 2008) or retinal dystrophies (Prokofyena et al. 2011).

The mechanisms underlying abnormal sensitivity to light remain elusive; however, it has long been proposed that trigeminal sensory nerves play a significant role (Lebensohn, 1951). This notion was supported recently by evidence of specific activation patterns to light stimulation in the trigeminal root ganglion (TRG) and brainstem in a patient with acute corneal abrasion by neuroimaging (Moulton et al., 2009). Trigeminal afferent nerves that supply the eye terminate in a distinctive bimodal pattern at the ventrolateral trigeminal subnucleus interpolaris (Vi) and caudalis (Vc) transition (Vi/Vc) and Vc/upper cervical spinal cord (Vc/C1) regions (Marfurt & del Toro, 1987; Panneton et al. 2010). Considerable evidence from studies using ocular surface stimuli suggests that neurons at the Vi/Vc transition and Vc/C1 regions serve different aspects of ocular function (Meng et al. 1997; Hirata et al., 1999; Bereiter et al., 2005; Tashiro et al., 2010). By contrast, little is known about the role of the Vi/Vc and Vc/C1 regions in processing sensory input from deep tissues in the eye. Recently, we reported that bright light increased the number of Fos-positive neurons at the Vi/Vc and Vc/C1 regions independent of ocular surface nerve activity suggesting an intraocular source of sensory input (Okamoto et al., 2009). Correspondingly, in recording studies, light-evoked Vc/C1 neural activity was not affected by local anesthesia of the ocular surface although it likely involved increased parasympathetic outflow consistent with a vascular-linked mechanism within the eye (Okamoto et al. 2010).

The goal of this study was to determine if ocular neurons at the Vi/Vc region were activated by bright light stimulation similar to ocular neurons recorded at the Vc/C1 region (Okamoto et al., 2010). Secondly, we sought to determine if bright light-evoked neural activity in the Vi/Vc region was necessary for ocular-specific autonomic reflexes such as lacrimation. The present study also applied vasoactive drugs locally within the eye to assess their effects on light-evoked changes in ocular blood flow (Laser Doppler flowmetry) and Vi/Vc neural activity. Tear volume was measured before and after synaptic blockade of the Vi/Vc or Vc/C1 region by microinjection of cobalt chloride to assess the contribution of each region in mediating ocular-specific reflexes evoked by bright light.

Materials and methods

The animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota (USA) and the Committee of Research facilities for laboratory Animal Science, National Defense Medical College (Japan) and conformed to the established guidelines set by The National Institutes of Health guide for the care the use of laboratory animals (PHS Law 99–158, revised 2002). All efforts were made to minimize the number of animals used for experiments and their suffering.

Electrophysiology procedures

Animals—Male rats (270–350 g, Sprague-Dawley, Harlan, Indianapolis, IN) were anesthetized initially with pentobarbital sodium (50 mg/kg ip). Catheters were positioned in the right femoral artery for monitoring blood pressure and jugular vein for drug infusion (gallamine triethiodide, 20 mg/kg/h, at the time of recording). After tracheostomy animals were respired artificially with oxygen-enriched room air and anesthesia was maintained with isoflurane (1.2–2.0%). Expiratory end-tidal CO_2 (3.5–4.5%), mean arterial pressure (MAP, 90–120 mmHg) and body temperature (38°C) were monitored continuously and kept within normal range. Animals were placed in a stereotaxic frame and portions of the C1 vertebra were removed to expose the lower brainstem and upper cervical dorsal horn. The exposed surface was bathed in warm mineral oil. Neurons recorded at the ventrolateral trigeminal subnucleus interpolaris/ caudalis transition (Vi/Vc) region were approached at an angle of

28° off vertical and 45° off midline, and 1.5–2.0 mm below the brainstem surface. Extracellular unit activity was recorded using tungsten microelectrodes (5–9 Mohm, Frederick Haer Inc., Bowdoinham, ME) and amplified, discriminated, stored and analyzed offline using a PowerLab interface and LabChart software (AD Instruments, Colorado Spring, CO).

Characterization of light-responsive ocular neurons—The search stimulus consisted of gently swiping a fine camelhair brush across the ocular surface (e.g., cornea surface and conjunctiva). All units included in this study were activated by mechanical stimulation of both the cornea and conjunctiva. Units with a convergent cutaneous receptive field (RF) were classified as wide dynamic range (WDR) or nociceptive specific (NS) based on the responses to a low force von Frey filament (1.2 g) and pinch with blunt forceps as described previously (Hirata et al., 1999). Neurons with no apparent cutaneous RF were classified as cornea only (CO). The ocular surface was kept moist with artificial tears throughout all experiments.

Light stimulation and experimental design—One neuron was recorded from each animal preparation. Experiments that involved intravitreal drug injection included only those neurons responsive to light stimulation. Light stimulation was delivered from a thermal-neutral fiber optic halogen source (150W, Cole Parmer, Vernon Hills, IL) positioned 5 cm from the left ocular surface under dim ambient light conditions. Light intensity was measured at the ocular surface with a lux meter (Control Co., Friendswood, TX). Light stimuli (30 s duration) were presented in a cumulative design of three intensities at 20 min interstimulus intervals: low (0.5×10^4 lux), moderate (1×10^4 lux) and high (2×10^4 lux).

Drug microinjections into the eye (intravitreal, ivt) or trigeminal root ganglion (TRG)—Light stimulation was presented before and 10 min after ivt injection of the selective $alpha_1$-adrenergic receptor agonist, phenylephrine (1 or 10 mM, pH 7.4, Sigma) or the nitric oxide synthase (NOS) inhibitor, L-N^G-nitro-arginine methyl ester (L-NAME, 1 or 10 mM, pH 7.4, Sigma). Activation of alpha 1 adrenergic receptors and inhibition of NOS are treatments known to decrease blood flow to the eye (Kawarai and Koss, 1998; Schmetterer and Polak, 2001). Previously, we reported that ivt phenylephrine inhibited light-evoked ocular Vc/C1 unit activity (Okamoto et al., 2009, 2010). Drugs were delivered from a 33 gauge needle inserted into the globe through the sclera posterior to the limbus. Intra-TRG injection of lidocaine (2%, pH 7) was delivered 10 min before light stimulation from a 33 gauge needle inserted through a 26 gauge guide cannula positioned vertically above the ganglion using the following coordinates: 3.1–3.3 mm caudal to bregma, 2.8–3.1 mm lateral and 9–10 mm ventral to the brain surface. Ivt phenylephrine was assessed on resting Vi/Vc neural activity and on the responses to low, moderate and high intensity light stimulation, while L-NAME effects were assessed before and after high intensity light stimulation. At least 20 min was allowed to elapse between successive light stimulus periods. All drugs were diluted in artificial cerebral spinal fluid (aCSF, 1 μl, pH 7.4) and injected in a volume of 1 μl. Control animals received an equivalent volume injection of aCSF.

Ocular blood flow procedures

In separate experiments Laser Doppler flowmetry (LDF) was used to measure blood flow from the anterior choroid in response to light stimulation adapted after the methods of Yamaguchi et al. (1999) and Kawarai and Koss (1998) with a non-contact Laser-Blood Flow meter (Omega flow FLO-C1EL, Omegawave, Japan). Male rats (320–400 g, Sprague-Dawley, SLC Japan, Shizuoka) were anesthetized with pentobarbital sodium (50 mg/kg, ip). Cannulae were positioned in the femoral artery (blood pressure) and jugular vein (drug infusion). Animals were placed in a stereotaxic frame and respired artificially. Anesthesia

was maintained with an infusion of thiopental (30–40 mg/kg/hr) and rats were paralyzed with gallamine triethiodide (10–30 mg/kg/hr, iv) given at the time of blood flow measurement. Expiratory end-tidal CO_2 (3.5–4.5%), mean arterial pressure (MAP, 100–120 mmHg) and body temperature (38°C) were monitored continuously.

Three light intensities (0.5×10^4, 1×10^4, 2×10^4 lux, 40 s) were presented at 20 min intervals under reduced ambient room light (< 100 lux). Relative changes in ocular blood flow evoked by high intensity light (2×10^4 lux, 40 s) were measured before and 10 min after ivt injection of phenylephrine (10 mM, 1 μl) or L-NAME (10 mM, 1μl) or vehicle (aCSF, 1 μl). The ocular surface was kept moist with artificial tears.

The LDF method is based on the concept that laser light reflects off moving blood cells to produce a Doppler frequency shift, dependent on cell concentration and velocity. The laser beam (0.5mm spot diameter) was irradiated at right angles to the eyeball surface several millimeters posterior to the limbus with care taken to avoid recording blood flow from any large external limbal blood vessels (Kawarai and Koss, 1998; Yamaguchi et al. 1999). Blood flow was recorded as arbitrary flux units, monitored continuously, stored and analyzed offline using a PowerLab interface board and LabChart software (PowerLab, AD Instruments, Castle Hill, Australia). Baseline blood flow was quantified as the area under curve (AUC) for a 40 s epoch sampled immediately prior to stimulation. The data were normalized to percentage of basal blood flow sampled for an equivalent 40 s epoch prior to light stimulation.

Tear volume

Male Sprague-Dawley rats (320–400 g, Harlan, Indianapolis, IN) were anesthetized initially with pentobarbital sodium (50 mg/kg ip) and a cannula positioned in the femoral artery (blood pressure monitor). After tracheostomy, animals were placed in a stereotaxic frame and respired artificially. Expiratory end-tidal CO_2 (3.5–4.5%), mean arterial pressure (MAP, 100–120 mmHg) and body temperature (38°C) were monitored continuously. Tear volume was measured under isoflurane anesthesia (1.0–1.5%) and low ambient light conditions (< 100 lux). Tear fluid was collected for 2 min into a calibrated glass capillary tube positioned at the lateral palpebral conjunctiva-limbus junction that did not directly contact the ocular surface. Tear volume was determined by fluid column length within the tube. Three series of experiments were performed to assess the contribution of the TRG, Vi/Vc and Vc/C1 regions on evoked tear volume. To assess TRG involvement, high intensity light (2×10^4 lux) was presented before and 10 min after lidocaine (2%, 1 μl, pH 7) injection delivered stereotaxically via a guide cannula and 33 gauge needle as noted above for neural recording. The involvement of the Vi/Vc and Vc/C1 regions was assessed by microinjection of the synaptic blocker, $CoCl_2$ (100 mM, 0.1 μl, pH 7.2), delivered via a glass micropipette ipsilateral to stimulated eye 10 min prior to high intensity light stimulus. Microinjections directed at the Vi/Vc used the same coordinates as for neural recording, while Vc/C1 sites were approached at an angle of 43° off vertical, 60° off midline, and a depth of 300 μm of the dorsal brainstem surface. Controls received an equivalent volume injection of aCSF (pH 7.4, 0.1 μl).

Data analysis

Neural recording data were acquired and displayed as peristimulus time histograms (PSTH) of spikes per 1 s bins, exported to a spreadsheet and analyzed off-line. Neural responses were analyzed as a response magnitude (Rmag) for each stimulus period defined as the cumulative sum of spikes for contiguous bins in which the spike count exceeded the mean + 2SD of the background activity (Hirata et al., 1999). The total Rmag was calculated for each stimulus period and can be thought of as the “area under the curve”. Neurons were defined

as light-responsive if the total Rmag exceeded a value of 10 independent of light intensity. Response duration was defined as the time interval after stimulus onset until three consecutive bins with a positive spike count occurred above background (initial latency) and until the value of three consecutive bins no longer exceeded the mean + 2SD above background activity. Response latency was defined as earliest time after stimulus onset for which three consecutive 1-s bins exceeded the mean + 2SD of background activity. The high-threshold convergent cutaneous RF area of light-responsive units was mapped onto standardized drawings of the rat face with a small blunt forceps, digitized and quantified by a planimetric method using NIH software (Image J). Choroidal blood flow was sampled continuously and values were rectified and stored as 1 s bins for off-line analyses. Average resting MAP was analyzed for 60 s prior to each stimulus. Neural activity, cutaneous RF area, ocular blood flow, tear volume, and MAP were assessed by ANOVA corrected for repeated measures. Significant treatment effects were further assessed by Newman-Keuls after ANOVA. Comparisons of frequency of occurrence across neuronal cell classes or recording locations were made by Chi-square or Fisher's Exact Probability tests. The data were presented as mean ± SEM and significance level set at $P = 0.05$.

Histology

At the end of the experiment, rats were given a bolus of pentobarbital sodium and perfused through the heart with saline followed by 10% formalin and the brain processed to recover the recording sites or microinjection sites at the Vi/Vc and Vc/C1 regions.

Results

General properties of Vi/Vc ocular neurons

A high percentage of ocular surface-responsive Vi/Vc units (80.9% or 38/47 units) were activated by high intensity light stimulation. All classes of units, based on cutaneous RF properties, were activated by bright light (NS = 17/23; WDR = 13/14 and CO = 8/10). All light-responsive Vi/Vc units, regardless of classification, displayed ongoing spontaneous activity (SA) prior to light stimulation (overall mean = 5.11 ± 0.78 spikes/s, n = 38). Recording sites for Vi/Vc units were similar to those we reported previously (Hirata et al., 1999, 2004).

Light intensity coding by Vi/Vc neurons

Light-responsive Vi/Vc units displayed an intensity-dependent increase in Rmag (see example in Fig 1A). Comparison across the three light intensities for all classes of Vi/Vc units revealed a highly significant progressive increase in total Rmag values ($F_{2,74} = 82.8$, $P < 0.001$, Fig 1B), whereas analyses across individual classes (i.e., NS, WDR and CO) found no differences ($F_{2,35} = 0.5$, $P > 0.1$). The low intensity light stimulus evoked responses (Rmag value >10) in 13 of 38 units, while nearly all units were activated by the moderate intensity stimulus (36/38 units) suggesting that under these experimental conditions, the threshold for light-evoked activation was in the range of $0.5–1\times10^4$ lux, although we cannot exclude that some units could have been activated by intensities lower than were applied here. Response duration increased significantly with increasing light intensity ($F_{2,74} = 61.6$, $P < 0.001$, Fig 1C), while response latency was decreased ($F_{2,74} = 63.3$, $P < 0.001$, Fig 1D). Comparisons across individual classes of Vi/Vc units found no inter-class differences (data not shown).

Trigeminal root ganglion (TRG) blockade prevents light-evoked Vi/Vc neural activity

Lidocaine injection into the TRG ipsilateral to the light stimulus blocked completely the light-evoked response of all Vi/Vc units tested (n = 5) compared vehicle controls (n = 4)

($F_{3,21}$ = 20.6, P < 0.001, Fig 2). By 10 min after lidocaine the evoked response was reduced to 96.7 ± 2.3% of the pre-injection value, while by 50 min it had recovered to 68.3 ± 13.1% of the pre-injection value. Intra-TRG lidocaine also blocked completely the convergent input from periorbital skin (data not shown). The light stimulus continued to evoke pronounced pupillary constriction after lidocaine blockade indicating that optic nerve function likely was not compromised. Intra-TRG injection of lidocaine did not affect resting MAP (data not shown).

Intraocular adrenergic and nitric oxide mechanisms contribute to light-evoked Vi/Vc neural activity

To determine if alpha-adrenergic receptor mechanisms within the eye influenced light-evoked neural activity, the potent vasoconstrictor, phenylephrine, was injected ivt. As seen in Fig 3B, light-evoked responses of Vi/Vc units were markedly inhibited by high dose phenylephrine ($F_{1,8}$ = 23.5, P < 0.001, n = 5), while the lower dose had no effect compared to responses prior to drug injection ($F_{1,6}$ = 0.4, P > 0.1, n = 4, Fig 3A). Resting discharge rates of Vi/Vc units were not affected by phenylephrine ($F_{3, 14}$ = 0.3, P > 0.1). Intraocular volume expansion alone did not alter Vi/Vc unit activity since ivt injection of aCSF did not affect resting discharge rates or light-evoked activity ($F_{1, 7}$ = 0.1, P > 0.1, n = 4).

To determine if nitric oxide-related mechanisms within the eye influenced light-evoked neural activity, the NOS inhibitor, L-NAME was injected ivt 10 min prior to high intensity light stimulation. As seen in Fig 3C, high intensity light-evoked responses of Vi/Vc units were reduced significantly after low dose (1 mM, −36 ± 9%, n = 5) and high dose (10 mM, −47 ± 11%, n = 5) L-NAME compared to vehicle controls ($F_{2,10}$ = 4.2, P < 0.05, n = 4), whereas resting discharge rates were not affected ($F_{2, 10}$ = 0.8 P > 0.1). The convergent RF area of light-responsive Vi/Vc units and systemic MAP were not altered by phenylephrine or L-NAME (data not shown), indicating that the effects of drugs given by the ivt route were not likely due to sites of action outside the eye.

Light-evoked changes in ocular blood flow

Ocular blood flow was measured from a probe positioned over a region of the anterior choroid before, during and after bright light stimulation using methods adapted from Yamaguchi et al. (1999) and Kawarai and Koss (1998). Bright light caused a prompt increase in blood flow that was maintained during the stimulus period and returned towards baseline level after stimulation (Fig 4A). Bright light increased the magnitude of blood flow (Fig 4B, $F_{2, 18}$ = 11.98, P < 0.001) and reduced the latency (Fig 4C, $F_{2, 18}$ = 4.67, P < 0.05) defined as a change of > 10% above baseline in an intensity-dependent manner (n = 10). The latency to observe blood flow changes after the low intensity light stimulus was variable since short latency responses (< 10s), similar to the example in Fig 4A, occurred in only 3 of 10 experiments, while in 3 other experiments low intensity light had no effect on blood flow. By contrast, high intensity light evoked short latency responses in 9 of 10 experiments. Chi-square analysis across all light intensities revealed a significant effect of intensity on the frequency to observe a latency of < 10 s ($\chi^2_{df\ 2}$ = 8.31, P < 0.015). In separate experiments, ivt phenylephrine ($F_{1, 3}$ = 29.7, P < 0.01, n = 4) or L-NAME ($F_{1, 3}$ = 28.4, P < 0.01, n = 4) significantly blunted high intensity light-evoked increases in blood flow (> 90% inhibition), while vehicle did not affect light-evoked blood flow ($F_{1, 2}$ = 0.7, P > 0.1, n = 3). Resting MAP was not affected by ivt phenylephrine ($F_{1, 3}$ = 0.5, P > 0.1) or L-NAME ($F_{1, 3}$ = 0.1, P > 0.1).

Trigeminal pathways and light-evoked lacrimation

Intact transmission through the TRG was necessary to observe normal reflex lacrimation since lidocaine (2%, 1 μl) injection into the TRG, ipsilateral to light stimulation, inhibited

(57 ± 13% reduction) light-evoked tear volume at 10 min with nearly complete recovery by 30 min (Fig 5A; $F_{3,12} = 9.49$, $P < 0.005$, n = 5). Light-evoked increases in tear volume from the contralateral eye were not affected by lidocaine blockade. Blockade of synaptic activity at the Vi/Vc region by $CoCl_2$ (100 mM, 0.1 μl) inhibited (66.8 ± 5% reduction) light-evoked increases in tear volume ($F_{3,15} = 42.04$, $P < 0.001$) that persisted for more than 30 min (Fig 5B, n = 4). By contrast, $CoCl_2$ blockade at the Vc/C1 region had no significant effect on light-evoked tear volume (Fig 5C; $F_{3,9} = 1.86$, $P > 0.1$, n = 4). Microinjection of an equal volume of aCSF injection into either the Vi/Vc (n = 4) or Vc/C1 (n = 3) region did not alter high intensity light-evoked tear volume ($F_{3,30} = 1.49$, $P > 0.1$). These data indicated that transmission through the TRG and synaptic integration at the Vi/Vc, but not the Vc/C1 region, was critical for bright light-evoked lacrimation. The sites of drug injections were similar to those for neural recording at the Vi/Vc and Vc/C1 regions (Fig. 5D).

Discussion

These results demonstrated that ocular neurons at the ventral Vi/Vc region encoded the intensity of bright light. Light-evoked neural activity was blocked completely by intra-TRG injection of lidocaine indicating that afferent input through trigeminal sensory neurons was required. Intraocular administration of drugs known to alter ocular blood flow also greatly reduced light-evoked Vi/Vc neural activity. Laser Doppler flowmetry revealed that bright light also caused a prompt (< 7 s) increase in ocular blood flow and, coupled with the long latency to observe light-evoked neural activity (>10 s), consistent with a transduction mechanism linked to vascular events in the eye. Synaptic blockade of the Vi/Vc, but not the more caudal Vc/C1 region, prevented light-evoked increases in tear volume suggesting the Vi/Vc region plays a significant role in ocular-specific reflexes.

The diversity of clinical conditions associated with abnormal enhanced sensitivity to light suggests that multiple mechanisms underlie this phenomenon (Amini et al. 2006; Noseda & Burstein 2011; Digre & Brennan 2012). Although it has long been proposed that the trigeminal nerve is critically involved in photophobia (Lebensohn 1951), the relationship between trigeminal nerve activity and luminance-evoked sensations has remained largely unresolved. Conditions in which trigeminal sensory and luminance information converge to induce abnormal enhanced photosensitivity appear to fall into two broadly defined, though not mutually exclusive, categories. In the first category, conditions such as migraine headache and subdural hemorrhage involve significant sensory input from extraocular trigeminal fibers. Sensory convergence in migraine headache has been proposed to occur supraspinally, in sensory thalamic regions reported to receive both somatic and luminance inputs (Noseda et al. 2010). In this model sensory convergence at higher centers may be sufficient to cause abnormal photosensitivity and exacerbate headache pain without enhancement of trigeminal nerve fiber activity coming from the eye. Alternatively, a second category, that includes many common primary eye conditions such as uveitis and dry eye disease, in which the properties of trigeminal fibers that innervate the eye may be altered, enhanced photosensitivity may involve quite different mechanisms than those of headache-related photophobia. In this category, convergence of somatic sensory and luminance information resulting in enhanced photosensitivity is proposed to rely more heavily on peripheral trigeminal mechanisms within the eye.

The present study assessed the light-evoked responses of Vi/Vc units identified by a vigorous response to ocular surface stimulation and would be most relevant in the context of ocular-related enhanced photosensitivity. Several lines of evidence suggested that light-evoked responses were linked to vascular events within the eye. First, light-evoked increases in Vi/Vc neural activity occurred only after a long delay (> 10 s) suggesting a mechanism involving non-neural elements. Second, intraocular administration of vasoactive drugs

significantly modified light-evoked Vi/Vc neural activity and ocular blood flow. The ocular circulation is well supplied by sympathetic and parasympathetic nerves that influence blood flow (see Klooster et al., 1996; Cuthbertson et al., 2003; Lutjen-Drecoll, 2006; Nickla & Wallman 2010; Neuhuber & Schrodl 2011). In his original description Lebensohn (1934) reported that instillation of epinephrine relieved photophobia in patients with conjunctivitis or iritis, whereas cervical sympathetic block greatly reduced light sensitivity in patients described as having photo-oculodynia and decreased tears (Fine & Digre 1995) or essential blepharospasm (McCann et al. 1999). These studies also supported the present results of a marked inhibition of light-evoked Vi/Vc units after intraocular administration of phenylephrine, a potent alpha-adrenergic receptor agonist, known to reduce ocular blood flow (Kawarai & Koss 1998; Takayama et al. 2009). The present results also demonstrated that light-evoked neural activity at the Vi/Vc transition was reduced significantly after acute inhibition of NOS by L-NAME consistent with parasympathetic involvement in this response. Previously we reported that light-responsive neurons at the Vc/C1 region were inhibited by blockade of the superior salivatory nucleus, a major source of parasympathetic outflow to the eye (Okamoto et al. 2010). Similarly, somatic stimulation-evoked increases in ocular blood flow were prevented by blockade of parasympathetic outflow in the rat (Shimura et al. 2002). Nitric oxide plays a significant role in regulating ocular blood flow (Schmetterer & Polak 2001; Schmidl et al. 2009) and other aspects of ocular function (Kiel et al. 2001; Wang et al. 2007). Third, the latency for light stimulation-evoked increases in ocular blood flow was intensity-related and occurred at latencies (~7 s) prior to those seen for changes in Vi/Vc neural activity. Others have reported similar latencies for changes in ocular blood flow after direct stimulation of the superior salivatory nucleus in the rat (Steinle et al. 2000). In humans, unilateral light-dark transitions evoked bilateral changes in choroidal blood flow consistent with a central neural reflex circuit (Fuchsjager-Mayrl et al., 2001).

Although these data were consistent with neurovascular coupling as a mechanism for light-evoked trigeminal neural activity, the exact mechanism is not yet known. Two general modes of transduction can be considered. One possibility is that light-evoked increases in choroidal blood flow and/or vessel caliber activates trigeminal nerves by mechanical deformation of nerve endings in close contact with ocular blood vessels. This may explain why vasoconstriction by phenylephrine as well as blockade of active vasodilatation by L-NAME was able to significantly reduced light-evoked Vi/Vc neural activity. Intraocular tissues, including blood vessels, are well supplied by peptidergic fibers consistent with sensory nerves (Terenghi et al., 1985; Stone et al., 1987; Schmid et al., 2006). At spinal levels, changes in vascular perfusion were sufficient to excite sensory nerves surrounding mesenteric blood vessels (Brunsden et al., 2007; Song et al. 2009). Similarly, trigeminal sensory fibers coursing in the long ciliary nerves were activated by changes in ocular blood flow (Gallar et al., 2003). Interestingly, humans that lack normal small fiber innervation of skin retained some sensations presumably due to sensory fiber innervation of cutaneous blood vessels (Bowsher et al. 2009). A second, though not mutually exclusive, mechanism predicts that release of neuroactive agents (e.g., NO, endothelin) released from postganglionic nerve endings or endothelial tissues after bright light stimulation was responsible for activation of trigeminal afferent nerves. Indeed, paravascular increases in NO caused pain sensation in humans (Holthusen and Arndt 1995), while application of cholinergic agonists was sufficient to excite a population of corneal afferents in rabbits that were not responsive to mechanical or thermal stimuli (Tanelian 1991). Recently, trigeminal sensitization to bright light, seen as enhanced air puff- or supraorbital nerve-evoked eye blink, was preserved in rats after optic nerve transection (Dolgonos et al. 2011). However, these data could not exclude the possibility of an intraocular vascular-related transduction mechanism.

The organization of the trigeminal brainstem complex that underlies bright light-evoked neural activity likely involves the ventral Vi/Vc and the Vc/C1 regions. Previously, we reported that bright light produced a bimodal distribution of Fos-positive neurons at the Vi/Vc and Vc/C1 regions (Okamoto et al. 2009) that was similar to that seen after ocular surface stimulation (Lu et al. 1993; Strassman & Vos 1993; Meng & Bereiter 1996; Chang et al. 2010). This suggested that afferents supplying the ocular surface and deeper tissues of the eye converged onto common second-order neurons in the trigeminal brainstem complex. Indeed, in the present study 81% of Vi/Vc units (present data) and 72% of units at the Vc/C1 region (Okamoto et al., 2010), identified by a response to mechanical stimulation of the ocular surface, also were excited by bright light. Although light-responsive units in both regions displayed several common features such an intensity-related increase in neural activity and marked inhibition after intraocular phenylephrine, a notable difference was the higher percentage of Vi/Vc units activated by the moderate intensity stimulus compared to Vc/C1 units (95% versus 63%, Fishers Exact Probability, $P < 0.001$). The basis for this apparent difference in luminance thresholds is not known and could be due to different populations of intraocular afferents projecting preferentially to the Vi/Vc and Vc/C1 regions. These populations may express a different set of receptors or, alternatively, are distributed differently within the deep tissues of the eye in relation to blood vessels. Evidence that select trigeminal afferent nerve populations projected differentially to the Vi/Vc and Vc/C1 regions was supported by our previous study in which neurons at the Vi/Vc, but not the Vc/C1 region, were sensitive to changes in the moisture status of the ocular surface (Hirata et al. 2004). Recently it has been reported that cold sensitive ocular afferents detect changes in ocular surface moisture status and mediate basal tear production (Parra et al., 2010; Hirata & Meng, 2010). Cold afferents are unique among trigeminal ocular afferents in that they display a high rate of background activity. In the present study light-responsive neurons at the Vi/Vc region (5.1 ± 0.8 spikes/s) had a resting discharge rate that was significantly higher than for Vc/C1 neurons (~ 2 spikes/s, Okamoto et al., 2010). The present results also found that light-evoked increases in tear volume depended on a neural relay through the TRG and Vi/Vc, but not the Vc/C1 region. This finding paralleled the results of an earlier study in which we reported that ocular surface-evoked tear volume required an intact Vi/Vc, but not Vc/C1 region (Hirata et al. 2004). The central neural circuitry underlying evoked eye blink appears to share a similar bimodal organization in that the ventral Vi/Vc region initiates evoked eye blinks, while the Vc/C1 region exerts mainly modulatory control (Henriquez & Evinger 2007). The basis for this regional specialization of function is not certain; however, it is possible that specialized classes of afferents such as moisture sensing primary afferents neurons terminate preferentially in the Vi/Vc region. However, these data cannot exclude the possibility that central neural mechanisms also play a role in processing ocular sensory information by Vi/Vc and Vc/C1 neurons and mediating ocular-specific reflexes.

It is well accepted that the main function of the trigeminal innervation of the eye is to mediate protective reflexes and preserve retinal function. The present study identified light-responsive neurons at the Vi/Vc region that displayed similar encoding properties and responses to vasoactive drugs as those reported previously for neurons at the Vc/C1 region. However, the selective role of light-responsive neurons at the Vi/Vc, but not Vc/C1 region, in mediating reflex tear volume strongly supported the hypothesis that neurons in each region serve different aspects of ocular function. The eye is arguably our most complex sense organ. The available evidence suggests that specialization of the central organization of the trigeminal system has developed to support this complexity.

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Abbreviations

aCSF	artificial cerebral spinal fluid
AUC	area under curve
CO	cornea only
ivt	intravitreal
L-NAME	L-N^G-nitro-arginine methyl ester
MAP	mean arterial pressure
NO	nitric oxide
NS	nociceptive-specific
RF	receptive field
Rmag	response magnitude
SA	spontaneous activity
TRG	trigeminal root ganglion
Vc	trigeminal subnucleus caudalis
Vc/C1	trigeminal subnucleus caudalis and upper cervical spinal cord junction
Vi	trigeminal subnucleus interpolaris
Vi/Vc	trigeminal subnucleus interpolaris and caudalis transition
WDR	wide dynamic range

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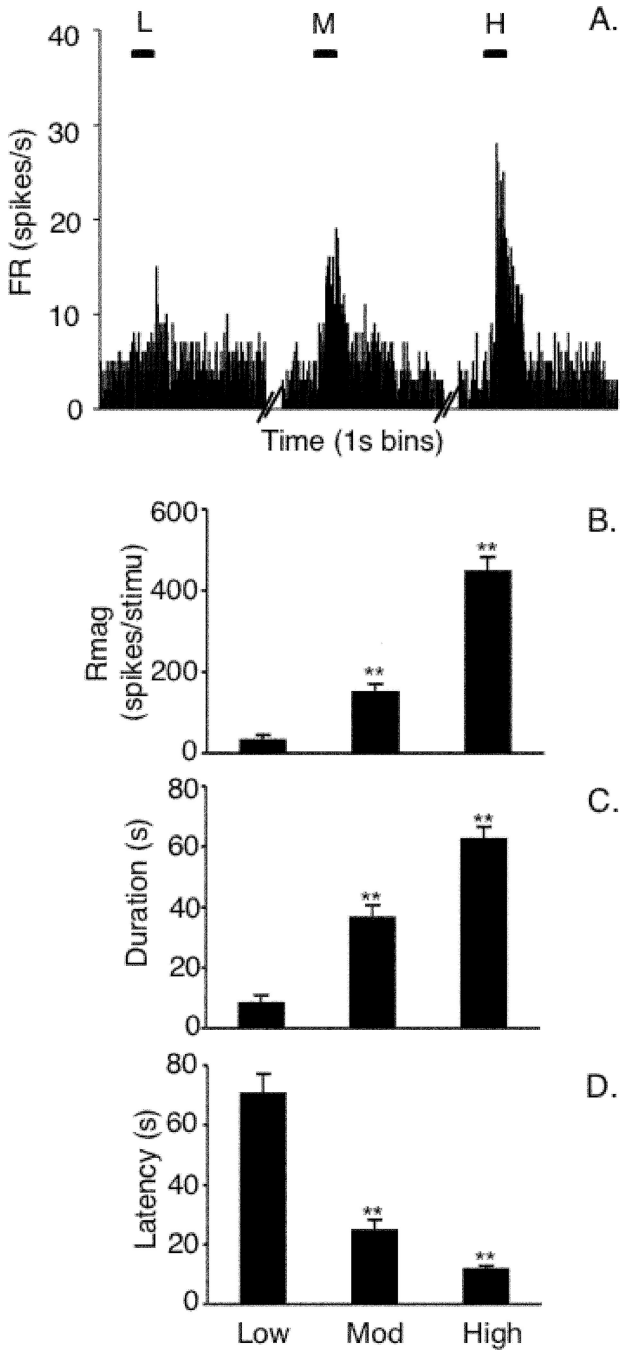

Figure 1.

Trigeminal neurons at the ventrolateral Vi/Vc region encode luminosity. Light stimuli were presented for 30 s duration at 20 min interval at low (L), moderate (M) and high (H) intensity (0.5×10^4, 1×10^4, 2×10^4 lux, respectively). Light-evoked peristimulus time histograms for neurons recorded at the Vi/Vc region (**A**). Mean values of response magnitude (Rmag, **B**), duration (**C**) and latency (**D**) to bright light stimulation. **P< 0.01 versus response to low intensity light (L).

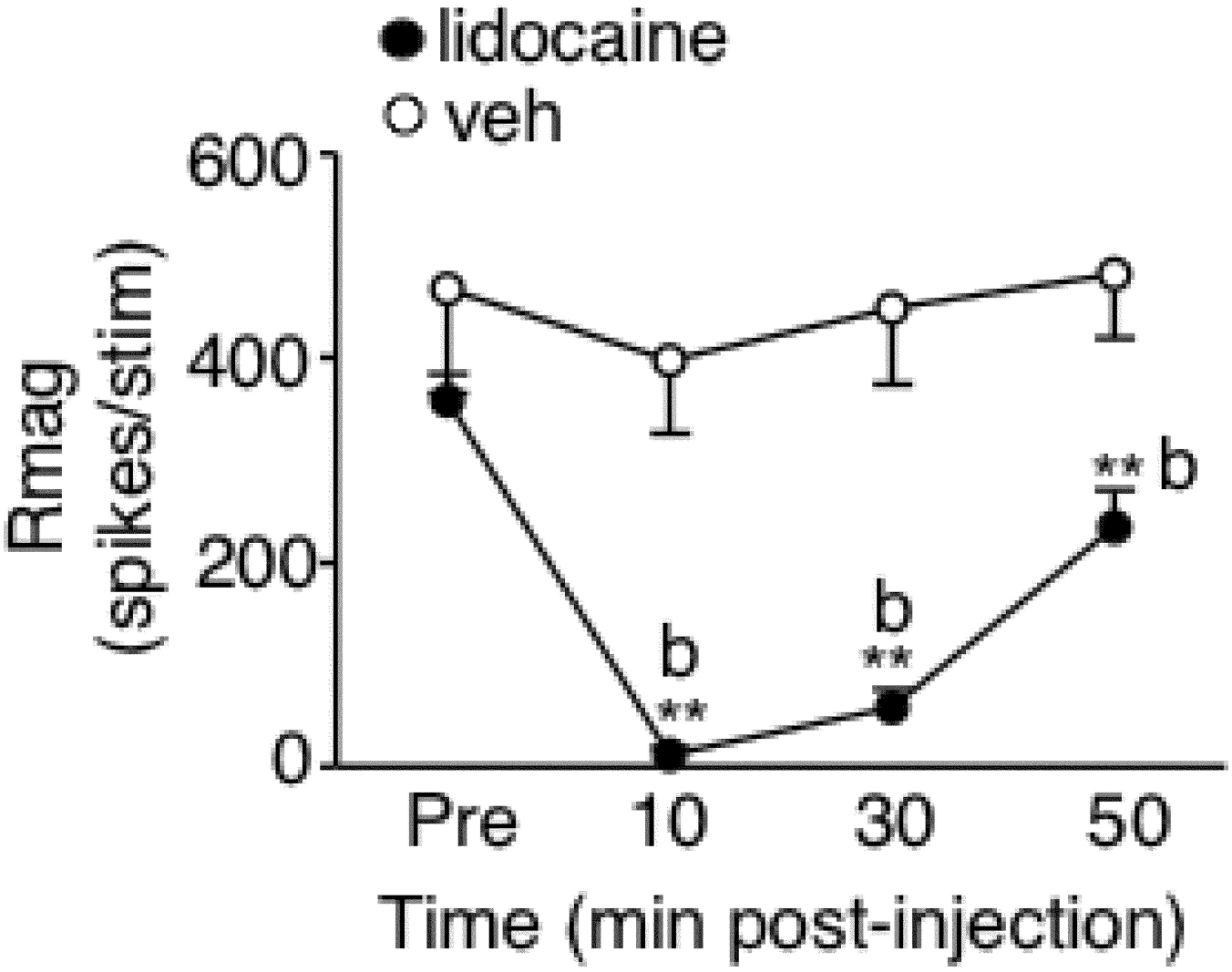

Figure 2.
Light-evoked Vi/Vc neural activity requires input through the trigeminal root ganglion (TRG). Intra-TRG injection of lidocaine (2%, 1μl) blocks completely the responses to high intensity light. ** P < 0.01 versus pre-lidocaine value. b = P < 0.01 vs. vehicle (veh) group.

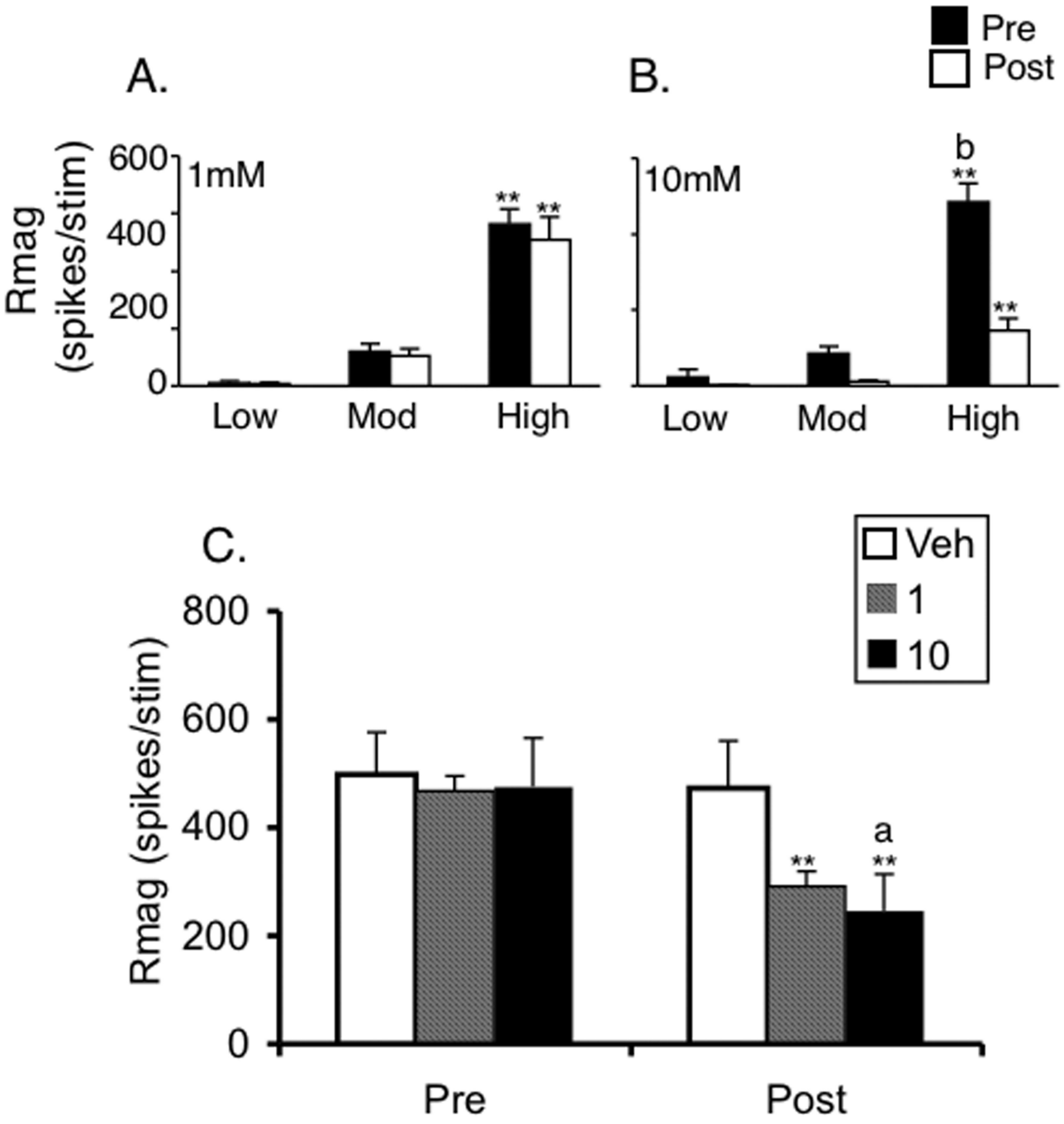

Figure 3.

Intraocular adrenergic and nitric oxide (NO) mechanisms contribute to light-evoked Vi/Vc neural activity. Ivt injection of the alpha adrenergic agonist, 1 mM (**A**) and 10 mM (**B**) phenylephrine (1μl) or the NO synthase inhibitor, L-NAME (1, 10 mM, 1μl, **C**), inhibits light-evoked responses of Vi/Vc units. **P < 0.01 versus response to low intensity light (**A, B**), and versus pre-drug value (**C**). a = P < 0.05 vs vehicle (Veh) group. b = P < 0.01 vs. post-drug value.

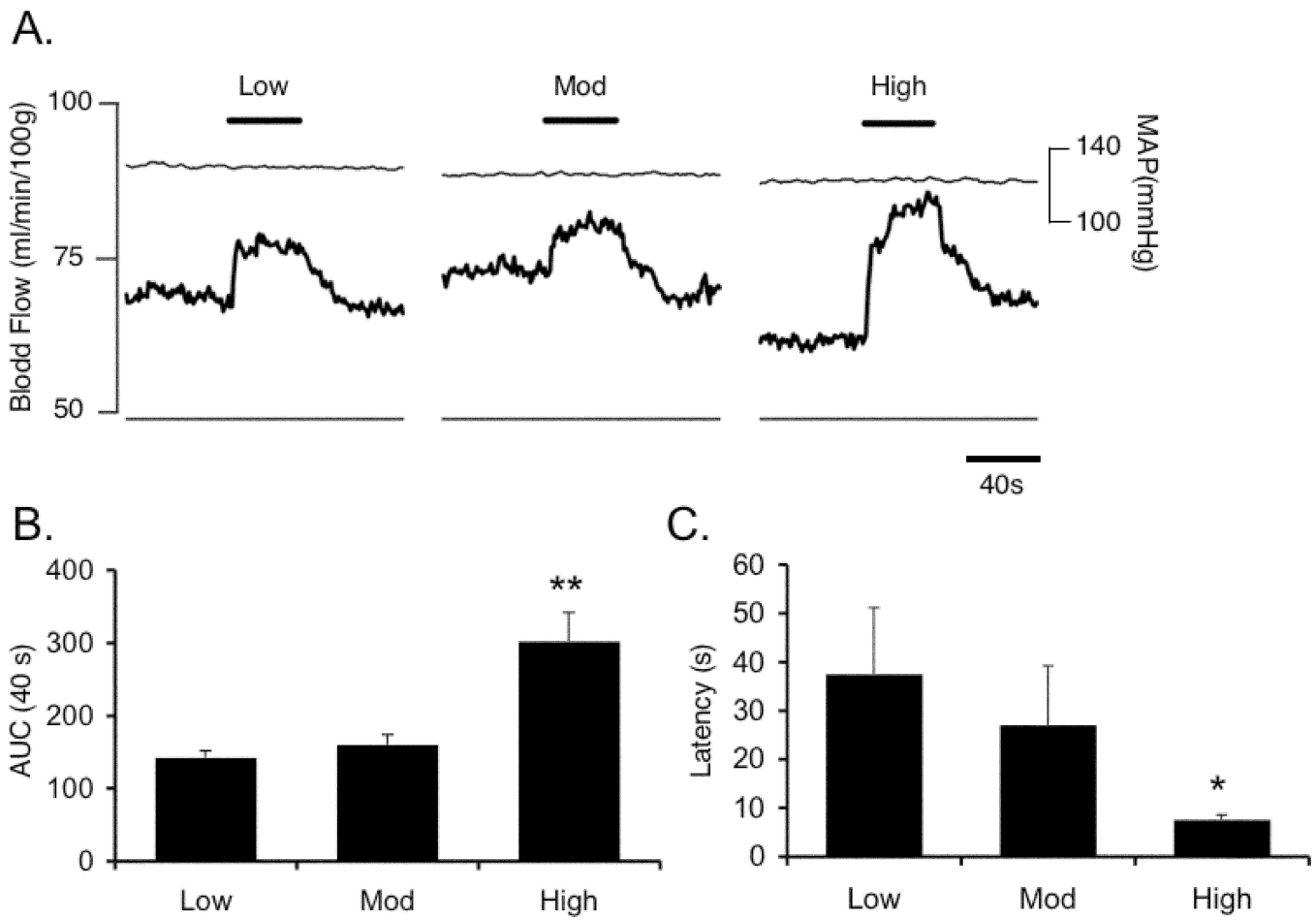

Figure 4.

Laser Doppler flowmetry (LDF) reveals light-evoked increases in ocular blood flow. **A**. Example recordings of LDF signal increases after acute exposure to light (0.5, 1.0, 2.0 × 10^4 lux, 40 s) and on mean arterial blood pressure (MAP). **B**. Summary of ocular blood flow responses to progressive increases in light intensity. **C**. Summary of latency to observe a >10% change in ocular blood flow to progressive increases in light intensity. Data are normalized to percentage of basal ocular flow sampled immediately prior to stimulation. *P < 0.05, ** P < 0.01 versus low intensity light stimulation.

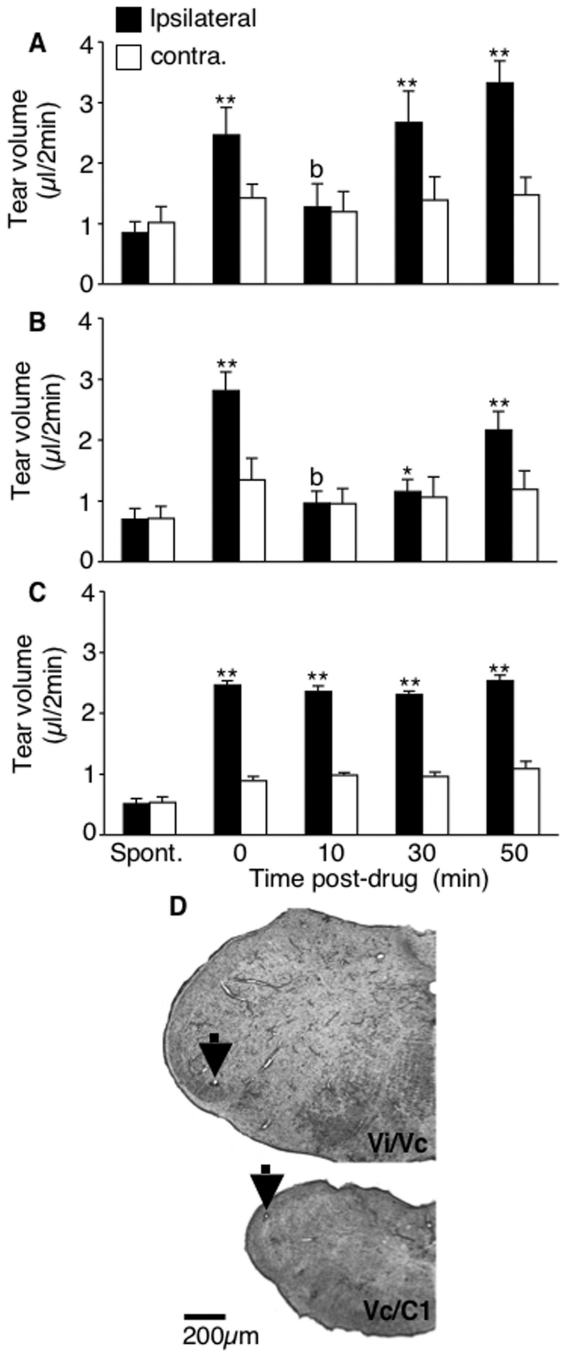

Figure 5.

The trigeminal root ganglion (TRG) and Vi/Vc regions, but not the Vc/C1 region, are critical for light-evoked tear production. Tear volume produced ipsilateral to the local injection of lidocaine (2%, 1μl) into the TRG (**A**) or $CoCl_2$ (100 mM, 100 nl) into the Vi/Vc (**B**), but not Vc/C1 (**C**) region reduced light-evoked tear formation. Light stimulus = 2.0×10^4 lux, 30 sec. *P < 0.05, **P < 0.01 versus spontaneous tears. b = P < 0.01 versus 0 min. **D.** Examples of drug injection sites (arrow) at the Vi/Vc (upper) and Vc/C1 (lower) regions.