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Trigeminal pathways for hypertonic saline and light-evoked corneal reflexes

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Abstract

Cornea-evoked eyeblinks maintain tear film integrity on the ocular surface in response to dryness and protect the eye from real or potential damage. Eyelid movement following electrical stimulation has been well studied in humans and animals; however, the central neural pathways that mediate protective eyeblinks following natural nociceptive signals are less certain. The aim of this study was to assess the role of the trigeminal subnucleus interpolaris/caudalis (Vi/Vc) transition and subnucleus caudalis/upper cervical cord (Vc/C1) junction regions on orbicularis oculi electromyographic (OOemg) activity evoked by ocular surface application of hypertonic saline or exposure to bright light in urethane anesthetized male rats. The Vi/Vc and Vc/C1 regions are the main sites of termination for trigeminal afferent nerves that supply the ocular surface, while hypertonic saline (saline = 0.15-5M) and bright light (light = 5-20k lux) selectively activate ocular surface and intraocular trigeminal nerves, respectively, and excite second-order neurons at the Vi/Vc and Vc/C1 regions. Integrated OOemg activity, ipsilateral to the applied stimulus, increased with greater stimulus intensities for both modalities. Lidocaine applied to the ocular surface inhibited OOemg responses to hypertonic saline, but did not alter the response to light. Lidocaine injected into the trigeminal ganglion blocked completely the OOemg responses to hypertonic saline and light indicating a trigeminal afferent origin. Synaptic blockade by cobalt chloride of the Vi/Vc or Vc/C1 region greatly reduced OOemg responses to hypertonic saline and bright light. These data indicate that OOemg activity evoked by natural stimuli known to cause irritation or discomfort in humans depends on a relay in both the Vi/Vc transition and Vc/C1 junction regions.

Keywords

corneal reflex; electromyography; orbicularis oculi; ocular pain; synaptic blocked; trigeminal brainstem

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Corneal reflexes are involuntary eyelid closures that can be evoked by mechanical or electrical stimulation of the ocular surface or by light flashes that serve mainly a protective function (Ongerboer de Visser, 1980, Mukuno et al., 1983, Cruccu et al., 1986). By contrast, eyeblink reflexes are critical for maintaining tear film integrity and can occur spontaneously, be evoked by diverse inputs of trigeminal or spinal origin as well as by conditioning stimuli (Evinger et al., 1991, Gruart et al., 1995, Delgado-Garcia et al., 2003, Dauvergne and Evinger, 2007, Kaminer et al., 2011). Although corneal reflexes and eyeblinks share several features and each results in excitation of orbicularis oculi (OO) motor units and lid closure, several lines of evidence suggest that the brain circuitry for corneal and blink reflexes are organized differently (Ongerboer de Visser, 1983, Berardelli et al., 1985, Cruccu et al., 1991).

Animal studies of brain pathways for cornea-evoked eyelid closure have relied mainly on results from electrical stimulation of the ocular surface (Henriquez and Evinger, 2005, 2007). While this approach allows for detailed analysis of the timing and pattern of orbicularis oculi electromyographic (OOemg) activity, electrical stimuli necessarily by-pass normal sensory transduction mechanisms. Tear osmolarity is a key factor in predicting severity in dry eye disease (Sullivan et al., 2010, Alex et al., 2013), while abnormal light sensitivity is a common symptom in dry eye (Pflugfelder, 2011) and blepharospasm (Adams et al., 2006, Hallett et al., 2008), conditions well associated with abnormal control of eyeblinks. Trigeminal sensory nerves that supply the eye and periocular tissues project centrally to terminate in two spatially discrete regions, the trigeminal subnucleus interpolaris/caudalis transition (Vi/Vc) and the trigeminal subnucleus caudalis/upper cervical cord junction (Vc/C1) regions (Marfurt, 1981, Marfurt and Del Toro, 1987, Marfurt and Echtenkamp, 1988, Panneton et al., 2010). Previously we reported that ocular neurons at the Vi/Vc and Vc/C1 regions encoded the concentration of hypertonic saline (Tashiro et al., 2010) and light intensity (Okamoto et al., 2010, Okamoto et al., 2012), whereas others have used electrical stimulation of the ocular surface and supraorbital nerve to assess the role of the Vi/Vc and Vc/C1 regions on corneal and blink reflexes, respectively (Pellegrini et al., 1995, Henriquez and Evinger, 2005, 2007). To better understand the organization of trigeminal pathways that mediate corneal reflexes evoked by physiological stimuli, OOemg activity was recorded in response to hypertonic saline or bright light before and after selective blockade of trigeminal sensory nerves or second-order trigeminal brainstem neurons at the Vi/Vc transition and Vc/C1 regions.

Experimental procedures

The animal protocol was approved by the Institutional Animal Care and Use committee of the University of Minnesota and conformed to the established guidelines set by The National Institute of Health guide for the care and 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.

Animal preparation

Adult male rats (240-270 g, $n = 72$, Harlan Sprague-Dawley, Indianapolis, IN) were anesthetized with urethane (1.2-1.5 g/kg, i.p.) and wound margins were infiltrated with 2% lidocaine. The left femoral artery was catheterized to monitor arterial blood pressure that was maintained at 90-110 mmHg. Body temperature was kept at 38°C with a heating blanket. The rat was positioned in a stereotaxic frame and in those experiments that involved microinjections into the Vi/Vc transition or Vc/C1 junction region, a small portion of the C1 vertebra was removed to expose the dorsal brainstem surface. A pair of Teflon-coated copper wires (0.12 mm diameter, 5 mm interpolar distance) was implanted by 26-gauge needle in parallel with muscle fiber at the lateral margin of the left orbicularis oculi (OO) muscle for electromyography (OOemg).

Experimental protocols

Hypertonic saline (0.15, 2.5 and 5 M NaCl, pH: 7.2, 20 μ l) was applied topically to the ocular surface from a microsyringe (Tashiro et al., 2010). Stimuli were applied in a cumulative dose design at 20 min intervals. Saline solutions remained on the eye during the 3 min sampling period and then washed out with artificial tears after each stimulus (total exposure time to NaCl = 3-4 min) to prevent desensitization or possible damage to the ocular surface.

Light (low: 0.5×10^4 lux, moderate: 1×10^4 lux, high: 2×10^4 lux) stimuli were applied from a thermal-neutral fiber optic halogen source (150W, Cole Parmer, Vernon Hills, IL) and the intensities were measured at the ocular surface with a lux meter (Okamoto et al., 2010). Light stimulation was applied for 30 s or 60 s in a cumulative intensity design delivered at 20 min intervals under low ambient light (< 100 lux).

In the initial series of experiments, OOemg was recorded in separate animals across a range of saline concentrations ($n = 6$), light intensities of 30 s ($n = 7$) or 60 s durations ($n = 5$). In this series and for protocols described below, stimuli were presented at 20 min intervals and OOemg was recorded continuously beginning 3 min before and until 3 min after the onset of each stimulus.

Two designs were used to examine the influence of trigeminal afferent nerves on hypertonic saline- or light-evoked OOemg activity. First, to address the issue of ocular surface nerves and evoked OOemg activity, hypertonic saline (2.5 M, $n = 4$) or light (20k lux, 60 s pulse, $n = 4$) was presented, OOemg activity was recorded and then lidocaine (2%, 20 μ l) was instilled onto the ocular surface. Hypertonic saline and light stimulation was repeated 10 min and 30 min after lidocaine. In a second series, hypertonic saline (2.5M, $n = 4$) or light (20k lux, 60 s pulse, $n = 4$) was presented, OOemg activity was recorded and then lidocaine (2%, 0.3 μ l) was injected via a 33-gauge needle inserted through a 26-gauge guide cannula positioned stereotaxically (3.1-3.3 caudal, 2.8-3.1 mm lateral to bregma, 9-10 mm ventral to cortical surface) over the left trigeminal ganglion (TG) (Okamoto et al., 2010). Hypertonic saline or light stimulation was repeated 10 and 30 min after lidocaine microinjection. This series assessed the more general role of trigeminal sensory neurons that include intraocular

afferents as well as afferent fibers that supply the ocular surface. The microinjection sites of drugs into TG were confirmed by microscopy (Fig 3C).

To assess the role of the Vi/Vc transition and Vc/C1 junction regions on hypertonic saline (2.5 M) or light (20k lux, 60 s pulse) synaptic activity was blocked by pressure microinjection of CoCl_2 (100 mM, 0.3 μl) (Hirata et al., 2003). A glass micropipette (40–80 μm tip diameter) filled with CoCl_2 , or vehicle (phosphate buffered saline, PBS) was directed at either the Vi/Vc transition (angle of 28° off vertical and 45° off midline, and 1.5–2.0 mm below the brainstem surface) or the Vc/C1 region (43° off vertical, 60° off midline, within 300 μm of the dorsal brainstem surface). Hypertonic saline (2.5 M) was applied and OOemg recorded. Next, either CoCl_2 ($n = 5$) or vehicle ($n = 4$) was injected into the Vi/Vc transition or Vc/C1 region (CoCl_2 , $n = 5$; vehicle, $n = 4$) followed by 2.5 M NaCl stimulus at 10, 30 and 50 min after injection. Similarly for light-evoked OOemg (20k lux) activity, after the initial stimulus period, microinjections were made into the Vi/Vc transition (CoCl_2 , $n = 5$; vehicle, $n = 4$) or the Vc/C1 region (CoCl_2 , $n = 5$; vehicle, $n = 6$) followed by three successive stimulus periods at 10, 30 and 50 min after microinjection. The microinjection sites of drugs were confirmed by microscopy (Fig 4C).

Data recording and analysis

OOemg activity was sampled at 1000 Hz, amplified ($\times 10\text{k}$), filtered (bandwidth 300–3000 Hz), displayed and stored online for later analyses (AD Instruments). OOemg activity was sampled continuously for 6 min beginning 3 min before until 3 min after each stimulus onset. OOemg activity was rectified and stored as 1 s bins for off-line analyses. Baseline activity was defined as an integrated area under the curve (AUC) for the 3 min epoch (μV per 3 min) sampled immediately prior to each stimulus. Ocular-evoked OOemg activity was calculated as AUC post-stimulus minus baseline AUC. The response latency (onset) was defined as the first time point at which OOemg exceeded the average baseline AUC.

The AUC and latency of OOemg and resting mean arterial pressure (MAP) were assessed by analysis of variance (ANOVA) corrected for repeated measures. Significant treatment effects were assessed by Newman-Keuls after ANOVA. The data were presented as mean $\pm$ SEM and the significant level set at $p < 0.05$.

Histology

For experiments that involved microinjections into the TG, Vi/Vc transition or Vc/C1 region, cresyl violet was delivered to confirm the site of injection. Animals were deeply anesthetized and perfused through the heart with the 0.9% saline and 4% paraformaldehyde, the brain was removed and sectioned at 30 μm .

Results

Stimulus intensity and OOemg activity

Normal and hypertonic saline applied to the ocular surface caused increases in OOemg activity (Fig 1A). The magnitude of the AUC increased with greater NaCl concentrations (Fig 1B, $F_{2,10} = 24.9$, $p < 0.001$), while the response latency was reduced significantly (Fig

1C, $F_{2,10} = 23.8$, $p < 0.001$). Although the timing for NaCl-evoked OOemg activity was much delayed compared to that seen after electrical stimuli (Henriquez and Evinger, 2005), the pattern of the response to hypertonic saline (i.e., 2.5 and 5 M) always was accompanied by a large early increase in activity that was absent after application of normal saline (Fig 1A). Eyelid movement was seen following application of 2.5 and 5 M solutions in all cases; however, we did not quantify these responses. Hypertonic saline did not affect the resting MAP (0.15 M = 104.2 ± 5.0 ; 2.5 M = 107.7 ± 4.0 ; 5 M = 106.3 ± 3.2 mmHg, $p < 0.1$).

Bright light increased OOemg activity after a long delay (>10 s) and was related to light intensity (Fig 2A). As seen in Fig 2B, increasing stimulus duration (30 s versus 60 s) as well as stimulus intensity significantly increased the light-evoked AUC ($F_{2,20} = 18.2$, $p < 0.001$). Similarly, response latency decreased with greater light intensity and duration (Fig 2C, $F_{2,20} = 11.1$, $p < 0.001$). Eyelid movement was seen infrequently to high intensity light stimulation, but these responses were not quantified. Bright light stimulation did not affect MAP (not shown).

Blockade of trigeminal sensory neurons and OOemg activity

Lidocaine (2%) applied to the ocular surface or microinjected into the TG caused a marked reduction in the AUC evoked by 2.5 M NaCl at 10 min post-drug with at least partial recovery by 30 min (Fig 3A, $F_{2,12} = 27.3$, $p < 0.001$). The latency of the evoked OOemg increased significantly ($p < 0.001$) from 3.0 ± 0.71 s before to 106.5 ± 43.3 s at 10 min after lidocaine applied to the ocular surface and from 4.5 ± 2.5 s before to 137.5 ± 42.5 s after lidocaine injection into the TG ($F_{2,12} = 40.1$, $p < 0.001$).

Lidocaine applied to the ocular surface did not affect OOemg activity evoked by high intensity light (20k lux, 60 s duration) (Fig 3B, $F_{2,12} = 2.3$, $p < 0.1$). By contrast, lidocaine microinjection into the TG completely prevented light-evoked OOemg activity at 10 min post-drug (Fig 3B, $F_{2,12} = 23.2$, $p < 0.001$) that recovered by 30 min. Similarly, lidocaine applied to the ocular surface did not affect the light-evoked response latency (before = 16.3 ± 2.4 s, +10 min = 16.0 ± 1.4 s, and +30 min = 16.8 ± 2.0 s, $F_{2,12} = 0.7$, $p > 0.1$), whereas lidocaine injection into TG caused a marked increase in latency (before = 30.3 ± 2.3 s; +10 min = 137.5 ± 36.1 s; +30 min = 20.0 ± 0.8 s, $F_{2,12} = 23.0$, $p < 0.001$). Resting MAP was not affected by lidocaine applied to the ocular surface or after microinjection into TG (data not shown).

Synaptic blockade of Vi/Vc or Vc/C1 region and saline-evoked OOemg activity

Microinjection of the nonselective synaptic blocking agent, CoCl_2 (100 mM, 0.3 μl), into the Vi/Vc transition region significantly reduced the OOemg response to hypertonic saline (2.5M) at 10 min with a gradual recovery by 50 min compared to vehicle injection (Fig 4A, $F_{3,21} = 6.8$, $p < 0.005$). Similarly, CoCl_2 injection into the Vc/C1 region also significantly reduced the NaCl-evoked OOemg response compared to vehicle injections (Fig 4B, $F_{3,21} = 4.8$, $p < 0.01$). Response latencies to NaCl stimulation were variable after CoCl_2 injection into the Vi/Vc transition or the Vc/C1 region and were not statistically significantly different from pre-drug values (data not shown).

Synaptic blockade of Vi/Vc or Vc/C1 region and light-evoked OOemg activity

Microinjection of CoCl_2 into the Vi/Vc transition region significantly reduced the light-evoked (20k lux) - evoked OOemg response at 10 min with a gradual recovery by 50 min compared to vehicle injection (Fig 5A, $F_{3,21} = 15.2$, $p < 0.001$). Similarly, CoCl_2 injection into the Vc/C1 region also significantly reduced the light-evoked OOemg response compared to vehicle injections (Fig 5B, $F_{3,27} = 5.9$, $p < 0.005$). Response latency to light increased significantly after CoCl_2 injection into the Vi/Vc transition ($F_{3,21} = 4.3$, $p < 0.025$) or into the Vc/C1 region ($F_{3,27} = 6.6$, $p < 0.005$) compared to vehicle injected controls. The light-evoked response latency increased significantly by 10 min after CoCl_2 microinjection into Vi/Vc transition with recovery by 50 min (before = 20.8 ± 3.2 s, +10 min = 96.2 ± 34.5 s, +30 min = 24.2 ± 4.8 s, +50 min after CoCl_2 = 24.4 ± 5.2 s, ($F_{3,21} = 7.4$, $p < 0.01$). Microinjection of CoCl_2 into Vc/C1 significantly increased latency by 10 and 30 min with recovery by 50 min (before = 22.0 ± 5.6 s, +10 min = 122.0 ± 35.7 s, +30 min = 62.8 ± 30.6 s, +50 min after CoCl_2 ; 22.4 ± 4.8 s; $F_{3,27} = 12.5$, $p < 0.001$). Resting MAP was not affected by CoCl_2 injection either into Vi/Vc transition or Vc/C1 region (data not shown).

Discussion

The main finding in this study was that OOemg activity evoked by ocular stimuli that cause pain and discomfort in humans required a relay through both the Vi/Vc transition and the Vc/C1 junction regions. This held true for two very different types of ocular stimuli, hypertonic saline and bright light. In the case of hypertonic saline, blockade of ocular surface nerve endings by topical application of lidocaine or by intra-ganglionic injection in the TG prevented the evoked OOemg response. By contrast, topical lidocaine had no effect on light-evoked OOemg activity, whereas injection into the TG completely prevented the evoked response. These results suggest that nociceptive signals originating from ocular surface nerve endings as well as from nerve branches that supply deeper structures of the eye can evoke OOemg activity and that both sources of input require integration at the Vi/Vc transition and Vc/C1 junction regions to cause eyelid closure.

Axonal tracing studies indicate that trigeminal nerves that supply the ocular surface terminate in two spatially discrete brainstem regions: the ventral Vi/Vc transition and Vc/C1 junction (Marfurt, 1981, Marfurt and Del Toro, 1987, Marfurt and Echtenkamp, 1988, Panneton et al., 2010) in agreement with the distribution of Fos protein seen after ocular surface stimulation (Lu et al., 1993, Strassman and Vos, 1993, Meng and Bereiter, 1996). Intraocular chemical stimuli (Chang et al., 2010) and bright light (Okamoto et al., 2009) produce a similar pattern of Fos-positive neurons suggesting that ocular surface and intraocular nerves project to similar trigeminal brain regions. However, several lines of evidence further suggest that ocular-responsive neurons at the Vi/Vc transition and Vc/C1 regions serve different aspects of ocular function. First, neural recording studies revealed that ocular surface-responsive neurons at the Vi/Vc transition and Vc/C1 regions displayed different encoding properties (Meng et al., 1997, Hirata et al., 1999) and responsiveness to opioid analgesics (Meng et al., 1998, Hirata et al., 2000) and different efferent projection targets. Second, inhibition of the Vi/Vc transition prevented reflex lacrimation to CO_2 pulses applied to the ocular surface, while blockade of the Vc/C1 region had no effect (Hirata et al.,

2004). Third, GABAergic inhibition of the Vi/Vc transition prevented the R1, but not the R2 component of the blink reflex evoked by supraorbital nerve stimulation, while blockade of the Vc/C1 region had the reverse effect (Pellegrini et al., 1995). Thus, it was somewhat unexpected to find that synaptic blockade of either the Vi/Vc or Vc/C1 region greatly reduced OOemg activity evoked by hypertonic saline or bright light.

Several possible explanations may account for the apparent shared contribution by the Vi/Vc transition and Vc/C1 region to ocular nociceptive-evoked OOemg activity. Stimulus intensity and/or modality may recruit different populations of neurons from each region to engage blink circuitry. Although sensations evoked by corneal stimulation are always perceived with an irritating component, increasing stimulus intensity produces further increases in visual analog scores (VAS) independent of stimulus modality (Acosta et al., 2001). Similarly, increasing the concentration of CO₂ applied to the ocular surface evoked increasing tear production (Hirata et al., 2004). Although electrical stimulation-evoked corneal reflexes increase with greater stimulus intensities (Accornero et al., 1980, Berardelli et al., 1985), the relationship between stimulus intensity and corneal reflex magnitude is not well defined. Recently, Wu et al. (2014) reported increased blink rate with increasing air flow rates applied to the ocular surface; however, the highest flow rate used evoked only minimal discomfort (VAS = 1-3 out of 10). Although the present study was performed under anesthesia, the osmotic concentrations of the saline stimuli used here were far above the discomfort threshold in humans (Liu et al., 2009) and would be expected to be painful in awake subjects. Since the threshold to activate ocular neurons at the Vi/Vc transition and Vc/C1 region by electrical (Meng et al., 1997) or chemical stimulation of the ocular surface (Hirata et al., 1999) were similar and since each region projects to the facial motor nucleus (Pellegrini et al., 1995, Morcuende et al., 2002), the relative contribution each region in mediating OOemg responses to ocular stimulation may depend on the strength of the applied stimulus.

Bright light evoked OOemg responses, though at much smaller magnitudes than those seen after hypertonic saline. The intensity of light used here (5k-20k lux) was within the range of intensities tolerated by normal awake subjects (Kowacs et al., 2001). Previously, we reported that ocular units at the Vi/Vc transition (Okamoto et al., 2012) and Vc/C1 region (Okamoto et al., 2010) encoded the bright light stimulus over the same range of intensities. The present results indicated that the magnitude of the OOemg response was enhanced with increasing light duration as well as intensity, whereas the response latency (>10 s) was decreased. Long latency OOemg responses to light also were reported by others in anesthetized rats (Dolgonos et al., 2011). Such long response latencies were consistent with the notion that light-evoked neural activity and corneal reflexes involve a neurovascular coupling within the eye and were independent of ocular surface nerve input (Okamoto et al., 2010). As we have proposed, the circuit for light-evoked trigeminal nerve activity involves light transduction by normal photoreceptors that activate accessory visual pathways to increase autonomic outflow to the eye. It is this increased outflow, possibly by increased blood flow, that indirectly activates trigeminal nerves and gains access to pain pathways (Okamoto et al., 2010, Okamoto et al., 2012). Interestingly, air puff-evoked OOemg responses were enhanced during periods of exposure to bright light (Dolgonos et al., 2011)

providing further support for the hypothesis that ocular surface-and light-evoked corneal reflexes involve overlapping trigeminal circuitry.

The relationship between the Vi/Vc transition and Vc/C1 junction regions and corneal reflexes may be complex. A characteristic feature of the trigeminal brainstem complex is the somatotopic representation of craniofacial structures at multiple rostrocaudal levels and an extensive system of longitudinal projecting fibers that connect these maps (Bereiter et al., 2009). Blockade of either the Vi/Vc transition or Vc/C1 region by microinjection of GABA_A receptor agonists markedly altered the encoding properties of ocular neurons (Hirata et al., 2003). Similarly, microstimulation of the Vc/C1 region significantly modified the OOemg response to electrical stimulation of the cornea (Henriquez and Evinger, 2005, 2007). Earlier it was reported that microinjection of the GABA_B receptor agonist, baclofen, into the Vi/Vc transition region prevented the R1 component of the electrically-evoked blink reflex, while injection into the Vc/C1 region prevented the R2 reflex in the guinea pig (Pellegrini et al., 1995). This result differs from the current study in which hypertonic-evoked OOemg activity was greatly reduced by synaptic blockade of either region. The reason for this difference is not clear but may be due the different circuitry underlying supraorbital nerve-evoked blinks and corneal reflexes. It also could be due to different stimulus intensities and modalities. It is difficult to make direct comparisons between results in anesthetized animals and the pathways that underlie blink and corneal reflexes in humans. However, patients with Wallenberg's syndrome, and lateral medullary infarctions, often display marked changes in eye blink reflexes (Vila et al., 1997). In a small study of patients followed over several months, initial testing revealed absent or delayed blink reflexes in most patients, however, when tested after several weeks blink reflex activity returned to normal, whereas imaging of the infarct area revealed no changes (Vila et al., 1997). Recovery of function despite similar signs of brain damage suggests that multiple pathways are involved in trigeminal-evoked eye muscle reflexes. The role of different trigeminal brainstem subnuclei in mediating blink and corneal reflexes in humans is not as well defined, although it may be distributed across multiple brainstem regions as seen in experimental preparations.

Conclusions

Eyeblinks and corneal reflexes have been widely used as diagnostic tools to assess neurological conditions (Ongerboer de Visser, 1980, Agostino et al., 1987, Basso and Evinger, 1996, Cruccu et al., 1997, Kofler and Halder, 2014). The present study suggests that protocols using natural physiological stimuli can be adapted for use in anesthetized animals to provide new information on trigeminal pain circuitry.

Acknowledgments

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Abbreviations

Vi/Vc	trigeminal subnucleus interpolaris/caudalis transition
Vc/C1	trigeminal subnucleus caudalis and upper cervical spinal cord junction
OOemg	orbicularis oculi electromyography
TG	trigeminal ganglion
AUC	area under the curve
MAP	mean arterial pressure
VAS	visual analog scores

Highlights

- Corneal reflex evoked by natural physiological stimuli (hypertonic saline and bright light).
- Hypertonic saline and bright light selectively activate ocular surface and intraocular trigeminal nerves, respectively.
- Corneal reflex evoked by physiological stimuli depends on the relay in both Vi/Vc transition and Vc/C1 junction regions.

Figure 1.

Measurement of OOemg activity in response to hypertonic saline solutions applied to the ocular surface. A. Examples of OOemg activity after 0.15, 2.5 and 5 M NaCl. Arrow indicates stimulus onset. NaCl solutions were flushed from the surface by artificial tears after each presentation. B. Magnitude of the OOemg response, defined as the integrated area over a 3 min sampling period minus background activity, to hypertonic saline. C. Response latency to hypertonic saline. $n = 6$; ** $p < 0.01$ versus response to 0.15 M NaCl.

Figure 2.

Measurement of the OOemg response to bright light. A. Examples of OOemg activity after high intensity light (20k lux) applied for 30 s or 60 s. B. Magnitude of the OOemg response, defined as the integrated area over a 3 min sampling period minus background activity, to light stimulation at low (5k lux), moderate (10k lux) and high intensity (20k lux). C. Response latency to light. 30 s duration, n = 7; 60 s duration, n = 5; *p<0.05, **p<0.01 versus response to low intensity light (5k lux); a = p<0.05, b = p<0.01 versus 30s.

A. Response to NaCl

C.

B. Response to light

Figure 3.

Effect of lidocaine applied to the ocular surface (OS) or microinjected into the trigeminal ganglion (TG) on evoked OOemg activity. A. Lidocaine blockade of OS or within TG greatly reduced the OOemg response to 2.5 M NaCl. Note that evoked OOemg activity returned to pre-drug levels by 30 min after lidocaine. B. Lidocaine blockade within TG prevented the OOemg response to light (20k lux, 60s duration), while lidocaine applied to the OS had no effect. C. Sites of drug injections into TG. $n = 4$ per treatment group; ** $p < 0.01$ versus pre-drug response; $b = p < 0.01$ versus response after OS lidocaine.

A. Vi/Vc transition region

C.

B. Vc/C1 region

Figure 4.

Effect of synaptic blockade at the Vi/Vc transition or the Vc/C1 region on the OOemg response to 2.5 M NaCl applied to the ocular surface. A. Microinjection of CoCl₂ (100 mM, 0.3 μl) into the Vi/Vc transition reduced the response to 2.5 M NaCl. B. Microinjection of CoCl₂ (100 mM, 0.3 μl) into the Vc/C1 region reduced the response to 2.5 M NaCl. C. Sites of drug injections into the Vi/Vc transition (top) and Vc/C1 region (bottom). n = 4 per treatment group; *p<0.05, **p<0.01 versus pre-drug response; a = p<0.05, b = p<0.01 versus response after vehicle injections.

A. Vi/Vc transition region

B. Vc/C1 region

Figure 5.

Effect of synaptic blockade at the Vi/Vc transition or Vc/C1 region on the OOemg response to bright light (20k lux, 60 s duration). A. Microinjection of CoCl₂ (100 mM, 0.3 μl) into the Vi/Vc transition prevented the response to light. B. Microinjection of CoCl₂ (100 mM, 0.3 μl) into the Vc/C1 region prevented the response to light. n = 4 per treatment group; **p<0.01 versus pre-drug response; a = p<0.05, b = p<0.01 versus response after vehicle injections.