Evidence for Facial Nerve–Independent Mechanisms of Blinking in the Rat

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Purpose. The rat facial nerve (CN VII) controls the orbicularis oculi (OO) muscle, which contracts to close the palpebral fissure during blinking. It was recently observed that rats are able to achieve nearly complete eye closure shortly after CN VII lesion, and hypothesized that the retractor bulbi (RB) muscle assumes an important compensatory role after CN VII lesion. This study was undertaken to determine the maintenance of rat corneal health and eye closure capability after lesion of the OO, RB, or both.

Methods. Twenty-two rats underwent RB transection; 12 of them had undergone complete unilateral CN VII transection (OO denervation) 15 weeks earlier. Corneal appearance and ability to blink in response to a corneal air puff was monitored weekly for 9 weeks. An additional 13 rats received CN VII transection and were video recorded (1000 frames/s) during elicited blinks at days 1, 3, 5/6, and 11 after surgery.

Results. Rats achieved nearly full or full eye closure after OO paralysis or RB myotomy, respectively. Ninety-two percent of rats maintained good corneal health after OO denervation over 9 weeks, consistent with compensatory eyelid movement served by the RB muscles. In contrast, only 40% of rats with loss of RB function alone and only 17% of rats with concurrent OO and RB paralysis were able to maintain corneal health by week 3.

Conclusions. Like other small mammals, the rat RB musculature can support nearly complete eye closure when CN VII is lesioned, and must be carefully considered when using blink as a functional recovery parameter of facial nerve lesion. (*Invest Ophthalmol Vis Sci.* 2010;51:179–182) DOI:10.1167/iovs.08-3371

The rat facial nerve (CN VII) controls the orbicularis oculi (OO) muscle, which is responsible for rapid eye closure during blinking. Division of the nerve results in an initial loss of complete eye closure during blinking, both spontaneously and in response to corneal stimulation.[1] We recently observed that rats regain their ability to close their eyes nearly completely within the first week of CN VII lesion,[1] well before regenerated axons would be expected to arrive to repopulate the neuromuscular junctions of the OO.[2] This observation, coupled with our prior findings that rats are able to maintain their corneal health throughout periods of facial paralysis,[3] indicates that corneal protection can be achieved in the absence of CN VII function. Although retractor bulbi (RB) muscles have been described in the rat,[4,5] their role in eyelid closure has not yet been described. Based on rat ocular anatomy and the time course of compensatory eyelid closure after facial nerve lesion, we hypothesized that rat eyelid closure may be under dual OO and RB control, as has been shown in some other small mammals.[6–8] In the present study, we report our observations of corneal health and elicited ocular closures after OO paralysis, RB myotomy, or both, and demonstrate multiple eye closure mechanisms in the rat. Such dual input to ocular closure behavior must be carefully considered by investigators who employ rodent models of facial nerve injury and recovery.[9–12]

Methods

Thirty-eight adult female Wistar rats weighing 200 to 300 g underwent surgical manipulations, in which anesthesia was induced by intramuscular injection of ketamine HCl and medetomidine HCl (60 mg/kg and 0.5 mg/kg, respectively). First they underwent implantation of a previously described titanium head fixation device[13] for facial nerve testing and behavioral conditioning to our previously described rodent facial nerve-testing apparatus.[1] In the first group (n = 12), rats underwent complete unilateral facial nerve transection to paralyze the OO, where the proximal nerve stump was buried in the sternomastoid muscle belly to prevent axonal resprouting to facial muscle targets. This OO paralysis group underwent weekly monitoring of corneal health as well as global facial nerve testing across postoperative weeks 3 to 12. Eyelid movement was measured by determining changes in infrared (IR) light reflection from the eye surface with dual IR emitter/detector units located in front of each eye.[1,14,15] Animal experimentation adhered to protocols approved by the Massachusetts Eye and Ear Infirmary IRB and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Fifteen weeks after CN VII transection, 11 of these rats underwent a second surgical manipulation, in which resection of the ipsilateral RB muscle was performed via a superior orbitotomy in the retrobulbar region, transitioning them to an OO paralysis and RB myotomy state. Eye surgery was performed by a team of two microsurgeons, under microsurgical magnification (40×) with a double-headed microscope (Wild M651; Leitz, Rockleigh, NJ), being careful not to damage the long ciliary nerves responsible for corneal sensation. The RB was lysed with a microsurgical dissecting needle as it inserted circumferentially onto the deep surface of the globe. Rats underwent weekly testing sessions and visual inspections of corneal health for an additional 9 weeks. Testing of particular rats was stopped if the IR tracing became uninterpretable because of corneal opacification or ulceration.

After head fixation device implantation and conditioning, a second set of rats (n = 10) underwent unilateral RB resection without facial nerve lesion, followed by the same 9-week monitoring of postoperative corneal health and induced blink. As in the other lesioned rats, testing

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was stopped if the IR tracing became uninterpretable due to deterioration of corneal health.

In a third set of rats ($n = 13$), elicited blinks were video recorded with high speed equipment after complete unilateral CN VII transection on days 1, 5, or 6, and 11 after surgery. Video centered on the eye at close range was captured at 1000 frames/s (240 × 256 pixels), and a single frame representing baseline (open) and maximal eyelid closure was analyzed using ImageJ software (available at http://rsb.info.nih.gov/ij/ developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). Eyelid closure was elicited by several instances of periocular stimulation with a blunt probe, while being careful to exclude instances in which this instrument may have influenced eyelid position. Analyzed still-frame images were thresholded to identify the exposed corneal surface, and the automated ellipse tool in ImageJ was used to objectively define and measure the shape of the exposed cornea within the palpebral fissure. Complete closure was not achieved by the OO paralyzed rats, and so there was measurable corneal surface in each trial. The major-over-minor axis length was measured and averaged among rats for each day and condition to represent average exposed corneal shape during attempted closure by postsurgical day. Averages were statistically compared in six two-tailed *t*-tests (α level of $P < 0.05$).

Results

General Health

All rats maintained normal weight and cage behavior throughout the study, with the exception of a single rat receiving dual OO/RB lesion, which developed evidence of systemic infection requiring euthanatization.

Corneal Health

Rats with OO paralysis maintained good ocular health, with only 1 (8%) rat of 12 demonstrating mild corneal desiccation by week 9 (Fig. 1). This rat was excluded from further manipulation. Four (40%) of 10 rats with RB myotomy alone, maintained good corneal health by week 3, with the remaining rats demonstrating physical changes in the cornea including corneal clouding, ocular bulging, and evidence of surface desiccation of the eye. These changes, when present, rendered their eyelid movement data uninterpretable in some rats (described later), based on loss of normal corneal translucence required for the IR reflectance measurement technique. By week 9, only two rats had a normal corneal appearance. In rats with combined OO/RB lesion, 9 (82%) of the 11 with previously healthy corneas developed obvious corneal disease by week 3, with only a single rat demonstrating normal corneal appearance by the conclusion of the 9-week testing period (92% diseased cornea rate).

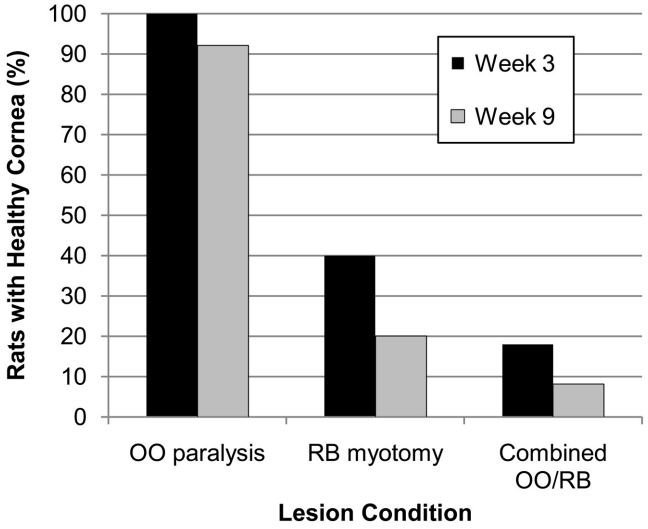

Figure 1. The percent of rats maintaining healthy cornea for their manipulated eye is shown at weeks 3 vs. 9 after OO paralysis ($n = 12$), RB myotomy ($n = 10$), or combined OO/RB lesions ($n = 11$).

Elicited Ocular Closures

Within days of surgery, rats receiving OO paralysis or RB myotomy alone were observed to move their eyelids (blink) on both the manipulated and control sides of the face in response to gentle air flow directed at the face during handling. Rats with combined OO/RB manipulations, however, had little to no observable eyelid movement at any point during the 9-week survival period. Quantification of eyelid movement using IR emitter/detector sensors at weeks 3 to 9 indicated complete eyelid closure in response to computer-controlled corneal air puff delivery in control eyes, as well as eyes after RB myotomy, although with a slightly lower and more variable sensor response on average in the latter group. Eyelid closure data did not appreciably change over the 9-week recovery period after RB myotomy, and the number of contributing rats decreased substantially over time due to changes in corneal surface IR reflectance associated with loss of corneal health, and therefore statistically analyzed data are presented for only the week-3 time point (Fig. 2). At week 3, the RB myotomy condition did not differ significantly from that in control rats (two-tailed *t*-test; $P = 0.19$), but the control and RB myotomy conditions had significantly greater eye closure based on IR sensor output compared with the combined RB/OO condition (two, two-tailed *t*-tests; $P < 0.01$).

The IR sensors failed to reliably register a voltage change representative of observed eyelid closure over the globe during blinking in rats with OO paralysis. The increase in IR reflectance normally recorded as the eyelids cover the relatively IR-translucent cornea was apparently offset by the retraction of the globe into the orbit (which pulls the reflective body surface away from the sensor), rendering the IR sensor output unreliable in OO-paralyzed rats. This inability of our IR testing apparatus to accurately assess eyelid closure after OO paralysis prompted the use of high-speed video recording during eye stimulation to test eyelid closure capabilities in a group of rats after transection and repair of the CN VII main trunk ($n = 13$). This subsequent group represented a manipulation frequently encountered in CN VII studies, and measurement of the exposed eye surface during attempted blink elicitation revealed substantial eye closure capabilities within the first week after OO paralysis (Fig. 3). The early appearance of eyelid movement and the fact that eye closure decreased between days 5 or 6 and day 11 ($P < 0.01$, two-tailed *t*-test), suggests that closure capability was not due to OO reinnervation within this period.

Discussion

The ability of mammals to retract the globe into the orbit during blinking has been described in many species.[6] When OO function is lost in some small mammals such as rabbits and cats, eyelid closure is achieved through compensatory contraction of the RB muscle system.[6–8] The muscle inserts circumferentially on the deep surface of the globe and retracts the globe into the orbit causing the eyelids to passively slide across the cornea to achieve eyelid closure.

In this study, the rat RB was shown to play a compensatory role when the facial nerve was lesioned, enabling substantial (yet incomplete) eyelid movements in response to periocular stimulation within the first week of OO paralysis. Moreover,

the RB may normally contribute to reflexive and spontaneous blinking, since RB myotomy slightly attenuated eyelid movement in response to corneal air puff, and corneal health was degraded in most of the RB-lesioned rats as soon as 3 weeks after myotomy. Both of these observations, however, could be attributable to ocular bulging after RB myotomy and the possible reduction of corneal sensation caused by sensory nerve injury to the eye (despite concerted attempts to protect the ciliary nerve), particularly in light of the fact that rats receiving RB myotomy typically achieved complete reflexive eyelid closure in our testing apparatus, yet usually failed to maintain ocular health. Nevertheless, the fact that elicited eyelid movements persisted after complete facial nerve lesion (OO paralysis) is consistent with the presence of RB musculature in rats[4,5] and the RB-mediated eye closure demonstrated in some other small mammals.[6–8]

Our identification of dual eyelid closure mechanisms with differing neural inputs in the rat has significant implications for those studying rodent facial nerve regeneration. Many investigators have depended on the rat vibrissal system alone in gauging functional recovery after nerve manipulation, although this represents only a single function of the multibranched facial nerve. Current research includes examining functional outcomes in multiple facial zones,[1,16] to address aberrant regeneration and synkinetic facial movements. The present findings show how it is quite possible to mistake RB-mediated eyelid movements for those controlled by the facial nerve. For example, some investigators have recently claimed that the appearance of a "semieyeblink" response in rats is the hallmark of early facial nerve recovery,[17] drawing conclusions about facial nerve manipulations based on this eyelid response without acknowledging the potential role of RB contraction.

Unfortunately, mitigating the RB contribution to eyelid movement after facial nerve lesion is not a simple process. With the current findings, we have established that rats cannot tolerate complete loss of blink reflex, as in our dual OO/RB lesion condition, because corneal exposure leads almost invariably to ulceration. Botulinum toxin injection of the orbital muscles can effectively paralyze the RB (Hadlock TA, unpublished observation, 2008), but leads to massive proptosis and globe herniation in the absence of OO function. It may be possible to identify and selectively lesion the brain stem motoneurons supplying the RB, but placement of chronic electromyographic electrodes in the OO muscles for investigating facial nerve-mediated eyelid movement is likely to be an easier approach. Future studies will be conducted to explore these possibilities, along with signal analysis techniques that differentiate OO from RB eyelid IR sensor responses characteristics with the goal of "subtracting" the RB contribution from the eyeblink measurements after facial nerve manipulation.

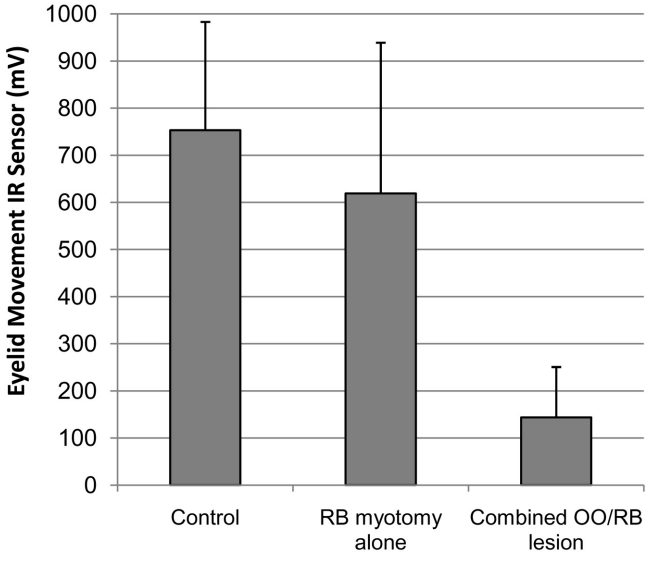

FIGURE 2. IR sensor output in head-fixed rats in response to corneal air puffs at week 3 for the RB myotomy rats ($n = 9$) and combined OO/RB rats ($n = 7$) are compared with contralateral (normal) eye closure ($n = 22$; ±SD). Complete eye closure typically produced an IR sensor response of ≥600 mV, which was often achieved in response to ocular air puff in eyes having received RB myotomy, but not nearly achieved in eyes receiving the combined OO/RB manipulation.

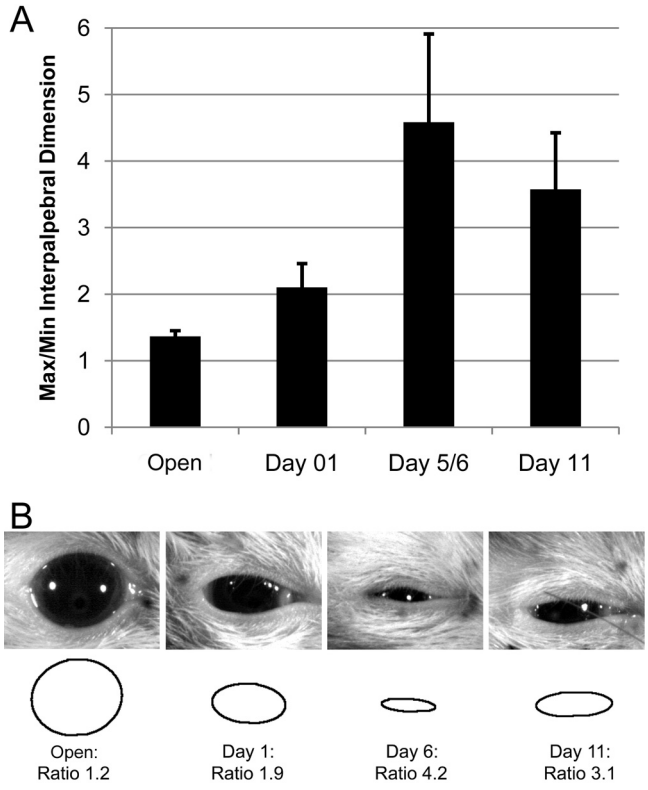

FIGURE 3. (**A**) The average ratio of maximum divided by minimum dimension (±SD) of the exposed cornea within the palpebral fissure during attempted eye closure after orbicularis oculi paralysis is shown at postsurgical days 1, 5 and 6 (combined), or 11 versus the baseline open state ($n = 13$ per data point). All average ratios significantly differed from one another ($P < 0.01$). (**B**) Representative high-speed video frames with automated ellipse calculations of exposed cornea and ellipse max/min ratio calculations are presented for the time points shown in (**A**). Examples for each time point are within 15% of average values. Note the retracted appearance of the eye surface during partial eyelid closure, particularly at day 1.

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