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The current study demonstrates that combining two mild alterations to the rat trigeminal reflex blink system reproduces the symptoms of benign essential blepharospasm, a cranial dystonia characterized by uncontrollable spasms of blinking. The first modification, a small striatal dopamine depletion, reduces the tonic inhibition of trigeminal reflex blink circuits. The second alteration, a slight weakening of the lid-closing orbicularis oculi muscle, begins an adaptive increase in the drive on trigeminal sensory-motor blink circuits that initiates blepharospasm. By themselves, neither of these modifications causes spasms of lid closure, but combined, they induce bilateral forceful blinking and spasms of lid closure. A two-factor model based on these rodent experiments may explain the development of benign essential blepharospasm in humans. The first factor, a subclinical loss of striatal dopamine, creates a permissive environment within the trigeminal blink circuits. The second factor, an external ophthalmic insult, precipitates benign essential blepharospasm. This two-factor model may also be applicable to the genesis of other cranial dystonias.

**METHODS**

All experiments were performed with strict adherence to all Federal, State, and University regulations governing the use of animals. Male Sprague-Dawley rats underwent one of three procedures: 1) a complete (>90%) unilateral 6-hydroxydopamine (6-OHDA) lesion (n = 5); 2) a unilateral lesion of the zygomatic branch of the facial nerve (n = 4); and 3) a small (<30%) unilateral 6-OHDA lesion followed by a unilateral zygomatic branch lesion (n = 5). Under general anesthesia and aseptic conditions, all rats were prepared for bilateral chronic recording of the lid-closing orbicularis oculi muscle electromyogram (Ooemg) and electrical stimulation of the supraorbital branch of the trigeminal nerve (SO). In the same surgery, rats in group 1 received a unilateral 6-OHDA injection to completely destroy the dopamine-containing cells in one substantia nigra pars compacta (see Basso et al. 1993 for details). Rats in group 3 were given a small, unilateral 6-OHDA injection to produce minimal destruction of compacta neurons. At least 20 days after the Ooemg and SO electrodes were implanted, the rats in groups 2 and 3 were anesthetized and the zygomatic branch of the facial nerve contralateral to the 6-OHDA injection was sectioned to weaken the orbicularis oculi. After rats recovered from the surgery, the minimal trigeminal threshold stimulus for evoking SO reflex blinks was established and the excitability of trigeminal reflex blinks was measured with the use of the paired stimulus paradigm. SO stimulation evokes both a short-latency R1 and a longer-latency R2 component. Two identical SO stimuli at 2 times blink threshold were presented at interstimulus intervals of 50–300 ms and blink excitability was quantified by comparing the magnitude of the Ooemg response to the second stimulus (test response) with the magnitude of the Ooemg response evoked by the first stimulus (condition response). Thirty-five to 50 days after the facial nerve transection, animals were deeply anesthetized and transectally perfused. Coronal sections of the brains were cut and reacted immunochemically with an antibody to tyrosine hydroxylase to identify dopamine-containing neurons in the substantia nigra pars compacta (see Basso et al. 1993 for details).

**RESULTS**

As demonstrated previously (Basso et al. 1993), 6-OHDA lesions that destroyed >90% of the dopamine-containing neurons in one substantia nigra pars compacta increased the R2 test/R2 condition ratio from normal values of <1 to values of 4–7.5, extreme trigeminal reflex blink hypereexcitability. The dopamine loss reduces tonic inhibition of the trigeminal sensory-motor transformation in blink circuits following a subclinical basal ganglia dysfunction produces blepharospasm that exhibits many of the characteristics of BEB.
Rat models of blepharospasm

Fig. 1. Basal ganglia regulation of trigeminal reflex blink excitability. A: schematic circuit linking a basal ganglia output nucleus, the substantia nigra reticulata (SNr), to the superior colliculus (SC), the nucleus raphe magnus (NRM), and the reflex blink circuits within the spinal trigeminal complex (V-RB). B: spasm of lid closure in a rat with complete (>90%) destruction of dopamine containing neurons in the right substantia nigra pars compacta. Stimulation of the left supraorbital branch of the trigeminal nerve (LSO Stim) initiated the spasm. The two records show the rectified orbicularis oculi electromyographic (OOemg) activity on the left (L OOemg) and right (R OOemg) during the spasm (top traces) and the reflex response at the onset of the spasm (inset).

Blind circuits (Fig. 1A). Complete unilateral 6-OHDA lesions also produced reflex spasms of lid closure triggered by SO stimuli (Fig. 1B). Just as occurs in some humans with severe Parkinson’s disease, spasms of lid closure occurred in response to trigeminal stimuli but did not occur spontaneously (e.g., Hotson and Bowman 1991). In contrast, 6-OHDA lesions that destroyed 30% of the nigral dopamine containing neurons in one substantia nigra slightly increased trigeminal reflex blink excitability to values of ~1 (Fig. 3D, ), but did not result in reflex-evoked or spontaneous spasms of lid closure.

Weakening the orbicularis oculi muscle also elevated trigeminal reflex blink excitability, but did not result in reflex induced or spontaneous spasms of lid closure. After VIIth nerve transection, trigeminal reflex blink excitability increased gradually over 1 wk to reach a level that remained constant (Fig. 2) for periods of up to 70 days. In addition to the increased excitability, the threshold for SO evoked OOemg activity of the ipsilateral and contralateral orbicularis oculi decreased in the 1st wk by a mean of 30 ± 3% (mean ± SE; t-test, P < 0.01). In contrast, the threshold for evoking ipsilateral and consensual reflex blinks through the SO nerve contralateral to the weakened orbicularis oculi did not change (6 ± 3%, mean ± SE; t-test, P < 0.049).

Weakening the orbicularis oculi muscle 20 days after a small (<30%) unilateral 6-OHDA lesion produced a dramatic increase in trigeminal reflex blink excitability and led to bilateral reflex-evoked (Fig. 3) and spontaneous spasms of lid closure. Within 24–48 h after a facial nerve transection in unilaterally 6-OHDA-lesioned rats, trigeminal reflex blink excitability increased. On the basis of the excitability determined at the 50-ms interstimulus interval, the trigeminal reflex blink excitability produced by combining the weak 6-OHDA and facial nerve lesions increased by a factor of 4 over the excitability increase caused by the small 6-OHDA lesion alone, and by a factor of 3.5 relative to rats that received orbicularis oculi weakening alone (Fig. 3D). In the same time period after the nerve lesion, the threshold for SO evoked ipsilateral and contralateral OOemg activity decreased by a mean of 68 ± 4% (t-test, P < 0.001), slightly more than twice the decrease caused by orbicularis oculi weakening alone. The SO threshold for evoking ipsilateral

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Effects of weakening the orbicularis oculi on excitability of trigeminal reflex blinks.

**A:** 2ND (Test) of a pair of identical stimuli delivered to the supraorbital branch of the trigeminal nerve (SO, arrowhead) with a 50-ms interstimulus interval evoked a smaller R2 response than the 1st (Condition) stimulus. Top traces: data collected on 2 separate days to illustrate the reliability of the suppression. Bottom trace: increase in the magnitude of the R2 response evoked by the 2nd stimulus relative to the 1st 32 days after the orbicularis oculi muscle was weakened. Each trace is the average of 10 rectified OOemg responses.

**B:** R2 test response magnitude / R2 condition response magnitude as a function of interstimulus interval before (●) and 3 (∆), 10 (●), and 32 (■) days after orbicularis oculi weakening. Each point is the mean of ≥10 responses.

DISCUSSION

Both dopamine depletion and orbicularis oculi weakening increase the excitability of trigeminal reflex blinks by modifying trigeminal sensory-motor blink circuits. The basal ganglia modulates the excitability of trigeminal reflex blinks via the substantia nigra pars reticulata inhibition of the superior colliculus. The superior colliculus excites tonically active, serotonergic neurons in the nucleus raphe magnus, which in turn inhibit reflex blink circuits within the spinal trigeminal complex (Fig. 3E) (Basso and Evinger 1996; Basso et al. 1996). Dopamine depletion increases nigral inhibition of the superior colliculus, which reduces the nucleus raphe magnus inhibition of trigeminal reflex blink circuits.

Orbicularis oculi weakening increases the excitability of SO evoked blinks and decreases the threshold for evoking blinks on the side ipsilateral to the orbicularis oculi weakening. The changes in threshold indicate an adaptive increase in the ability of trigeminal sensory stimuli to engage motor activity, i.e., motor learning (Evinger and Manning 1988). These changes have the effect of elevating the slope of the relationship between stimulus magnitude and evoked blink size to SO stimuli ipsilateral to the weakened orbicularis oculi. If orbicularis oculi weakening produced a general increase in blink excitability or an increase in the axotomized facial nucleus, then the threshold for eliciting a trigeminal blink should have decreased for the OOemg response ipsilateral to the nerve cut regardless of which SO nerve was cut.
FIG. 3. Effect of combining a small 6-hydroxydopamine (6-OHDA) lesion with weakening of the orbicularis oculi on trigeminal reflex blink excitability. A: after a small (<35%) unilateral 6-OHDA lesion, the response to the 2nd stimulus to the SO (arrowhead) was unaffected by the occurrence of the 1st stimulus and reflex blink evoked 100 ms earlier. B: 20 days after weakening of the orbicularis oculi muscle of the rat shown in A, the trigeminal reflex blink was extremely hyperexcitable. Records in A and B are averages of 5 rectified OOemg responses. C: 21 days after orbicularis oculi weakening, an SO stimulus produced prolonged spasms of lid closure. Single rectified record. D: R2 test response magnitude/R2 condition response magnitude as a function of interstimulus interval before lesioning ( ), Pre), after a small 6-OHDA lesion ( ), 6-OHDA), and 20 days after orbicularis oculi weakening in addition to the 6-OHDA lesion ( ), BLEPH). Each point is the mean of ≥10 responses from each of 5 rats. E: change in the slope of the line relating OOemg activity with stimulus intensity. Slope was established by calculating the change in A/D units of OOemg activity (ADU) as a function of SO stimulus current (μA). Slope increased for all 5 rats from the small 6-OHDA lesion condition (6-OHDA) to the small 6-OHDA lesion combined with orbicularis oculi weakening (BLEPH).
stimulated. Thus these data argue that orbicularis oculi weakening primarily modified trigeminal sensory-motor circuits ipsilateral to the facial nerve transection.

We hypothesize that the increased inhibitory output of the basal ganglia’s substantia nigra pars reticulata created a permissive condition in the trigeminal sensory-motor blink circuits that made the rats susceptible to the induction of blepharospasm. In this permissive environment, the normally adaptive increase in drive on the trigeminal sensory-motor circuits to compensate for lid weakness became too strong and altered trigeminal circuits to produce blepharospasm. Thus the transformation of sensory stimulus magnitude to motor drive continued to shift toward increased sensitivity as if its ‘set point,’ the correlation between sensory magnitude and evoked blink size, was dramatically increased or was no longer recognized in the permissive trigeminal environment. This rodent model also accounts for the characteristics of BEB in humans: 1) onset usually during the 5th–7th decades of life; 2) hyperexcitable trigeminal reflex blinks; 3) involvement of the basal ganglia; and 4) beginning usually with an ophthalmologic insult, e.g., dry eye.

The factors of aging, trigeminal reflex blink excitability, and basal ganglia dysfunction are interrelated. Decreasing striatal dopamine levels elevates trigeminal reflex blink excitability (Basso et al. 1993; Kimura 1973). Because there is progressive dopamine cell loss in the substantia nigra pars compacta with aging (e.g., Fearnley and Lees 1991), it is not surprising that trigeminal reflex blink excitability increases with age. Trigeminal reflex blink excitability escalates during normal aging, and starting in the fifth decade of life these increases become most pronounced (Evinger et al. 1995). Our model predicts that when a subclinical increase in basal ganglia output significantly reduces nucleus raphe magnus inhibition of the trigeminal complex, an individual will become susceptible to blepharospasm that can be initiated by an external ophthalmic insult.

Ophthalmic insults, such as dry eye, can initiate adaptive increases in the drive on trigeminal blink circuits. In the case of dry eye, the nervous system changes the set point of the trigeminal blink system so that a given trigeminal stimulus now evokes a larger blink to better wet the cornea (Pita-Salorio and Quintana-Conte 1988). In otherwise normal individuals, treatment of the dry eye eliminates the excessive blinking associated with dry eye. However, when basal ganglia dysfunction creates a permissive trigeminal sensory-motor blink environment, the adaptive process initiated by the dry eye appears to lose its set point or the set point continually increases, which results in blepharospasm.

Our model may also account for development of other cranial dystonias. BEB often expands from the eyelids to the orofacial region. This spreading may be induced by joint displacement. We hypothesize that this would cause an increase in the permissive trigeminal blink environment, which is consistent with the clinical observation that dystonia associated with blepharospasm often spreads to the orofacial region. Thus the increased excitability of the trigeminal blink system is likely to be a primary contributor to the development of idiopathic blepharospasm.

We thank D. M. Schmidt for expert technical assistance. We also acknowledge the crucial insights gathered at symposia organized by the Essential Blepharospasm Research Foundation.

This work was supported by National Eye Institute Grant EY-07391 to C. Evinger and Parkinson’s Disease Summer Fellowships to M. A. Basso.

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Received 9 October 1996; accepted in final form 22 January 1997.

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