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Reflex Excitability Regulates Prepulse Inhibition

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Presentation of a weak stimulus, a prepulse, before a reflex-evoking stimulus decreases the amplitude of the reflex response relative to reflex amplitude evoked without a preceding prepulse. For example, presenting a brief tone before a trigeminal blink-eliciting stimulus significantly reduces reflex blink amplitude. A common explanation of such data are that sensory processing of the prepulse modifies reflex circuit behavior. The current study investigates the converse hypothesis that the intrinsic characteristics of the reflex circuit rather than prepulse processing determine prepulse modification of trigeminal and acoustic reflex blinks.

Unilateral lesions of substantia nigra pars compacta neurons created rats with hyperexcitable trigeminal reflex blinks but normally excitable acoustic reflex blinks. In control rats, presentation of a prepulse reduced the amplitude of both trigeminal and acoustic reflex blinks. In 6-OHDA-lesioned rats, however, the same acoustic prepulse facilitated trigeminal reflex blinks but inhibited acoustic reflex blinks. The magnitude of prepulse modification correlated with reflex excitability.

Humans exhibited the same pattern of prepulse modification. An acoustic prepulse facilitated the trigeminal reflex blinks of subjects with hyperexcitable trigeminal reflex blinks caused by Parkinson's disease. The same prepulse inhibited trigeminal reflex blinks of age-matched control subjects. Prepulse modification also correlated with trigeminal reflex blink excitability. These data show that reflex modification by a prepulse reflects the intrinsic characteristics of the reflex circuit rather than an external adjustment of the reflex circuit by the prepulse.

Key words: prepulse modification; acoustic startle; reflex blink; Parkinson's disease; trigeminal; 6-hydroxydopamine

Presentation of an innocuous sensory stimulus, a prepulse, before a reflex-eliciting stimulus, transiently modifies reflex magnitude (Graham, 1975; Sanes and Ison, 1979; Hoffman and Ison, 1980; Anthony, 1985; Blumenthal and Gescheider, 1987; Braff and Geyer, 1990). Typically, a prepulse preceding a reflex-evoking stimulus by >50 msec reduces reflex magnitude, whereas a prepulse occurring <50 msec before the reflex facilitates the response (for review, see Hackley and Boelhouwer, 1997). Graham (1975) proposes that prepulse inhibition occurs because the nervous system reduces its sensitivity to sensory stimuli presented after the prepulse to protect sensory processing of the prepulse. Because such processing should automatically reduce responsiveness to subsequent sensory stimuli, the reflex-eliciting stimulus "appears" weaker after a prepulse and evokes a smaller response. Studies of prepulse inhibition of the acoustic startle reflex in schizophrenic humans (Swerdlow et al., 1994) and in rodents (Braff et al., 1990) are consistent with the idea that prepulse processing determines prepulse inhibition.

Hypotheses about the neural substrates producing prepulse inhibition and facilitation exist for blink reflexes (for review, see Hackley and Boelhouwer, 1997). Prepulse inhibition occurs because processing of the prepulse transiently inhibits brainstem interneurons involved in the generation of reflex blinks. For example, cholinergic neurons in the pedunculopontine tegmental nucleus projecting to startle reflex interneurons could produce prepulse inhibition (Koch et al., 1993; Koch, 1999). Prepulse facilitation occurs because of subliminal facilitation of facial motoneurons. These explanations assume that modification in the sensory processing of the prepulse controls the effect of a prepulse on subsequent reflex responses. These hypotheses predict that presenting the same prepulse before a reflex blink-evoking stimulus to a normal or an abnormal reflex blink circuit should produce short-lasting excitation and long-lasting inhibition of the reflex response, regardless of the state of the reflex circuit. The prepulse data, however, are also consistent with the hypothesis that the intrinsic characteristics of the reflex circuit rather than imposition of higher level prepulse processing on a reflex circuit determines the effect of a prepulse. This hypothesis predicts that the same prepulse will produce different patterns of prepulse modification for the normal and the abnormal reflex circuit. The present investigation tests this hypothesis by examining prepulse modification of normal and hyperexcitable reflex blinks.

To investigate how changes in reflex excitability influence prepulse modification, it is necessary to characterize the reflex circuit excitability. Presenting pairs of identical blink-eliciting stimuli and comparing the magnitude of the response to the second stimulus to that evoked by the first stimulus, the paired stimulus paradigm, estimates the excitability of reflex blink circuits (Kimura, 1983; Powers et al., 1997). In normal subjects, the response to the second stimulus is smaller than the response to the first stimulus for interstimulus intervals <600 msec. In hyper-

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excitable subjects, the response to the second stimulus can even be greater than the response to the first stimulus. Trigeminal reflex blink hyperexcitability occurs with Parkinson's disease (PD) in humans (Penders and Delwaide, 1971; Kimura, 1973) and with unilateral 6-hydroxydopamine (6-OHDA) lesions of dopamine-containing neurons in rats (Basso et al., 1993). In contrast to the trigeminal reflex blink hyperexcitability caused by dopamine loss, both Kinney et al. (1999) and the current study demonstrate that 6-OHDA lesions do not alter acoustic reflex blink excitability. The present study tests prepulse modification of hyperexcitable trigeminal and normally excitable acoustic reflex blinks using the same acoustic prepulse.

MATERIALS AND METHODS

Rat experiments

All experiments were performed in strict adherence to all federal, state, and university regulations governing the use of animals. Male Sprague Dawley rats were randomly assigned to one of two groups, a control nonlesioned group ($n = 6$), or a unilateral 6-OHDA-lesioned group ($n = 7$).

Under general anesthesia (xylazine 10 mg/kg and ketamine 90 mg/kg) and following aseptic procedures, all rats were prepared for bilateral stimulation of the supraorbital branch of the trigeminal nerve (SO) and electromyographic recordings of orbicularis oculi muscle activity (OOemg). The details of these procedures are presented elsewhere (Evinger et al., 1993). Rats were alert and eating within 24 hr of surgery, but were not tested until at least 7 d after surgery. During the same surgery in which OOemg and SO nerve cuffs were implanted, rats in the 6-OHDA lesion group also received a unilateral dopamine cell lesion with 6-OHDA using the procedure of Brundin et al. (1988).

Blink evocation and measurement. Reflex blinks were evoked with acoustic and trigeminal stimuli. The acoustic blink-evoking stimulus was a 95 dB (SPL), 10 kHz pure tone lasting 50 msec with a 0.1 msec rise time. A Coulbourn precision signal generator, amplified through an Optimus integrated stereo amplifier, and delivered through an Optimus 50 W loudspeaker produced the acoustic startle stimulus. SO blink-evoking stimuli were constant current, 70 μS pulses. For each rat, the lowest SO stimulus intensity that reliably elicited a blink was designated as threshold (T). All testing was conducted using an intensity of 2T. The range of threshold intensities was 0.1–1 mA for all rats. These 2T stimulation parameters produced clear R1 and R2 components of the blink response. Weak, nonreflex-evoking SO stimuli (0.7T) were also used as prepulses for three control and four 6-OHDA rats.

Testing procedures. The experimental procedure consisted of four prepulse trial types in which a prepulse was presented 50, 150, 300, or 600 msec before the reflex-eliciting stimulus, and a control trial, of only the reflex-eliciting stimulus. The order of trial-type presentation was pseudorandomly determined, with each of the five trial types being presented five times. The intertrial interval was 25 ± 5 sec. Prepulse stimuli were 60 dB [sound pressure level (SPL)] 10 kHz tones, lasting 1 msec with a 0.1 msec rise time. These stimuli did not evoke a blink. Rats were tested in a dimly lit room with a background noise of ~40 dB (SPL). Animals were brought into the recording room, allowed to habituate to the environment for 5 min, and then tested to establish their SO threshold. All animals underwent at least 3 d of preliminary testing to establish baseline response levels and to acclimate them to the experimental procedure. Data collection for animals with 6-OHDA lesions did not begin until 7 d after the lesion. To insure maximum lesion effectiveness, only data acquired 14 d after the 6-OHDA lesion were compared with data from the control rats.

Histology. At the end of all procedures, each 6-OHDA rat was deeply anesthetized with xylazine and ketamine and intracardially perfused with 4% paraformaldehyde in phosphate buffer. Details of the histological and anatomical procedures are presented elsewhere (Basso et al., 1993). Brain tissue was incubated in primary antibody against tyrosine hydroxylase (TH). The total number of TH-positive neurons in the substantia nigra pars compacta were counted in five consecutive sections through the center of the substantia nigra. Comparing the number of TH-positive neurons on the lesioned side with the number of TH-positive neurons counted on the intact side estimated lesion size. The 6-OHDA-lesioned rats contained an average of 37 ± 8.5% fewer TH-labeled neurons in the lesioned substantia nigra pars compacta relative to the intact side.

Human experiments

Five patients diagnosed with PD, two subjects diagnosed with dry eye, and five control subjects were used in this study. The average age of the PD, dry eye, and control subjects was 68.2 ± 1.3, 65.0 ± 7, and 56.5 ± 3.9, respectively. All subjects gave informed consent for their participation in the study. All experiments were performed in strict accordance with federal, state, and university regulations regarding the use of humans in experiments and received approval of the university's Institutional Review Board.

Blink evocation and measurement. We measured upper eyelid movement using the magnetic lid coil procedure and recorded concomitant OOemg activity (for details, see Evinger et al., 1991). To monitor upper eyelid position, a thirty turn, 2 mm diameter lid coil was taped to the center of the lower margin of each upper eyelid. Pretarsal OOemg was recorded with a pair of silver plate electrodes (<2 mm diameter) taped to the medial and lateral sides of both upper eyelids. The OOemg signal was filtered 0.3–2 kHz, (−3 dB). An electrode affixed to the center of the forehead served as ground. SO stimuli were delivered through a pair of gold-plated surface electrodes. The first electrode was placed immediately above the supraorbital notch, and the other was attached 1 cm above the first. For all subjects, SO stimulus intensity was set at 2T with a 170 μS duration. Threshold intensities ranged from 1–4 mA. Acoustic prepulses were a 1 msec, 60 dB click presented through a loudspeaker located 3 m from the subject's head. Subjects watched videotape during the experimental session.

Testing procedures. The experimental procedure consisted of two trial types in which subjects were exposed to either a prepulse that was presented 150 msec before the SO stimulus, or a control trial, containing the SO stimulus alone. The order of presentation of each of the two trial types was pseudorandomly determined, with each trial type presented six times. The intertrial interval was 25 ± 5 sec.

Data collection and analysis

Rodent OOemg data were digitized at 4 kHz/channel (Data Translation, 12 bit accuracy) and stored for off-line analysis. Laboratory-written software allowed the user to integrate rectified OOemg activity and determine latencies. OOemg amplitude was calculated by integration of the rectified OOemg responses. Human eyelid position and OOemg activity were digitized at 2 kHz/channel and stored for off-line analysis. Laboratory-written software allowed the user to measure blink amplitude, duration, and peak velocity as well as integrated OOemg activity. One-way ANOVA tests were used for all statistical analyses. P values < 0.05 were deemed significant.

RESULTS

Excitability of reflex blink circuits

Presentation of two identical reflex-evoking stimuli, the paired stimulus paradigm, provides a measure of reflex circuit excitability. In normal humans and rats, the response evoked by the second stimulus (test response) is smaller than the response evoked by the first stimulus (condition response; Pellegrini et al., 1995; Powers et al., 1997). A ratio of less than one indicates that the first stimulus suppresses the second response, and a ratio greater than one implies that the condition stimulus facilitates the test response, hyperexcitability.

With presentation of a pair of identical SO stimuli, the test stimulus evoked a smaller reflex blink than the condition stimulus for control rats. The test response, however, was usually larger than the condition response for 6-OHDA rats (Fig. 1*A*). Although there were no significant differences between the R1 excitability of control and 6-OHDA rats ($F_{(1,10)} = 1.4, 0.08, 1.74, 2.59$; $p > 0.05$ at all intervals), the difference in R2 excitability between control and 6-OHDA rats was significant at all intervals ($F_{(1,10)} = 30.18, 10.95, 15.61, 6.52$; $p < 0.03$ at all intervals; Fig.

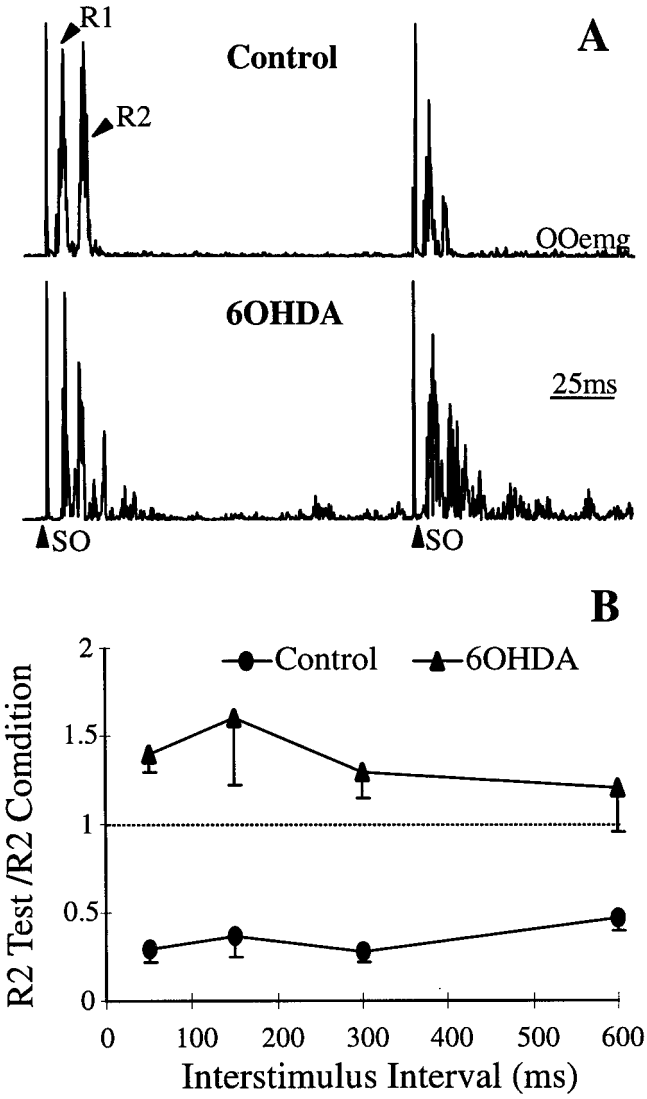

Figure 1. 6-OHDA lesions increase the excitability of trigeminal reflex blinks. *A,* Rectified orbicularis oculi EMG activity (OOemg) evoked by two identical supraorbital nerve stimuli (▲, *SO*) with an interstimulus interval of 150 msec in a control and a 6-OHDA-lesioned rat. Each trace is a representative response plotted at the same scale. *B,* Excitability of trigeminal reflex blinks estimated from the ratio of the magnitude of the R2 component of the blink evoked by the second SO stimulus (test) to the R2 magnitude evoked by the first SO stimulus (condition) for control (●) and 6-OHDA-lesioned rats (▲). Each point is the average and SEM of data from 10 trials (from two test sessions) for six control and seven 6-OHDA rats.

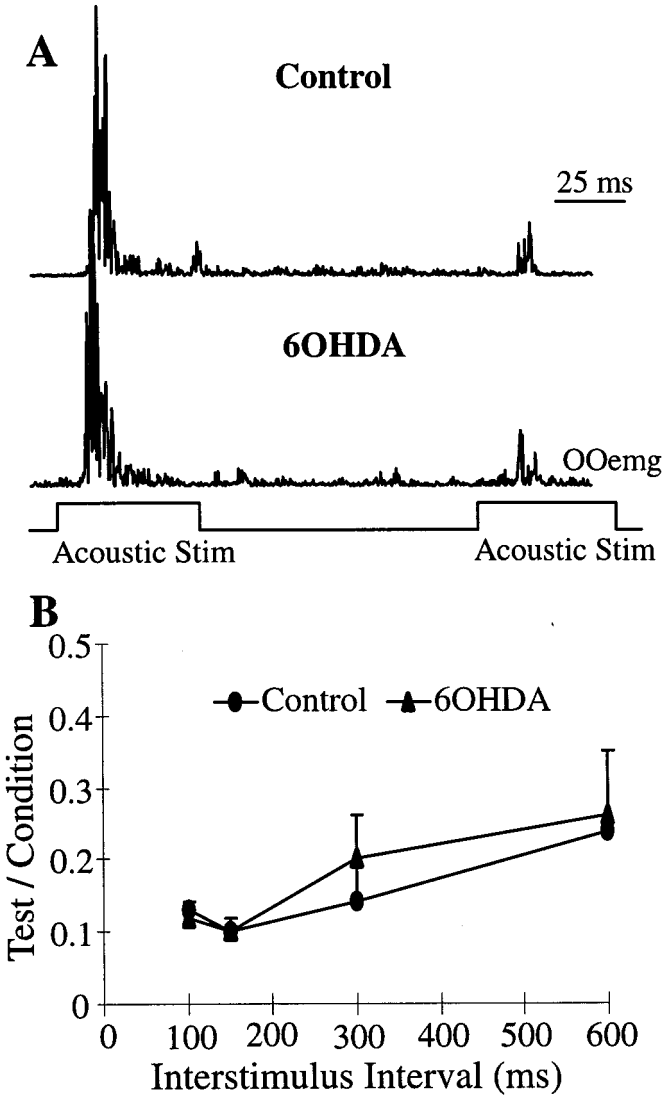

Figure 2. 6-OHDA lesions do not increase the excitability of acoustic reflex blinks. *A,* Rectified orbicularis oculi EMG activity (OOemg) evoked by two identical acoustic stimuli (*Acoustic Stim*) with an interstimulus interval of 150 msec in a control and a 6-OHDA-lesioned rat. Each trace is a representative response plotted at the same scale. *B,* Excitability of the acoustic blink reflex estimated from the ratio of the magnitude of the OOemg activity evoked by the second acoustic stimulus (*Test*) to the magnitude evoked by the first acoustic stimulus (*Condition*) for control (●) and 6-OHDA-lesioned rats (▲). Each point is the average and SEM of data from 10 trials (from 2 test sessions) for six control and seven 6-OHDA rats.

1*B*). These R2 excitability data demonstrated that the trigeminal reflex blink circuit was hyperexcitable in 6-OHDA lesioned rats, as reported previously (Basso et al., 1993; Schicatano et al., 1997).

Presenting pairs of identical acoustic blink-evoking stimuli, however, evoked smaller blinks to the second stimulus in both control and 6-OHDA-lesioned rats (Fig. 2*A*). There were no significant differences between control and 6-OHDA rats in response to pairs of acoustic blink-evoking stimuli ($F_{(1,10)} = 0.04$, 0.02, 0, 0.05; $p > 0.5$ at all intervals). The second acoustic reflex blink was strongly suppressed relative to the condition response at all interstimulus intervals tested (Fig. 2*B*). Thus, 6-OHDA lesions increased the excitability of the trigeminal reflex blink circuit but did not affect acoustic reflex blink excitability.

Acoustic prepulse: trigeminal reflex blinks

We investigated whether the excitability of the reflex circuit regulated prepulse modification of the reflex by presenting the same weak, acoustic prepulse before trigeminal and acoustic reflex blink-evoking stimuli in the same rats. Presentation of a weak acoustic prepulse significantly modified the R2 component of SO-evoked blinks (Fig. 3*A*). In control rats, an acoustic prepulse suppressed the amplitude of the R2 component of the blink

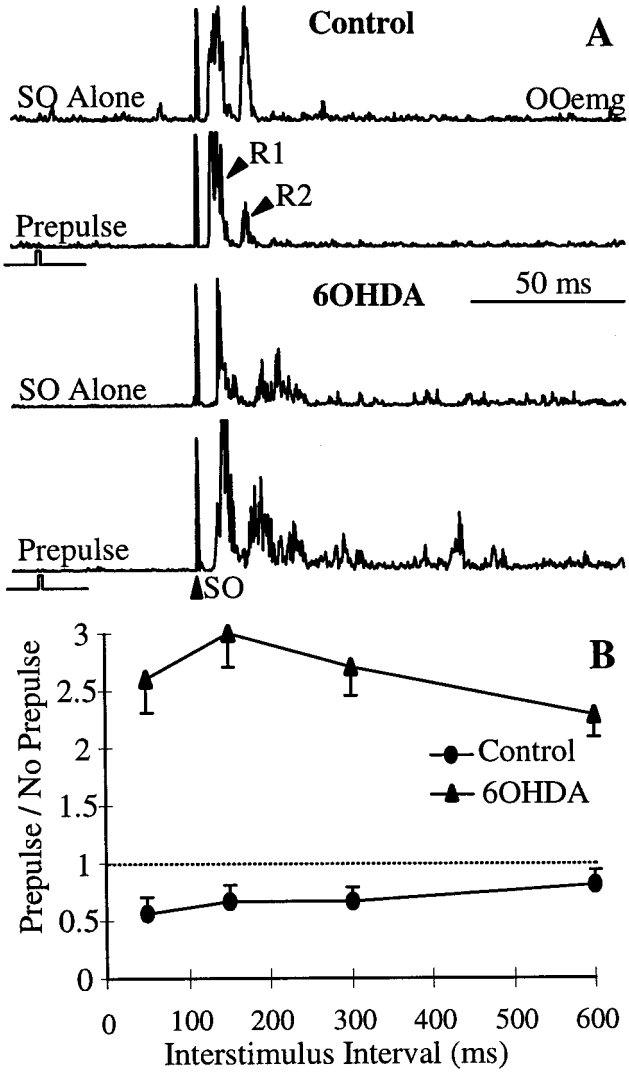

Figure 3. Acoustic prepulses produce opposite effects on the trigeminal reflex blinks of control and 6-OHDA-lesioned rats. *A,* For both control (*top traces*) and 6-OHDA records (*bottom traces*), the first trace shows the rectified orbicularis oculi EMG (OOemg) activity evoked by a supraorbital nerve stimulus (▲, *SO*) alone. The second trace shows the OOemg response elicited by a SO stimulus when preceded by an acoustic prepulse (*Prepulse*). Each trace is a representative response plotted at the same scale. *B,* For control (●) and 6-OHDA-lesioned rats (▲), the *y*-axis shows the ratio of orbicularis oculi (OOemg) activity evoked by a supraorbital nerve stimulation (*SO*) preceded by an acoustic prepulse to OOemg activity evoked by the SO stimulus alone. The *x*-axis is the interval between the prepulse and the SO stimulus. Each data point is the average and SEM of 10 trials (from 2 test sessions) for six control and seven 6-OHDA rats.

reflex at interstimulus intervals up to 300 msec (Fig. 3*B*). In contrast, the same acoustic prepulse facilitated R2 amplitude in 6-OHDA-lesioned rats. There was a significant difference between control and 6-OHDA rats in R2 reflex amplitude modification at all interstimulus intervals ($F_{(1,10)} = 7.39, 31.7, 7.96, 4.99$; $p < 0.05$). In control rats, an acoustic prepulse did not modify R1 amplitude of the trigeminal blink reflex. In 6-OHDA-lesioned rats, however, the acoustic prepulse significantly facilitated R1 amplitude at all interstimulus intervals except 600 msec ($F_{(1,10)} = 8.0, 7.82, 27.25$; $p < 0.02$; data not shown). Thus, a weak acoustic prepulse inhibited the R2 component of SO-evoked blinks in control rats, but facilitated the R1 and R2 components of SO-evoked blinks in 6-OHDA-lesioned rats.

Acoustic prepulse: acoustic reflex blinks

We further investigated the role of reflex circuit excitability in regulating prepulse modification by presenting the same weak, acoustic prepulse before an acoustic reflex-evoking stimulus. Because 6-OHDA lesions did not change acoustic blink circuit excitability (Fig. 2), prepulse modification of acoustic blinks should be similar in control and 6-OHDA rats if intrinsic reflex excitability determines prepulse modification. In control and 6-OHDA rats, a weak acoustic prepulse inhibited acoustic reflex blinks at all interstimulus intervals tested (Fig. 4). There were no significant differences in prepulse inhibition between the control and 6-OHDA rats ($F_{(1,10)} = 0.08, 1.03, 0.91, 1.17$; $p > 0.05$ at all intervals).

These data demonstrate that the 6-OHDA lesions did not modify the effectiveness of the acoustic prepulse. The loss of prepulse inhibition of trigeminal reflex blinks in rats with 6-OHDA lesions cannot be attributed to disruption of acoustic prepulse processing by dopamine depletion because the acoustic prepulse inhibited acoustic reflex blinks in dopamine-depleted rats. The conversion of prepulse inhibition to facilitation of trigeminal reflex blinks must result from changes within the trigeminal reflex blink circuit.

Because reflex excitability exhibits variability between subjects within a condition as well as between conditions, it was possible to correlate reflex excitability with prepulse modification (Fig. 5). Plotting the change in reflex blink magnitude when preceded by the acoustic prepulse as a function of maximum reflex circuit excitability for acoustic (◇) and trigeminal (◆) reflex blinks revealed that prepulse modification varied monotonically with excitability regardless of whether the rat was in the 6-OHDA or the control group. This result further supports the conclusion that intrinsic reflex excitability rather than modifications in prepulse processing caused by dopamine loss that regulates prepulse modification.

Weak SO prepulse: trigeminal reflex blinks

Because weak acoustic prepulses did not modify acoustic reflex blinks in 6-OHDA rats, it is possible that when the prepulse is the same modality as the reflex-evoking stimulus, it is impossible to facilitate reflex blinks regardless of reflex excitability. In this case, weak, nonreflex-evoking SO prepulses should suppress subsequent SO-evoked blinks. On the average, a weak SO prepulse inhibited both the R1 and R2 components of the blink reflex in control rats ($n = 3$; Fig. 6). In 6-OHDA rats ($n = 4$), however, the same SO prepulse facilitated both R1 and R2 components of the blink reflex at all interstimulus intervals on the average. Because of the small number of rats, only R2 responses at 50 and 150 msec intervals were significantly different between control and 6-OHDA rats ($F_{(1,6)} = 16.01$ and 5.68; $p < 0.05$). Thus, a weak prepulse of the same modality as the reflex-evoking stimulus facilitated reflex blinks evoked through a hyperexcitable circuit. This observation argues that reflex circuit excitability rather than differences between the modality of the prepulse and the reflex-evoking stimulus regulates prepulse modification.

Acoustic prepulse: SO reflex blinks in humans

Just as occurred after 6-OHDA destruction of dopamine neurons in rodents (Basso and Evinger, 1996), patients with Parkinson's disease exhibited trigeminal reflex blink hyperexcitability (Penders and Delwaide, 1971; Kimura, 1973). Similar to the 6-OHDA-

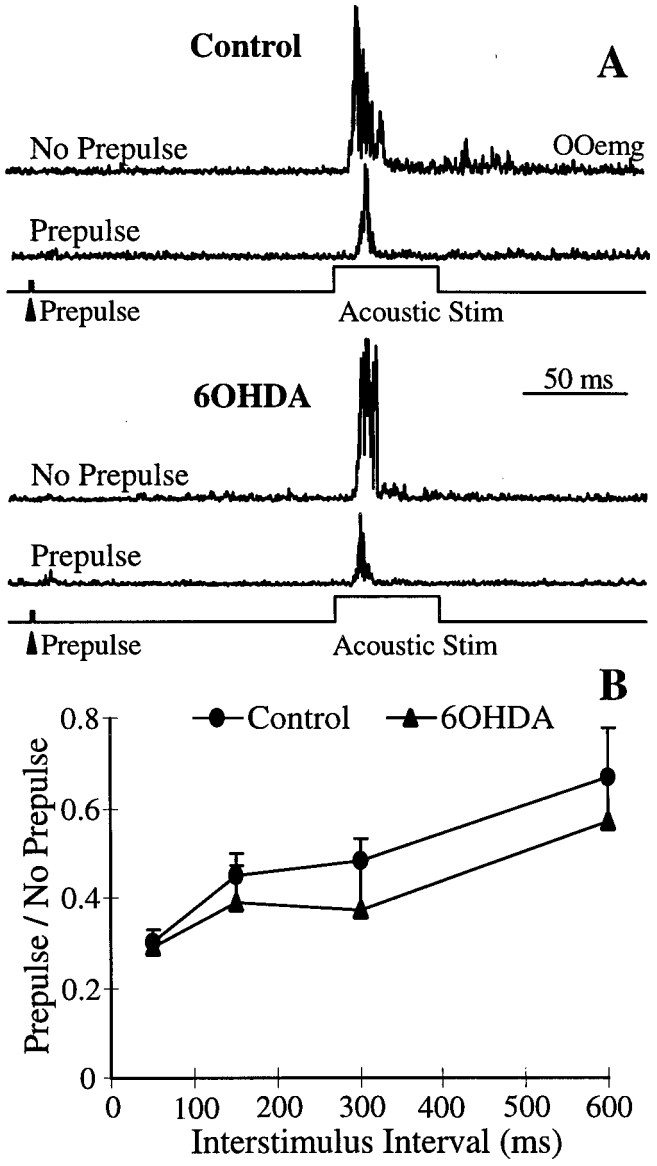

Figure 4. Acoustic prepulses inhibit acoustic reflex blinks of control (*top traces*) and 6-OHDA-lesioned (*bottom traces*) rats. *A,* For both control and 6-OHDA records, the first trace shows the rectified orbicularis oculi EMG (OOemg) activity evoked by an acoustic startle stimulus (*Acoustic Stim*), and the second trace shows the OOemg response elicited by an acoustic startle stimulus when preceded by an acoustic prepulse (▲, *Prepulse*). Each trace is a representative response plotted at the same scale. *B,* The *y*-axis shows the ratio of OOemg activity evoked by an acoustic startle stimulus preceded by an acoustic prepulse to OOemg activity evoked by the acoustic startle stimulus alone for control (●) and 6-OHDA-lesioned (▲) rats. The *x*-axis is the interval between the prepulse and the acoustic startle stimulus. Each data point is the average and SEM of 10 trials (from 2 test sessions) for six control and seven 6-OHDA rats.

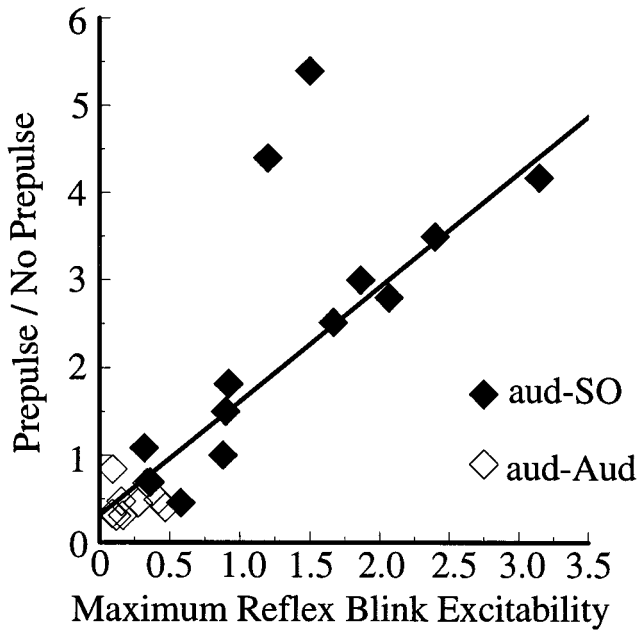

Figure 5. Acoustic prepulse modification of reflex blinks varies monotonically with the reflex blink excitability of acoustic (◇) and trigeminal (◆) reflex blinks. Each point is the average maximum prepulse modification and the maximum excitability measured for individual rats. The points are from both control and 6-OHDA rats. The regression line is calculated from the pooled values of the two data sets ($Y = 1.49X + 0.31$; $r = 0.82$).

lesioned rat data, an acoustic prepulse facilitated subsequent SO blinks in Parkinson's disease patients (Fig. 7). There was a significant difference between control humans and Parkinson's disease patients for trigeminal reflex blink amplitude and duration after an acoustic prepulse ($F_{(1,5)} = 13.65$; $p < 0.01$). In control subjects, the same acoustic prepulse decreased the amplitude of lid closing an average of 67% but increased the amplitude of lid closing by 30% in Parkinson's patients.

To investigate whether the prepulse facilitation shown by Parkinson's disease patients resulted from an increase in trigeminal reflex blink excitability caused by dopamine loss, we measured prepulse modification as a function of reflex excitability (Fig. 8). Plotting the ratio of the SO blink amplitude preceded by a prepulse to blink amplitude of SO alone trials as a function of trigeminal reflex blink excitability revealed that trigeminal reflex blink excitability for control (◆) and Parkinson's disease patients (◇) accurately predicted prepulse modification. As a further test, we included two subjects with dry eye (✶). These subjects had normal dopamine levels, but one exhibited hyperexcitable and the other had normally excitable trigeminal reflex blinks. Regardless of condition, prepulse modification increased monotonically with trigeminal reflex blink excitability. Thus, trigeminal reflex blink excitability regulated prepulse effects in humans as well as rodents.

DISCUSSION

A widely accepted explanation for prepulse modification of reflex blinks is that the nervous system transiently decreases its sensitivity to subsequent stimuli to insure uninterrupted sensory processing of the prepulse (Graham, 1975). This hypothesis implies that a descending signal determines prepulse effectiveness and that prepulse modification is relatively independent of the intrinsic reflex circuit characteristics. In this model, the most significant reflex circuit contribution to prepulse modification is reflex magnitude. In contrast, the current study investigates the hypothesis that a prepulse initiates the intrinsic excitatory and inhibitory processes produced by a reflex-evoking stimulus. In our model, intrinsic reflex circuit characteristics rather than external inputs determine prepulse modification of the circuit.

The paired stimulus paradigm, presentation of two identical blink-evoking stimuli, reveals the intrinsic excitatory and inhibi-

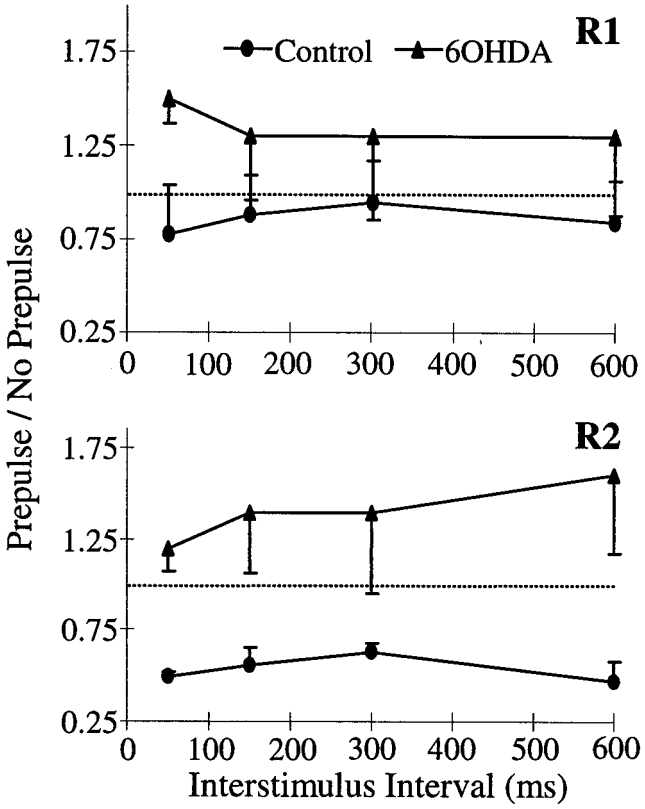

Figure 6. A weak trigeminal prepulse produced the same effect on trigeminal reflex blinks as an acoustic prepulse in control (●) and 6-OHDA-lesioned (▲) rats. The *y*-axis is the ratio of trigeminal reflex blink orbicularis oculi EMG (OOemg) activity evoked by supraorbital nerve stimulation (SO) when preceded by a trigeminal prepulse to OOemg activity elicited by SO stimulation alone. The *x*-axis is the interval between the weak trigeminal prepulse and the SO blink-evoking stimulus. Each data point is the average and SEM of 10 trials (from two sessions) for four 6-OHDA and three control rats.

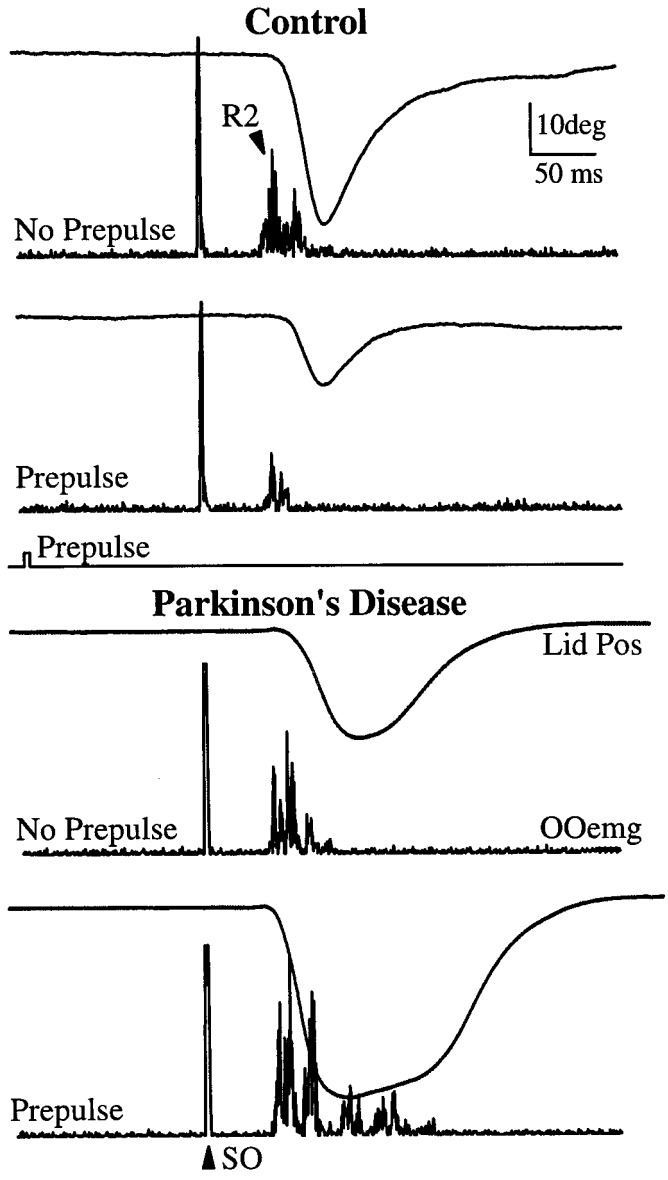

Figure 7. An acoustic prepulse produced different effects on the trigeminal reflex blinks of control and Parkinson's disease subjects. For both control and Parkinson's disease subjects, the *top trace* shows upper eyelid position (*Lid Pos*) and the rectified orbicularis oculi EMG (OOemg) activity evoked by a supraorbital nerve (▲, SO) stimulation alone (*SO Alone*). The *second trace* shows the trigeminal blink evoked by the same SO stimulus when preceded by an acoustic prepulse (*Prepulse*). Each trace is a representative record.

tory processes of a reflex circuit. The initial blink-evoking stimulus initiates an excitatory process that generates the reflex blink followed by a subthreshold excitatory period. To prevent the corneal and eyelash activation produced by lid closure from initiating another trigeminal reflex blink, the blink-evoking stimulus also initiates a transient inhibitory process. In normal subjects, excitatory processes initially dominate the reflex circuit, whereas inhibitory processes dominate later. Presenting a second, blink-evoking stimulus during the dominant inhibitory phase of reflex circuit activity initiated by the first stimulus results in a smaller blink magnitude evoked by the second stimulus. In normal subjects, this inhibitory phase dominates for hundreds of milliseconds (Figs. 1, 2). Such a reflex circuit has a normal state of excitability. After a reduction in the inhibitory processes caused by disease states, however, the subliminal, excitatory process dominates the inhibitory process after a stimulus. In this condition, presenting a second, blink-evoking stimulus during the dominant excitatory phase of circuit activation initiated by the first stimulus increases blink magnitude evoked by the second stimulus. This reflex circuit is hyperexcitable as occurs with trigeminal reflex blink circuits after dopamine cell loss (Fig. 1; Basso et al., 1993).

Loss of substantia nigra pars compacta dopamine neurons increases trigeminal reflex blink excitability through a well-established circuit (Basso and Evinger, 1996; Basso et. al., 1996). Dopamine cell loss increases substantia nigra pars reticulata inhibition of the superior colliculus that in turn reduces excitation of the nucleus raphe magnus. Because the nucleus raphe magnus tonically inhibits trigeminal reflex blink circuits within the trigeminal complex, the reduction in raphe input leads to more excitable trigeminal reflex blink circuits. It is reasonable that increased auditory reflex blink excitability does not occur after dopamine depletion (Fig. 2; Kinney et al., 1999) because the only circuit elements shared by the auditory and trigeminal reflex blink circuits are orbicularis oculi motoneurons.

The current data demonstrate that the intrinsic patterns of inhibitory and excitatory processes generated in a reflex blink

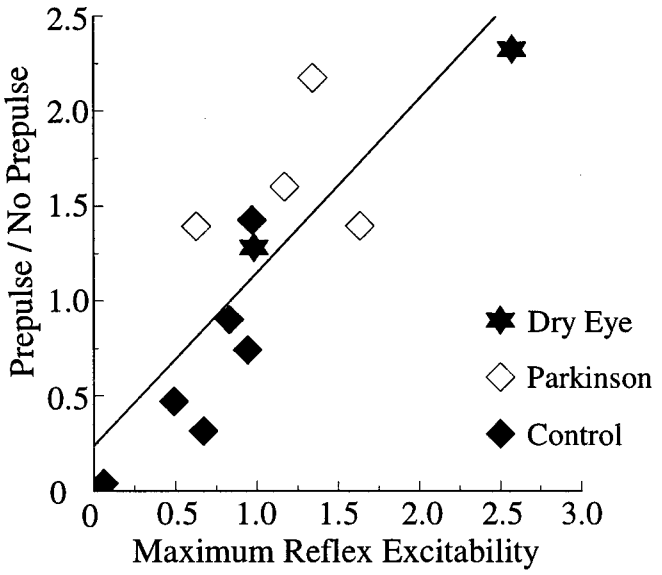

Figure 8. The prepulse modification of trigeminal reflex blinks increases with reflex blink excitability for human control subjects (◆), Parkinson's disease patients (◇) and dry eye patients (✶). The *x*-axis is the maximum trigeminal reflex blink excitability. The *y*-axis is the amplitude of the lid movement evoked by an SO stimulus with a 150 msec auditory prepulse divided by the lid amplitude of the trigeminal reflex blinks without a prepulse. Each point is the averaged data from one subject. The regression line is calculated from the pooled values of all three data sets ($Y = 0.915X + 0.24$; $r = 0.82$).

circuit determine prepulse modification of reflex blinks. First, the same prepulse that inhibits normally excitable reflex blink circuits facilitates hyperexcitable reflex blink circuits (Figs. 3, 4, 7). If sensory processing of the prepulse controls prepulse modification by varying the strength of an external signal to reflex blink circuits, then relative prepulse modification of normal and hyperexcitable reflex blink circuits should be similar. Second, reflex circuit excitability measured with the paired stimulus paradigm predicts the magnitude and direction of prepulse modification of reflex blinks (Figs. 5, 8). Previous studies of prepulse modification support this observation. Similar to the current results, Nakashima et al. (1993) report a reduction in acoustic prepulse inhibition of trigeminal reflex blinks in Parkinson's disease patients relative to control subjects. Gille de la Tourette syndrome causes hyperexcitable trigeminal reflex blinks (Smith and Lees, 1989) and reduces prepulse inhibition of these blinks (Castellanos et al., 1996). Similarly, benign essential blepharospasm is associated with hyperexcitable trigeminal reflex blinks (Berardelli et al., 1985) and reduced prepulse inhibition of trigeminal reflex blinks produced by both acoustic (Gomez-Wong et al., 1998) and photic (Katayama et al., 1996) prepulse stimuli. The sensory processing hypothesis of prepulse modification does not predict these results. The simplest explanation for the present observations is that the prepulse activates the same pattern of intrinsic excitatory and inhibitory processes, as does a reflex-evoking stimulus.

The reflex excitability established with the paired stimulus paradigm may be an excellent predictor of prepulse modification because the paired stimulus paradigm is a special case of prepulse modification. The first reflex blink-evoking stimulus acts as a prepulse for the blink evoked by the second stimulus. Inhibition or facilitation of the response to the second stimulus occurs because the first stimulus initiates the pattern of excitatory and inhibitory processes intrinsic to the reflex circuit. The present data demonstrate that a prepulse stimulus does not need to be blink-evoking to initiate these intrinsic processes because a nonblink-evoking SO prepulse stimulus modifies subsequent trigeminal evoked blinks in a manner qualitatively identical to a blink-evoking stimulus (Figs. 1, 6; Pellegrini and Evinger, 1995). Thus, nonblink-evoking stimuli can initiate the same excitatory and inhibitory processes intrinsic to a reflex blink circuit that a blink-evoking stimulus activates.

The hypothesis that a nonblink-evoking prepulse initiates excitatory and inhibitory processes intrinsic to the reflex blink circuit even when the prepulse and blink-evoking stimuli are different modalities requires that different modality stimuli have access to the reflex circuit. There is abundant evidence that the trigeminal system receives auditory inputs that could provide prepulse information. Neurons in the trigeminal complex exhibit a short-latency response to tones (McCormick et al., 1983; Richards et al., 1991; Clark and Lavond, 1996). Presentation of pure 60 dB tones activates cFos in the ventral border of the trigeminal complex (Sato et al., 1993). Similarly, the auditory system receives trigeminal inputs. Trigeminal complex neurons project to both the cochlear nucleus and the inferior colliculus (Weedman et al., 1996; Li and Mizuno, 1997). Thus, at least these and probably other prepulse modalities clearly have access to reflex blink circuits.

Prepulses of a variety of modalities initiate the subthreshold excitatory and inhibitory processes intrinsic to that reflex blink circuit. This hypothesis explains why the same prepulse facilitates a hyperexcitable reflex circuit yet inhibits a normally excitable reflex blink circuit. This hypothesis also makes it clear why the excitability of the reflex accurately predicts prepulse modification of the reflex blink. Thus, reflex blink modification after a prepulse primarily reflects the intrinsic properties of the reflex blink circuit rather than sensory processing of the prepulse.

There is a wealth of data on prepulse modification of whole-body startle elicited by an acoustic stimulus (for review, see Koch, 1999). Comparisons with the current data are difficult, however, because absolute reflex response magnitude instead of the paired stimulus paradigm is the typical measure of reflex circuit excitability in startle studies. In our hypothesis, the absolute magnitude of the response is less important than the intrinsic excitatory and inhibitory processes within the reflex circuit generated by the first stimulus. Nevertheless, data from drug and lesion treatments that alter prepulse modification are consistent with the possibility that these treatments achieve their effect by modifying the intrinsic excitatory and inhibitory processes of the whole-body reflex startle circuit rather than altering a descending prepulse stimulus. The simplest startle circuit organization is that primary auditory afferents activate dorsal cochlear root neurons that in turn excite neurons in the nucleus reticularis pontis caudalis. These reticular neurons project to the spinal cord to produce the short-latency, whole-body startle (Lee et al., 1996) (for review, see Koch, 1999) There is evidence that prepulse control of this circuit arises from peduculopontine nucleus neurons onto nucleus reticularis pontis caudalis neurons (Koch et al., 1993; Koch, 1999). Just as the tonic basal ganglia modulation of trigeminal reflex blink circuits alters prepulse effects (Basso et al., 1993, 1996: Basso and Evinger, 1996), pharmacological and lesion-induced modifications of peduculopontine activity could tonically alter the intrinsic startle circuit properties to modify prepulse effects. Consistent with this interpretation, lesions of the pedunculopontine nucleus do not block prepulse modification of whole-body startle (Koch, 1999).

Thus, like the blink reflex, prepulse modification of other reflex circuits may result from intrinsic properties of the reflex circuit rather than modifications of an external input to the reflex circuit.

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