Trigeminal high-frequency stimulation produces short- and long-term modification of reflex blink gain

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1Department of Neurobiology and Behavior, Stony Brook University, Stony Brook, New York; 2Department of Psychology, Stony Brook University, Stony Brook, New York; 3SUNY Eye Institute, Stony Brook, New York; and 4Program in Neuroscience, Stony Brook University, Stony Brook, New York

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Ryan M, Kaminer J, Enmore P, Evinger C. Trigeminal high-frequency stimulation produces short- and long-term modification of reflex blink gain. J Neurophysiol 111: 888 – 895, 2014. First published November 27, 2013; doi:10.1152/jn.00667.2013.—Reflex blinks provide a model system for investigating motor learning in normal and pathological states. We investigated whether high-frequency stimulation (HFS) of the supraorbital branch of the trigeminal nerve before the R2 blink component (HFS-B) decreases reflex blink gain in alert rats. As with humans (Mao JB, Evinger C. J Neurosci 21: RC151, 2001), HFS-B significantly reduced blink size in the first hour after treatment for rats. Repeated days of HFS-B treatment produced long-term depression of blink circuits. Blink gain decreased exponentially across days, indicating a long-term depression of blink circuits. Additionally, the HFS-B protocol became more effective at depressing blink amplitude across days of treatment. This depression was not habituation, because neither short- nor short-term blink changes occurred when HFS was presented after the R2. To investigate whether gain modifications produced by HFS-B involved cerebellar networks, we trained rats in a delay eyelid conditioning paradigm using HFS-B as the unconditioned stimulus and a tone as the conditioned stimulus. As HFS-B depresses blink circuits and delay conditioning enhances blink circuit activity, occlusion should occur if they share neural networks. Rats acquiring robust eyelid conditioning did not exhibit decreases in blink gain, whereas rats developing low levels of eyelid conditioning exhibited weak, short-term reductions in blink gain. These results suggested that delay eyelid conditioning and long-term HFS-B utilize some of the same cerebellar circuits. The ability of repeated HFS-B treatment to depress trigeminal blink circuit activity long term implied that it may be a useful protocol to reduce hyper-excitability blink circuits that underlie diseases like benign essential blepharospasm.

MAMMALS SHARE a common motor control plan for trigeminal reflex blinks (Basso et al. 1993, 1996; Basso and Evinger 1996; Gnadt et al. 1997) and exhibit nearly identical patterns of muscle activity for all other types of blinking (Arumideh et al. 1994; Evinger et al. 1984, 1991; Evinger and Manning 1993; Kaminer et al. 2011). These homologies make trigeminal reflex blinks an ideal model system for investigating motor learning and its relationship with movement disorders. For example, pathological reductions in lid motility and eye irritation initiate motor learning in trigeminal blink circuits (Evinger and Manning 1988; Kassem and Evinger 2006; Schicatano et al. 2002) and eyelid dystonias appear to be exaggerations of this normally adaptive motor learning (Evinger et al. 2002; Hallett et al. 2008). It is possible to investigate these interactions between motor learning and disease states with high-frequency stimulation (HFS) of the supraorbital branch of the trigeminal nerve (SO) to create a short-term modification of reflex blink gain (Mao and Evinger 2001). The HFS paradigm identifies motor learning abnormalities in Parkinson’s disease, dystonia, and Tourette syndrome (Battaglia et al. 2006; Crupi et al. 2008; Quartarone et al. 2006; Suppa et al. 2011).

Determining how these neurological disorders disrupt HFS gain modifications requires identification of the neural bases of this form of motor learning. Short-term HFS-induced modifications occur in trigeminal blink circuits only on the side of the brain receiving HFS. For example, after HFS treatment of the left SO to increase blink gain, stimulating the left SO evokes larger than normal blinks whereas right SO stimulation elicits normal-amplitude blinks. This pattern is analogous to other blink adaptations initiated by reduced eyelid motility (Cosso et al. 1999; Evinger et al. 1989; Evinger and Manning 1988; Kassem and Evinger 2006; Manca et al. 2001; Schicatano et al. 2002; VanderWerf et al. 2007). It is unknown, however, whether HFS treatment reproduces the long-term changes in blink gain that occur with reductions in lid motility with facial palsy (Abell et al. 1998; Manca et al. 2001; VanderWerf et al. 2007). We address the long-term effects by examining the consequence of multiple days of HFS treatment on blink reflex gain. Given the role of the cerebellum in gain modifications of blinking and other motor systems (Blazquez et al. 2004; Boyden et al. 2004; Evinger et al. 1989; Hopp and Fuchs 2004; Pellegrini and Evinger 1997; Raymond et al. 1996; Scudder and McGee 2003; Soetedjo et al. 2008), we delineate some of the critical neural circuits in HFS blink modification by combining HFS treatment designed to depress blink amplitude with cerebellum-dependent delay eyelid conditioning that increases blink circuit activity (Christian and Thompson 2003; De Zeeuw and Yeo 2005; McCormick and Thompson 1984; Thompson 2005; Topka et al. 1993). We posit that if delay conditioning and HFS-induced modifications utilize overlapping cerebellar circuits, then some occlusion will occur between the two forms of learning.

MATERIALS AND METHODS

Experiments were performed on 20 male Sprague-Dawley rats (175–550 g) maintained on a reversed 12:12-h light-dark cycle and fed ad libitum. Data were collected during the rats’ subjective night. All experiments received approval by the Stony Brook University Institutional Animal Care and Use Committee and complied with all

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federal, state, and university regulations regarding the use of animals in research.

**Surgery.** Under general anesthesia (90 mg/kg ketamine and 10 mg/kg xylazine), rats were prepared for chronic recording of the orbicularis oculi (OO) EMG (OOemg) and SO stimulation (Dauvergne and Evinger 2007; Evinger et al. 1993). To record the OOemg, a pair of Teflon-coated wires (0.003-in. diameter bare, 0.0055 in. coated; no. 791000, A-M Systems, Everett, WA) with ~1 mm exposed at the tip were implanted in the OO muscle below the lateral canthus. To stimulate the SO nerve, a nerve cuff containing a pair of stainless steel wires with the insulation removed (0.003-in. diameter bare, 0.0055 in. coated; no. 791000, A-M Systems) encased in Teflon tubing (1-mm diameter; no. 163300, Small Parts, Miami, FL) was placed around the SO branch of the trigeminal nerve. Wires were led subcutaneously to a connector embedded in a dental acrylic platform on the skull. The platform was attached to the skull by four stainless steel screws. A silver wire connected to one of the stainless steel screws served as the ground. Rats received an analgesic (ketorolac, 7 mg/kg) for at least 24 h after the surgery. Rats were alert and eating within 24 h of the surgery. The experiments began at least 1 wk after the surgery.

**Stimulation paradigm.** The SO stimulus in all experiments was relative to the minimum current at which a unipolar 100-μs stimulus reliably elicited the R1 component of a reflex blink, threshold (T). This current was determined at the beginning of each day for each rat and was held constant throughout that day’s experiment. Across all subjects and days tested, the range of threshold currents was 100–900 μA, with a median of 250 μA. For each rat, threshold varied little across days. The range of coefficients of variation for thresholds across all rats was 0.025–0.35, with a mean of 0.13 ± 0.03. All data were collected at twice threshold (2T), a stimulus intensity that evoked a strong R1 response and an inconsistent and small R2 component in rats (Fig. 1A) (Basso et al. 1993; Dauvergne and Evinger 2007; Evinger et al. 1993). The HFS train was five 2T SO stimuli presented at 400 Hz.

Each day’s data collection consisted of five blocks: 1) before treatment, 2) treatment condition, 3) immediately after treatment, 4) 30 min after treatment, and 5) 60 min after treatment (Fig. 1E). Pre- and posttreatment blocks were the same for all experiments. In these blocks, rats received 20 trials of paired 2T SO stimuli with an interstimulus interval of 100 ms. Thus two blinks were evoked in each nontreatment trial. The first evoked blink was called the condition blink, and the second blink was termed the test blink. The intertrial interval for all data blocks varied pseudorandomly over the range of 20 ± 5 s. Each rat received at least 10 days of a given treatment.

**Treatment conditions.** One group (n = 11) underwent a blink motor learning paradigm to depress blink amplitude (Mao and Evinger 2001). As with the human study, rats in this treatment group received 60 trials of high-frequency SO stimulation that occurred during the R1 but before the R2 component of the blink (HFS-B). Each treatment

![Fig. 1. Treatment protocols. A: supraorbital branch of the trigeminal nerve (SO)-evoked reflex blink with R1 and R2 components. B: high-frequency stimulation (HFS) presented before R2 (HFS-B). C: HFS presented after R2 (HFS-A). D: right: HFS Cond trial, in which HFS-B coterminated with a 300-ms tone. Left: expansion of the same record to show the HFS-B stimulus. E: experimental paradigm. Triangles show a twice-threshold 100-μs supraorbital nerve stimulus. T0, T30, T60, immediately, 30 min, and 60 min after treatment.](http://jn.physiology.org/)

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trial consisted of a single SO stimulus at 2T to evoke a reflex blink followed by five 2T SO stimuli delivered at 400 Hz before the onset of the R2 component of the Ooemg activity (Fig. 1B). The latency between first SO stimulus and HFS was adjusted for each rat based on their average R2 latency.

The second group (n = 8) underwent delay eyelid conditioning (HFS Cond) in which the HFS-B stimulus served as the unconditioned stimulus (US) and a tone served as the conditioned stimulus (CS) (Fig. 1D). Three rats in the HFS Cond group also contributed to the HFS-B group data. Each of the 60 paired trials consisted of a 300-ms CS with a 40-ms rise time that coterminal with the HFS-B US. Four rats were trained with a 2-kHz tone and the other four with an 8-kHz tone. Both tones were 85 dB. Two rats in each CS group developed robust conditioning (see Fig. 4A). During the HFS Cond treatment each day, rats received 10 paired CS-US trials followed by a CS alone on the twelfth trial. We presented 6 of these 12 trial blocks each day, delivering a total of 60 CS HFS-B, 6 CS alone, and 6 SO alone trials. Thus rats in the HFS-Cond treatment received the same number of HFS-B stimuli as rats in the HFS-B alone condition.

In the third group (n = 4), HFS occurred after the R2 component of the blink reflex (HFS-A). This protocol did not modify blink amplitude in humans (Mao and Evinger 2001) and served as a control for the number of stimuli delivered in the other treatment conditions. Rats received 60 trials of stimulation in which the HFS occurred after termination of the R2 blink component (Fig. 1C). Latency between first SO blink evoking stimulus and HFS was adjusted for each rat based on the average latency and duration of the R2 component.

Data collection and analysis. Reflex and conditioned blink magnitude and timing. Goodwin et al. (1976) and Evinger et al. (1976, 1983) developed methods to measure blink amplitude and latency before and after HFS. Amplitude of blink activity was used as an index of blink gain, whereas the R2 response is inconsistent and provides very little lid closure in primates (Evinger et al. 1991; Snow and Frith 1989). In mammals other than primates, however, the R1 component provides most of the lid closure (Pellegrini et al. 1995), whereas the R2 response is weak and inconsistent (Anor et al. 1996, 2000; Basso et al. 1993; LeDoux et al. 1997; Pellegrini et al. 1995). Thus we investigated changes in R1 magnitude.

Averaged across all rats and days, HFS-B treatment significantly depressed subsequent condition R1 and test R1 responses by 27.2 ± 3.0% before HFS-B each day to the median R1 blink amplitude. The second CR was defined as Ooemg activity 5 standard deviations above the baseline Ooemg activity determined from the 100 ms of Ooemg activity prior to tone onset. Trials in which Ooemg baseline activity was disrupted by other activities, e.g., face cleaning, were not analyzed. Percent CRs was calculated as the number of trials exhibiting a CR divided by the total number of useable CS trials.

Statistical tests of significance (P < 0.05) were performed with SPSS software (SPSS, Chicago, IL) using an independent-samples t-test or a paired-samples t-test. Data are presented as means ± SE. Regressions were performed with linear, power, logarithmic, and exponential curves. The function generating the highest correlation was employed to describe the data.

RESULTS

In humans, the HFS-B condition depressed the R2 component of subsequent blinks (Mao and Evinger 2001). The R1 response, however, is not reported in this study because the R1 response is inconsistent and provides very little lid closure in primates (Evinger et al. 1991; Snow and Frith 1989). In mammals other than primates, however, the R1 component provides most of the lid closure (Pellegrini et al. 1995), whereas the R2 response is weak and inconsistent (Anor et al. 1996, 2000; Basso et al. 1993; LeDoux et al. 1997; Pellegrini et al. 1995). Thus we investigated changes in R1 magnitude. Averaged across all rats and days, HFS-B treatment significantly decreased subsequent condition R1 and test R1 responses by 27.2 ± 3.0% before HFS-B each day to the median R1 blink amplitude. The second CR was defined as Ooemg activity 5 standard deviations above the baseline Ooemg activity determined from the 100 ms of Ooemg activity prior to tone onset. Trials in which Ooemg baseline activity was disrupted by other activities, e.g., face cleaning, were not analyzed. Percent CRs was calculated as the number of trials exhibiting a CR divided by the total number of useable CS trials.

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test R1 showed a similar, but smaller, change in relative gain across days of treatment ($r^2 = 0.14$; Fig. 2C). Repeated days of HFS-B decreased the probability of an R2 occurring before HFS-B treatment ($r^2 = 0.73, P < 0.001$; Fig. 2D). Thus the decreasing R1 blink amplitude and R2 probability demonstrated that repeated HFS-B treatment reduced trigeminal drive on blink circuits. In addition, repeated HFS-B treatment made daily HFS-B treatment more effective at decreasing blink gain.

Consistent with the absence of short-term effects (Fig. 2A, right), repeated days of HFS-A treatment failed to produce long-term changes. Repeated days of HFS-A treatment did not change pretreatment amplitude relative to day 1 pretreatment blink amplitude for condition R1 ($r^2 = 0.03, P > 0.05$; Fig. 2E) or test R1 ($r^2 = 0.02, P > 0.05$; Fig. 2E). Likewise, R2 probability did not change with repeated days of HFS-A treatment ($r^2 = 0.07, P > 0.05$; Fig. 2D). There were also no consistent modifications of relative change across days for condition R1 ($r^2 = 0.03, P > 0.05$; Fig. 2F) or test R1 ($r^2 = 0.27, P > 0.05$; Fig. 2F). Indeed, the variability of the HFS-A data demonstrated that when the HFS occurred after the R2 component it produced no consistent effect on blink amplitude (Fig. 2, E and F). Thus the absence of long-term modifications with HFS-A treatment demonstrated that the long-term reductions in blink gain created by HFS-B treatment were the result of the timing of HFS relative to the R2 and not generalized habituation.

Repeated HFS-B or HFS-A treatment did not modify blink threshold (Fig. 3A). For each rat and condition, every day’s reflex blink threshold was normalized to the threshold current on the first day of treatment. These relative thresholds were averaged across rats for each day of treatment. Blink threshold did not change significantly with repeated days of HFS-B treatment ($r^2 = 0.32, P > 0.05$; Fig. 3A), indicating that the long-term changes in trigeminal blink amplitude were not the result of a change in blink threshold. Likewise, repeated HFS-A treatment did not alter blink threshold ($r^2 = 0.13, P > 0.05$; Fig. 3A). Thus the HFS-B paradigm produced both short- and long-term decrease in the gain of trigeminal reflex blinks without affecting blink threshold.

Delay eyelid conditioning (HFS Cond). The cerebellum plays a critical role in eyelid conditioning (Christian and Thompson 2003; De Zeeuw and Yeo 2005; Raymond et al. 1996; Thompson 2005) and trigeminal reflex blink adaptation (Chen and Evinger 2006; Evinger and Manning 1988; Pellegri n and Evinger 1997). If short- and long-term HFS-B depression of trigeminal reflex blinks involves some of the same cerebellar neurons responsible for delay eyelid conditioning, then some occlusion should occur when HFS-B treatment and delay eyelid conditioning are combined, because HFS-B depresses blink circuits (Fig. 2A, left) whereas eyelid conditioning increases the activity in blink circuits. If occlusion occurred between the two paradigms, then we expected that rats failing to develop eyelid conditioning would generate small gain decreases whereas rats that developed robust eyelid conditioning would not show blink gain changes with HFS Cond treatment. Using 70% CRs as a threshold for the develop-
opment of robust eyelid conditioning, we found four rats that consistently achieved >70% CRs (Fig. 4A) and four rats that failed to attain this level of conditioning (<70% CRs; Fig. 4A). Averaged across all days and rats that exhibited <70% CRs, condition R1 amplitude decreased by 11.8 ± 5.3% after HFS Cond \( t_{(49)} = 2.25, P < 0.05 \); Fig. 4B). As predicted, this decrease in condition R1 amplitude was significantly smaller than the depression produced by HFS-B treatment alone \( t_{(43)} = 3.1, P < 0.01 \). For rats that achieved >70% CRs, however, neither condition (R1C) nor test (R1T) R1 amplitude changed significantly \( t_{(50)} = 0.002, P > 0.05 \); R1C; \( t_{(50)} = 1.2, P > 0.05 \); R1T; Fig. 4B, >70%). Comparing blink depression caused by HFS-B treatment with that caused by HFS Cond treatment in the three rats that participated in both experiments reinforced the differences between the two treatments on blink gain. The blink depression with HFS-B treatment for these three rats was not different from that for rats that received HFS-B treatment alone \( [R1C, t_{(104)} = -0.6, P > 0.05]; R1T, t_{(104)} = -1.5, P > 0.05]; Fig. 4B, HFS-B*]. In the HFS Cond treatment, these rats exhibited less blink depression than they developed in the HFS-B treatment and were similar to the <70% group (Fig. 4B, HFS-Cond*). Thus combining the HFS-B stimulus with eyelid conditioning disrupted both forms of motor learning.

The long-term changes with repeated HFS Cond treatment were smaller than those with HFS-B treatment. Looked at across days of HFS Cond treatment, neither the <70% nor the >70% group exhibited a significant change in pre-HFS condition R1 amplitude (Fig. 4C; <70% \( r^2 = 0.04, P > 0.05 \); >70% \( r^2 = 0.04, P > 0.05 \)). Blink threshold also did not change across days of HFS Cond (Fig. 3B; \( r^2 = 0.28, P > 0.05 \), <70%; \( r^2 = 0.22, P > 0.05 \), >70%). There was a trend for relative change to become more negative across days of HFS Cond treatment for the <70% group (Fig. 4D; \( r^2 = 0.08, P > 0.05 \)) but to become more positive for the >70% group (Fig. 4D; \( r^2 = 0.12, P > 0.05 \)). The trend for larger relative changes across days of treatment for the <70% group was confirmed by averaging relative change across days (Fig. 4E). Averaged over the first 4 days of HFS Cond treatment there was no short-term relative change for rats in the <70% group \( [0.03 ± 0.11, t_{(15)} = -0.26, P > 0.05] \); Fig. 4E, <70%], but averaged over days 8–12 the relative change was \(-0.23 ± 0.08 [t_{(15)} = 3.0, P < 0.01] \); Fig. 4E, <70%]. Thus it took at least 8 days of HFS Cond treatment before these rats exhibited a significant short-term decrease in condition R1 blink gain. In contrast, HFS-B treatment produced a relative change in condition R1 blink gain of \(-0.21 ± 0.04 [t_{(43)} = 4.9, P < 0.001] \); Fig. 4E, HFS-B] in the first 4 days of treatment, and by days 8–12 the mean relative change was \(-0.31 ± 0.05 [t_{(23)} = 6.1, P < 0.001] \) across all of the rats receiving HFS-B. In rats that achieved >70% CRs and rats receiving HFS-A treatment, there were no significant changes in condition R1 gain in either the first 4 or the last 4 days of treatment (Fig. 4E).

Consistent with occlusion occurring between HFS-B and eyelid conditioning, rats that failed to acquire robust eyelid conditioning only slowly developed short-term gain reductions and never exhibited long-term changes and rats that acquired robust eyelid conditioning never exhibited short- or long-term reductions in blink gain.

**DISCUSSION**

HFS treatment in which the 400-Hz SO stimulus train occurs before the R2 component of a SO-evoked blink produces both short- and long-term decreases in trigeminal reflex blink amplitude (Fig. 2, Fig. 4E) independent of short- or long-term habituation (Abel et al. 1998; Ferguson et al. 1978; Hirano et al. 1996; Sanes and Ison 1983). For example, HFS-A matched for the number of HFS-B trains does not cause short (Fig. 2A)- or long (Fig. 2, E and F)-term decreases in blink amplitude. Further evidence against habituation causing the blink depression demonstrated here is that potentiation rather than habituation develops when the HFS occurs during the R2 blink component in humans (Battaglia et al. 2006; Crupi et al. 2008; Kranz et al. 2013; Mao and Evinger 2001; Quararone et al. 2006). Independent of the number of HFS trains, however, habituation can occur when the intertrial interval used to collect pre- and post-HFS data is 10 s (Zeuner et al. 2010) instead of the 20-s intertrial intervals used in this and other HFS studies. Thus the timing of the HFS relative to the R2 is critical in creating the short- and long-term decreases in blink reflex amplitude. This reduction is a modification of reflex blink gain, because blink amplitude decreases while the blink-evoking stimulus remains constant (Fig. 3A).

HFS treatment in humans (Battaglia et al. 2006; Crupi et al. 2008; Mao and Evinger 2001; Quararone et al. 2006; Suppa et al. 2011; Zeuner et al. 2010) focuses exclusively on changes in the R2 component of SO-evoked blinks (Evinger et al. 1991; Snow and Frith 1989). In this study, we primarily measured changes in the R1 component because it produces most of the lid closure in nonprimate mammals (Pellegrini et al. 1995). Our data demonstrated that the R1 showed the same pattern of gain changes as the R2 in human HFS paradigms. The demonstration that R1 and R2 responses of SO-evoked blinks arise from
HFS-B treatment, trigeminal blink drive fell exponentially, with trigeminal blink gain modification (Chen and Evinger 1997; Rambold et al. 2002; Raymond et al. 1996; Lisberger 1988; Miles and Lisberger 1981; Pellegrini et al. 1995) suggests that the blink changes created by HFS treatment reflect modifications in second-order trigeminal neurons and/or OO motoneurons. Repeated HFS-B treatment produced a cumulative depression of trigeminal reflex blink circuits. Over days of HFS-B treatment, trigeminal blink drive fell exponentially, so that a 2T SO stimulus evoked smaller blinks (Fig. 2B). HFS-B treatment also became increasingly effective at reducing blink gain over days of HFS-B treatment (Fig. 2C). Just as facial palsy creates a long-term enhancement of blink circuits (Cossu et al. 1999; Manca et al. 2001; VanderWerf et al. 2007), repeated HFS-B treatment produced a long-term depression of blink circuits. Thus repeated HFS-B treatment created long-term modifications of trigeminal blink circuits and blink gain equivalent to those caused by facial palsy.

The cerebellum plays a major role in gain modification of trigeminal reflex blinks, saccadic eye movements, and the vestibuloocular reflex (Blazquez et al. 2004; Boyden et al. 2004; Lisberger 1988; Miles and Lisberger 1981; Pellegrini and Evinger 1997; Rambold et al. 2002; Raymond et al. 1996; Robinson et al. 2002; Scudder and McGee 2003; Soetedjo and Fuchs 2006; Straube et al. 2001). The present data suggest that the cerebellum also participates in gain modification created by HFS-B treatment. The activity of interpositus neurons changes with trigeminal blink gain modification (Chen and Evinger 2006) and delay eyelid conditioning (Freeman and Nicholson 2000; Gould and Steinmetz 1996; Kim and Thompson 1997; Nicholson and Freeman 2004). If HFS-B gain modification also involves some of the same pools of interpositus neurons as delay eyelid conditioning, then competition between HFS-B treatment to depress blink circuits and delay eyelid conditioning to excite blink circuits should affect both types of learning. In one group of rats, <70%, robust conditioning did not occur, as evidenced by the inability to exhibit at least 70% CRs in 12 days of conditioning. Thus the excitatory drive created by delay conditioning competed only partially with the decreased drive of HFS-B on blink gain. This competition slowed the development of short-term changes in blink gain and prevented long-term changes (Fig. 4). Rats developing >70% CRs exhibited rates of eyelid conditioning similar to those reported in other studies of eyelid conditioning (Freeman et al. 2007; Green et al. 2000; Lee and Kim 2004; Weiss and Thompson 1991). In these rats, eyelid conditioning won the competition for neural resources, blocking short- and long-term blink depression normally produced by HFS-B treatment.

The long-term changes created by repeated days of HFS-B treatment suggest the possibility of reducing trigeminal activity in the focal dystonia benign essential blepharospasm (BEB), involving trigeminal overactivity (Hallett et al. 2008) and cerebellar abnormalities (Kerrison et al. 2003; Obermann et al. 2007). The visually disabling spasms of lid closure that characterize BEB appear to be an exaggeration of blink adaptations to dry eye or eye irritation (Evinger et al. 2002; Hallett et al. 2008) initiated in trigeminal blink circuits (Henriquez and Evinger 2007; Schicatano et al. 2002) and probably sustained by 10.220.33.6 on October 28, 2016 from http://jn.physiology.org/ Downloaded by

\[ \text{Fig. 4. A: } \% \text{ CRs as a function of days of HFS Cond for rats that achieved } <70\% \text{ CRs and rats that achieved } >70\% \text{ CRs over 12 days of HFS Cond treatment. B: average relative change in RIC and RIT blink amplitude following HFS treatment for all HFS-B rats, HFS-B treatment for the 3 rats that participated in both HFS-B and HFS Cond treatments (HFS-B*), HFS Cond treatment for the 3 rats that participated in both HFS-B and HFS Cond treatments (HFS-Cond*), and for all } <70\% \text{ and all } >70\% \text{ rats. C: mean pre-HFS blink amplitude relative to median day 1 pre-HFS blink amplitude as a function of days of HFS Cond treatment for rats that achieved } <70\% \text{ CRs and rats achieving } >70\% \text{ CRs. D: mean relative change as a function of days of HFS Cond treatment for rats in the } <70\% \text{ and } >70\% \text{ groups. Lines are best fit linear regressions. E: mean relative change over days 1–4 and days 8–12 for all rats in the HFS-B, HFS-A, <70%, and } >70\% \text{ groups. Error bars are SE. *P < 0.05, **P < 0.01, ***P < 0.001.} \]
by modifications in cerebellar activity (Evinger et al. 1989; Pellegrini and Evinger 1997). Other characteristics of BEB, photophobia and excessive blinking, also reflect exaggerated excitability of trigeminal blink circuits (Dolgonos et al. 2011; Kaminer et al. 2011). A single day of HFS-B treatment, however, does not produce significant clinical changes in lid symptoms, although patients report feeling considerably better after HFS-B (Kranz et al. 2013). The lack of a significant reduction in lid abnormalities with a single treatment may reflect the inability of one HFS-B treatment to modify cerebellar circuits significantly. Repeated HFS-B treatment, however, clearly modifies cerebellar circuits to create an exponential reduction in the activity of trigeminal blink circuits and with repetition becomes increasingly effective at short-term gain decreases. This long-term change may be effective at reducing spasms of lid closure and other trigeminal abnormalities. Because BEB patients exhibit enhanced learning in HFS paradigms (Quartarone et al. 2006), repeated HFS-B treatment may produce more long-term blink circuit depression than occurs in normal subjects.

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