Long-Term Potentiation of the Human Blink Reflex

Jian-Bin Mao and Craig Evinger

Departments of Neurobiology and Behavior and Ophthalmology, State University of New York at Stony Brook, Stony Brook, New York 11794-5230

The trigeminal reflex blink is an ideal system to investigate whether stimulus paradigms that produce long-term potentiation (LTP) *in vitro* modify motor learning in humans. Presentation of 12 trains of low-intensity, high-frequency stimuli (HFS) to the supraorbital branch of the trigeminal nerve (SO) modified subsequent reflex blinks of human subjects. When HFS occurred concurrently with reflex blinks, the procedure potentiated subsequent blinks for >1 hr. Combining HFS with feedback from the lid movement was critical for this facilitation because presenting HFS immediately after the blink did not alter subsequent blinks. When HFS preceded the blink, however, this treatment suppressed subsequent blinks for 30 min. These effects appear to occur within the trigeminal reflex blink circuits rather than at motoneurons, because stimulation of the previously HFS-treated SO evoked altered blinks in both eyelids, whereas stimulation of the untreated SO elicited unaltered blinks in both eyelids. The modified blink amplitude resulted from altering the response to A-fiber inputs to the trigeminal nerve because all stimuli were too weak to activate C-fibers. The data suggest that HFS produce LTP- and long-term depression (LTD)-like effects on wide dynamic range neurons in the trigeminal reflex blink circuit. The data also support the hypothesis that LTP and LTD mechanisms play a role in adaptive modification of human reflex blinks.

Key words: LTP; LTD; blinking; wide dynamic range neurons; adaptive gain control; trigeminal

Wide dynamic range (WDR) neurons are key elements of reflex circuits. These neurons respond to both noxious, C-fiber and innocuous, A-fiber stimuli. Both the A- and C-fiber inputs elicit reflexes. For example, stimulation of the A-fibers of the supraorbital branch of the trigeminal nerve (SO) evokes reflex blinks by activating WDR neurons (Pellegrini et al., 1995; Ellrich and Treede, 1998). Wide dynamic range neurons also participate in the limb withdrawal reflex elicited by C-fiber stimulation (Schouenborg and Sjölund, 1983; Schouenborg et al., 1995; Morgan, 1998). Wide dynamic range neurons exhibit different forms of plasticity for C- and A-fiber inputs. Windup in WDR neurons is an example of short-lasting modification specific to C-fibers. Presentation of 8–16 repetitive electrical stimuli intense enough to activate C-fibers at a frequency of 0.5–3 Hz facilitates the response of WDR neurons to C-fiber stimuli for 1–5 min without altering the response of the neuron to innocuous A-fiber stimuli (Mendell and Wall, 1965; Herrero et al., 2000). Wide dynamic range neurons also appear to support long-term potentiation (LTP) for both A- and C-fiber inputs. Presentation of 20 2 sec, 100 Hz suprathreshold C-fiber stimulus trains facilitates the response of WDR neurons to A- and C-fiber stimuli for >1 hr if the animal is not paralyzed (Svendsen et al., 1997, 1998). Paralysis, however, prevents the development of A-fiber potentiation in WDR neurons (Svendsen et al., 1997). These observations reveal that WDR neurons can support A-fiber as well as C-fiber plasticity.

The observation of Svendsen et al. (1997) suggests that combining feedback from the muscle contraction with WDR neuronal depolarization rather than combining muscle feedback with C-fiber activation may be the critical component producing A-fiber potentiation in WDR neurons. This hypothesis makes two strong predictions. First, because the only requirement for producing A-fiber plasticity is combining WDR neuron depolarization with muscle feedback, A-fiber activation alone should be sufficient to modify the response of WDR neurons to A-fiber inputs. Second, the coincidence of movement feedback and neuronal depolarization should be critical in potentiating WDR neurons. Human trigeminal reflex blinks fulfill the requirements to test these predictions about A-fiber input plasticity. First, electrical stimulation of the SO that only activates A-fibers evokes a reflex blink (Pellegrini et al., 1995; Ellrich and Treede, 1998). Second, feedback from the lid movement enters the blink circuit through the SO nerve (Evinger et al., 1989; Pellegrini and Evinger, 1997). Third, WDR neurons are part of the trigeminal reflex blink circuit (Ellrich and Treede, 1998). Moreover, because movement feedback begins ~40 msec after a blink evoking SO stimulus, it is possible to vary

Received Jan. 10, 2001; revised April 4, 2001; accepted April 5, 2001.

This work was supported by National Eye Institute Grant EY07391 to C.E. We thank V. M. Henriquez and A. S. Powers for their valuable comments on earlier versions of this manuscript.

Correspondence should be addressed to Craig Evinger, Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, NY 11794-5230. E-mail: levinger@notes.cc.sunysb.edu.

Copyright © 2001 Society for Neuroscience 0270-6474/01/210001-04$15.00/0

This article is published in *The Journal of Neuroscience*, Rapid Communications Section, which publishes brief, peer-reviewed papers online, not in print. Rapid Communications are posted online approximately one month earlier than they would appear if printed. They are listed in the Table of Contents of the next open issue of JNeurosci. Cite this article as: JNeurosci, 2001, 21:RC151 (1–4). The publication date is the date of posting online at www.jneurosci.org.

http://www.jneurosci.org/cgi/content/full/5354

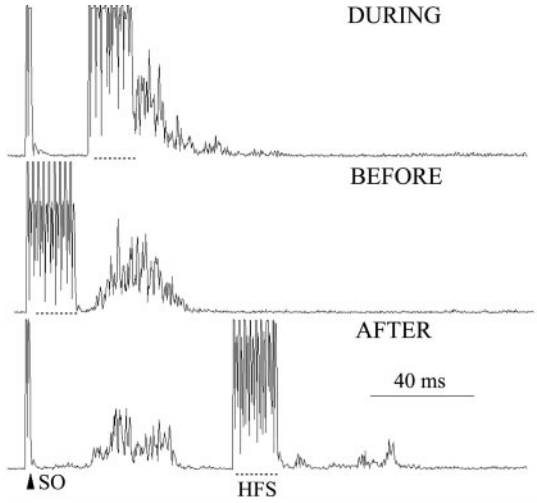

Figure 1. Example of the three treatment conditions from one subject. The 2T supraorbital nerve stimulus (▲) evoked a blink, and HFS, 2T SO stimuli (*dashed line*) took place at different times relative to the R2 OOemg activity. In the *DURING* condition, a single SO stimulus evoked a blink, and HFS occurred at the onset of OOemg activity. In the *BEFORE* condition, HFS preceded the reflex blink. In the *AFTER* condition, a single SO evoked a reflex blink, and the HFS were delivered after the OOemg activity. Each *trace* is the average of four rectified OOemg responses from the first treatment block.

the timing between WDR neuronal depolarization and movement feedback.

MATERIALS AND METHODS

All experiments were conducted in accordance with federal, New York state, and university regulations regarding the use of human subjects. Bilateral stimulation of the SO and recording of the orbicularis oculi electromyogram (OOemg) were performed on three female and two male human subjects without eye or eyelid disorders. As detailed previously (Evinger et al., 1991), pairs of gold-plated electrodes were affixed over each SO, and another pair were taped to each lower eyelid. Unilateral SO nerve stimulation can evoke three components of OOemg activity in humans. Supraorbital nerve stimulation just greater than the threshold for perceiving the stimulus evokes a bilateral, long-latency response (R2) (Figure 1, *AFTER*) that occurs ~40 msec after the SO stimulus. In addition to the bilateral R2, higher intensity SO stimuli evoke a short-latency, unilateral R1 component in the eyelid ipsilateral to the SO stimulus (data not shown). Pain sensations evoked by SO stimulation occur only at stimulus intensities five times greater than the threshold intensity for eliciting the R2 component (Ellrich and Treede, 1998). At these stimulus intensities, the activated C-fibers in the SO nerve elicit a bilateral, R3 component with an 84 msec latency (Ellrich and Hopf, 1996). In the current experiments, the minimum intensity for a 170 μsec stimulus required to evoke a reliable R2 reflex blink component was determined for each SO. For all experiments, stimulus intensity was set at two times this threshold (2T) to evoke an R2 response. There were no indications of C-fiber activation by SO stimuli in the current experiments.

Each treatment consisted of three blocks of high-frequency SO stimuli (HFS) with a 5 min interblock interval. Each block consisted of four HFS trains (nine 2T SO stimuli, 400 Hz) separated by 10 sec (Fig. 1). With the DURING treatment, a single 2T SO stimulus evoked a blink, and the HFS began near the onset of OOemg activity. With the BEFORE treatment, the HFS terminated before the onset of OOemg activity. With the AFTER treatment, a single 2T SO stimulus evoked a blink, and the HFS occurred after the OOemg activity. In the CONTROL condition, the subject did not receive any SO stimuli for a period equal to the duration of the treatment.

Each experiment consisted of five blocks: (1) pretreatment; (2) one of the three treatments or the CONTROL condition; (3) immediately after treatment; (4) 30 min after treatment; and (5) 60 min after treatment. For each of the 30-trial, nontreatment blocks, a pair of identical, 2T SO stimuli with a 750 msec interstimulus interval were presented alternately to the left and right SO every 25 ± 5 sec. Each subject participated in all four conditions with at least 72 hrs between each experiment. OOemg records were amplified, filtered (1–5 kHz), digitized (4 kHz/channel), and stored for off-line analysis. OOemg amplitude was calculated by integrating the rectified OOemg response using laboratory-written software. For each data block, the median of the integrated OOemg amplitude was calculated for each eyelid and normalized to the median blink amplitude of that eyelid pretreatment. For each treatment condition, statistical significance was tested with a one-way repeated-measure ANOVA. In cases of significance, Dunnett's treatment versus control test was conducted to compare each post-treatment block with the pretreatment block.

RESULTS

High-frequency stimulation modified the blinks evoked by subsequent 2T SO stimuli (Figure 2). To determine whether these modifications occurred at the motoneurons ipsilateral to the treated SO or at circuit elements within the trigeminal complex, the response of the same eyelid evoked by stimulation of the treated SO was compared with that evoked by the untreated SO. If HFS modified motoneurons, then the motoneurons should exhibit modified responses to stimulation of either SO nerve. In contrast, if modifications occurred within the trigeminal complex, stimulation of the treated SO should modify responses in both eyelids, whereas responses evoked by stimulation of the untreated SO should be unchanged.

The data revealed that modifications occurred within trigeminal circuits rather than from generalized excitability changes at the motoneurons. For example, HFS delivered during the blink potentiated subsequent blinks evoked by stimulation of the treated, but not the untreated, SO nerve (Fig. 2, *DURING*). For each eyelid, the normalized R2 amplitude evoked by stimulation of the untreated SO was subtracted from the normalized R2 amplitude evoked by the treated SO. For the DURING condition, HFS significantly increased the difference between the responses of the eyelid ipsilateral to the treated SO evoked by treated and untreated SO stimulation ($F_{(4,12)} = 8.32$; $p < 0.01$). Relative to pretreatment, this increase was 33 ± 14% ($p < 0.01$), 29 ± 6% ($p < 0.01$), and 18 ± 6% ($p < 0.05$) immediately, 30 min, and 60 min after HFS, respectively (Fig. 3, ■). For the eyelid contralateral to the treated SO, the difference between the responses to untreated and treated SO stimuli increased 10–17% relative to pretreatment levels, but this potentiation did not achieve statistical significance ($F_{(4,12)} = 0.91$; $p > 0.05$).

The effect of HFS also depended on the relative timing between HFS and feedback from the eyelid movement. HFS delivered BEFORE the onset of OOemg activity decreased the amplitude of blinks evoked by subsequent stimulation of the treated, but not the untreated, SO (Fig. 2, *BEFORE*). For the eyelid contralateral to the treated SO, the difference in blink amplitude evoked by stimulation of the treated and untreated SO decreased significantly ($F_{(4,12)} = 4.66$; $p < 0.05$). This decrease was 30 ± 3% ($p < 0.05$), 20 ± 13% ($p > 0.05$), and 42 ± 17% ($p < 0.01$) immediately, 30 min, and 60 min after HFS, respectively. For the eyelid ipsilateral to the treated SO, the difference in R2 amplitude evoked by treated and untreated SO stimuli approached significance ($F_{(4,12)} = 2.8$; $p < 0.08$), but achieved significance only at 30 min after HFS (−27 ± 11%; $p < 0.05$) (Fig. 3, ▲). Neither presentation of the HFS AFTER the OOemg response nor the CONTROL condition altered the response to subsequent SO stimuli (Fig. 2, *AFTER*, *CONTROL*). There were no significant differences between blinks evoked before and after the AFTER (ipsilateral eyelid, $F_{(4,12)} = 0.99$; contralateral eyelid, $F_{(4,12)} = 0.83$; $p > 0.05$) (Fig. 3, ●) or CONTROL (ipsilateral eyelid,

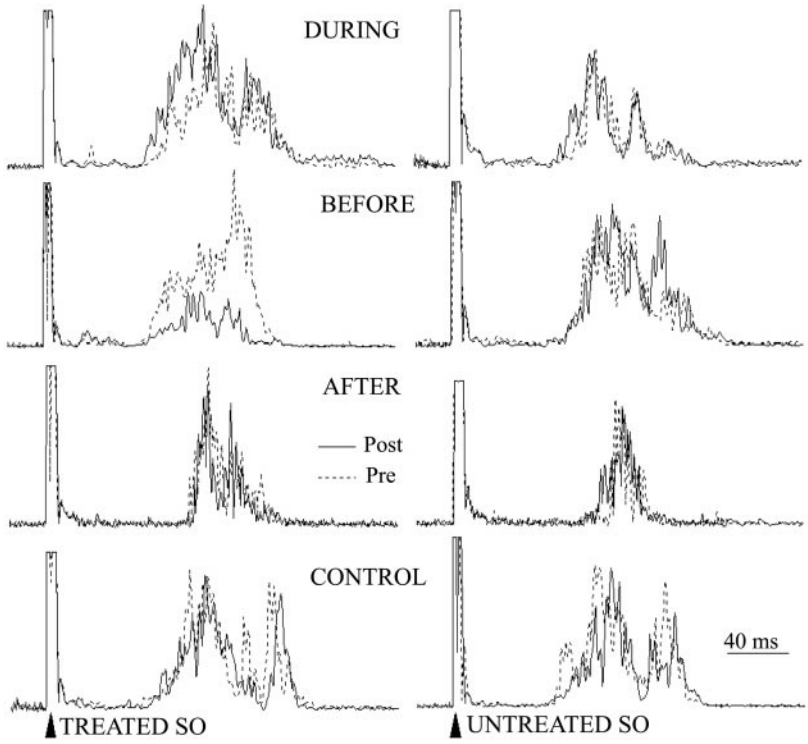

Figure 2. Shown is the average of five rectified blinks from the eyelid ipsilateral to the treated SO before treatment (*Pre*, *dotted line*) and five blinks immediately after treatment (*Post*, *solid line*) for one subject evoked by stimulation of the treated (*TREATED SO*) and untreated (*UNTREATED SO*) supraorbital nerve.

$F_{(4,12)} = 0.34$; contralateral eyelid, $F_{(4,12)} = 0.33$; $p > 0.05$) (Fig. 3, ♦) conditions. Thus, HFS that activated only A-fibers modified reflex magnitude, and the polarity of this modification depended critically on the timing between movement feedback and trigeminal neuron depolarization produced by HFS.

DISCUSSION

The results demonstrate that A-fiber HFS to the SO nerve produce LTP- and long-term depression (LTD)-like effects on human trigeminal reflex blinks. This blink reflex modification is not a generalized excitability alteration of orbicularis oculi motoneurons. After HFS treatment, stimulation of the treated SO elicits modified blinks in both eyelids, whereas stimulation of the untreated SO evokes unmodified blinks in both eyelids (Fig. 2), nor is the altered blink response a generalized reaction to HFS. With the three treatments, the same HFS increased blink amplitude with the DURING treatment, decreased it with the BEFORE treatment, and had no effect on blink amplitude with the AFTER treatment. Blink modification produced by A-fiber HFS most likely results from synaptic changes of trigeminal neurons experiencing modified movement feedback caused by HFS. These blink modifications may enable the nervous system to regularize the relationship between stimulus intensity and neuronal depolarization for individual neurons and to modify the gain of the blink reflex.

A single neuron receiving a feedback signal proportional to blink magnitude in addition to the input evoking a blink can use the feedback input to regulate the synaptic strength of the blink-evoking input. Normally, the amount of trigeminal neuron depolarization initiated by a blink-evoking stimulus should correlate with the amount of movement feedback. For example, a strong SO stimulus will evoke a large blink that will produce substantial movement feedback. A small SO stimulus will evoke a smaller blink that will create less feedback. Mismatches between movement feedback strength and neural depolarization produced by the blink-evoking stimulus could generate LTP- and LTD-like processes to modify the synaptic strength of the input eliciting the blink.

The current data support the hypothesis that combining HFS with movement feedback produces LTP- and LTD-like phenomena for the blink-evoking input to trigeminal blink reflex neurons. With the DURING treatment, repeated summation of trigeminal neuron depolarization generated by the initial SO stimulus with the increase in the feedback from the eyelid movement created by the addition of HFS could produce sufficient depolarization to allow Ca^{2+} influx to cause LTP (Artola et al., 1990; Mulkey and Malenka, 1992; Hansel et al., 1997). Because no summation of

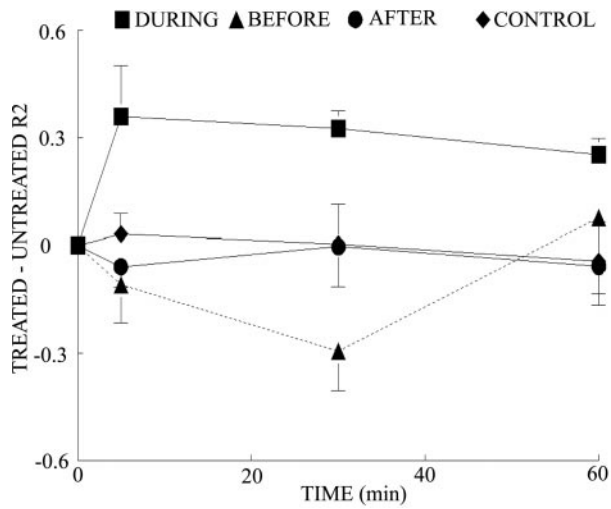

Figure 3. Normalized R2 amplitudes of the eyelid ipsilateral to the treated SO. Data are presented as the difference of R2 amplitudes evoked by stimulation of the treated and untreated SO as a function of the time after *DURING* (■), *BEFORE* (▲), *AFTER* (●) treatments and *CONTROL* (♦) condition. Error bars are SEM.

trigeminal feedback and HFS occurs with the AFTER treatment, there is no drive to alter A-fiber synaptic input that evokes a blink. The decrease in blink magnitude after the BEFORE treatment would occur because this condition functionally reduces the blink feedback input to trigeminal neurons. A blink-evoking trigeminal stimulus normally reduces the sensitivity of the trigeminal system to subsequent trigeminal stimuli (Pellegrini and Evinger, 1995; Powers et al., 1997). This decreased sensitivity to trigeminal stimuli is apparent in the inability of HFS to evoke a blink in the AFTER condition (Fig. 1, *AFTER*). With the BEFORE treatment, the HFS preceding the blink transiently decreases the amount of trigeminal feedback from the eyelid movement during neuronal depolarization. In this decreased movement feedback condition, reduced Ca^{2+} influx could generate LTD (Artola et al., 1990; Mulkey and Malenka, 1992; Hansel et al., 1997; Aizenman et al., 1998). Thus, temporal interactions between HFS and feedback from the lid movement appear to instigate LTP- and LTD-like mechanisms in neurons within the trigeminal reflex blink circuits.

These LTP- and LTD-like mechanisms can ensure that all neurons generating the blink reflex exhibit a similar relationship between stimulus magnitude and neural activity. Because the sensory feedback from the lid movement reflects the summed activity of all the neurons generating the blink, the feedback that each neuron receives should be proportional to the amount of neuronal depolarization producing the blink. This situation does not occur, however, for a neuron in the population that possesses a significantly different relationship between stimulus magnitude and neural activity than do the other neurons in the circuit. For example, because the feedback signal returning to a neuron reflects the summed activity of all the neurons in the circuit, if a specific stimulus magnitude evokes significantly less depolarization of an individual neuron than that shown by the other cells in the population, then feedback will be larger than expected for the depolarization of that neuron. For this neuron, the increased feedback is equivalent to the experience of the entire population of blink neurons with the DURING treatment. LTP-like mechanisms should increase the synaptic strength of the A-fiber blink-evoking input to this neuron. Conversely, a neuron that exhibits more neural activity to a stimulus than the other neurons in the population will experience less sensory feedback than appropriate for its depolarization. By resolving mismatches between stimulus intensity and movement feedback, the nervous system could use sensory feedback to calibrate the response of each neuron to A-fiber blink-evoking stimuli.

Wide dynamic range neurons of the trigeminal blink circuits could support these LTP-like synaptic modifications. Svendsen et al. (1997) demonstrated that C-fiber intensity HFS facilitates the response of WDR neurons to A-fiber inputs when movement occurs and that this potentiation lasts >1 hr. The current data indicate that WDR neurons potentiate their response to A-fiber inputs without activating C-fibers when movement feedback sums with increased neuronal depolarization produced by HFS activation of A-fibers. Wide dynamic range neurons also appear to depress their response to A-fiber inputs when feedback is reduced relative to the amount of depolarization generated in producing the blink.

In addition to stabilizing the relationship between stimulus magnitude and neural discharge for individual neurons, the LTP and LTD mechanisms of WDR neurons may contribute to adaptive gain modification of reflex blinks. Under normal circumstances, trigeminal reflex blinks exhibit a stable relationship between stimulus intensity and neuronal discharge and consequent OOemg magnitude, i.e., a constant gain. Making it more difficult to blink by reducing lid motility, however, initiates a rapid, compensatory increase in blink gain so that each trigeminal stimulus evokes a larger OOemg response (Evinger and Manning, 1988; Evinger et al., 1989; Pellegrini and Evinger, 1997). These adaptive gain processes depend on feedback from the eyelid movement. Eliminating feedback from the lid movement not only prevents increases in adaptive gain; it also decreases blink magnitude (Evinger et al., 1989). This decrease corresponds to the blink amplitude decrease caused by the BEFORE treatment. One mechanism for rapidly altering the gain of the stimulus magnitude and neuronal discharge relationship is to modify the synaptic strength of the feedback input rather than the strength of the blink-evoking input. Because the cerebellum is critical for these adaptive gain modifications, this structure could play a role in modifying the synaptic strength of the feedback input. Thus, the current studies support the hypothesis that adaptive processes in human blink circuits use LTP- and LTD-like mechanisms to maintain the appropriate relationship between stimulus intensity and blink magnitude.

REFERENCES

Aizenman CD, Manis PB, Linden DJ (1998) Polarity of long-term synaptic gain change is related to postsynaptic spike firing at a cerebellar inhibitory synapse. Neuron 21:827–835.

Artola A, Bröcher S, Singer W (1990) Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. Nature 347:69–72.

Ellrich J, Hopf HC (1996) The R3-component of the blink reflex: normative data and application in spinal lesions. Electroencephalogr Clin Neurophysiol 101:349–354.

Ellrich J, Treede R-D (1998) Characterization of blink reflex interneurons by activation of diffuse noxious inhibitory controls in man. Brain Res 803:161–168.

Evinger C, Manning KA (1988) A model system for motor leaning: adaptive gain control of the blink reflex. Exp Brain Res 70:527–538.

Evinger C, Pellegrini JJ, Manning KA (1989) Adaptive gain modification of the blink reflex: a model system for investigating the physiological bases of motor learning. In: Modulation of defined neural circuits (Davis M, Jacobs BL, Schoenfeld RL, eds), pp 87–100. New York: Ann NY Acad Sci.

Evinger C, Manning KA, Sibony PA (1991) Eyelid movements: mechanisms and normal data. Invest Ophthalmol Vis Sci 32:387–400.

Hansel C, Artola A, Singer W (1997) Relation between dendritic Ca^{2+} levels and the polarity of synaptic long-term modifications in rat visual cortex neurons. Eur J Neurosci 9:2309–2322.

Herrero JF, Laird JMA, Lopez-Garcia JA (2000) Wind-up of spinal cordJMA neurons and pain sensation: much ado about something? Prog Neurobiol 61:169–203.

Mendell L, Wall PD (1965) Responses of single dorsal cells to peripheral cutaneous unmyelinated fibers. Nature 206:97–99.

Morgan MM (1998) Direct comparison of heat-evoked activity of nociceptive neurons in the dorsal horn with the hindpaw withdrawal reflex in rat. J Neurophysiol 79:174–180.

Mulkey RM, Malenka RC (1992) Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

Pellegrini JJ, Evinger C (1997) Role of the cerebellum in adaptive modification of reflex blinks. Learn Memory 3:77–87.

Pellegrini JJ, Horn AKE, Evinger C (1995) The trigeminally evoked blink reflex: I. Neuronal circuits. Exp Brain Res 107:166–180.

Powers AS, Schicatano EJ, Basso MA, Evinger C (1997) To blink or not to blink: inhibition and facilitation of reflex blinks. Exp Brain Res 113:283–290.

Schouenborg J, Sjölund BH (1983) Activity evoked by A- and C-afferent fibers in rat dorsal horn neurons and its relation to a flexion reflex. J Neurophysiol 50:1108–1121.

Schouenborg J, Weng H-R, Kallomäki J, Holmberg H (1995) A survey of spinal dorsal horn neurons encoding the spatial organization of withdrawal reflexes in the rat. Exp Brain Res 106:19–27.

Svendsen F, Tjølsen A, Hole K (1997) LTP of spinal Aβ and C-fibre evoked responses after electrical sciatic nerve stimulation. NeuroReport 8:3427–3430.

Svendsen F, Tjølsen A, Hole K (1998) AMPA and NMDA receptor-dependent spinal LTP after nociceptive titanic stimulation. NeuroReport 9:1185–1190.