

Neuronal Correlates of Conditioned Inhibition of the Eyeblink Response in the Anterior Interpositus Nucleus

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Conditioned inhibition (CI) of the rat eyeblink response and the neuronal correlates of CI in the cerebellar interpositus nucleus were examined in the present study. In Experiment 1, CI was established with a novel, 3-group design. In Experiment 2, neuronal activity in the anterior interpositus nucleus was recorded during CI training and testing. Each rat was given 2 training phases and then tested for CI with summation and retardation tests. Rats given CI training showed behavioral inhibition compared with rats in 2 control groups. Neuronal activity in the anterior interpositus nucleus correlated with behavioral responding during discrimination training and during the summation test. The results suggest that neurons in the cerebellar anterior interpositus nucleus may participate in the acquisition or expression of CI.

Much progress has been made toward delineating the neural systems and processes underlying simple excitatory classical conditioning (Alkon, 1984; Davis, 1992; Disterhoft, Coulter, & Alkon, 1986; Fanselow & LeDoux, 1999; Kapp, Wilson, Pascoe, Supple, & Whalen, 1990; Moyer, Thompson, & Disterhoft, 1996; Schneiderman et al., 1987; Thompson & Krupa, 1994). Identifying the neural systems responsible for inhibitory classical conditioning has proved to be less successful. The scarcity of evidence regarding the neural substrates of inhibitory learning is surprising, given its importance in influential models of associative learning (Pearce & Hall, 1980; Rescorla & Wagner, 1972; Wagner & Larew, 1985). The most clearly defined form of inhibitory classical conditioning is conditioned inhibition (CI).

The nature of CI has been studied since Pavlov's initial experiments (Konorski, 1948; Marchant, Mis, & Moore, 1972; Pavlov, 1927; Rescorla, 1969). A four-phase procedure can be used for establishing CI, consisting of two training phases and two testing phases. In Phase 1 of training, the subject receives pairings of a conditioned stimulus (CS_A) and an unconditioned stimulus (US) until some criterion level of responding is reached. In Phase 2 of CI, subjects receive feature-negative discrimination training. Feature-negative discrimination training uses two different types of trials. On half of the trials, CS_B is paired with the US. On the other half of the trials, CS_B is presented in compound with CS_X and is never paired with the US. After reaching a criterion level of discrimination, the subject proceeds to the two testing phases: summation and retardation. Phase 3 is the summation test, which

consists of two trial types: CS_A -alone trials, and CS_A - CS_X compound trials. If CS_X has been established as a conditioned inhibitor, then the subject should exhibit fewer conditioned responses (CRs) to the CS_A - CS_X compound than to CS_A alone. Phase 4, the retardation test, consists of CS_X -US pairings in order to establish the putative conditioned inhibitor as a conditioned excitor. Slower acquisition of excitatory CRs during CS_X -US pairings indicates that CS_X was established as a conditioned inhibitor during feature-negative discrimination training (Phase 2).

A recent series of studies has identified some of the circuitry necessary for CI of the fear-potentiated startle response in rats (Falls & Davis, 1997). Lesions of the central nucleus of the amygdala (Falls & Davis, 1995), perirhinal cortex (Falls, Bakken, & Heldt, 1997), or medial prefrontal cortex (Gewirtz, Falls, & Davis, 1997) did not affect CI. However, posttraining lesions of the superior colliculus (SC; Waddell, Pistell, Heldt, & Falls, 2000), or the medial geniculate body (MG; Heldt & Falls, 1998; Heldt, Falls, & Coover, 2000) interfered with feature-negative discrimination. Neurons in the MG and SC possess firing characteristics that could play a role in CI. Learning-related plasticity in the MG and other auditory areas has been demonstrated during feature-negative discrimination (McIntosh & Gonzalez-Lima, 1993, 1994, 1995, 1998), discriminative avoidance conditioning (Gabriel, 1993; Gabriel, Saltwick, & Miller, 1975; Poremba & Gabriel, 2001), and classical conditioning (O'Connor, Allison, Rosenfield, & Moore, 1997; Ryugo & Weinberger, 1978; Weinberger, Imig, & Lippe, 1972). Neurons in the SC are capable of polymodal processing (Meredith & Stein, 1985), and some SC neurons respond more strongly to polymodal stimuli than to unimodal stimuli (Cooper, Miya, & Mizumori, 1998). The MG and SC are not necessary for fear-potentiated startle (Heldt et al., 2000; Meloni & Davis, 1999; Waddell et al., 2000) but appear to be necessary for CI of fear-potentiated startle. These findings suggest that the MG and SC receive relevant unimodal and polymodal stimulus information and are capable of learning-related neuronal plasticity that may act on the neural system expressing conditioned fear during presentations of the conditioned inhibitor. However, it is unknown whether the amygdala, which is critical for conditioned excitation, but not inhibition, of the fear-potentiated startle response (Falls &

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Davis, 1995), demonstrates differential activity during feature-negative discrimination.

Current theories regarding the neural basis of CI posit that the conditioned inhibitor influences the behavioral response system in one of three ways (Britton & Farley, 1999; Falls & Davis, 1995, 1997; Freeman & Nicholson, 1999; Mis, 1977): (a) CI is produced by acting on the neural pathways responsible for expression of the behavioral response (output pathway), (b) CI is produced by acting on the neural pathways responsible for CS input to the neural system underlying learning-related plasticity (input pathway), or (c) CI is produced in the same neural system underlying the memory formation for conditioned excitation of a behavioral response.

Studying CI of the classically conditioned eyeblink response provides a unique opportunity to examine the mechanisms of behavioral inhibition because the neural circuitry underlying excitatory eyeblink conditioning has been characterized extensively (Kim & Thompson, 1997; Lavond, Kim, & Thompson, 1993; Thompson & Krupa, 1994). A detailed analysis of neuronal activity within the excitatory eyeblink circuitry during CI will aid in the evaluation of the three possible mechanisms underlying CI (Freeman & Nicholson, 1999). The cerebellum and brainstem are essential components underlying the eyeblink CR (Kim & Thompson, 1997; Lavond et al., 1993; Thompson & Krupa, 1994). The CS pathway includes mossy fiber projections from the pontine nuclei to the cerebellar cortex and deep nuclei (Bao, Chen, & Thompson, 2000; Gould, Sears, & Steinmetz, 1993; Lewis, LoTurco, & Solomon, 1987; Solomon, Lewis, LoTurco, Steinmetz, & Thompson, 1986; Steinmetz, 1990; Steinmetz et al., 1987; Steinmetz, Lavond, & Thompson, 1985, 1989; Steinmetz, Rosen, Chapman, Lavond, & Thompson, 1986; Tracy, Thompson, Krupa, & Thompson, 1998). The US pathway includes climbing fiber projections from the inferior olive to the cerebellar cortex and deep nuclei (De Zeeuw, Van Alphen, Hawkins, & Ruigrok, 1997; Gould et al., 1993; Mauk, Steinmetz, & Thompson, 1986; McCormick, Steinmetz, & Thompson, 1985; Mintz, Lavond, Zhang, Yun, & Thompson, 1994; Steinmetz et al., 1989; Yeo, Hardiman, & Glickstein, 1986). Learning-related plasticity occurs in both the deep cerebellar nuclei and the cerebellar cortex (Freeman, Scharenberg, Olds, & Schreurs, 1998; Gould & Steinmetz, 1996; Gruart & Yeo, 1995; Hardiman, Ramnani, & Yeo, 1996; Katz & Steinmetz, 1997; McCormick & Thompson, 1984b; Schreurs, 2000; Schreurs, Gusev, Tomsic, Alkon, & Shi, 1998; Schreurs, Sanchez-Andres, & Alkon, 1991; Schreurs, Tomsic, Gusev, & Alkon, 1997; Steinmetz, Lavond, Ivkovich, Logan, & Thompson, 1992), which some theories of cerebellar function posit to be crucial for memory formation (Mauk, 1997; Mauk & Donegan, 1997; Raymond, Lisberger, & Mauk, 1996; Yeo & Hesslow, 1998). The CR pathway includes cerebellar efferents through the superior cerebellar peduncle to the red nucleus (Chapman, Steinmetz, Sears, & Thompson, 1990; Clark & Lavond, 1993; Krupa & Thompson, 1995; Krupa, Thompson, & Thompson, 1993; McCormick, Guyer, & Thompson, 1982; Rosenfield & Moore, 1983) and efferents from the red nucleus to the facial motor nuclei (Krupa, Weng, & Thompson, 1996). The extensive characterization of the neural and behavioral components of excitatory eyeblink conditioning makes it an ideal preparation for the analysis of dynamic interactions within a neural system (Freeman & Nicholson, 2000; Gormezano, Kehoe, & Marshall, 1983; Nicholson & Freeman, 2000a; Stanton, 2000;

Woodruff-Pak & Steinmetz, 2000) and facilitates the interpretation of results from neural studies of CI (Blazis & Moore, 1991; Freeman & Nicholson, 1999; Mis, 1977) by providing a framework for identifying specific mechanisms of inhibitory learning within the eyeblink conditioning circuitry.

Studies using the eyeblink response have had mixed success in identifying brain structures necessary for acquisition and expression of CI. The lateral septum has been shown to exhibit neural correlates of CI of some behaviors (Yadin & Thomas, 1981). However, hippocampal lesions (Solomon, 1977) or neocortical lesions (Moore, Yeo, Oakley, & Russell, 1980; Yeo, Hardiman, Moore, & Russell, 1983) did not abolish retention or acquisition of eyeblink CI. Stimulation and lesion studies have indicated that several brainstem nuclei, including the nucleus of Darkschewitsch, the interstitial nucleus of Cajal, and the anterior red nucleus, play transient roles in the acquisition and expression of CI (Berthier & Moore, 1980; Blazis & Moore, 1991; Mis, 1977).

Recently, Freeman and Nicholson (1999) reported that the activity of neurons in the anterior interpositus nucleus and lateral pontine nuclei mirrors the behavioral discrimination observed during feature-negative discrimination training of the eyeblink CR. It was suggested that the "neuronal discrimination" may be a critical factor in suppressing CRs to the inhibitory compound CS. However, in the Freeman and Nicholson (1999) study, it was not clear whether the discriminative cerebellar neuronal activity was attributable to the absence of a behavioral response or active suppression of cerebellar neurons during presentations of the inhibitory compound CS (e.g., fewer CRs during inhibitory trials will lead to a lower mean neuronal response). In addition, because Freeman and Nicholson (1999) did not include a summation test, it was unclear whether the conditioned inhibitor would modulate cerebellar neuronal activity to a previously conditioned tone CS during presentations of the inhibitory compound, or whether the conditioned inhibitor alone would modulate neuronal activity relative to baseline. The presence of neural correlates during CI training and testing would indicate that plasticity within the interpositus nucleus may be involved in the acquisition and expression of CI of the eyeblink CR. However, the absence of neural correlates during CI training and testing would indicate that CI may be produced by some other neural system (i.e., not the brainstem-cerebellum eyeblink circuit).

The present study used eyeblink classical conditioning to investigate the neural correlates of CI in the cerebellar interpositus nucleus. In Experiment 1, three groups of rats received feature-negative discrimination training with a four-phase procedure designed to establish a light CS as a Pavlovian conditioned inhibitor (Rescorla, 1969). Feature-negative discrimination has been shown to produce CI during eyeblink conditioning in rabbits (Marchant et al., 1972), but it has not been as extensively tested in rats. In Experiment 2, neuronal activity was recorded in the cerebellar interpositus nucleus during CI training and testing.

Experiment 1

The feature-negative discrimination procedure used in a previous experiment from our laboratory (Freeman & Nicholson, 1999) has been shown to produce CI of the eyeblink response in rabbits (Marchant et al., 1972). However, CI of the eyeblink response in rats has not been as extensively tested. This experiment was

designed to determine whether the feature-negative discrimination training in the present study's four-phase procedure established the light CS as a conditioned inhibitor in the experimental group (Group CI; see Table 1). Control Group 1 (CTL1) was used to control for inhibition caused by presenting a CS (CS_X) in a nonreinforced compound that did not include a reinforced CS (see Table 1). That is, CTL1 controlled for any inhibitory value that the light may have acquired by being an element in a nonreinforced compound stimulus during standard differential conditioning. Control Group 2 (CTL2) was used to control for any differential inhibition caused by presenting a CS (CS_X) that was never reinforced and never presented in compound with a previously reinforced CS (see Table 1).

Method

Subjects. Subjects were 36 male Long-Evans rats (250–350 g). The rats were housed in the animal colony in Spence Laboratories at the University of Iowa. All rats were maintained on a 12-hr light–dark cycle with light onset at 6:30 a.m. and were given ad lib access to food and water.

Surgery. One week before training, rats were removed from their home cages and anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg, Abbot Laboratories, Chicago, IL) and atropine sulfate (0.45 mg/kg). At the onset of anesthesia, the rats were fitted with differential electromyograph (EMG) electrodes that were implanted in the left upper eyelid muscle (orbicularis oculi) and a ground electrode that was attached to a stainless steel skull screw. The EMG electrode leads terminated in gold pins in a plastic connector, which was secured to the skull with dental acrylic. A bipolar stimulating electrode (Plastics One, Roanoke, VA) for delivering the shock US was implanted subdermally, immediately caudal to the left eye. The bipolar electrode terminated in a plastic connector that was secured to the skull by dental acrylic. The surgical site was closed with sutures on both sides of the electrode connectors. All rats were given 1 week to recover from surgery before training began.

Apparatus. The conditioning apparatus consisted of four small-animal sound-attenuating chambers (BRS/LVE, Laurel, MD). Within each sound-attenuating chamber was a small-animal operant chamber (BRS/LVE) in which the rats were kept during conditioning. One wall of the operant chamber was fitted with two speakers that independently produced tones of up to 120 dB (SPL), with a frequency range of approximately 1000–9000 Hz. The back wall of the sound-attenuating chamber was equipped with a small light. The connectors for the EMG electrodes and bipolar stimulating electrode were connected to lightweight cables that allowed the rats to move freely during conditioning. The electrode leads from the rat's headstage were connected to peripheral equipment and a Pentium computer. Custom-written computer software controlled the delivery of stimuli and the recording of eyelid EMG activity. One circuit permitted the delivery of

a shock stimulus (2–3 mA, depending on the threshold for eliciting an unconditioned response; 60 Hz; constant current) through a stimulus isolator (Model 365A, World Precision Instruments, Sarasota, FL). EMG activity was recorded differentially, filtered, amplified, rectified, and integrated by equipment that was similar to that used in previous studies (Freeman & Nicholson, 1999; Nicholson & Freeman, 2000b; Stanton, Freeman, & Skelton, 1992).

Conditioning procedure. The experimental design is illustrated in Table 1. All of the rats in this experiment received two phases of training (Phases 1 and 2) and two phases of testing (Phases 3 and 4). The CI group received a four-phase procedure designed to establish a light CS as a conditioned inhibitor. The two control groups (CTL1 and CTL2) received training procedures designed to distinguish two other types of inhibition, generalization decrement (Pavlov's external inhibition) and differential inhibition, from CI. All rats received the same summation test during Phase 3 and retardation test during Phase 4. For all rats in this study, Tone 1 (T_1) was an 8-kHz tone; Tone 2 (T_2) was a 2-kHz tone; the light CS (L) was provided by the illumination of a houselight in the middle of the back wall of the conditioning chamber; and the US was a 25-ms, 60-Hz constant current periorbital shock. All compound stimuli were presented simultaneously. A plus sign after a CS indicates that it was paired with the US; a minus sign indicates that it was not paired with the US. The duration of each CS was 400 ms, which coterminated with the 25-ms US, yielding an interstimulus interval of 375 ms.

The three groups in this experiment received different training procedures during Phases 1 and 2 (training phases). During Phase 1, Group CI was given classical delay eyeblink conditioning training (T_1+) until they reached a criterion of 80% CRs in a half session for two consecutive 100-trial sessions. In Phase 2, Group CI received 100 trials per day of feature-negative discrimination training, which consisted of 50 T_2+ trials and 50 T_2L- trials. Each rat in Group CI received feature-negative discrimination training until it reached a criterion of 40% discrimination for two consecutive 100-trial sessions. Previous studies of CI have shown that 40% discrimination approximates the asymptotic level of discriminative responding (Freeman & Nicholson, 1999; Mis, 1977; Moore et al., 1980; Solomon, 1977; Yeo et al., 1983).

During Phase 1, each rat in Group CTL1 sat in the conditioning chamber, yoked to a rat in Group CI. During Phase 2, CTL1 rats received a yoked number of sessions, each of which consisted of 50 T_1+ trials and 50 T_2L- trials. This condition controls for any inhibitory value that the light may acquire by being an element in a nonreinforced compound stimulus during classical differential conditioning.

During Phase 1, rats in Group CTL2 were given classical delay eyeblink conditioning training (T_1+) until they reached a criterion of 80% CRs in a half session for two consecutive 100-trial sessions. In Phase 2, CTL2 rats were yoked to rats in Group CI and received a yoked number of sessions, each of which consisted of 50 T_2+ trials, 50 T_2- trials, and 50 $L-$ trials. This condition controls for any inhibitory value that the light may acquire by being a discriminative CS that is never reinforced (Bull & Overmier, 1968; Hammond, 1968; Rescorla & Wagner, 1972; Wagner & Rescorla, 1972). The intertrial interval (ITI) was adjusted such that rats in each condition spent approximately the same amount of time in the conditioning chamber (~1 hr).

During Phases 3 and 4 (the testing phases), Groups CI, CTL1, and CTL2 all received the same testing sessions. Phase 3 (summation test) consisted of two parts. Each rat was required to exhibit 80% CRs in a 10-trial block during T_1+ reminder presentations. None of the rats needed more than two blocks to reestablish criterion performance levels. The rats were then allowed to sit in the chamber for 5 min, after which the summation test trials were presented. The summation test consisted of 40 T_1- trials and 40 T_1L- trials. Phase 4 testing (retardation test) consisted of two 100-trial sessions of $L+$ training. If the light had been established as a conditioned inhibitor, then Group CI rats should have responded less to T_1L- presentations in Phase 3 (summation) and exhibited fewer CRs during $L+$

Table 1
Experimental Design for Experiments 1 and 2

Group	Phase 1 (Acq)	Phase 2 (FN Discr)	Phase 3 (Summ)	Phase 4 (Ret)
CI	B+	A+/AX-	B-/BX-	X+
CTL1	Sit	B+/AX-	B-/BX-	X+
CTL2	B+	A+/A-/X-	B-/BX-	X+

Note. Acq = acquisition; FN = feature-negative; Discr = discrimination; Summ = summation; Ret = retardation; CI = conditioned inhibition; B = 8-kHz tone conditioned stimulus (CS); A = 2-kHz tone CS; X = light CS; + = shock unconditioned stimulus (US); - = no shock US; CTL = control group.

training in Phase 4 (retardation) than either of the two control groups (Rescorla, 1969).

Results and Discussion

Only Groups CI and CTL1 received classical conditioning during Phase 1 (see Table 1). There was no significant difference between CI and CTL1 in the number of sessions required to reach criterion, $F(1, 26) = 0.06, p > .05$, nor in percentage of CRs in the final session of Phase 1, $F(1, 26) = 0.79, p > .05$, which indicates that these two groups were identical in their level of conditioning to T_1+ .

All three groups (CI, CTL1, and CTL2) received training in Phase 2 that can be analyzed in terms of a discrimination between a reinforced CS (CS_A for CI and CTL2, CS_B for CTL1) and a CS that is never reinforced (CS_A-CS_X for CI and CTL1, CS_X for CTL2; see Table 1). Group CI required an average of 5.1 sessions to reach criterion levels of discrimination. There was a between-subjects effect of response percentage, $F(2, 33) = 7.94, p < .01$, which post hoc analysis indicated was attributable to lower overall responding in CTL2 (Tukey-Kramer's honestly significant difference, $p < .05$). There was a within-subjects effect of stimulus, $F(1, 33) = 242.56, p < .01$, which was attributable to all groups responding more to the reinforced CS than to the nonreinforced CS ($ps < .05$). Analyses of CR amplitudes and CR onset latencies on reinforced and nonreinforced trials yielded significant within-subjects effects of stimulus, $F(1, 33) = 114.43, p < .01$, and $F(1, 33) = 96.86, p < .01$, respectively. Post hoc tests indicated that the significant effects of stimulus were attributable to lower CR amplitudes and longer CR latencies during presentations of the nonreinforced stimulus ($ps < .05$). There was no Stimulus \times Group interaction for sessions required to acquire the discrimination in Phase 2, $F(2, 33) = 3.20, p > .05$. There was also no effect of group, nor was there a Stimulus \times Group interaction for CR amplitude and onset latencies ($ps > .05$). These analyses indicate that all three groups showed similar levels of discrimination between the reinforced CS ($CS+$) and the nonreinforced CS ($CS-$; see Figure 1, Phase 2). Moreover, the significantly lower CR amplitudes and longer CR onset latencies during nonreinforced CS

trials suggest that the topography of the CR during nonreinforced CS trials was different.

All three groups received a summation test consisting of 40 presentations of a previously reinforced CS (T_1) and 40 presentations of that CS presented in compound with a light CS ($T_1 L$), none of which were reinforced (see the *Method* section). There was a significant Stimulus \times Group interaction, $F(2, 33) = 10.61, p < .01$. Post hoc analyses ($p < .05$) indicated that Group CI responded more on T_1- trials than did Groups CTL1 and CTL2 but that Groups CTL1 and CTL2 did not differ, and that Group CTL1 responded more on $T_1 L-$ trials than did Groups CI and CTL2 but that Groups CI and CTL2 did not differ (see Figure 1, Phase 3). Group CI was the only group to significantly discriminate between presentations of T_1 and $T_1 L$ ($p < .05$). That is, Group CI was the only group in which the light CS had a subtractive effect on CR percentage.

All three groups received a retardation test consisting of two 100-trial sessions of $L+$ training. There was a significant between-subjects effect of group, $F(2, 33) = 4.30, p < .05$. Post hoc analysis indicated that Group CI exhibited fewer CRs overall than did Groups CTL1 and CTL2 during retardation testing but that the control groups did not differ ($ps < .05$). That is, the acquisition of an excitatory CR to the light CS was retarded in Group CI compared with the control groups (see Figure 1, Phase 4). There was also a within-subjects effect of session, $F(1, 33) = 119.37, p < .01$, which was attributable to the increase in CR percentage from Session 1 to Session 2 that occurred in all three groups.

The four-phase procedure with which Group CI was trained in the present experiment established the light CS as a conditioned inhibitor (Marchant et al., 1972; Rescorla, 1969). The presence of a small amount of light-induced inhibition in Group CTL2 generally replicated previous studies that investigated the inhibition produced by discrimination training (Bull & Overmier, 1968; Hammond, 1967, 1968), even though the light CS in CTL2 was only an element in a compound discriminative $CS-$. However, the inhibition produced by the conditioning procedures used in Group CTL2 was much weaker than the inhibition produced with CI procedures. The differences between Group CI and Groups CTL1

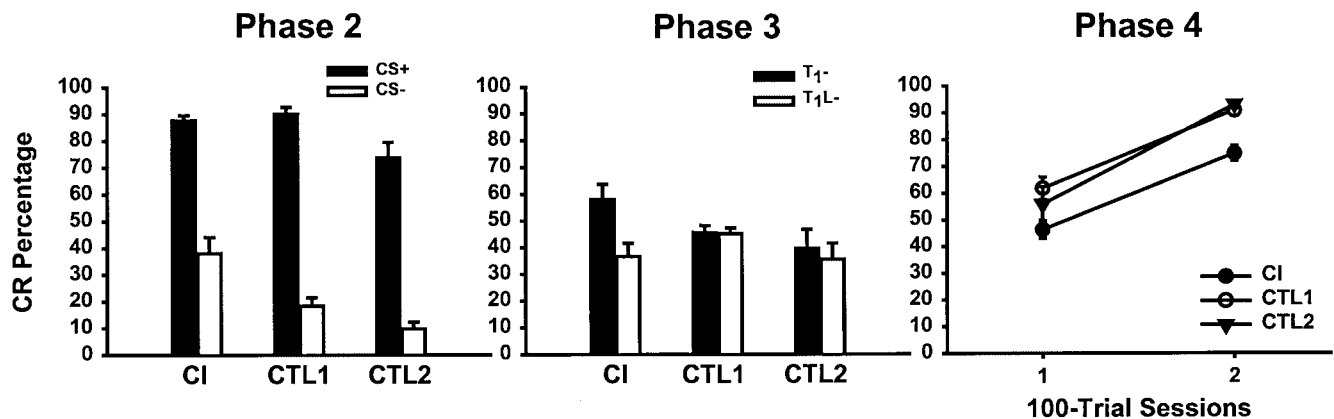


Figure 1. Mean (\pm SEM) percentages of conditioned responses (CRs) for the three groups in Experiment 1 during Phases 2 (feature-negative discrimination training), 3 (summation test), and 4 (retardation test). CS = conditioned stimulus; T_1 = 8-kHz tone CS; L = light CS; CI = conditioned inhibition; CTL = control; + = shock unconditioned stimulus (US); - = no shock US.

and CTL2 during the retardation (Phase 3) and summation (Phase 4) tests suggest that this experiment successfully distinguished between the CI produced by feature-negative discrimination procedures and the inhibition produced by discrimination procedures that do not use feature-negative discrimination (e.g., Bull & Overmier, 1968; Hammond, 1967, 1968; Wagner & Rescorla, 1972).

Experiment 2

There is a large body of literature which suggests that the brainstem and cerebellum are components of the necessary and sufficient circuitry for excitatory eyeblink conditioning (see the introduction). Permanent or temporary lesions of neurons in the cerebellar anterior interpositus nucleus prevented the acquisition and retention of the classically conditioned eyeblink CR (Chapman et al., 1990; Clark, Zhang, & Lavond, 1992; Krupa et al., 1993; Krupa & Thompson, 1997; Lavond, Hembree, & Thompson, 1985; McCormick & Thompson, 1984a). Extracellular recordings of neuronal activity within the interpositus nucleus have shown conditioning-related increases in firing that precede the onset of the CR (Berthier & Moore, 1990; Freeman & Nicholson, 1999, 2000; Gould & Steinmetz, 1996; McCormick & Thompson, 1984b; Steinmetz, 1990). Moreover, the activity of neurons in the interpositus nucleus models the behavioral CR within and across trials, suggesting that the interpositus neurons are producing the CR (Kim & Thompson, 1997; Lavond et al., 1993; Thompson & Krupa, 1994). These findings indicate that the plasticity necessary for excitatory classical eyeblink conditioning is localized within the cerebellum (Thompson & Krupa, 1994).

The brainstem and cerebellum may also be involved in inhibitory classical eyeblink conditioning (Britton, Brown, & Steinmetz, 2000; Brown, Britton, & Steinmetz, 2000; Freeman & Nicholson, 1999). Neuronal activity in the lateral pontine and interpositus nuclei decreased during inhibitory trials compared with excitatory trials (Freeman & Nicholson, 1999). One possible explanation for this "discriminative" neuronal activity is that the interpositus cells were being actively suppressed or inhibited, which would have the indirect effect of decreasing neuronal activity in the lateral pontine nuclei through its excitatory feedback loop (Bao et al., 2000; Clark, Gohl, & Lavond, 1997). The present experiment was conducted to elucidate the role of the anterior interpositus nucleus in CI through extracellular recording during CI training and testing. The presence of neural correlates of CI training and testing would suggest that cerebellar plasticity plays a significant role in the acquisition and expression of CI of the eyeblink CR.

Method

Subjects. Subjects were 5 male Long-Evans rats (250–350 g). The rats were housed in the animal colony in Spence Laboratories at the University of Iowa. All rats were maintained on a 12-hr light–dark cycle with light onset at 6:30 a.m. and were given ad lib access to food and water.

Surgery. The surgical methods were identical to those used in Experiment 1, except for the chronic implantation of a bundle of eight stainless steel microwire electrodes into the left cerebellar anterior interpositus nucleus under stereotaxic and electrophysiological guidance (Freeman & Nicholson, 1999, 2000). The stereotaxic coordinates relative to bregma were AP -11.5 , ML $+2.3$, and DV -5.7 . The electrodes were held in place by a microelectrode connector and dental acrylic applied to the skull. The surgical site was closed with sutures on both sides of the electrode

plug. The connectors for the EMG electrodes, bipolar stimulating electrode, and microwire electrodes were connected to lightweight cables that allowed the rats to move freely during conditioning.

Apparatus. The conditioning apparatus was the same as that used in Experiment 1, except that the small-animal sound-attenuating chamber was electrically shielded.

Conditioning procedure. The conditioning procedure was the same as that used in Experiment 1, with the exception that L– probe trials were included in the summation test (Phase 3) to determine the interpositus neuronal activity during presentations of the conditioned inhibitor alone (see Table 1). Also, each rat received pretraining presentations of T₁, T₂, and L-alone trials to test for any pretraining sensory bias that would have confounded the interpretation of differences in neuronal activity during training and testing. There was a slight excitation to presentations of the L alone in this pretraining sensory session (data not shown). Previous studies in this laboratory indicated that the number of pretraining presentations of the three stimuli (50 per stimulus) is not sufficient to produce any observable adverse effects on conditioning, for example, latent inhibition (Nicholson & Freeman, in press).

Neuronal recording procedure. The activity of each microwire electrode was initially passed through a unity gain JFET preamplifier (NB Labs, Denison, TX). The outputs of the JFET preamplifier were fed into an eight-channel programmable amplifier (Lynx-8, Neuralynx, Tucson, AZ), filtered between 300 and 6000 Hz, and amplified at a gain of 10,000. The outputs of the amplifier were fed at 20 kHz per channel into a computer-controlled acquisition system (Workbench-32, Datawave Technologies, Longmont, CO), where thresholding was used to detect and extract waveforms of units with signal-to-noise ratios of at least 2:1. The waveforms were saved as separate, 32-point data chunks. Two voltage thresholds were used. Waveforms that crossed the lower threshold but did not cross the upper threshold within the 32 sample points were recorded to computer disk. Custom-written software was then used to separate the multiple-unit records. This software displays all of the waveforms that were recorded during a particular data collection period (usually a training session). The user may examine any individual spike waveform that was recorded during the training session. A template-matching program was used to identify all of the spikes with similar waveform characteristics. This technique effectively isolated single units from multiunit records (Freeman & Nicholson, 1999, 2000; Nicholson & Freeman, 2000a). Peristimulus-time histograms of the firing rates of each unit were created for the CS– period of the conditioning trials (375 ms) for the last sessions of training in Phase 1 and Phase 2 and the summation test in Phase 3. Neuronal activity was not recorded in a control group during the retardation test in Phase 4, which precluded an evaluation of neuronal retardation. The neuronal activity during the CS period was analyzed with Wilcoxon's signed rank tests. The neuronal activity from the last 125 ms after CS onset in Phase 1 (excitatory classical eyeblink conditioning) was compared with that from a baseline period immediately preceding CS onset. The first 375 ms after CS onset in Phase 2 (feature-negative discrimination) and the whole CS period in Phase 3 (summation test) were compared between the different types of training and testing trials for three (Phase 2) or four (Phase 3) time epochs of equal duration (e.g., first time epoch of T+ vs. first time epoch of T L–; Freeman & Nicholson 1999, 2000; Kubota, Wolske, Poremba, Kang, & Gabriel, 1996; Nicholson & Freeman, 2000a). The absence of the US and its artifact in the neuronal records allowed the analysis of the entire CS period during Phase 3.

Histology. After training, the rats were killed with a lethal injection of sodium pentobarbital (90 mg/kg) and transcardially perfused with 100 ml of physiological saline followed by 300 ml of 3% (wt/vol) Formalin. After perfusion, the brains were postfixed in the same fixative for a minimum of 24 hr and subsequently sectioned at 50 μ m with a sliding microtome. Sections were then stained with cresyl violet. The locations of the recording electrodes were confirmed by examining serial sections. Two rats received stimulation (a train of ten 50- μ s pulses at 100 Hz) through the

recording electrodes to confirm that the recording electrodes were within the eye region of the interpositus nucleus (Freeman & Nicholson, 2000).

Results and Discussion

Electrode placement. The electrode bundles of all 5 rats were placed in the anterior interpositus nucleus (see Figure 2).

Behavioral data. All rats reached criterion levels of performance in Phases 1 and 2 that did not significantly differ from those of Group CI in Experiment 1 (see Figure 3, Phase 1 and Phase 2). During feature-negative discrimination training (Phase 2), the rats performed significantly more CRs to the reinforced CS (T_2+) than to the nonreinforced compound CS (T_2L-), $t(4) = 6.82$, $p < .01$ (see Figure 3, Phase 2). Moreover, CR onset latencies were significantly shorter, $t(4) = 3.93$, $p < .02$, and CR amplitudes were significantly higher, $t(4) = 8.85$, $p < .01$, on T_2+ trials compared with T_2L- trials, indicating that CRs that occurred on T_2L- trials had an altered topography (see Figure 4). During the summation test (Phase 3), all rats demonstrated that the presentation of the inhibitory L in compound with the excitatory T_1 had an inhibitory effect on responding (see Figure 3, Phase 3). One rat did not receive $L-$ trials during summation testing. This rat did discriminate between T_1- and $T_1 L-$ trials but was excluded from the behavioral analysis. During summation, rats performed significantly more CRs on T_1- trials than on $T_1 L-$ trials, $t(3) = 5.40$, $p < .04$, and $L-$ trials, $t(3) = 7.60$, $p < .02$ (all ps Bonferroni adjusted). There were more CRs performed on $T_1 L-$ than on $L-$ trials, but this difference was not significant, $t(3) = 1.87$, $p > .15$ (Bonferroni adjusted). Comparison of the performance during the retardation test with that of a control group (CTL2) from Experiment 1 demonstrated a significant between-subjects effect, $F(1, 8) = 19.09$, $p < .01$, which post hoc tests (Tukey's honestly significant difference, $p < .05$) showed to be attributable to lower CR percentages for the 5 rats in the present experiment during retardation testing (see Figure 3, Phase 4). A significant within-subjects effect of session, $F(1, 8) = 50.75$, $p < .01$, was attributable to the increase in CR percentage from Session 1 to Session 2 in both groups (comparisons $p < .05$; see Figure 3, Phase 4). The presence of CR suppression during $T_1 L-$ trials in summation testing (Phase 3) and the performance of fewer CRs than those in a control group during $L+$ retardation testing (Phase 4) demonstrate that this experiment established the light CS as a conditioned inhibitor.

Neuronal data. The total numbers of neurons recorded during Phase 1, Phase 2, and Phase 3 were 92, 106, and 104, respectively. Table 2 summarizes the distribution of different types of learning-related plasticity during Phases 1, 2, and 3. Table 3 summarizes the different types of CR-related modification that occurred during Phase 2. During the last session of Phase 1 training, 71.7% of the neurons showed significantly more activity than baseline, 25.0% did not differ, and 3.3% showed less activity than baseline. These results indicate that a majority of the interpositus neurons exhibited learning-related plasticity that parallels the learned behavioral CR (see Figure 5, Phase 1).

During the last session of Phase 2 training, 33% of the neurons exhibited significantly greater neuronal activity during T_2+ trials compared with T_2L- trials. The last CS epoch exhibited the largest number of "discriminating" units (see Table 2, Phase 2). Figure 6 shows the activity of two representative units during T_2+ and T_2L- trials. The top two panels of Figure 6 show data from a discriminating unit. There is a dramatic increase in neuronal activity preceding the onset of the US during T_2+ trials, which mirrors the behavioral CR. However, there is a meager amount of excitatory modulation during presentations of the inhibitory compound. The bottom two panels of Figure 6 show data recorded from a "nondiscriminating" unit during the two trial types. As can be seen, there is no modulation of neuronal activity as a result of trial type in this unit. The functional contributions of the massive discrimination exhibited in the discriminating units are seen most clearly in the mean activity of all units (see Figure 5, Phase 2). It is clear from this figure that the presence of discriminating units has a large impact on the neuronal ensemble response of all units. An analysis of CR-related modifications in neuronal activity revealed a massive disparity in the number of units that demonstrated CR-related excitation between the two trial types. CR-related increases in firing were at least twice as likely during T_2+ trials compared with T_2L- trials, in all three CS epochs (see Table 3). This disparity offers an explanation for the significantly lower CR amplitudes during T_2L- trials compared with T_2+ trials (see Figure 4, behavioral data). Figure 7 shows the neuronal activity during the four different trial types for a single representative unit. Although CR-related excitation was present in both T_2+ and T_2L- trials, there was substantially more excitation during T_2+ trials. This can be seen most clearly in a graph of the mean unit activity (see Figure 8). Although suppression of firing (compared

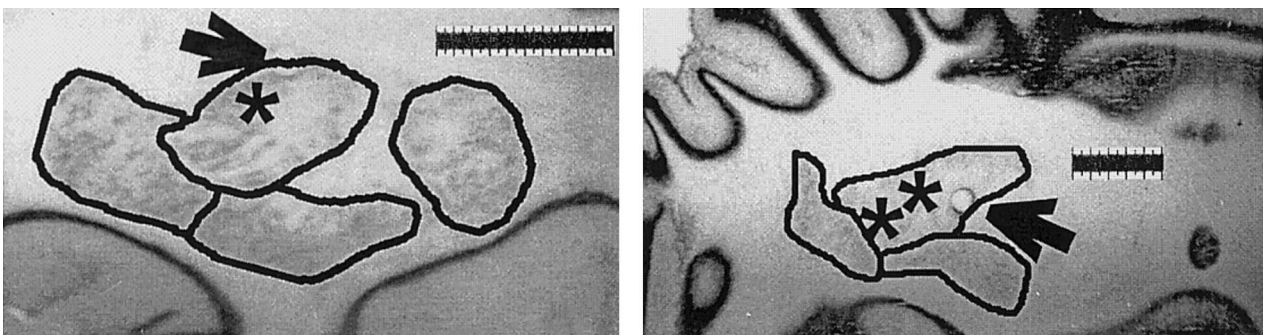


Figure 2. Digitized photomicrographs of two electrode bundle placements (the arrows are pointing to the electrode tracks) that represent the two most peripheral of the five placements in the anterior interpositus nucleus. Asterisks indicate the placements in the remaining 3 rats. Scale bars = 1 mm.

with T_2+ trials) during T_2L- trials occurred regardless of whether or not a CR occurred, more inhibition occurred during the T_2L- trials on which there was no CR. The presence of gradations of inhibition in neuronal discharge during T_2L- trials is similar to gradations in the inhibition of the CR during T_2L- trials (see Figure 4).

During Phase 3 testing, the majority of the neuronal discrimination occurred in the last two CS epochs (see Table 2, Phase 3). Figure 9 shows the data from a single unit during the three trial types in Phase 3 summation testing. The unit shown in Figure 9 clearly demonstrated higher levels of activity during T_1- trials, compared with both T_1L- and $L-$ trials. The greatest differences among the three trial types appear in the second half of the CS period, when most of the CRs began. The presence of neuronal discrimination among the three trial types is apparent in the mean unit activity, which also exhibits the largest differences in the second half of the CS period (see Figure 10). The large differences in the neuronal activity between T_1- trials and both T_1L- and $L-$ trials mirrors the differences in CR percentages among these three trial types. Note also the presence of less neuronal activity during $L-$ trials compared with T_1L- trials. That is, the magnitudes of the mean neuronal activity for each trial type differ in the same direction in which the behavior differs (compare Figure 3, Phase 3 with Figure 10).

Stimulation. Two rats received a 100-ms, 100-Hz train of stimulation through the recording electrodes (range = 10–200 μ A; Freeman & Nicholson, 2000). Eyelid, eyeball, and vibrissae movements and full eyeblinks were elicited.

General Discussion

In Experiment 1, a four-phase procedure was used to establish a light CS as a conditioned inhibitor. Experiment 1 demonstrated, for the first time during eyeblink conditioning in rats, that the inhibition produced by feature-negative discrimination training (Group CI) was different from the inhibition produced by simply presenting a CS in a nonreinforced compound (Group CTL1) and the differential inhibition produced by presenting a CS that was never reinforced (Group CTL2; see Table 1). That is, Experiment 1

demonstrated that feature-negative discrimination training established the light CS as a conditioned inhibitor. Experiment 2 found that neuronal activity in the cerebellar anterior interpositus nucleus during CI training and testing provided an accurate neuronal model of the production and suppression of CRs. That is, the neuronal activity was greater during presentations of an excitatory CS (e.g., T_1) than during presentations of that CS in compound with the inhibitory CS (e.g., T_1L) or the inhibitory CS (i.e., L) alone. The discriminative neuronal activity was present in the single-unit activity and also in the population activity. The last 250 ms of the CS had the largest number of discriminative and CR-related units. Moreover, the large magnitudes of the neuronal discrimination and CR-related modification at the level of individual single units provides an explanation for how a subpopulation of cerebellar neurons can account for the behavioral discrimination in CI.

The presence of neuronal discrimination during feature-negative discrimination training (Phase 2) replicated the results of a previous study (Freeman & Nicholson, 1999). However, the present study included analyses of learning- and CR-related modifications of neuronal activity during training and testing that elaborate on some of the possible mechanisms by which CI can occur within brainstem–cerebellum circuitry. The three most important results from these analyses are that (a) the discriminative neuronal activity during feature-negative discrimination training (Phase 2) and the summation test (Phase 3) mirrored the behavioral CI, (b) CR-related modifications were twice as likely during $T+$ trials as during $TL-$ trials (see Tables 2 and 3), and (c) learning- and CR-related modifications tended to be clustered within the last half of the CS period.

Several models of eyeblink conditioning propose that the memory underlying the eyeblink CR is formed by means of an interaction between the cerebellar cortex and deep nuclei (Choi, 1999; Hesslow, 1994; Hesslow, Svensson, & Ivarsson, 1999; Mauk & Donegan, 1997; Medina, Garcia, Nores, Taylor, & Mauk, 2000; Medina & Mauk, 1999, 2000; Ohyama & Mauk, 2001; Raymond & Lisberger, 1998; Svensson & Ivarsson, 1999; Yeo & Hesslow, 1998). In general, these models suggest that cortical plasticity is primarily responsible for correct CR timing and that plasticity

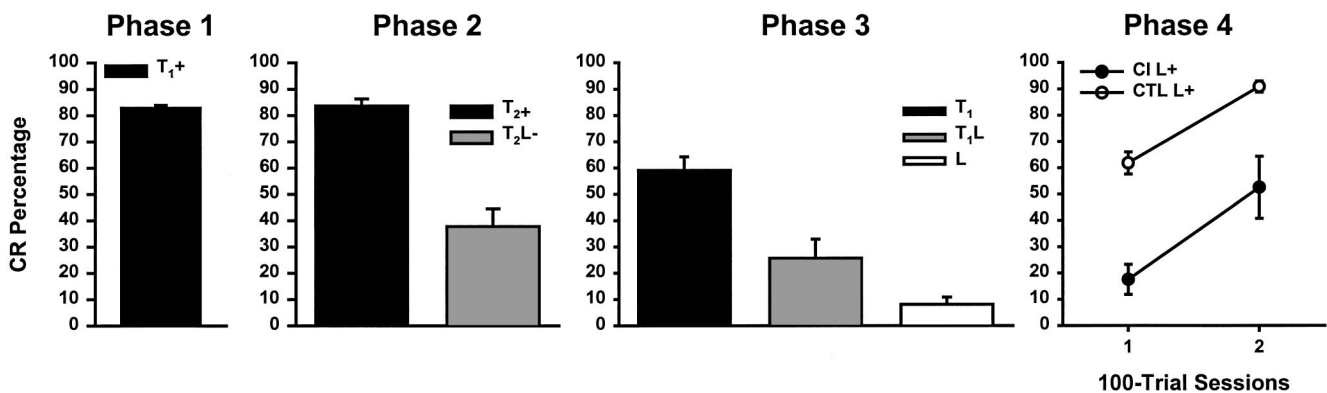


Figure 3. Mean (\pm SEM) percentages of conditioned responses (CRs) for the rats in Experiment 2 during Phases 1 (excitatory conditioning), 2 (feature-negative discrimination training), 3 (summation test), and 4 (retardation test). T_1 = 8-kHz tone conditioned stimulus (CS); T_2 = 2-kHz tone CS; CI = conditioned inhibition; L = light CS; CTL = control; + = shock unconditioned stimulus (US); - = no shock US.

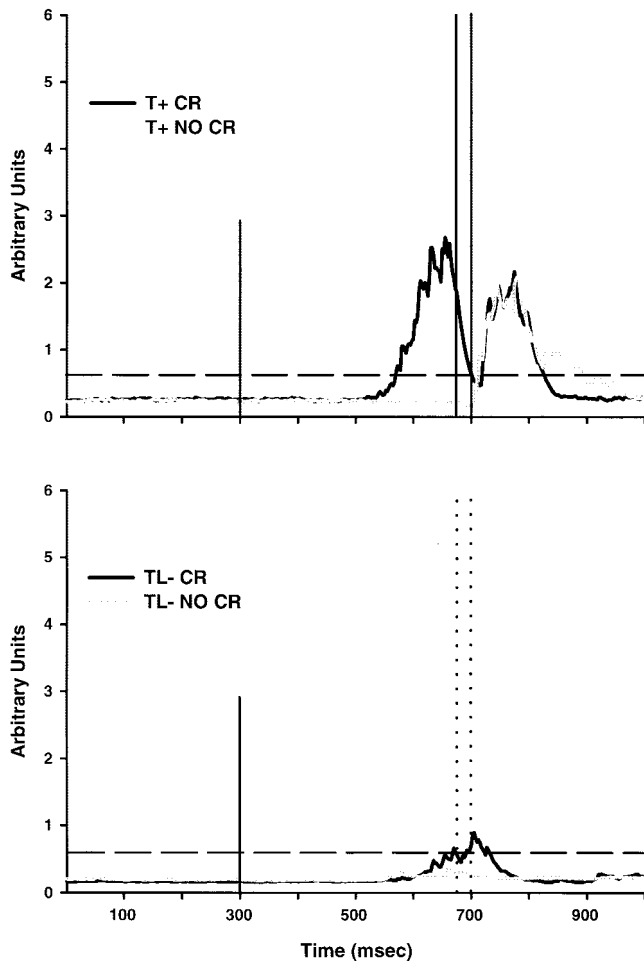


Figure 4. Integrated electromyograph (EMG) activity from individual T+ and TL- trials during Phase 2 from a rat in Experiment 2. The short vertical lines indicate conditioned stimulus (CS) onset. The pair of vertical lines later in the trial indicate the unconditioned stimulus (US) onset and offset period during T+ and TL- trials. Black lines in each graph indicate a trial on which a conditioned response (CR) was recorded, and gray lines in each graph indicate a trial on which a CR was not recorded. The horizontal black dashed line indicates the threshold that the EMG activity must cross to be counted as a CR. The EMG trace for the T+ CR trial (top graph, black line) decreased during the US period because the EMG amplifier was turned off during this period to avoid saturation associated with shock artifact. T = 8- or 2-kHz tone CS; L = light CS; + = shock US; - = no shock US.

within the deep nuclei is responsible for CR production. A recent study by Medina, Garcia, et al. (2000) demonstrated that the anterior lobe provides an important inhibitory component that suppresses responding in the early part of the CS period (e.g., first half). These results were interpreted as supporting a model of CR production by disinhibition of cells in the deep nuclei, a process that exhibits a latency of ~ 100 ms (Hesslow, 1994). It was proposed that performance of adaptively timed CRs is the result of strong Purkinje cell suppression of the deep nuclei in the early portions of the CS period (i.e., suppressing responding to produce Pavlov's inhibition of delay), followed by disinhibition (by cortical

Table 2
Number of Neurons That Exhibited Significant Changes in Activity During CI Training and Testing in Experiment 2

Comparison	More excited	Equal	Less excited
Phase 1	66	23	3
Phase 2 T+ vs. TL-			
1st 125 ms	5	94	7
2nd 125 ms	14	89	3
3rd 125 ms	29	65	12
Phase 3 T- vs. TL-			
1st 100 ms	4	93	7
2nd 100 ms	3	97	4
3rd 100 ms	16	87	1
4th 100 ms	18	83	3
Phase 3 T- vs. L-			
1st 100 ms	60	22	0
2nd 100 ms	48	30	4
3rd 100 ms	50	32	0
4th 100 ms	54	28	0

Note. CI = conditioned inhibition; T = 8- or 2-kHz tone conditioned stimulus (CS); L = light CS; + = shock unconditioned stimulus (US); - = no shock US.

long-term depression) and potentiation (by deep nuclear long-term potentiation) in the later portions of the CS period (see also Katz, Tracy, & Steinmetz, 2001; Kistler, van Hemmen, & De Zeeuw, 2000; Mauk & Donegan, 1997; Medina & Mauk, 1999, 2000; Raymond & Lisberger, 1998; Yeo & Hesslow, 1998). According to this view, the eyeblink CR is formed by interactions between the cerebellar cortex and deep nuclei. Preliminary evidence indicates that cerebellar cortical inhibition is not required for the expression of CI (Nolan, Nicholson, & Freeman, 2000). However, cortico-nuclear interactions may be required for *acquisition* of CI (Logan & Thompson, 1991).

What mechanisms are responsible for the neuronal discrimination reported in the present study? There are at least three possibilities. First, it is possible that the neuronal discrimination in interpositus neurons is a reflection of plasticity that is induced in an extra-cerebellar structure within the excitatory eyeblink circuit (see the introduction). Recent studies have indicated that neuronal activity in the pontine (Freeman & Nicholson, 1999) and red

Table 3
Conditioned Response (CR) Related Modulation of Neuronal Activity During Feature-Negative Discrimination Training

Comparison	More excited	Equal	Less excited
Phase 2 T+ CR vs. no CR			
1st 125 ms	15	78	2
2nd 125 ms	35	60	0
3rd 125 ms	34	60	1
Phase 2 TL- CR vs. no CR			
1st 125 ms	8	83	4
2nd 125 ms	10	82	3
3rd 125 ms	17	77	1

Note. T = 8- or 2-kHz tone conditioned stimulus (CS); L = light CS; + = shock unconditioned stimulus (US); - = no shock US.

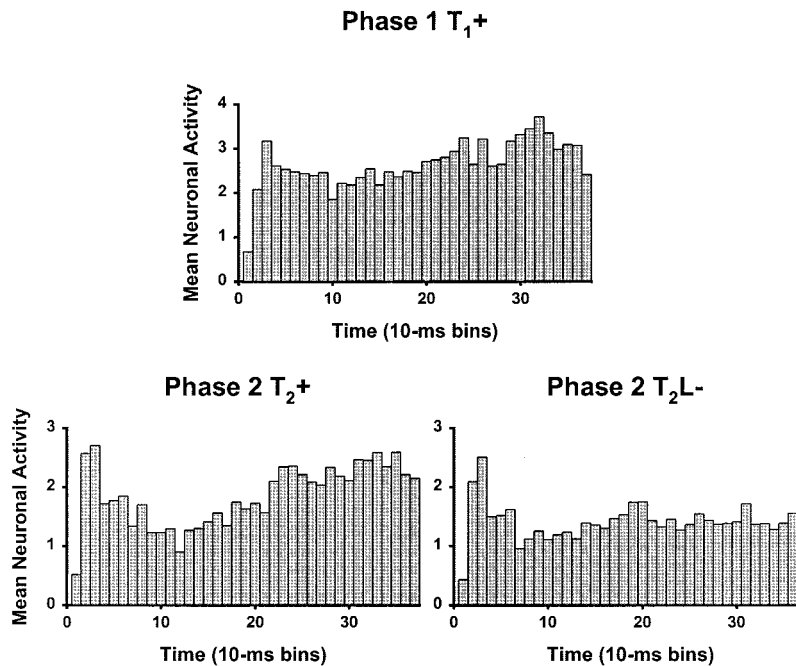


Figure 5. Mean neuronal activity (normalized to baseline) of all single units during the conditioned stimulus (CS) period (375 ms) from Phase 1 training and the excitatory (T_2+) and inhibitory (T_2L-) trials during Phase 2 feature-negative discrimination training. T_1 = 8-kHz tone CS; T_2 = 2-kHz tone CS; L = light CS; + = shock unconditioned stimulus (US); - = no shock US.

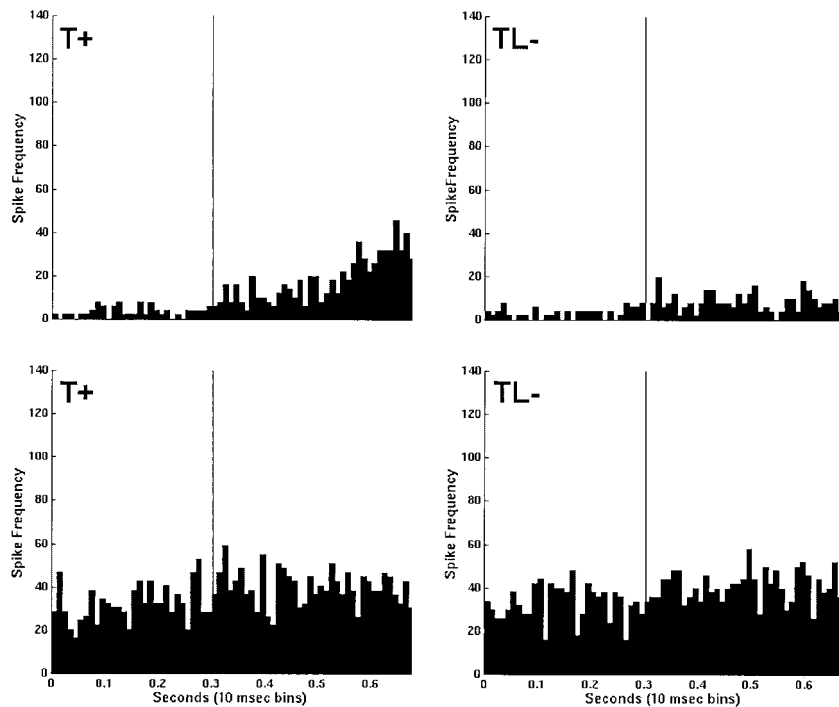


Figure 6. Histograms from the baseline and conditioned stimulus (CS) periods of representative units recorded in the anterior interpositus nucleus during feature-negative discrimination training for excitatory trials ($T+$) and inhibitory trials ($TL-$). The top two panels are from a representative discriminating unit. The bottom two panels are from a representative nondiscriminating unit. The black vertical lines indicate CS onset. Frequency is expressed in spikes per second. T = 8- or 2-kHz tone CS; L = light CS; + = shock unconditioned stimulus (US); - = no shock US.

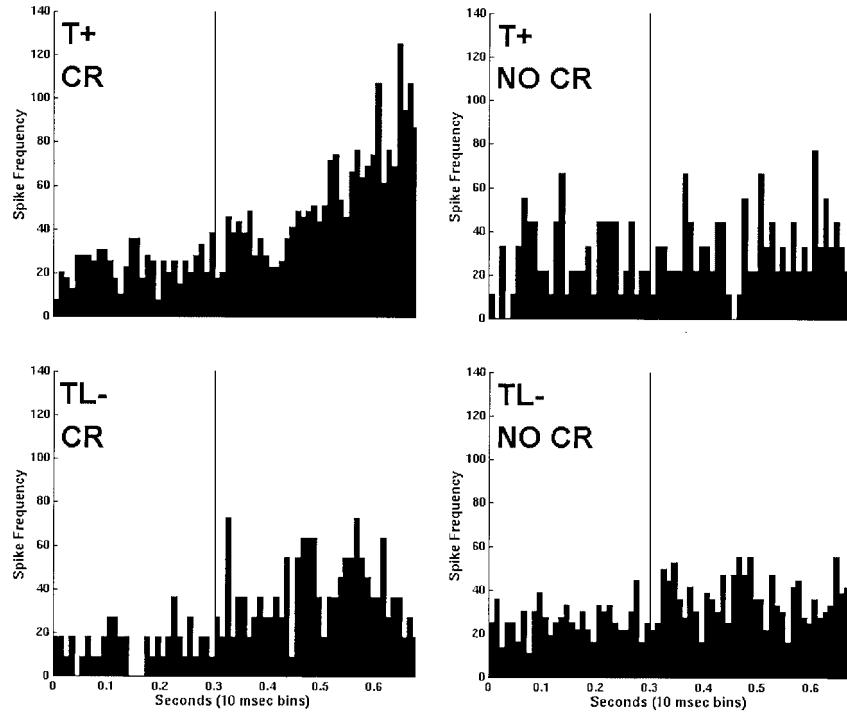


Figure 7. Histograms from the baseline and conditioned stimulus (CS) periods of a representative unit during feature-negative discrimination training (Phase 2). The top two panels are from excitatory (T+) trials on which an eyeblink conditioned response (CR) was performed or not performed. The bottom two panels are from inhibitory trials (TL-) on which an eyeblink CR was performed or not performed. The black vertical lines indicate CS onset. Frequency is expressed in spikes per second. T = 8- or 2-kHz tone CS; L = light CS; + = shock unconditioned stimulus (US); - = no shock US.

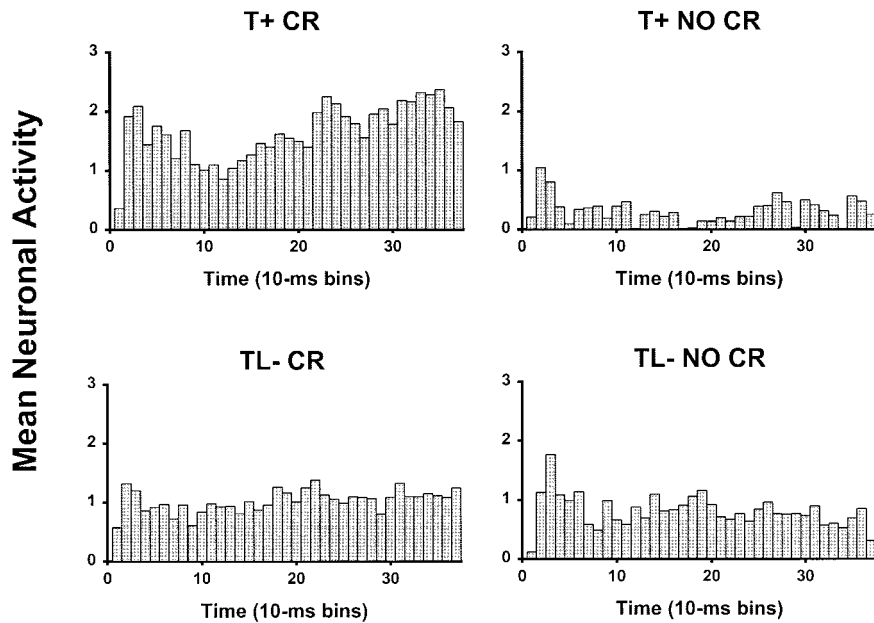


Figure 8. Mean neuronal activity (normalized to baseline) of all single units during the conditioned stimulus (CS) period (375 ms) during Phase 2 feature-negative discrimination training. The top two panels are from excitatory trials (T+) on which a conditioned response (CR) was or was not performed. The bottom two panels are from inhibitory trials (TL-) on which a CR was or was not performed. T = 8- or 2-kHz tone CS; L = light CS; + = shock unconditioned stimulus (US); - = no shock US.

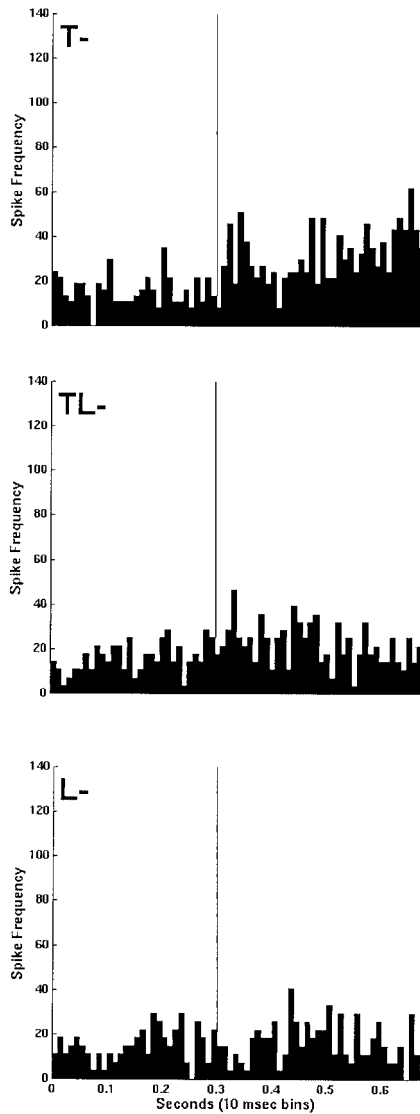


Figure 9. Histograms from the baseline and conditioned stimulus (CS) periods of a representative discriminating single unit during the summation test (Phase 3). The black vertical lines indicate CS onset. Frequency is expressed in spikes per second. T = 8- or 2-kHz tone CS; L = light CS; - = no shock unconditioned stimulus.

(Brown et al., 2000) nuclei is strongly correlated with behavioral inhibition during CI training. It is possible that the cerebellar pontine or red nucleus afferents provide discriminative information about the CSs such that cerebellar neurons passively reflect their discriminative plasticity. The red nucleus and pontine nuclei exhibit substantial learning-related activity during excitatory classical eyeblink conditioning and comprise feed-forward circuits with the interpositus nucleus (Bao et al., 2000; Chapman et al., 1990; Clark et al., 1997; Desmond & Moore, 1991; McCormick, Lavond, & Thompson, 1983; Mihailoff, 1994; Nakamura, Kitao, Moriizumi, & Kudo, 1987; Tracy et al., 1998). Desmond and Moore (1991), using discriminative classical eyeblink conditioning, demonstrated that some neurons in the red nucleus exhibit discriminative neuronal activity locked to the CS or CR. They proposed that the CS- and CR-locked activity could act as triggering inputs within the rubrocerebellar loop, which could serve to generate (e.g., to the CS+) or inhibit (e.g., to the CS-) a CR. A similar situation may occur within the pontocerebellar loop (Clark et al., 1997; Tracy et al., 1998). Therefore, these neurons could drive discriminative neuronal activity in the cerebellum. It is important to note, however, that the induction and maintenance of learning-related activity exhibited in the red nucleus and pontine nuclei depend on cerebellar feedback (Bao et al., 2000; Chapman et al., 1990; Clark et al., 1997).

Second, discriminative plasticity could be induced within the interpositus nucleus. The single-unit (Figures 5 and 9) and mean neuronal (Figures 6 and 10) activity both demonstrate maximum suppression immediately preceding the inhibitory CS offset. One possible mechanism underlying this neuronal discrimination is through cerebellopontine (Berretta, Bosco, Smecca, & Perciavalle, 1991; Tsukahara, Bando, Kitai, & Kiyohara, 1971; Watt & Mihailoff, 1983) or cerebellorubral (Daniel, Billard, Angaut, & Baitini, 1987; Desmond & Moore, 1991; Keifer, 1996; Keifer & Houk, 1994; Nakamura et al., 1987) connections. The feed-forward connections, in conjunction with olivonuclear input (Oldenbeuving, Eisenman, De Zeeuw, & Ruigrok, 1999; Ruigrok & Voogd, 2000), may provide interpositus neurons with more synaptic excitation during T+ trials (due to higher neuronal activity in the interpositus nucleus driving the CR and increasing excitation in the pontocerebellar and rubrocerebellar loops) and less excitation during T L- trials. The enhancement of synaptic excitation during T+ trials may induce potentiation of the mossy fiber synapses that

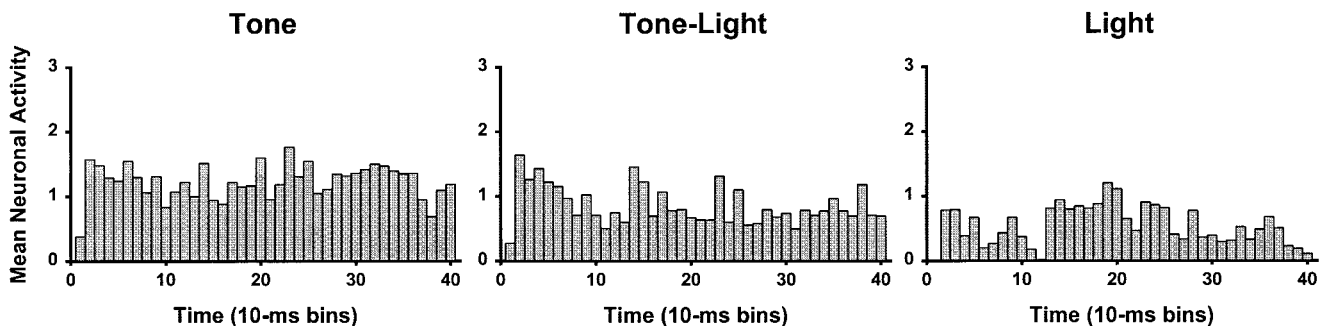


Figure 10. Mean neuronal activity (normalized to baseline) of all single units during the conditioned stimulus period (400 ms) during the summation test (Phase 3) for trials on which the tone, tone-light compound, or light conditioned inhibitor alone were presented.

provide tone input to the cerebellum (Medina & Mauk, 1999; Thompson & Krupa, 1994; Tracy et al., 1998), whereas the mossy fiber inputs for the L- stimulus (the inhibitor) may become inhibitory or not change. According to this hypothesis, the cerebellum provides the triggering inputs to the cerebellopontine or cerebellorubral loop.

An alternative possibility is that GABAergic cerebellopontine projections (Berretta et al., 1991; Border, Kosinski, Azizi, & Mihailoff, 1986; Border & Mihailoff, 1985) could influence the CS input to the cerebellum from the pontine nuclei. Such inhibitory feedback neurons may modulate CI by selectively inhibiting pontocerebellar CS inputs for the inhibitory stimulus during the compound CS, resulting in a weaker mossy fiber input to the interpositus nucleus (Berretta et al., 1991; Tolbert, Bantli, & Bloedel, 1978; Tracy et al., 1998; Tsukahara et al., 1971). The mechanism of plasticity would therefore involve an increase in the output of inhibitory interpositus neurons during the compound CS (perhaps the less excited neurons seen in Tables 2 and 3).

Third, structures that are not necessary for excitatory eyeblink conditioning may modulate the brainstem-cerebellum eyeblink circuitry to produce CI. Both the SC (Wells, Hardiman, & Yeo, 1989) and the lateral geniculate nucleus (Ribak & Peters, 1975; Wells et al., 1989) project to the pontine nuclei. Moreover, the SC is necessary for the expression of CI of fear-potentiated startle (Waddell et al., 2000). It is possible that these structures provide modulated CS input via pontocerebellar projections during CI training or testing.

The experiments in this study are part of an ongoing analysis of the neural mechanisms of CI. The findings indicate that discriminative plasticity within the interpositus nucleus is a possible mechanism underlying CI of the eyeblink response. In each of the training and testing phases, neuronal activity in the interpositus nucleus was correlated with behavioral excitation and inhibition. It is plausible that a subpopulation of discriminating units in the cerebellar interpositus nucleus supports CI by maintaining near-baseline levels of activity, thereby decreasing cerebellar output during presentations of the inhibitory compound or the conditioned inhibitor alone. The single-unit data also indicate that the small percentage of low-amplitude CRs during presentations of the inhibitory compound CS could be the result of fewer neurons showing weaker and later increases in neuronal activity compared with the activity during presentations of the excitatory CS. Although the data suggest that discriminative plasticity occurs in the cerebellum, other neural structures that have been demonstrated to participate in conditioned inhibition of fear-potentiated startle (e.g., SC or sensory thalamus) may contribute to the behavioral inhibition of CRs through the modulation of cerebellar inputs.

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