Pharmacological analysis of cerebellar contributions to the timing and expression of conditioned eyelid responses

Keith S. Garcia, Michael D. Mauk *

Department of Neurobiology and Anatomy, University of Texas Medical School, 6431 Fannin, Houston, TX 77030, USA

Accepted 22 March 1998

Abstract

Contradictory results have been reported regarding the effects of cerebellar cortex lesions on the expression of conditioned eyelid responses—either no effect, partial to complete abolition of responses, or disruption of response timing. This uncertainty is increased by debates regarding the region(s) of cerebellar cortex that are involved, by the likelihood that cortex lesions can inadvertently include damage to the interpositus nucleus or other pathways necessary for response expression, and by potential confounds from the degeneration of climbing fibers produced by cerebellar cortex lesions. We have addressed these issues by reversibly blocking cerebellar cortex output via infusion of the GABA antagonist picrotoxin into the interpositus nucleus. After picrotoxin infusion, conditioned responses are spared but their timing is disrupted and their amplitude diminished. In the same animals, conditioned responses were abolished by infusion of the GABA agonist muscimol and were unaffected by infusion of saline vehicle. These results are consistent with the hypothesis that (i) plasticity in the interpositus nucleus contributes to the expression of conditioned responses, as suggested by the responses seen with the cortex disconnected, and (ii) plasticity in the cerebellar cortex also contributes to conditioned response expression, as suggested by disruption of response timing. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: Classical conditioning; Plasticity; Nictitating membrane; Muscimol; Picrotoxin; Reversible lesion

1. Introduction

Evidence from lesion, recording, and stimulation studies indicates that Pavlovian eyelid conditioning is mediated by plasticity in the cerebellum (Thompson, 1986; Mauk and Donegan, 1997). Briefly, mossy fiber and climbing fiber afferents to the cerebellum are activated respectively by the conditioned stimuli (CS) and unconditioned stimuli (US) used for Pavlovian eyelid conditioning (Aitkin and Boyd, 1978; Sears and Steinmetz, 1991). Electrical stimulation of mossy fibers and climbing fibers will support learning when substituted for the CS and US respectively (Mauk et al., 1986; Steinmetz et al., 1986, 1989; Steinmetz, 1990), and lesions of these afferent pathways affect conditioning in ways identical to omitting the CS or US (McCormick et al., 1985; Lewis et al., 1987; Steinmetz et al., 1987). Similar studies have demonstrated that output from the cerebellar (anterior) interpositus nucleus is necessary and sufficient for the expression of conditioned eyelid responses. Neurons in the interpositus nucleus show increased activity that precedes the conditioned response (McCormick and Thompson, 1984a,b; Chapman et al., 1990), stimulation in the same region in untrained animals elicits eyelid responses (McCormick and Thompson, 1984a,b), and lesions of this nucleus permanently abolish conditioned response expression (Clark et al., 1984; McCormick and Thompson, 1984a,b; Lavond et al., 1985; Yeo et al., 1985b). Together these data suggest that converging mossy fiber and climbing fiber inputs induce plasticity in the cerebellum, and that this plasticity allows the CS to activate cerebellar output and drive the expression of conditioned responses (Mauk, 1997).

Although there is, based on this sort of evidence, general agreement that eyelid conditioning is mediated by the cerebellum (see Welsh and Harvey, 1991; Bloedel, 1992; Llinas et al., 1997 for alternative views), there remain debates regarding the specific cerebellar
Two of the more contentious issues have been the relative contributions of the cerebellar cortex and cerebellar nuclei, and the exact regions of cerebellar cortex that are involved. Surgical ablation of the cerebellar cortex or cerebellar nuclei, and more recently reversible pharmacological inactivation of the cerebellar nucleus, have been employed to address these issues. There is general agreement that both surgical lesions and reversible inactivation of the cerebellar anterior interpositus nucleus abolish the expression of conditioned responses (Clark et al., 1984; McCormick and Thompson, 1984b,a; Lavond et al., 1985; Yeo et al., 1985b; Welsh and Harvey, 1991; Krupa et al., 1993; Nordholm et al., 1993; Hardiman et al., 1996; Ivarsson et al., 1997; Welsh and Harvey, 1989).

Results from lesions of the cerebellar cortex have been far less clear. Following the original studies showing that lesions of the cerebellar cortex have no effects (McCormick and Thompson, 1984a), Yeo et al. reported that lesions of the HVI region of cerebellar cortex abolish conditioned responses (Yeo et al., 1984, 1985a). Subsequent studies from this group and others report less robust and less persistent effects of these lesions (Lavond et al., 1987; Lavond and Steinmetz, 1989; Yeo and Hardiman, 1992; Clark and Lavond, 1994). Lesions or genetic defects of the cerebellar cortex have also been reported to produce only modest effects on the acquisition of conditioned responses, and no effect on the extinction of previously learned responses (Lavond et al., 1987; Lavond and Steinmetz, 1989; Chen et al., 1996).

In previous studies we have reported quite different effects of cerebellar cortex lesions. When made in previously trained animals, lesions that included the anterior lobe of the cerebellar cortex did not abolish conditioned responses, but diminished their amplitude and disrupted their learned timing. Whereas normally the learned responses are delayed to peak near the onset of the US, post-lesion responses displayed a fixed, short latency to onset (Perrett et al., 1993). We have subsequently shown that these same lesions prevent the extinction of previously learned responses (Perrett and Mauk, 1995), and prevent the acquisition of responses using a second CS (Garcia and Mauk, 1995). Lesions of HVI without damage to the anterior lobe had no effect on response timing or amplitude and had no measurable effects on acquisition or extinction. These studies raise important issues regarding both the regions of cerebellar cortex that are involved in eyelid conditioning, and regarding sites of cerebellar plasticity.

On the basis of these and other studies, we have proposed that plasticity in both the cerebellar cortex and cerebellar anterior interpositus nucleus are involved in the acquisition and expression of conditioned eyelid responses (Raymond et al., 1996; Mauk and Donegan, 1997; Mauk et al., 1997). Plasticity in the cerebellum and not extra-cerebellar sites is suggested by both the abolition of conditioned responses by lesions or inactivation of the interpositus nucleus as well as by the ability of stimulation of mossy fibers and climbing fibers to substitute for the CS and US respectively in producing normal response acquisition. Plasticity in the interpositus nucleus is suggested by the expression of (improperly timed) conditioned responses following lesions of the cerebellar cortex. Plasticity in the cerebellar cortex is suggested by the ability of cortex lesions to abolish learned response timing.

Although these hypotheses are consistent with existing lesion data and provide concrete ideas regarding conditioned response acquisition and expression, testing these ideas has been hampered by two key limitations. The first of these is the continued debate regarding the exact regions of cerebellar cortex that may be involved. This issue is exacerbated by the inherent difficulty in determining the exact functional, and not just anatomical, extent of surgical ablations or electrolytic lesions. For example, it is difficult on the basis of histology alone to exclude the possibility that cerebellar cortex lesions abolish conditioned response expression due to inadvertent damage to input or output pathways that are necessary for response expression. Secondly, and perhaps more seriously, studies of the cerebellar cortex are potentially confounded by the degeneration of the climbing fibers that such lesions produce (Yeo et al., 1984, 1985a). Indeed, this is a specific example of a more general concern regarding the potential for permanent lesions to produce their effects through unintended compensatory mechanisms rather than the strict absence of the cerebellar cortex. Thus, it is always possible with permanent lesions that post-lesion responding is mediated by neuronal processes that differ from normal (see Mauk and Thompson, 1987).

Here, we attempt to address these issues by using microinfusion of GABA antagonists into the interpositus nucleus to produce a rapid and reversible block of the GABA-mediated inhibitory input. Since this inhibitory projection represents the sole output from the cerebellar cortex, infusion of picrotoxin produces a reversible disconnection of the cerebellar cortex from the output cells of the interpositus nucleus. Moreover, infusion of the GABA agonist muscimol through the same cannula can be used to produce reversible inactivation of the interpositus nucleus neurons. Since these pharmacological manipulations are rapid and reversible, this approach obviates previous confounds from damage to climbing fibers, or from lesion-induced compensatory mechanisms. Moreover, these infusions obviate debates regarding which sites of cerebellar cortex are crucial since the abolition of responses with muscimol indicates the critical nucleus follower cells are affected, regardless of the anatomical source of their inhibitory Purkinje cell inputs.
We report that infusion of picrotoxin into the anterior interpositus nucleus diminishes the amplitude and disrupts the learned timing of conditioned eyelid responses. Infusion of the GABA agonist muscimol through the same cannula completely abolishes the expression of responses, and infusion of saline has no effect.

2. Method

2.1. Animals

Data were obtained from four male New Zealand albino rabbits (Oryctolagus cuniculus), weighing 2.5–3.0 kg each. The animals were individually housed and had free access to food and water. Treatment of the animals and surgical procedures were in accordance with an approved animal welfare protocol.

2.2. Surgical preparation

Animals were first prepared with a cannula implanted in the anterior interpositus nucleus and with a head bolt cemented to the skull. Animals were pre-anesthetized with 5 mg/kg acepromazine, and their skulls were immobilized in a stereotaxic restrainer. Anesthesia was maintained with halothane (1–2% mixed in oxygen), and sterile procedures were used during the placement of the cannulae. After exposing the skull, four holes were drilled to accommodate screws that would be used to affix a bolt to the skull. A craniotomy was drilled just lateral to lambda and covered with bone wax. The head was positioned with lambda 1.5 mm ventral to bregma. A cannula (Plastics One, Roanoke) consisting of a 26-gauge stainless steel guide sheath and a 33-gauge internal cannula that projected 1.2 mm beyond the tip of the guide sheath was placed at stereotaxic co-ordinates corresponding to the nucleus (0.7 mm anterior, 4.9 mm left lateral, and 14.0 mm ventral to lambda). Following placement, the cannula assembly and head bolt were secured to the skull with dental acrylic, and the skin was sutured. Finally, two stainless steel stimulating electrodes were chronically implanted in the periorbital muscles rostral and caudal to the eye. Antibiotics, intravenous fluids, and analgesics were administered post surgically as needed, and animals were allowed approximately 1 week to recover.

2.3. Conditioning procedures

Training involved a standard Pavlovian conditioning delay protocol with a 500-ms interstimulus interval. Each training session consisted of 12 nine-trial blocks. Each block was comprised of eight paired presentations of the CS and US and one presentation of the CS only. The CS (a 1-kHz, 85-dB tone) was presented for 550 ms during CS-alone trials, and co-terminated with a 50 ms train of constant current pulses (200 Hz, 1 ms pulse width, 2–6 mA) delivered to the periorbital electrodes during paired trials. Trials were separated by a 30 s intertrial interval. Asymptotic conditioned responding was established with at least ten training sessions.

Stimulus presentation and data acquisition were controlled by a computer using custom software. Movement of the unrestrained eyelid was recorded by measuring the reflectance of an infrared LED aimed at the eyelid. Voltage responses were determined to be linearly related to eyelid movement and were calibrated for each animal daily. Digitized responses (1 point/ms) were analyzed using custom software to determine latencies to onset and to peak, as well as peak amplitudes.

2.4. Drug infusion

Three test sessions were conducted. Each test session began with three blocks of training to establish a baseline of responding prior to drug infusion. To keep the infusion volumes as small as possible, the following procedure was employed. During the first test session, 2 μl of picrotoxin (100 μM) was infused at a rate of 1 μl/min. If this initial infusion failed to affect response timing in the first two trials after resuming training, the session was paused and an additional 2 μl were infused. One animal required a single 2 μl infusion and the remaining three required two infusions for a total of 4 μl. During the second and third test sessions, animals were infused with saline vehicle and with muscimol (1.0 mM), respectively. For each animal, the volumes infused during these sessions were the same as that of the picrotoxin infusion in the first session. Animals were allowed at least 12 h to recover between test sessions.

2.5. Histology

Following training, the location of the cannula tip was determined for each animal using standard histological procedures. Briefly, the infusion site was marked by passing a DC current (200 μA for 20 s) through a small wire cut to the length of the internal cannula and exposed at the tip. Animals were sacrificed with an overdose of sodium pentobarbital and perfused intracardially with 1.0 l of 10% formalin. The brains were removed and stored in 10% formalin for several days. Brains were embedded in an albumin gelatin mixture, and the cerebellum was sectioned using a freezing microtome (80 μm sections). Tissue was mounted, and stained with cresyl violet and counterstained with Prussian blue.
2.6. Data analysis and statistical tests

Peak response amplitude and onset latency were calculated by custom software. Digitized sweeps corresponded to the 200 ms prior and 2300 ms after the CS onset. Once calibrated, peak amplitude was measured relative to an average of the 200 ms baseline collected prior to CS onset. Onset latency was determined by calculating the point at which the response slope deviated by two standard deviations from baseline. In order to be counted as a conditioned response, onset latency had to follow CS onset, and the movement amplitude had to reach 0.3 mm prior to US onset during paired trials. This criterion was relaxed for CS-alone trials in which movements were counted as conditioned responses if they reached a 0.3 mm amplitude at any time following CS onset. Amplitude measures were determined only for the single CS-only trial presented each block. Trials in which there was greater than 0.3 mm of movement during the baseline were excluded from further analysis.

After determining amplitude and onset latency for each trial of a test session, block by block response rates and mean onset latencies were calculated. For these block data, separate two-way repeated-measures ANOVAs were performed on the response rate, amplitude, and onset latency data. Post-hoc comparisons were made using an F-test for simple effects with a significance criterion of \( P < 0.01 \).

3. Results

As shown in Fig. 1, infusion of picrotoxin into the nucleus significantly decreased the amplitude and onset latency of conditioned responses without affecting the rate of responding. In contrast, muscimol infusion abolished conditioned responses altogether. A two-way, repeated measures ANOVA demonstrated significant effects on the percentage of conditioned responses for trial blocks \([F(3,1) = 178.51, P < 0.001]\), drug treatment \([F(2,6) = 89.13, P < 0.001]\), and a significant blocks by treatment interaction \([F(2,6) = 73.65, P < 0.001]\).

![Fig. 1](image-url)

Fig. 1. Group data for the effects of picrotoxin (■), muscimol (▲), and saline (○) infused into the interpositus nucleus (n = 4). (A) Infusion of muscimol abolished the responses almost completely as illustrated by its effects on the percentage of trials in which conditioned responses are seen. Infusion of picrotoxin and saline had no significant effects on the likelihood of conditioned responses. (B) Both picrotoxin and muscimol infusion significantly decreased the amplitude of the responses. Picrotoxin produced a decrease in amplitude that was significantly different from saline and from the effects of muscimol, which abolished conditioned responses altogether. (C) Picrotoxin significantly decreased the latency to onset of the conditioned responses. Data for the muscimol infusions are not shown since so few responses were seen, but the latencies of the few responses seen were comparable to controls. (D) Average responses of the nine CS-alone trials following infusion of picrotoxin, muscimol, and vehicle (from the same animal as shown in Fig. 2). Bold lines correspond to times at which the CS and US were present.
$F$-test for simple effects performed on the post-infusion training blocks revealed muscimol significantly decreased the rate of responding relative to picrotoxin and vehicle treatment ($P < 0.01$). There were no significant differences in the response rates of animals during vehicle and picrotoxin treatment sessions. The ANOVA performed on response amplitude data demonstrated significant trial blocks [$F(1,3) = 342.02, P < 0.001$], treatment [$F(2,6) = 36.97, P < 0.001$], and blocks by treatment interaction [$F(2,6) = 79.50, P < 0.001$] effects.

In contrast to measures of percent responding, post-hoc analysis revealed a difference among all drug treatment comparisons ($P < 0.01$). Finally, an ANOVA was performed to compare onset latency data between vehicle and picrotoxin infusion sessions. The muscimol session was omitted from this comparison due to the low response rate. This analysis revealed a significant treatment [$F(1,3) = 33.88, P < 0.025$] and blocks by treatment interaction [$F(1,3) = 26.58, P < 0.025$] effect. There was no significant effect of trial blocks [$F(1,3) = 9.44$]. An $F$-test for simple effects comparing post-infusion blocks demonstrated picrotoxin treatment significantly decreased onset latencies relative to injection vehicle control ($P < 0.01$). Average sweeps for one animal from the nine CS-alone trials presented after control, picrotoxin, and muscimol injections are depicted in Fig. 1D.

Robust effects produced by infusion of picrotoxin and muscimol are clearly apparent in the individual sweeps collected during CS alone trials, as shown for a typical animal in Fig. 2. Picrotoxin infusion rapidly decreased the onset latencies and amplitude of the conditioned responses. Responses were normally timed at the beginning of the second test session 12 h later. In this session, neither response timing, nor amplitude were affected by injection of an equal volume of saline vehicle. In the last session, injection of muscimol abolished conditioned responses completely. The infusion site for this animal is shown in the inset of Fig. 2. The infusion sites for all four animals in the study are shown in Fig. 3. Each is in the proximity of the ipsilateral anterior interpositus nucleus.

---

**Fig. 2.** Individual CS alone trials for all three infusion sessions from a representative animal. For each session the three pre-infusion responses are shown in bold. The inset indicates the cannula placement for this animal (same as that shown at the top of Fig. 3).
4. Discussion

We have demonstrated that pharmacological block of cerebellar cortex output reversibly disrupts the timing and decreases the amplitude of conditioned eyelid responses. In contrast, pharmacological block of cells in the cerebellar interpositus nucleus abolishes responses completely, whereas infusion of equal volumes of vehicle has no measurable effects. These results replicate previous studies that demonstrated reversible inactivation of the interpositus nucleus abolishes the expression of conditioned eyelid responses. More importantly, the effects of picrotoxin-induced blockade of cerebellar cortex output to the interpositus nucleus parallel previous findings produced with surgical ablations or electrolytic lesions of the anterior lobe of cerebellar cortex.

The approach we have employed, by pharmacologically removing the cerebellar cortex from the eyelid response circuit, obviates many sources of uncertainty that have made it difficult to determine the contribution of the cerebellar cortex to eyelid conditioning. Perhaps most importantly, interpretation of the present results does not depend on the resolution of ongoing debates regarding the precise region of cerebellar cortex that is involved in eyelid conditioning. The complete abolition of responses with muscimol ensures that the infusions in the present study affected the neurons in the interpositus nucleus that are responsible for the expression of conditioned responses. Infusion of picrotoxin through the same cannula therefore removed the inhibitory action of the critical Purkinje cells, whatever the location of their cell bodies in the cerebellar cortex. The possibilities that our results are due to inadvertent damage of interpositus nucleus or other key pathways, or to damage or degeneration of climbing fibers, are excluded both by the lack of effect from vehicle infusions and by the immediate and complete recovery of the conditioned responses the day following picrotoxin infusion. Based on these arguments, the present data strongly support previous findings that lesions of the cerebellar cortex in well trained animals do not abolish conditioned responses, but disrupt their timing and diminish their amplitude (Perrett et al., 1993).

Our results appear to contradict a previous study in which infusions of high concentrations of picrotoxin into the interpositus nucleus abolished expression of conditioned nictitating membrane responses in well trained animals (Mamounas et al., 1987). However, this previous study reported only a decrease in response amplitude with an amount of picrotoxin similar to that used in the present study (previous study: 500 μM at 0.75 μl = 0.38 nmol/present study: 100 μM at 4 μl = 0.4 nmol). Because response timing was not examined in the previous paper, it seems possible that similar effects were produced in each case. Since high concentrations of picrotoxin (> 0.5 mM) have been shown to depolar-
ize axons to the point of inactivation of voltage-dependent conductances (Freeman, 1973), it also seems possible that the abolition of responses seen at higher concentrations (1.0 mM) were due to blockade of action potential activity in the nucleus cells. If so, then the abolition of responses seen at higher concentration of picrotoxin in the previous study parallel our results with infusion of muscimol.

The effects of pharmacological disconnection of the cerebellar cortex on response timing that we report here differ in one interesting way from the effects of surgical ablation of the cerebellar cortex. With ablations, we have generally seen post-lesions responses that are short latency and short duration (e.g. Perrett et al., 1993; Perrett and Mauk, 1995). As seen in Fig. 2, picrotoxin infusion produces responses that are similarly short in latency to onset, but are of longer duration than the typical ablation effect. Our data do not provide an explanation for this difference and thus we can only speculate as to the cause. This may reflect the differences between long-term removal of the cortex—with the attendant possibility of compensatory processes—and short term disconnection where such processes may be absent. Alternatively, this difference may reflect the removal of only the Purkinje cell inhibitory inputs (ablations) versus blockade of all GABA_A-mediated inhibition in the nucleus, which probably includes local inhibitory interneurons as well. Whatever the explanation, this difference does not affect the basic conclusions to be gleaned from the results, and with further work may provide hints as to the functional significance of local inhibitory circuitry in the cerebellar nuclei.

The reversible inactivation results we have presented are consistent with the hypothesis that conditioned eyelid responses are mediated by interactions between the cerebellar cortex and the output cells of the interpositus nucleus. The abolition of responses by muscimol infusion indicates further that activity of cells in the cerebellar interpositus nucleus is necessary for the expression of the conditioned eyelid responses. Combined with previous demonstrations that stimulation of the interpositus nucleus in untrained animals can elicit responses, including eyelid responses, our data suggest that CS-evoked increases in activity in the appropriate cells of the interpositus nucleus are both necessary and sufficient for the expression of conditioned eyelid responses.

Our results with picrotoxin infusion into the interpositus nucleus are consistent with the hypothesis that in a well trained animal the CS has acquired the ability to elicit activity in the interpositus cells through training-induced plasticity in both the cerebellar cortex and the interpositus nucleus (Fig. 4A). That responses are spared by picrotoxin infusions is consistent with the hypothesis that training induces plasticity in the interpositus nucleus, presumably a potentiation of the mossy fiber synapses activated by the CS. The only alternative is that learning-induced plasticity upstream of the cerebellum is responsible for the short-latency responses. The best evidence to the contrary comes from the ability of mossy fiber stimulation (as the conditioned stimulus) to support conditioning and the inability of this stimulation to elicit responses prior to training (Steinmetz et al., 1986, 1987, 1989; Steinmetz, 1990; Ivarsson et al. 1997).

The disruption of the timing seen with picrotoxin also provides further evidence that the cerebellar cortex contribution to the conditioned responses involves both plasticity and an ability for temporal coding during the CS. Since the timing of the responses is learned and does not simply depend on the strength of the association (Millenson et al., 1977; Mauk and Ruiz, 1992), response timing requires some form of temporal coding combined with synaptic plasticity. This is consistent with recordings from Purkinje cells showing decreases in activity that are also delayed to begin just before the onset of the conditioned responses (Hesslow and Ivarsson, 1994). Similarly, the need for decreases in Purkinje activity to elicit conditioned responses is supported by findings that stimulation of Purkinje cells suppress conditioned response expression (Hesslow, 1994). Previous computer simulations have suggested the type of temporal coding of the CS that might occur in the cerebellar cortex to allow Purkinje cells to learn an appropriately timed decrease in activity during the CS (Buonomano and Mauk, 1994). Thus, we suggest that conditioned responses in a well trained animal are mediated by increases in the strength of mossy fibers synapses in the interpositus nucleus that are activated by the CS, and by plasticity in the cerebellar cortex that mediates a learned, and appropriately-timed decrease in Purkinje cell activity during the CS. In this context, infusion of picrotoxin into the interpositus nucleus would block Purkinje cell modulation of nucleus cells and unmask short latency responses mediated only by the strengthened mossy fiber synapses in the nucleus itself (Fig. 4B). The complete abolition of responses by infusion of muscimol is, of course, also consistent with these hypotheses (Fig. 4C).

Analysis of the neural basis of adaptation of the vestibulo-ocular reflex (VOR) has yielded surprisingly similar ideas regarding cerebellar mechanisms of motor learning (Raymond et al., 1996). Like eyelid conditioning, VOR adaptation requires the paired occurrence of a stimulus that comes to elicit the response (tone for eyelid conditioning or moving the head for VOR) with an error stimulus (the US in eyelid conditioning or visual errors for VOR). The mossy fiber and climbing fiber afferents appear to play parallel roles in the two systems in terms of conveying these stimuli to the cerebellum. The effects of cerebellar cortex lesions are also quite similar for VOR and eyelid conditioning. In
Fig. 4. (A) A schematic representation of the synaptic organization of the cerebellum and its hypothesized involvement in Pavlovian eyelid conditioning. Paired presentation of a tone and a reinforcing US leads to the acquisition of a conditioned eyelid response. Previous results have shown that (1) the cerebellar nuclei are necessary for the expression of the learned responses, (2) the tone is conveyed to the cerebellum via mossy fibers, and (3) the US is conveyed to the cerebellum via climbing fibers (gray pathway). Based on previous results, we hypothesize that the expression of conditioned responses is mediated by LTD at the granule to Purkinje synapses, and by LTP at the mossy fiber synapses in the cerebellar nuclei. We also hypothesize that time varying activation of different granule cells during the CS allows the LTD to produce a properly timed decrease in Purkinje activity during the tone. Since the only output of the Purkinje cells is GABA-mediated inhibition of the nucleus cells, pharmacological block of this synapse can be used to produce reversible disconnection of the cerebellar cortex. (B) A schematic representation of the effects hypothesized for infusion of picrotoxin into the interpositus nucleus. This infusion should reversibly disconnect the cerebellar cortex by blocking Purkinje cell inhibition of the nucleus cells. We hypothesize that conditioned responses will still be present, due to the learning-induced LTP at the mossy fiber synapses in the nucleus. However, the responses should no longer be delayed appropriately and instead should display a fixed, short latency to onset. (C) With infusion of muscimol into the interpositus nucleus, responses should be abolished completely due to strong hyperpolarization of the output cells whose activity is required for response expression.
both systems, lesions abolish some but not all of the memory for previous learning, and completely prevent any further learning (Robinson, 1976; Raymond et al., 1996). Thus, evidence in both systems is consistent with plasticity in both the cerebellar cortex and nuclei, and with the need for intact cerebellar cortex input for the induction of plasticity in the nuclei. The similarity of results between the two systems suggests that the mechanisms implied are not specific to eyelid conditioning or VOR adaptation, but rather are general features of cerebellar mechanisms of motor learning.

Acknowledgements

We thank Dodie Baccigalopi for assistance with surgery and post-surgical care of the animals. This work was supported by NIMH 46904-06.

References


Steinmetz, J.E., 1990. Classical nictitating membrane conditioning in rabbits with varying interstimulus intervals and direct activation of cerebellar mossy fibers as the CS. Behav. Brain Res. 38, 97–108.


