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Localization of the Cerebellar Cortical Zone Mediating Acquisition of Eyeblink Conditioning in Rats

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Abstract

Delay eyeblink conditioning is established by paired presentations of a conditioned stimulus (CS) such as a tone or light and an unconditioned stimulus (US) that elicits eyelid closure before training. The CS and US inputs converge on Purkinje cells in the cerebellar cortex. The cerebellar cortex plays a substantial role in acquisition of delay eyeblink conditioning in rabbits and rodents, but the specific area of the cortex that is necessary for acquisition in rodents has not been identified. A recent study identified an eyeblink microzone in the mouse cerebellar cortex at the base of the primary fissure (Heiney, Kim, Augustine, & Medina, 2014). There is no evidence that the cortex in this eyeblink microzone plays a role in rodent eyeblink conditioning but it is a good candidate region. Experiment 1 examined the effects of unilateral (ipsilateral to the US) lesions of lobule HVI, the lateral anterior lobe, or the base of the primary fissure on eyeblink conditioning in rats. Lesions of either the anterior lobe or lobule HVI impaired acquisition, but lesions of the base of the primary fissure produced the largest deficit. Experiment 2 used reversible inactivation with muscimol to demonstrate that inactivation of the putative eyeblink microzone severely impaired acquisition and had only a modest effect on retention of eyeblink conditioning. The findings indicate that the base of the primary fissure is the critical zone of the cerebellar cortex for acquisition of eyeblink conditioning in rats.

Keywords

associative learning; cerebellum; cerebellar cortex; eyeblink conditioning

1. Introduction

The cerebellum is necessary for acquisition and retention of associative eyeblink conditioning (McCormick, Clark, Lavond, & Thompson, 1982). Delay eyeblink conditioning consists of a conditioned stimulus (CS; e.g., a tone) paired with an unconditioned stimulus (US) that elicits an eyelid closure unconditioned response (UR) before training. Repeated CS–US pairings result in the development of an eyelid closure

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conditioned response (CR) which precedes the onset of the US. Numerous studies demonstrated the essential role of the cerebellum in acquisition and retention of the CR using lesions, reversible inactivation, electrical stimulation, unit recording, genetic manipulations, neuropharmacology, and quantitative neuroanatomy (for review see J. H. Freeman & Steinmetz, 2011).

Acquisition and retention of the eyeblink CR are completely abolished by lesions or inactivation of the anterior interpositus nucleus (Clark, Zhang, & Lavond, 1992; J. H. Freeman Jr, Halverson, & Poremba, 2005; Krupa, Thompson, & Thompson, 1993; Lavond, Hembree, & Thompson, 1985; Lincoln, McCormick, & Thompson, 1982; McCormick et al., 1982; Steinmetz, Logue, & Steinmetz, 1992; Steinmetz, Lavond, Ivkovich, Logan, & Thompson, 1992; Yeo, Hardiman, & Glickstein, 1985a). Lesions or inactivation of the cerebellar cortex also produce deficits in acquisition; however, the severity of the deficit has differed between studies (Attwell, Rahman, & Yeo, 2001; Garcia, Steele, & Mauk, 1999; Hardiman, Ramnani, & Yeo, 1996; Lavond & Steinmetz, 1989). Mice with the Purkinje cell degeneration mutation (*pcd*) which lose most of their Purkinje cells during development are impaired during acquisition, but show an increase in CRs across training that is significantly greater than unpaired controls (Chen, Bao, Lockard, Kim, & Thompson, 1996; Chen, Bao, & Thompson, 1999). A similar set of results was found in rats that had Purkinje cells destroyed by OX7-saporin, an immunotoxin, as adults (Nolan & Freeman, 2006). The findings from the *pcd* studies in mice and the OX7-saporin study in rats suggest that the cerebellar cortex plays a substantial role in acquisition of eyeblink conditioning but the cerebellar interpositus nucleus can support modest learning without the cortex (Chen et al., 1999; Nolan & Freeman, 2006). Indeed, lesions of the interpositus nucleus abolish CRs in *pcd* mice (Chen et al., 1999). The rodent studies were informative regarding the role of the cerebellar cortex in eyeblink conditioning but they did not identify the area within the cortex that plays a critical role in acquisition.

A recent study that used optogenetic inhibition of Purkinje cells in mice that were not given eyeblink conditioning identified an “eyeblink microzone” at the base of the primary fissure (Heiney et al., 2014). Electrophysiology, electrical stimulation, and optogenetics were used to demonstrate that inhibition of Purkinje cells in this area produced eyelid closure. This mouse eyeblink microzone is therefore a very good candidate for the cortical area that is critical for acquisition of eyeblink conditioning in rodents. The current study used lesions (Experiment 1) and reversible inactivation (Experiment 2) to localize the area of the cerebellar cortex that is critical for acquisition of eyeblink conditioning in rats. Electrolytic lesions were made in the base of the primary fissure (PFb) or in the lobules on either side of the primary fissure: hemispheric lobule VI (HVI) or lateral anterior lobules IV/V (AIV/V). Reversible inactivation was used to determine whether the base of the primary fissure plays a role in acquisition and retention of eyeblink conditioning in a within subjects design.

2. Materials and Methods

2.1 Subjects

Subjects were 78 male Long-Evans rats, 250-350 g at the beginning of the experiment. The rats were housed in Spence Laboratories of Psychology at the University of Iowa with a 12 hr light-dark cycle and were given ad libitum access to food and water.

2.2 Surgery

One week prior to the onset of training, rats were removed from their home cage and anesthetized with isoflurane. For Experiment 1, unilateral lesions of the cerebellar cortex were produced by passing 1.0 mA of DC current for 10 sec through an insect pin insulated with Epoxylite into the left cerebellar cortex. Rats in the control group had electrodes lowered into the cerebellar cortex but no current was passed. In Experiment 2, a 23-gauge guide cannula was implanted 1.0 mm dorsal to the targeted area in the cerebellar cortex. A 30-gauge stylet was inserted into the guide cannula and extended 1.0 mm from the end of the guide. The stereotaxic coordinates taken from bregma were 11.2 mm posterior, 2.6 mm (AIV/V), 3.0 mm (PFb), or 3.4 mm (HVI) lateral, and 3.2 mm ventral to the skull surface. For both experiments rats were implanted with differential electromyographic (EMG) electrodes in the left upper eyelid muscle (orbicularis oculi) and a ground electrode attached to a stainless steel skull screw. The EMG electrode leads terminated in gold pins held in a plastic connector, which was secured to the skull with dental acrylic. A bipolar stimulating electrode (Plastics One, Inc.) for delivering the periorbital stimulation US was implanted immediately caudal to the left eye. The bipolar electrode terminated in a plastic connector that was secured to the skull with dental acrylic.

2.3 Infusions

Prior to infusions, the 30-gauge stylet was removed and replaced with a 30-gauge infusion needle that extended 1.0 mm past the tip of the guide cannula. A 10 μ L syringe (Hamilton, Reno, NV) was connected to the infusion needle by a polyethylene tube (PE 10, 110-120 cm) and the syringe was secured to an infusion pump (Harvard Apparatus, Hilliston, MA). Rats were infused with .5 μ L of either muscimol (2.0 mM) or PBS at a rate of 6.0 μ L/hr 30 min in each site prior to the beginning of training.

2.4 Apparatus

The conditioning apparatus consisted of four small-animal sound attenuation chambers (BRS/LVE, Laurel, MD). Within each sound attenuation chamber was a small animal operant chamber (BRS/LVE, Laurel, MD) where the rats were located during conditioning. One wall of the operant chamber was fitted with two speakers through which the CS was presented. The electrode leads from the rat's headstage were connected to peripheral equipment. Computer software controlled the delivery of stimuli and the recording of eyelid EMG activity (JSA Designs, Raleigh, NC). The US (2-3 mA, DC constant current) was delivered through a stimulus isolator (Model number 365A, World Precision Instruments, Sarasota FL). EMG activity was recorded differentially, filtered (500–5000 Hz) and

integrated by equipment (JSA Designs, Raleigh, NC) as described in other reports (J. H. Freeman Jr et al., 2005).

2.5 Conditioning procedures

Rats recovered from surgery for 1 week prior to the initiation of training. Rats completed daily sessions of paired training. Each session consisted of 10 blocks of 9 paired CS-US presentations and 1 CS alone probe trial. The CS was a 400 ms tone (2 kHz; 85 dB). The CS terminated with a 25 ms periorbital stimulation US. The shock intensity was adjusted in each rat to elicit a blink and slight head movement prior to the first session and then it was unchanged for the remaining sessions (range = 2 – 3 mA). For Experiment 1, rats completed daily sessions until they reached two consecutive sessions at or above 80% CRs. Training was terminated after 15 sessions if the rat never reached criterion. In Experiment 2, rats underwent 12 paired sessions. Thirty minutes before each of the first 5 sessions, rats received infusions of muscimol or vehicle (0.5 μ l). During the next five sessions rats received training without infusions in order to examine possible savings following infusions or to make sure that all rats reached asymptotic conditioning. On sessions 11 and 12 rats received muscimol or vehicle to assess inactivation effects on retention. CRs were defined as EMG activity that exceeded a threshold of 0.4 units (amplified and integrated units) above the baseline mean during the CS period after 80 ms. EMG responses that exceeded the threshold during the first 80 ms of the CS period were defined as startle responses to the CS. On CS-alone probe trials, the duration for scoring CRs was extended beyond the CS to the end of the trial period (1.0 s). URs were defined as responses that crossed the threshold after the onset of the US.

2.6 Histology

Following the last training session, rats were euthanized with a lethal injection of sodium pentobarbital (150 mg/kg) and perfused transcardially with 0.9% saline followed by 10% buffered formalin. Brains were extracted and then post-fixed and cryo-protected in a 30% sucrose formalin solution and subsequently sectioned at 50 μ m with a sliding microtome. Sections were mounted on gel-coated slides and stained with thionin. The extent of lesions and cannula placements were then verified using light microscopy.

3. Results

3.1 Experiment 1: Cerebellar Cortical Lesions before Acquisition

Unilateral electrolytic lesions were made in the cerebellar cortex ipsilateral to the conditioned eye prior to acquisition of delay EBC. Following histological examination, rats were placed into one of five groups depending on the location of the lesion: control (n=10), anterior lobe (AIV/V; n=5), lobule HVI (HVI; n=4), base of the primary fissure (PFb; n=13), or cerebellar cortex and anterior interpositus nucleus (CCTX/AIN; n=8). Two rats within the AIV/V group and 1 within the HVI group contained lesions that included small portions of the base of the primary fissure. However, their behavioral data did not significantly differ from other rats in their groups without lesions to the PFb. Four of the rats in the PFb group had extensive lesions extending to both AIV/V and HVI. All behavior measures of learning were examined and no significant differences were found when

comparing the extensive PFb lesions to the non-extensive PFb rats and were thus combined for further analysis. The data from 6 rats was not included in the analysis due to loss of their head-stage prior to completion of training.

Rats were trained daily until they reached criterion (two consecutive sessions at or above 80% CRs). Training concluded if rats did not reach the criterion within 15 sessions. Rats that received lesions of the cerebellar cortex or anterior interpositus nucleus exhibited marked impairments in the number of sessions to reach criterion regardless of the location of the lesion. None of the rats with lesions that included the anterior interpositus nucleus reached criterion and only one rat with a lesion in the PFb reached criterion. In contrast, all of the rats in the HVI and AIV/V groups reached criterion. A representative rat's histology and behavioral data from each group is shown in Figure 1. A one-way analysis of variance (ANOVA) revealed a significant Group effect [$F(4,39)=5.240$, $p < .001$]. Post-Hoc tests (Tukey-Kramer) indicated that control group learned significantly faster than the AIV/V, HVI, PFb, and CCTX/AIN groups ($p < .05$). The PFb group did not significantly differ from the CCTX/AIN group, and both groups took significantly longer to reach criterion than the AIV/V and HVI groups ($p < .05$). Additionally, the AIV/V and HVI groups did not differ from each other (Figure 2A). To further examine the effects of the lesions CR percentage during the last two sessions of training was analyzed (Figure 2B). A one-way ANOVA revealed a significant main effect of Group on CR percentage [$F(4,39)=4.515$, $p < .001$]. Post-hoc tests (Tukey-Kramer) revealed that control group learned to a higher percentage than the PFb and CCTX/AIN groups, but there were no significant differences between the control and HVI or AIV/V groups. Also, PFb rats learned to a higher CR percentage than the CCTX/AIN group. These results together indicate that lesions of the cerebellar cortex significantly impaired learning. However, when the lesion included the base of the primary fissure the impairment was more severe than with lesions confined to the AIV/V or HVI.

The amplitude, onset latency, and peak latency of the CR were examined for each of the lesion and control groups. These measures were collected using both paired and CS-alone trials in which a CR occurred in order to have sufficient data for statistical analyses for the PFb and CCTX/AIN groups. Data from the last two sessions were averaged together in order to have an adequate sample size. A one-way ANOVA revealed a significant main effect of Group for CR amplitude [$F(4,39)=3.430$, $p < .001$]. Post-hoc tests (Tukey-Kramer) indicated that the PFb and CCTX/AIN groups had significantly lower amplitude CRs than the control, HVI, or AIV/V groups (Figure 2C). The PFb and CCTX/AIN groups did not differ from one another and the control, HVI, and AIV/V groups did not differ. CR onset and peak latencies were also examined in order to determine the effects of cerebellar cortex lesions on CR timing. One-way ANOVAs were completed for both onset and peak latencies (Figure 2 D,E). There was no significant effect of group on onset latency but there was a significant main effect of group for peak latency [$F(4,39)=5.393$, $p < .01$]. Post-hoc tests indicated that the PFb and CCTX/AIN groups had significantly earlier peak latencies as compared to the control group (Figure 2E). No other significant differences were observed.

Four rats in the PFb group were additionally implanted with a cannula into the anterior interpositus nucleus. Following 15 sessions of training without reaching criterion these rats received muscimol infusions and vehicle infusions on different sessions (order counter-

balanced) in to determine whether or not the shorter latency CRs in the PFb group depended on the interpositus nucleus. A repeated measures ANOVA examining session 15, the muscimol session, and the vehicle session revealed a significant main effect of Session [$F(2,6)=6.094$, $p < .05$]. Post-hoc tests (Tukey HSD) found that there was a lower percentage of CRs in the muscimol session than either session 15 or the vehicle session (Figure 2F). The CR percentage during the muscimol sessions was at the level typically seen with unpaired training. These data indicate that the anterior interpositus nucleus is necessary for the CRs that were produced without the critical zone of the cerebellar cortex.

The results of Experiment 1 indicate that the base of the primary fissure is the area of the cerebellar cortex critical for acquisition of eyeblink conditioning in rats. This area is the same anatomically as the eyeblink microzone identified in mice (Heiney et al., 2014). The results of this lesion study therefore suggest that the base of the primary fissure is the putative eyeblink conditioning microzone in rodents.

3.2 Experiment 2: Inactivation of the Eyeblink Conditioning Microzone during Acquisition and Retention

Reversible inactivation of the cortex was given during acquisition and retention of eyeblink conditioning to further examine the role(s) of the eyeblink conditioning microzone (ECM). Lesions of the ECM may have damaged axonal projections from Purkinje cells in both HVI and AIV/V to the anterior interpositus nucleus, which could account for the more severe deficit seen with ECM lesions relative to HVI or AIV/V lesions. It was therefore important to further investigate the role of the ECM in acquisition using muscimol inactivation, which has no effect on axons. Muscimol or vehicle was infused into the ECM prior to each of 5 sessions of acquisition followed by 5 sessions without infusions, which were given to establish asymptotic eyeblink conditioning in all rats. The rats were then given muscimol or vehicle just prior to retention tests (order counter-balanced).

A summary of the cannula placements is presented in Figure 3. Rats were further subdivided after histology, based on cannula placements, into either the ECM group ($n=10$), an outside the ECM group (oECM; $n=19$), or a vehicle control group ($n=8$). The border of the ECM was defined based on the lesion results as a 0.7 mm radius surrounding the base of the primary fissure in the coronal plane at 10.4 and 10.8 mm posterior to bregma (Figure 3, black circle).

Reversible inactivation of the ECM produced a severe impairment in acquisition (Figure 4). A Group (3) x Phase (2, Infusions vs. No Infusions) x Session (5) repeated measures ANOVA indicated a significant 3-way interaction, [$F(8, 64) = 10.121$, $p < .001$]. Post-hoc tests (Tukey-Kramer) found that the vehicle group had significantly more CRs than the ECM group (sessions 2-9) or the oECM group (sessions 2-5). Moreover, the ECM group exhibited significantly fewer CRs than the oECM group on sessions 3-8. Reversible inactivation therefore severely impaired acquisition of eyeblink conditioning only when infusions were localized to the ECM.

Amplitude and timing of the CR were also examined with 3-way ANOVAs. For CR amplitude, there were main effects of Group [$F(2, 20) = 2.283$, $p < .05$], Phase [$F(1, 80) =$

5.518, $p < .05$], and Session [$F(4, 80) = 9.454$, $p < .001$], but no interactions. Post-hoc tests indicated that the vehicle group had higher amplitude CRs than the ECM group, but no other differences were found. There was a significant Group x Infusion x Session interaction [$F(8, 58) = 2.903$, $p < .05$] for the CR peak latency data. Post-hoc tests revealed that ECM group had significantly later timed CRs than the vehicle group (Sessions 2-8), and the oECM group (Sessions 4-6). There were no significant group effects found with the CR onset latency data.

Following Session 10 rats were given muscimol or vehicle infusions in separate CS-US sessions to examine the effects of inactivation of cerebellar cortex on retention of eyeblink conditioning. A Group (2) X Session (3, Session 10, Muscimol, Vehicle) repeated measures ANOVA was conducted and revealed a significant Group x Session interaction [$F(2, 32) = 8.030$, $p < .001$]. There was a significant decrease in CR percentage for the ECM group during muscimol sessions relative to session 10 and the vehicle session. The oECM and vehicle groups did not exhibit a significant decrease in CR percentage following muscimol infusions. Additionally, a significant Group x Session interaction was found for CR amplitude [$F(2, 32) = 4.972$, $p < .05$] and CR peak latency [$F(2, 32) = 3.143$, $p < .05$], but not for CR onset latency. Post-hoc tests indicated that the ECM group had a significant decrease in CR amplitude and an increase in CR peak latency during the muscimol test relative to the control group.

4. Discussion

The goal of the current study was to identify the area of the cerebellar cortex that is critical for acquisition of eyeblink conditioning in rats. Lesions or inactivation of the cerebellar cortex ipsilateral to the trained eye resulted in impaired acquisition of eyeblink conditioning. Lesions that included the base of the primary fissure produced the most severe impairment. The small amount of conditioning observed in rats with lesions of the base of the primary fissure was abolished by inactivation of the interpositus nucleus. Reversible inactivation of the base of the primary fissure also severely impaired acquisition and partially impaired retention of eyeblink conditioning. The findings indicate that the base of the primary fissure is the area of the cerebellar cortex that is critical for acquisition of eyeblink conditioning in rats.

A recent study localized an “eyeblink microzone” at the base of the primary fissure in the mouse cerebellar cortex (Heiney et al., 2014). This eyeblink microzone was defined based upon a combination of electrophysiology, electrical stimulation, and optogenetic inhibition of Purkinje cells. Optogenetic stimulation of cerebellar cortical interneurons caused inhibition of Purkinje cell firing and resulted in eyelid closure. However, the role of this eyeblink microzone in eyeblink conditioning has not been determined in mice. The results of the current study are consistent with the findings from Heiney et al. (2014), indicating that impairing function within the same area of the cortex in rats severely impaired eyeblink conditioning. Thus, findings from two different rodent species, using completely different methods, converge to indicate the base of the primary fissure is the critical microzone for eyeblink conditioning. It is therefore hypothesized that lesions or inactivation of this putative eyeblink conditioning microzone will impair eyeblink conditioning in mice as well.

Previous studies using large aspirations of the cerebellar cortex or global loss of Purkinje cells in *pcd* mice or with OX7-saporin in adult rats found a deficit in the rate of acquisition of eyeblink conditioning, but moderate levels of conditioning were evident with extended training (Chen et al., 1996; Lavond & Steinmetz, 1989; Nolan & Freeman, 2006). In the current study we found that cerebellar cortex lesions localized to the base of the primary fissure severely impaired conditioning but did not prevent it from occurring (~50% after 15 d). Even rats with large lesions that included significant portions of both lobule HVI and the lateral anterior lobe still showed a small amount of conditioning. The magnitude of the impairment with lesions of the base of the primary fissure was equivalent to the impairment following global loss of Purkinje cells following exposure to OX7-saporin (Nolan & Freeman, 2006), suggesting that the modest levels of learning were not due to incomplete lesions. The small amount of eyeblink conditioning in the rats given lesions at the base of the primary fissure was abolished by muscimol inactivation of the anterior interpositus nucleus. This finding suggests that the anterior interpositus nucleus can support weak conditioning in the absence of the cerebellar cortex (Chen et al., 1999; Nolan & Freeman, 2006).

Inactivation of the cerebellar cortex also impaired acquisition of eyeblink conditioning, most severely when infusions of muscimol were within the base of the primary fissure. Although analysis of cannula placements indicated that all infusions were made within the cerebellar cortex, it is possible that a small amount of muscimol spread to the anterior interpositus nucleus. However, it is unlikely that the anterior interpositus nucleus was inactivated directly since the white matter between the infusion site and the nucleus would have been a barrier to the spread of muscimol (Allen et al., 2008). Moreover, the deficit in retention was very minor, whereas infusions of muscimol directly into the anterior interpositus nucleus abolish CRs (Campolattaro & Freeman, 2009; Freeman, Halverson, & Poremba, 2005; Garcia & Mauk, 1998). Thus, the severe acquisition deficit in Experiment 2 cannot be attributed to the spread of muscimol from the cortical infusion site to the anterior interpositus nucleus. Nevertheless, we cannot completely rule out the possibility that the spread of muscimol from the cortical infusion site had a slight effect on the anterior interpositus nucleus.

The inactivation results are generally consistent with the lesion results with one notable exception: CR peak latency was shorter with lesions but longer with inactivation relative to the respective control groups. Previous studies in rabbits showed that lesions of the cerebellar cortex produce impairments in CR timing (Garcia et al., 1999; Perrett, Ruiz, & Mauk, 1993). Rabbits with lesions in the anterior lobe show short latency CRs that are associative and depend on the interpositus nucleus (Ohshima, Nore, Medina, Riusech, & Mauk, 2006). The current lesion results are consistent with this finding but we did not see shorter latency CRs with muscimol inactivation. A definitive explanation for the difference in the effects of lesions and inactivation on CR timing is elusive, but it is possible that hyperpolarizing the network of cortical neurons with muscimol is less disruptive to downstream mechanisms than completely disconnecting the cortex from the deep nuclei. Indeed, pharmacological disconnection of the cerebellar cortex from the nuclei with GABA antagonists has been shown to severely impair CR timing in rabbits (Garcia & Mauk, 1998;

Medina, Garcia, & Mauk, 2001). Another possible explanation for the differential effects of lesions and inactivation on CR timing is that cerebellar cortical lesions produce degeneration in the inferior olive (Lavond, Steinmetz, Yokaitis, & Thompson, 1987; Yeo, Hardiman, & Glickstein, 1985b) and the subsequent loss of climbing fiber input to the deep nuclei may produce effects that are as severe as pharmacological disconnection of the cortex from the interpositus nucleus. Support for this idea comes from examining the effects of deleting Purkinje cells globally with OX7-saporin where there was no inferior olive degeneration and no deficit in CR timing (Nolan & Freeman, 2005; Nolan & Freeman, 2006). A third possibility is that there are species differences in the degree to which the cerebellar cortex is necessary for CR timing. This explanation seems unlikely since optogenetic inactivation of Purkinje cells affects the kinematics of eyelid movement in mice (Heiney et al., 2014). A resolution to the issue of why there are differential effects of cerebellar cortical lesions and inactivation on CR timing in rats may come with further analysis of the cellular mechanisms underlying learning-specific plasticity within the base of the primary fissure.

Retention of eyeblink conditioning was partially impaired by infusions of muscimol into the base of the primary fissure but not by infusions into neighboring lobules. Previous studies of the effects of post-acquisition lesions or inactivation of the cerebellar cortex on eyeblink conditioning in rabbits have found either no impairment or a severe impairment in retention (Attwell, Rahman, Ivarsson, & Yeo, 1999; Harvey, Welsh, Yeo, & Romano, 1993; Lavond et al., 1987; Perrett et al., 1993; Woodruff-Pak, Lavond, Logan, Steinmetz, & Thompson, 1993; Yeo et al., 1985b; Yeo & Hardiman, 1992). In some cases CRs could be re-established with additional training (Harvey et al., 1993; Lavond et al., 1987; Yeo & Hardiman, 1992). Lesions restricted to the anterior lobe and pharmacological disconnection of the cerebellar cortex from the deep nuclei impair CR timing but do not impair CR percentage in rabbits (Garcia & Mauk, 1998; Garcia et al., 1999; Medina et al., 2001; Ohyama et al., 2006). Inactivation of the lateral anterior lobe, HVI, or the base of the primary fissure did not affect CR timing in rats in the current study. A small deficit in CR percentage during retention was only seen in the rats given inactivation of the base of the primary fissure. It is not clear why the results of the current study differ from some of the previous results from some of the studies with rabbits. Additional studies are needed to determine the extent to which the putative eyeblink conditioning microzone in rats has the same physiological properties as the rabbit cortex.

The current results indicate that the cerebellar cortical area at the base of the primary fissure is critical for acquisition of eyeblink conditioning in rats. Localization of this putative eyeblink conditioning microzone lays the groundwork for studies that examine the cellular and molecular mechanisms underlying memory formation and maintenance within the cerebellum. Future studies can now more readily identify Purkinje cells that are specifically involved in producing the eyelid closure CR and determine the mechanisms underlying synaptic and excitability changes during learning.

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Highlights

- Unilateral cerebellar cortex lesions impair delay eyeblink conditioning in rats
- Lesions of the base of the primary fissure produce the most severe impairment
- Inactivation of this area severely impairs acquisition of eyeblink conditioning
- The base of the primary fissure is the eyeblink conditioning microzone in rats

Figure 1.

Mean ($\pm$ SEM) conditioned response (CR) percentage during 100-trial sessions of eyeblink conditioning for individual rats that were given lesions in lobule HVI (HVI), the lateral anterior lobe (AIV/V), the base of the primary fissure (PFb), a portion of the cerebellar cortex plus the dorsal part of the anterior interpositus nucleus (CCTX/AIN), or control surgery (Control). Histology from each rat is shown on the left.

Figure 2.

Group results from rats given lesions of either lobule HVI (HVI), anterior lobe (AIV/V), the base of the primary fissure (PFb), or cerebellar cortex and anterior interpositus nucleus (CCTX/AIN). **A:** Mean ($\pm$ SEM) sessions to reach a criterion of 2 consecutive sessions at or above 80% conditioned responses (CR). All of the rats in the HVI and AIV/V groups reached criterion prior to the 15th session, whereas only 1 rat in the PFb group and 0 rats in the CCTX/AIN group reached criterion. The last two sessions of data were combined for CR percentage (**B**), CR amplitude (**C**), CR onset latency (**D**), and CR peak latency (**E**). **F:** Following 15 sessions of training a subset of rats with PFb lesions received muscimol infusions into the anterior interpositus nucleus (Mus) followed by vehicle infusions (Veh) the next day.

Figure 3.

Cannula placements for rats in the vehicle group (black triangles), the group with placements outside of the eyeblink conditioning microzone (white circles), and the group with placements within the eyeblink conditioning microzone (white squares). The black circle indicates the extent of the eyeblink conditioning microzone.

Figure 4.

Mean ($\pm$ SEM) conditioned response (CR) percentage for rats given vehicle (Veh) infusions into the cerebellar cortex, rats given muscimol (Mus) into the eyeblink conditioning microzone (ECM), and rats given Mus infusions outside of the ECM (oECM) during the first 5 acquisition session (P1-P5). All rats were then trained without infusions for 5 additional training sessions (P6-P10). Rats in all three groups were then given muscimol or vehicle infusions during retention tests (order counter-balanced).