Single-Unit Evidence for Eye-Blink Conditioning in Cerebellar Cortex is Altered, but Not Eliminated, by Interpositus Nucleus Lesions

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Abstract

Many theories of motor learning explain learning-related changes in motor behavior in terms of plasticity in the cerebellar cortex. Empirical evidence, however, does not always appear to be consistent with such formulations. It is the anterior cerebellar interpositus nucleus (aINP) that seems to be essential for acquisition and retention of conditioned eye-blink responses under most circumstances and it has been therefore suggested that the aINP is the critical site of learning-related plasticity during eye-blink conditioning. Supporting this conclusion are studies demonstrating that multiple-unit conditioning-related neural activity patterns observed in many brain regions disappear after aINP lesion. The possibility that the cerebellar cortex may be involved in forming these patterns has not been assessed adequately, however.

In the current study, trained rabbits received kainic acid lesions of the INP. After recovery, the animals underwent additional sessions of conditioning during which single-unit activity was recorded from the cerebellar cortex. Our results suggest that the aINP is not the sole site of plasticity during eye-blink conditioning, as a subset of the neurons recorded from lesioned animals demonstrated conditioning-related firing patterns. The lesions did change the character of these firing patterns from those

observed in saline controls, however, in ways that can be generally described as a loss of organization. The normal tendency for the population of cortical cells to change firing rate together, for instance, was significantly less noticeable in lesioned animals. These results suggest that the aINP may be involved in the production of important features of conditioned responding, such as system timing function, therefore suggesting the need for more models that incorporate aINP and brain stem feedback as integral to the production of organized neural and behavioral responses.

Introduction

In 1969, David Marr presented a hypothesis of motor learning whereby sensory or cerebrally guided movement commands are associated with teaching or error signals within the cerebellum (Marr 1969). This hypothesis, later modified by James Albus (1971), invokes circuitry intrinsic to cerebellar cortex to explain changes in motor responses in relation to environmental contingencies. The mossy/parallel fiber system is postulated to code movement-related signals, and climbing fibers are postulated to code teaching and error signals by which the success of movement is evaluated. According to the Marr-Albus theory, the result of covariance between these two signals is a loss of efficacy at Purkinje cell-parallel fiber synapses; this plasticity is presumed to code learned movement.

The appeal of the Marr-Albus formulation is such that many, if not most, of the later proposed

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models of cerebellar function are in some way instantiations of the original theory (for review, see Chapeau-Blondeau and Chauvet 1991; Kawato and Gomi 1992; Ito 1993). All of the cited references postulate that long-term changes in synaptic efficacy develop somewhere in the mossy fiber–Purkinje cell system, and that it is in particular the mossy fiber changes in response to covariant climbing fiber input that underlies motor learning. Most models of eye-blink conditioning rely similarly on the architecture of the cerebellar cortex (and its associated input pathways) to explain the development of an association between a tone conditioned stimulus (CS) and an air puff unconditioned stimulus (US), and to explain the well-timed quality of the resultant conditioned eye blink (Moore and Blazis 1989; Buonomano and Mauk 1994). These models share the assumption that the occurrence of climbing fiber activity may down-regulate the efficacy of the particular subpopulation of granule cells or parallel fibers active around that time (when active in the absence of climbing fiber activity, parallel fiber synapses are thought to either remain unchanged or increase in efficacy). The repeated pairing of a particular tone with an air puff, at a particular delay after tone onset, therefore changes the system response in the vicinity of that particular latency. Although some theorists continue to question the relative importance of the cerebellum for eye-blink conditioning (Bloedel 1992; Llinás and Welsh 1993; Gruart et al. 1994; Bloedel and Bracha 1995), and although alternative theories of olivocerebellar function continue to emerge (e.g., Bower and Kassel 1990; Bloedel et al. 1993; Welsh et al. 1995), it is widely agreed that the cerebellar cortex is well-suited to serve as the substrate of motor timing demonstrated in the conditioned eye-blink. Specifically to the case of eye-blink conditioning, the timing-specific reduction of Purkinje cell output is thought to release the interpositus nucleus from inhibition at the appropriate time for response production.

Given that so many of these theories emphasize the involvement of cerebellar cortex, it would be reasonable to expect that the evidence supporting the cerebellar cortex as the site of learning-related changes in eye-blink conditioning is unimpeachable. Surprisingly, this is not the case. In fact, it has proven difficult to demonstrate conclusively a necessary relationship between the cerebellar cortex and the induction of motor learning in the eye-blink conditioning paradigm. In eye-blink conditioning, evidence has instead consistently

implicated the deep cerebellar nuclei—the anterior interpositus (aINP), in particular—as integral for the occurrence and maintenance of learning (Thompson 1986; Steinmetz et al. 1992a). Lesions of the aINP eliminate previously conditioned responses (CRs) for as long as 8–10 months (Steinmetz et al. 1992b) and make naive animals untrainable (Lavond et al. 1985; Steinmetz et al. 1992a; Thompson and Steinmetz 1992; Krupa et al. 1993). Lesions of the cerebellar cortex, meanwhile, have had highly variable results on conditioned responses, ranging from little effect (Woodruff-Pak et al. 1993) to destabilization of response timing and amplitude (Lavond and Steinmetz 1989; Perrett et al. 1995) to abolition (Hardiman and Yeo 1992). Given that it is difficult to lesion the cerebellar cortex extensively without causing ancillary damage to the deep nuclei, these lesion data do not strongly support theories that explain conditioning in terms of cortical processing.

Particularly interesting in relation to this issue are electrophysiological studies that implicate the aINP in the maintenance of learning-related neural activity elsewhere in the brain. Modulations of single- and multiple-unit firing rates precede and “model” the closing of the eyelid in the aINP, cerebellar cortex, red nucleus, trigeminal nucleus, motor abducens, thalamic nuclei, basal ganglia, and hippocampus (for review, see Anderson and Steinmetz 1994; Steinmetz 1996). All studies to date, however, suggest that lesions of the aINP eliminate learning-related patterns of firing in other areas. Recent work, for instance, has demonstrated that multiple-unit CR models in the red nucleus and trigeminal complex vanish (along with the CR itself) when the aINP is inactivated by cooling or lidocaine infusion (Chapman et al. 1990; Clark et al. 1992; Clark and Lavond 1996). Similar findings have linked conditioned eye-blink predictive firing in the thalamus and hippocampus to aINP integrity (Sears and Steinmetz 1990; Sears et al. 1996). The aINP models, meanwhile, do not appear to depend on the integrity of the red nucleus (Chapman et al. 1990). These studies do not demonstrate conclusively that plasticity in the aINP is solely responsible for conditioning-related activity, but they certainly demonstrate a repeated failure to prove otherwise.

The literature discussed above does not conclusively rule out the occurrence of non-aINP localized plasticity, however. All studies in which neural activity was seen to be entirely dependent

on the aINP involved multiple-unit recording. It is becoming increasingly clear that such recordings may obscure subtle patterns of firing observable in single-unit spike trains. For instance, single-unit recordings in cerebellar cortex made during eye-blink conditioning reveal a subpopulation of Purkinje cells that show CR-related firing-rate decreases (“inhibitory models”); these cells are invisible in multiple-unit records (Gould and Steinmetz 1996). The above studies, then, may have missed conditioning-related activity that survived aINP lesion. It is possible that the relative properties of the population had changed as a result of the lesion, such that inhibitory and excitatory models canceled each other out in the multiple-unit record.

It is also possible that the catastrophic effect that aINP lesions have on eye-blink conditioning (and on extracerebellar firing patterns) may reflect, at least in part, isolation of cerebellar cortical processing. That is, the effect of aINP lesions may not solely reflect intrinsically deep nuclear processing, but may reflect changes in the impact of cortical activity on system output (it has also been suggested that the result of aINP lesions may, in part, reflect changes in the activity of brain stem nuclei; e.g., Bloedel and Bracha 1995). The deep nuclei do serve as a localized “bottleneck” in the outflow of a highly distributed cerebellar cortical system that in itself may be difficult to lesion completely. Cutting of this outflow path could result in massive learning decrements without these decrements entailing destruction of the entire learning substrate. Studies concerning the effects of temporary inactivation on learning and performance of the conditioned eye blink (e.g., Krupa et al. 1993; Nordholm et al. 1993) strongly suggest that at least some of the plastic machinery of learning is intrinsically deep nuclear, but in both studies the researchers noted probable diffusion into the cerebellar cortex, and specifically into the region shown to be involved in eye-blink conditioning (Steinmetz and Sengelaub 1992; Van Ham and Yeo 1992; Gould et al. 1993). It remains possible that the cerebellar cortex (or even some extracerebellar structure) undergoes learning-related plasticity during eye-blink conditioning.

Solid evidence that the cerebellar cortex is intrinsically involved in learning of the conditioned eye-blink requires the documentation of conditioning-related cortical activity recorded in the absence of the driving force that seems to be provided by an intact aINP. Appropriate lesions of the deep

nucleus would be expected to eliminate all behavioral traces of conditioning, but it is conceivable that non-naïve patterns of single-unit neural activity in response to CS presentation might survive in the cerebellar cortex. These are the data that are presented in this paper.

In this study, rabbits were trained to criterion (>75% CRs) in forward-paired delay conditioning of the eye-blink reflex. After reaching criterion for two sessions, animals received either kainic acid or sham saline lesions of the aINP, and then additional sessions were run in concert with single-unit recording from the overlying cerebellar cortex. Permanent excitotoxic lesions were used because: (1) They spare fibers of passage, such that axon collaterals from the pons and inferior olive are left intact; (2) they have proven effective in eliminating behavioral evidence of conditioning (Lavond et al. 1985); (3) simultaneous inactivation of the aINP and recording from the cerebellar cortex is technically difficult given the anatomical arrangement of the cortical/aINP complex; and (4) it is possible that aINP-controlled conditioning-related firing patterns in cerebellar cortex could briefly survive aINP inactivation—therefore the removal of aINP cells entirely, followed by a 7-day recovery period, tests more strongly the hypothesis that the cerebellar cortex maintains conditioning-related neural activity independently of the aINP.

Our data suggest that the cerebellar cortex does in fact support conditioning-related patterns of neural activity following aINP lesion, but that such activity tends to be less well-organized than that seen in a population of cortical cells under normal conditions. The data also suggest that excitatory conditioning-related activity exists in neurons that, in US-alone trials, produce complex or simple spikes in response to the air puff. We interpret these results as evidence of a distributed system of learning, and suggest that the intact system is required for the operation of appropriate timing mechanisms. Specifically, we argue that the aINP is an integral part of a circuit that organizes the activity of the distributed system into a coherent whole, capable of driving a precisely timed eye-blink CR. The excitatory firing patterns produced during the function of this system seem integrally involved in the production of eye blinks, despite the fact that such activity should inhibit the aINP. The implications of these findings for cerebellar learning theories are discussed below.

Materials and Methods

SUBJECTS

Nineteen male, New Zealand white rabbits (Myrtle's Rabbitry, Thompson Station, TN) weighing >1.75 kg at the start of training served as the subjects in this experiment. Twelve eventually served as kainic acid-lesioned subjects and the remaining seven became sham-lesioned controls. Before and between sessions, animals were housed individually in clean cages with ad lib access to water and food. The housing area maintained rabbits on a 12hr light-dark schedule.

PRESURGERY TRAINING

Naive rabbits were adapted to the sound-attenuating conditioning chamber, and the Plexiglas restraint box placed inside the chamber, for 90 min (spread over two sessions). The conditioning chamber was equipped with a fan that produced background noise (~59 dB) and a speaker to deliver the tone CS. In the restraint box, a metal plate that fit just over the rabbit's head was equipped to hold the air puff US/eye-blink monitor assembly. This assembly held a nozzle attached to an air tank that delivered the air puff US, and also an infrared emitter/detector that measured changes in diffraction of a beam of infrared light (Thompson et al. 1994); these measured changes were amplified and calibrated to reflect millimeters of eye closure in response to unconditioned and conditioned stimuli. The entire assembly hung directly (4–5 mm) in front of the animal's left eye during conditioning.

On the third day, forward-paired delay conditioning sessions began, one per day. Sessions included 12 blocks of 10 trials each: nine pairings of a 350 msec CS tone [1 kHz, 85 dB sound pressure level (SPL)] coterminating with a 100-msec US (3 psi) air puff directed at the eye; one CS-alone trial in which no air puff was delivered. The air puff strength was sufficient to produce a reliable reflex eye blink in all animals. An additional block of 10 US-alone trials was presented at the beginning and end of each session. Trials were separated by an intertrial interval (ITI) that varied randomly between 20 and 30 sec.

Animals were trained in this fashion until they produced CRs that is, >0.5-mm blinks that began before air puff delivery, on 75% of the paired trials in two sessions.

SURGERY

Surgeries were performed under aseptic conditions. Rabbits were anesthetized with a combination of ketamine [60 mg/kg i.m. (intramuscularly)] and xylazine [6 mg/kg s.c. (subcutaneously)], and maintained throughout surgery on 1 ml i.m. injections of a ketamine/xylazine mixture administered every 45 min. Once anesthetized, rabbits were secured in a standard stereotaxic head holder, and the skull over the left cerebellum was drilled away.

Lesions were placed in the aINP via microinjections of kainic acid (0.2 μ l of 25 nmoles of kainic acid per microliter of saline), according to standard coordinates reported in Lavond et al. (1985). A Hamilton syringe containing the infusate was lowered through the skull hole (+0.7 AP and +5.5 ML relative to λ) to a depth of 14.5 mm below λ , where it sat untouched for 2 min. The 0.2 μ l of solution was then pressure injected over the course of 8 min. After an additional 2 min during which the acid was allowed to diffuse away from the needle tip, the syringe was raised out of the skull over a 1-min period. A sham-lesioned control group received similar penetrations, but normal saline was infused instead of kainic acid.

After lesioning, the skull hole was filled with bone wax, and a cylindrical base capable of supporting a micromanipulator was cemented over Larsell's Lobule H-VI of the cerebellar cortex (H-VI) with dental acrylic; the base was filled with petroleum jelly to protect the brain surface. Two head bolts were cemented anterior to the base to facilitate securing the rabbit's head during recording sessions, and also as an anchor from which to hang the air puff/eyelid measurement assembly. The scalp was then sutured around the headstage, and povidone-iodine ointment was applied liberally to the wound. In-cage water was replaced with sulmet for the 5 days following surgery. Rabbits were allowed one full week of recovery before training resumed.

RECORDING

During the five sessions of post-surgery training, single-unit activity was recorded from the cerebellar cortex. An acid-etched, varnish-insulated stainless-steel rod (3–5 M Ω) was set in a hydraulic micromanipulator that was then attached to the base cemented into the headstage. As the electrode was lowered into the cortex, cells were isolated

auditorially and visually (using a 2-to-1 signal-to-noise criterion). Amplitude-discriminated action potentials were sent as TTL pulses to the run-time computer, and stored to computer disk, whereas the waveform was saved onto VCR tape; more recently, a commercially available hardware/software package (Spike2, from CED, Ltd; Cambridge, England) allowed us to digitize raw neural signals, and to store them directly onto a computer hard disk. After collection of data during two to four blocks of trials, the electrode was lowered until a new cell was isolated, and the process was repeated. A subset of neurons were held through an additional one or two blocks of US-alone trials. The last penetration was marked with a current lesion (100 microamps for 10 sec), as was any penetration in which more than two obviously conditioning-related neurons were isolated, and the locations of lesions were noted.

HISTOLOGY

Following the final session, each rabbit was overdosed with an intravenous injection of pentobarbital (4 ml) and perfused transcardially with saline and formalin. The brains were removed, fixed in a sucrose formalin solution, embedded, sliced coronally to 40- μ g thickness, stained with Prussian blue to obtain a precise measure of electrode placement, and counterstained with cresyl violet for visualization of cell bodies.

Lesion effectiveness was measured both behaviorally and through examination of slices in the planes 0.5, 1.0, and 1.5 mm anterior to λ . The aINP was visualized under a light microscope at 400 $\times$ magnification, and the number of neurons in the dorsal aspect of the nucleus was counted. A similar count was made of the contralateral aINP for direct within-rabbit comparison. Centering of the microscope field was done in relation to anatomical landmarks in a standardized fashion across hemispheres and animals. Neuron counts were based on standard morphological criteria, including cell size, shape, and nonhomogeneity of staining.

DATA ANALYSIS

Peristimuli time histograms of neural activity, constructed from amplitude-discriminated spike counts or from discriminated spike trains produced via template matching algorithms on the

Spike2 system, were organized into time bins. Each of three same-length trial periods—the time before CS onset (pre-CS period), the time between CS and US onsets (CS period), and the time after US onset (US period)—were divided into five time bins. Spike count standardization procedures established whether significant learning-related activity occurred. Briefly, firing rates within bins in the CS and US periods were compared with appropriate bins in the pre-CS period, and paired *t*-tests on the different scores provided the standardized firing rate. Significant trial-related activity was recognizable as *t*-scores less likely to occur by chance than 5%; corrections that took the pre-CS period variability into account limited the likelihood of type-I errors.

Cells showing significant activity levels within a trial were categorized using the following scheme: (1) CS-onset cells, showing phasic bursts of activity briefly following and sharply time-locked to tone presentation; (2) US-onset cells, showing phasic bursts of activity briefly following air puff presentation; (3) movement-related cells, showing increased activity after the onset of eyelid closure; and (4) conditioning-related cells, showing increases or decreases in firing rates between CS and US or blink onset that were not time-locked to either of the stimuli, and that showed broader firing rate peaks. Typically, eye-blink generative responses show significant changes in firing rate between 60 and 30 msec before blink onset and model the CR; in this case, however, there was expected to be no post-lesion CRs for the activity to model. Therefore, this slightly more indirect method was used for classification.

Descriptive analyses were used to summarize the number of cells showing learning-related activity in the sessions immediately following lesion of the aINP. Subsequent χ^2 tests (along with related effect-size measurements) and *t*-tests revealed (1) whether learning-related activity found in the cerebellar cortex in the absence of the aINP changed with time post-lesion; (2) whether the amount of activity found in sham-lesioned animals was similar to that found in aINP-lesioned animals; and (3) whether the distributions of single-cell response timings, and the proportion of firing-rate increases and decreases differed between the lesioned and nonlesioned groups. For the purpose of these analyses, the peristimulus time histograms were transformed into firing rate running averages. Standardized firing rates (standardized this time on the entire pre-CS period variability) were smoothed on

a Gaussian curve and the result—a relatively smoothly changing curve—was plotted as a function of time. Onsets and peaks of activity were easily identifiable from these plots.

Results

BEHAVIORAL EFFECTS OF KAINIC ACID aINP LESIONS

Figure 1 shows how kainic acid infusion into the aINP affected conditioning. On the *x*-axis are the last 4 days of presurgery training and the 5 days of postsurgery training for both kainic acid- and sham-lesioned groups. As has been observed repeatedly in earlier studies (Lavond et al. 1985; Thompson 1988; Clark et al. 1992; Steinmetz et al. 1992a,b; Krupa et al. 1993; Bracha et al. 1994), aINP lesions effectively reduced the occurrence of eye-blink CRs to chance blinking levels.

A 2×5 mixed-effect ANOVA with group as the two-level between-group effect and post-surgery session as the five-level within-group effect confirmed that the kainic acid- and sham-lesioned groups performed to significantly different levels of conditioning following surgery, [$F(1,16) = 45.3$; $P < 0.001$]. A similar 2×4 ANOVA on pre-surgery sessions showed only an effect of session [$F(3,36) = 30.7$; $P < 0.001$] and not of group ($F < 1$). After surgery the sham-lesioned animals did show a temporary decrease in level of conditioning (interaction of group with session, [$F(4,64) = 4.664$; $P < 0.005$] that was probably at-

Figure 1: Percent CRs made by kainic acid-lesioned and sham-lesioned animals in the last four sessions of presurgery training (a1–a4) and in the five sessions of postsurgery training (r1–r5). Bars, standard error.

Figure 2: Anatomical results of excitotoxic lesions. Photomicrographs of a single kainic acid-lesioned animal's lesioned (*left*) and control (*right*) aINPs.

tributable either to tiny lesions caused by the pressure injections themselves or to the week after surgery spent untouched in the home cages; by post-surgery day 5, however, the sham group performed indistinguishably from the last day before surgery ($t < 1$).

The proportion of CRs made per session in the kainic acid-lesioned group (collapsed across days) was also compared by *t*-test with that predictable due to spontaneous blink rates (five per session). The difference (which was virtually nonexistent) did not approach significance ($t < 1$). The lesions eliminated the animals' ability to perform conditioned eye blinks. This pattern was consistent for all but one rabbit in the kainic acid-lesioned group.

ANATOMICAL EXAMINATION OF LESION EFFECTIVENESS

Figure 2 presents a pair of photomicrographs, showing the left and right interpositus–dentate regions from a kainic acid-lesioned rabbit. Notice the near absolute neuron loss in the left aINP and dentate nuclei, and its replacement with heavy accumulations of glial cells.

Neurons were counted in single microscope fields centered on the medial aspect of the dentate–interpositus triangle and set to magnify the tissue 400 $\times$. Counts were taken from injected (left) hemispheres and from the same location in control hemispheres of representative sections (~ 0.5 , 1.0, and 1.5 mm anterior to λ); the average (and standard error) cell counts for both groups are dis-

played in Figure 3. Comparisons of experimental and control hemispheres confirmed what is obvious from the behavioral results and from Figure 2, that kainic acid decimated the region of deep cerebellar nucleus traditionally considered to be essential for eye-blink conditioning.

The difference between groups was analyzed by computing the proportion of cells spared by the lesion. That is, the number of neurons counted within the injected (left) aINP was divided by the number of neurons counted within intact (right) aINP. These proportions were then compared between groups. Proportions were calculated, rather than simple differences, to control for the possibility that cells would be lost: (1) in the sham-lesioned hemispheres as a result of the saline injection; and (2) in control hemispheres as a result of connections between the left and right cerebella. If there was some cell loss in both groups, and in both hemispheres of the kainic acid-lesioned animals, then simple differences between left and right hemisphere cell counts might cause an underestimation of the effect of kainic acid infusion. The reported results were replicated in difference measurements and absolute cell number measurements, however; in fact, the small difference between cell counts in the control hemispheres of the kainic acid- and sham-lesioned groups proved insignificant ($t < 1$).

In sham-lesioned animals, the left-to-right cell count proportion averaged 74%, whereas in kainic acid-lesioned animals, it averaged 17%. The difference between groups on this measure was significant [$t(16) = -4.33$; $P < 0.001$]. The impact of

kainic acid lesions was to more than quadruple the cell loss attributable to the injection.

It is clear that even the saline injection destroyed some cells; the left-to-right cell count proportion in control animals was significantly smaller than unity [$t(6) = -3.72$; $P < 0.001$].

CORRELATION OF CELL LOSS AND CR EXECUTION IN LESIONED ANIMALS

The variability in kainic acid-related cell sparing proportions was related to the proportion of post-surgery trials in which eye-blink CRs were observed. As expected, the two measures correlated positively ($r = 0.78$). This correlation was largely driven by one animal that was an outlier on both measures, showing fewer cells destroyed and higher proportion of CRs than the mean. With this animal removed from the analysis, the correlation between cell loss and CR likelihood became insignificant, and in fact slightly negative. The lack of correlation reflects the lack of variability (i.e., the floor effect) seen in percent CRs produced (and to some extent in the number of cells found). The few cells remaining were incapable of driving any sort of CR, and thus there was no correlation between behavioral performance and cell proportion. The animal with spared cells was excluded from subsequent analyses comparing the sham- and kainic acid-lesioned group. Exclusion of this animal from the cell count analysis reduced the average left-to-right cell sparing proportion to 10%.

SINGLE-UNIT RESPONSES IN KAINIC ACID- AND SHAM-LESIONED ANIMALS

In total, recordings were taken from 293 neurons in cerebellar cortex. Examination of electrode tracks and lesions revealed that the majority of these neurons were located in Larsell's lobule H-VI or the anterior lobe directly anterior to H-VI. Figure 4 shows the general region from which all neurons were recorded, and Figure 5 shows a representative H-VI slice, with an electrode tract and marking lesion indicated.

In analyses published previously of cerebellar single-unit activity related to eye-blink conditioning (e.g., Berthier and Moore 1986; Gould and Steinmetz 1996), neurons were classified as either nonmodulated in the conditioning context, responsive to the onsets of stimuli, or conditioning-

Figure 3: Average (and standard errors) of cell sparing, measured as the left to right proportion of cell numbers counted in the aINP, for the kainic acid-lesioned and sham-lesioned groups.

Figure 4: Shaded regions of coronal cerebellar schematics showing the region in which most electrode tracks were located; numbers to the *left* represent distance anterior of λ . (DE) Dentate nucleus; (f) fiber tract; (FA) fastigial nucleus; (IC) inferior colliculus; (IN) interpositus nucleus.

related. We began our neural analysis by preparing the same descriptions, according to the criteria set forth in the Materials and Methods. Figure 6A presents the classificatory breakdown for the 110 neurons recorded from the cerebellar cortex of sham-lesioned animals. The proportion of neurons in each category (nonmodulated, CS-onset, US-onset, onset of both stimuli, and conditioning-related) largely replicates the results of the above-mentioned studies. Slightly under 25% of neurons recorded in the vicinity of H-VI showed patterns of activity that were related to the behavioral CR. This replication serves to validate the slight differences in statistical methods used to quantify the firing patterns.

Figure 6B presents a similar breakdown for the 183 neurons recorded from the cerebellar cortices of kainic acid-lesioned animals. The similarity between Figure 6A and B, is such that the graphs may look identical under casual examination. We found no statistical differences between the groups in terms of the proportion of neurons that fit each general classification. Most importantly, similar proportions of cells appeared to be conditioning-

related in sham- and kainic acid-lesioned samples. Effective lesions of the aINP did not eliminate the occurrence of conditioning-related firing patterns in single neurons.

CORRELATION BETWEEN aINP CELL LOSS AND CONDITIONING-RELATED SINGLE-UNIT ACTIVITY

It is important to assess the possible contribution of spared aINP to the activity observed in kainic acid-lesioned animals. Therefore, the raw number of cells remaining in the lesioned aINP of each kainic acid-lesioned animal was related to the percentage of recorded neurons that had been classified as conditioning-related in that same animal. A striking lack of relationship between these measurements was present ($r = 0.04$). The subject with the least spared INP provided the highest proportion of conditioning-related neurons, and the next highest proportion of conditioning-related neurons came from an animal with a relatively spared aINP. This latter subject is the animal, discussed above, that produced a sizeable number of CRs; with this

Figure 5: Coronal section through left Larsell's lobule H-VI showing representative electrode track. The white line from entry mark to lesion mark is the path in which the electrode traveled; note that the track skims the Purkinje cell layer.

Figure 6: Breakdown of neurons recorded into those not modulated by conditioning trials, those responsive to the CS, the US, or both stimuli, and those designated as conditioning-related. (A) Sham-lesioned animals; (B) kainic acid-lesioned animals.

subject's data removed, the correlation between spared aINP and the probability of recorded neurons being conditioning-related became somewhat (but not significantly) negative, $r = -0.52$. Although this result is surprising and difficult to interpret, it certainly re-affirms the point that the conditioning-related activity patterns observed were not supported by spared aINP.

POPULATION DISTRIBUTION PROPERTIES OF CONDITIONING-RELATED ACTIVITY IN KAINIC ACID-LESIONED AND SHAM-LESIONED ANIMALS

Closer examination of the subsamples of neurons classified as conditioning-related revealed substantial differences between the experimental and control groups. Kainic acid lesions changed the proportions of neurons showing firing rate inhibition relative to excitation. Researchers have reported previously that conditioning-related firing patterns seem to fall into two types, those that

show increases in firing rate preceding CR-onset (for the remainder of the paper, these will be referred to as “on” cells), and those that show decreases in firing rate preceding CR-onset (“off” cells). In agreement with Berthier and Moore (1986), we have typically found that excitatory cells outnumber inhibitory cells by a ratio of ~2:1 (e.g., Gould and Steinmetz 1996). As shown in the left half of Figure 7, this result was replicated in our sham-lesioned group. Figure 8 shows examples of summary peri-stimulus time histograms of these two basic types of neural responses. The right half of Figure 7, however, reveals that on cells and off cells were equally likely after kainic acid lesion. The likelihood of an isolated neuron being an on cell was significantly different for the two groups [$t(16) = 2.46$, $P < 0.03$].

In addition, a type of firing profile that could not be classified as simply increasing or decreasing was observed in neurons from kainic acid-lesioned animals, and not from those in sham-lesioned animals. These neurons were reliably bi- or triphasic, turning, for example, off and then on. Figure 9 shows a traditional on cell found in a kainic acid-lesioned animal, as well as a more unusual triphasic discharging neuron. The kainic acid lesion changed the shapes of individual firing patterns observed, as well as the proportion of on cells to off cells.

These differences were studied in finer detail. The modulations of firing rate were characterized roughly with regard to the onsets and peaks of

Figure 7: Kainic acid lesions of the aINP changed properties of observed single-unit cortical firing patterns. Proportions of the conditioning-related subpopulation of recorded neurons that showed excitatory modulation (white bars), inhibitory modulation (lightly shaded bars), or some combination of the two, that is, bi- and triphasic cells, (darkly shaded bars) for kainic acid- and sham-lesioned groups.

Figure 8: Summary peristimulus time histograms from two representative neurons observed in control animals; time is the x-axis for both (time of CS and US onsets are marked).

modulation. Firing rate modulations were identifiable as hills or valleys in the firing rate running averages. The time at which the hill or valley reached its most extreme point was designated the peak modulation time (PMT; in the case of certain off cells, it was necessary to designate the center time of a flat bottomed valley as the PMT, because of the obvious floor effect of a zero firing rate), and the point halfway between base line (inflection point into the modulation) and peak was designated as onset of modulation time (OMT).

Figure 10A shows the population distribution of OMTs for the control group, and Figure 10B shows the population distribution of OMTs for the kainic acid-lesioned group. The ordinate on each is proportion of the total sample for the group, and the abscissa is modulation onset time post CS-onset binned into 50-msec segments. It is clear from this presentation that, as a population, neurons from kainic acid-lesioned animals tended to modulate earlier in the CS period, and tended to be more variable in OMT. The OMTs for neurons recorded

from sham-lesioned animals tended to cluster around the middle of the CS period, a result that is not surprising given the often cited finding that normally observed firing rate modulations precede CR-onset by 30–50 msec. A χ^2 test for association revealed that the distributions of OMT for the two groups were at least marginally significantly different [$\chi^2(4) = 8.57$; $P = 0.07$]. Cohen's text on power analysis suggests that detection of even a medium-sized difference between distributions would have required recording from many more cells than was feasible in the present context (Cohen 1992). Given this fact, it is likely that the failure to quite reach $P = 0.05$ reflects small sample size, not small effect size. Effect size analysis support this possibility (Rosenthal and Rosnow 1991). Calculations of w , a standard measure of effect size in the χ^2 test, demonstrated that the difference between OMT distributions was larger than that described by Cohen (1992) as a medium-sized difference ($w = 0.355$).

It is important to note that, although the kainic

Figure 9: Summary peristimulus time histograms from two representative neurons recorded from lesioned animals; time is the x-axis for both (time of CS and US onsets are marked). Note that the second neuron showed abnormal (i.e., triphasic) modulation.

Figure 10: Distribution of OMT for the sham- and kainic acid-lesioned animals. (A) Sham-lesioned group; (B) kainic acid-lesioned group.

acid-lesioned group's distribution of OMTs was not as tightly grouped as that from the sham-lesioned group, it was also far from flat [$\chi^2(4) = 9.90$; $P < 0.05$]. Timing in the lesioned animals was changed by the lesion, but it was not totally eliminated.

A similar analysis was performed on the PMTs, as displayed in Figure 11 A and B. Here the broadening of population timing organization is even more obvious. Neurons from control animals show the expected tendency to cluster ~200 msec after tone onset; neurons in the kainic acid-lesioned group, however, distributed without bias into each of the bins between 50 and 250 msec after tone onset. Again, the χ^2 test for association revealed a marginally significant difference between the groups [$\chi^2(3) = 6.44$; $P = 0.09$]. Once again, the effect size for the difference between the distributions was on the large size ($w = 0.315$).

CORRELATION BETWEEN DAYS OF POSTLESION TRAINING AND CONDITIONING-RELATED SINGLE-UNIT ACTIVITY

Patterns of training-related modulation in cerebellar cortex have been shown to emerge in circumstances that do not lead to behavioral changes. For instance, subpopulations of Purkinje cells respond to the tone and air puff in a non-naïve fashion after trials of backward pairing (that is, air puff followed briefly by tone). The animal fails to condition to these parameters, but activity in the cerebellar cortex does change (Gould and Steinmetz 1996) with the training. It was therefore necessary to consider the possibility that the patterns of conditioning-related activity observed in the present study actually developed over the course of post-lesion training, a situation in which conditioning does not occur

Figure 11: Distribution of PMT for both groups. (A) Sham-lesioned group. (B) Kainic acid-lesioned group.

but in which cerebellar cortex activity might change.

To assess this possibility, the samples of conditioning-related neural patterns were broken down in relation to the day of training on which they were found. The proportions of the total sample recorded on any one day of post-lesion training was not significantly different from that found on any other [$F(4,20) = 1.53$, $P > 0.2$]. It does not appear that patterns of conditioning-related activity developed over the course of postlesion training. Instead, the evidence once again suggests that the observed patterns represent plasticity induced before the lesion that survived lesion of the aINP.

FIRING RATE MODULATIONS OF CONDITIONING-RELATED NEURONS ON US-ALONE TRIALS

An issue related to the observation of extra-interpositus nucleus plasticity in the system is the relationship between cortical firing rate modulation and stimulus convergence. It is assumed that neurons demonstrating conditioning-related firing patterns represent a subset of those neurons that receive convergent input from the CS and US, although direct confirmation of this assumption has not been reported. In fact, it is possible that such an assumption may be misguided, particularly with regard to neurons demonstrating excitatory modulations in the conditioning context (on cells). As Purkinje cells in cerebellar cortex inhibit the deep nuclei, on cells would seem to influence the aINP in the wrong direction for CR production, inhibiting activity rather than promoting it. It is possible that 'on' cells may not be connected to the upper facial musculature at all, being involved instead with ancillary responses that develop to the tone in the conditioning context (e.g., other movements). Given the topographic nature of cerebellar input-output patterns, such neurons would not be expected to receive sensory input from the region around the eye.

Although this study was not designed to rigorously test this possibility, we did record from several Purkinje cells, both conditioning-related and not, during US-alone trials. A subset of this sample proved air puff responsive. A total of 46 neurons from either sham-operated or kainic acid-lesioned rabbits were isolated and recorded from under both paired trial (two blocks) and unpaired trial

(one block) conditions. Of these neurons, 37% ($n = 17$) showed changes in simple spike activity that appeared to be related to CR execution. Of these 17 neurons, seven showed complex spiking evoked by the air puff on US-alone trials. This sample of 46 cells included some neurons that showed excitatory CR-related activity and some cells that showed inhibitory CR-related activity. For Purkinje cells in which simple spikes showed an excitatory modulation during paired trials, complex spike air puff responses were typically only observed during US-alone trials. This finding is consistent with earlier work suggesting that responses to the tone can inhibit olivary responses to the air puff (Sears and Steinmetz 1991).

It should be noted that many conditioning-related neurons did not respond to the air puff delivered alone, and that many neurons that were not classified as conditioning-related did. Still, these observations suggest that excitatory modulations may in some way be directly involved with CR production in the normal animal.

Discussion

The main findings of the present study can be summarized as follows: (1) Kainic acid lesions of the aINP effectively eliminate the majority of cell bodies normally found there, and reduce the incidence of behavioral CRs to chance levels; (2) despite this lack of behavioral response to the tone CS, neurons in cerebellar cortical lobule H-VI demonstrate tone-modulated patterns of activity that are not simple sensory responses, and are therefore likely to be learning-related responses sustainable independently of the aINP. These neurons are found in similar proportions to those in control animals, in fact; (3) the ratio of on cells to off cells observed after kainic acid lesion is changed greatly from the control condition. Whereas normally excitatory models outnumber inhibitory models 2:1, the ratio in lesioned animals is ~1:1; (4) in addition, neurons appear in the lesioned sample that show more than one peak of modulation, a pattern not observed in neurons from the control sample; (5) the onset and peak times of firing-rate modulations tend to be earlier and far more variable in animals with lesioned aINPs than in sham-lesioned animals; and (6) in both groups, CS period modulation was sometimes associated with a strong tendency to fire either complex or simple spikes in relation to the air puff on US-alone trials.

The first conclusion that can be reached on the basis of these data is that neuronal plasticity related to basic eye-blink conditioning exists extrinsic to the aINP. Earlier studies, in which neural models in the red nucleus, trigeminal nucleus, ventromedial thalamus, and hippocampus vanished after aINP inactivation or lesion, have at times been interpreted as evidence of localization of memory to the aINP (Chapman et al. 1990; Sears and Steinmetz 1990, 1991; Stotler et al. 1990; Clark et al. 1992; Clark and Lavond 1996). By the same logic, the present data preclude the possibility of complete engram localization within the aINP. Despite near total decimation of the ipsilateral aINP, firing patterns in the same side cerebellar cortex reflected the training that the animal had undergone before the lesion.

It could be argued, however, that our results reflect either (1) our failure to make complete lesions; or (2) statistical artifact. These two possibilities will be discussed in turn.

It is conceivable that some scrap of intact aINP mediated the conditioning-related activity that we observed in cortex. This, then, would leave untouched the possibility that all eye-blink plasticity resides in the aINP. We reject this possibility for several reasons. First of all, the behavioral evidence is consistent with complete aINP loss. Previous studies have used the abolition of responding as proof of lesion efficacy (Lavond et al. 1985; Lavond et al. 1990; Steinmetz et al. 1991; Clark et al. 1992). By this criterion, our lesions were effective and complete. More importantly, the anatomical evidence suggests that our lesions were highly effective. It has been suggested that the primary site of plasticity is quite localized to the dorsal INP; in fact, our experience has suggested that even simple electrode or cannula placement in this region can disrupt conditioning. Cell counts demonstrated that the kainic acid-lesioned animals were nearly neuron free in these very regions. Further examination suggests that damage extended far ventrally, laterally, and medially of the infusion sites themselves.

In addition, the lack of relationship found between aINP cell numbers and probability of finding conditioning-related firing patterns suggests that leftover neurons are not what allows for the occurrence of conditioning-related firing patterns. If eye-blink conditioning memory was fully localized to the aINP, it would be reasonable to expect some sort of relationship between the amount of spared tissue and amount of conditioning evidence re-

corded in the brain. This relationship was entirely lacking; the probability of encountering conditioning-related neurons did not decrease as cell counts declined. It is highly unlikely, therefore, that the observed neural patterns originated in the aINP.

Alternatively, it might be argued that we did not observe conditioning-related patterns of firing at all. It's true that the subsample of conditioning-related neurons recorded from our experimental group were not as coherent as those culled from control animals; the timing of modulation varied widely between neurons. Given that only one quarter of the neurons in the total sample were conditioning-related to begin with, it is conceivable that some of these supposedly significant patterns were in fact chance modulations in firing. We recognized and attempted to minimize this possibility, only classifying neural activity patterns as conditioning-related if the CS-period variability (e.g., conditioning-related modulation) was more than twice the magnitude of pre-CS-period variability according to the running averages, and the statistically significant bins of CS-period activity could not be explained in terms of pre-CS variations in the opposite direction. Therefore, many patterns of activity that were 'significantly' conditioning-related were rejected as nonmodulated. Others were classified as CS-related, on the basis of being time-locked to the CS and only phasically active; in fact, the percentage of neurons so classified was equivalent in the kainic acid- and sham-lesioned groups. These considerations make it highly likely that the remaining patterns were true evidence of conditioning-related plasticity in animals with aINP lesions.

Other considerations add to our confidence. Importantly, the characterization of the control and experimental neural samples are not consistent with the hypothesis that our kainic acid lesion data represent "noise". If such patterns were truly random, then (1) the control group should have contained similar proportions of such patterns, on top of the typical neural models; and (2) the distribution of OMTs should have been flat for the kainic acid lesion group. Neither of these descriptions fit the distributions that we observed. Instead, the proportions of conditioning-related cells were remarkably similar for the two groups, and the OMT distribution for the kainic acid-lesioned group was shifted and broadened, but not even close to statistically flat. The patterns of activity demonstrated by cerebellar cortical neurons in lesioned animals were not random; they were, in fact, responses

developed through sessions of presurgery eye-blink conditioning.

This finding does not necessarily imply that eye-blink-related patterns of cortical activity can develop with the aINP lesioned. The fact that conditioning-related activity survives aINP removal does not mean that it can be induced without an intact aINP. It may well be, as is suggested by work cited earlier (e.g., Krupa et al. 1993), that plasticity related to eye-blink conditioning does not get laid down in the absence of a fully functioning aINP; the obvious test of this possibility would of course be an acquisition study, in which the aINP is lesioned before training and single-unit recording begins. These data are evidence, however, that eye-blink conditioning-related plasticity, once laid down, is not restricted to the aINP.

The second major implication of this data set is that conditioning-related cortical activity is abnormal in the absence of an intact aINP. The patterns observed in the two samples of cells differ in a number of ways that can perhaps be summarized in one word—disorganization. After a kainic acid lesion, the normal 2:1 preponderance of on over off cells was lost. In addition, single-unit firing patterns occasionally failed to present single organized peaks (or troughs) of modulation, and the sample as a whole failed to cohere into an organized entity with particular timing parameters. Loss of the aINP defocused the conditioning-related activity such that the neural population was no longer a single functional unit. It may be tentatively concluded that organization of the conditioned eye-blink population code is not possible without an intact aINP.

Poorly organized timing of the neural response to the tone CS was a large part of the overall disorganization observed in the kainic acid-lesioned animals. The majority of the neurons recorded did not respond to the tone in a fashion amenable to the production of a well-timed CR. To some extent this finding is similar to earlier reports of single-unit cortical activity recorded during backward and unpaired conditioning (Gould and Steinmetz 1996). In this earlier project, unpaired or backward conditioning led to a broader range of conditioning-related changes than were observed following paired conditioning. Importantly, aINP firing rates showed no modulation to either stimulus in these regimens. It was suggested that the cortical changes were not organized in a fashion that could induce aINP plasticity or, therefore, conditioned eye blinks.

In this study the reported disorganization does not relate to differences in conditioning paradigm, but rather to differences in brain system organization. Neurons that were (presumably) involved previously in an organized, well-timed process of response generation were, after aINP lesion, no longer so. This result is somewhat surprising, as most theorists have ascribed the task of generating motor timing to either cerebellar cortical, pontocerebellar, or hippocampal circuitry (Schmajuk and Moore 1985; Moore and Blazis 1989; Chapeau-Blondeau and Chauvet 1991; Grossberg and Merrill 1992; Kawato and Gomi 1992; Ito 1993; Buonomano and Mauk 1994). These models do not attach functional significance to output pathways of the aINP, and therefore none should predict such changes in activity following aINP lesion. Our data strongly suggest that the models presented in these publications do not fully capture the mechanism of conditioned eye-blink timing. Somehow the flow of information through the aINP shapes the single-unit and population responses in cerebellar cortex, a finding not predicted by the above models.

The neural circuitry underlying eye-blink conditioning has been laid out to differing degrees of complexity in several recent review articles (Anderson and Steinmetz 1994; Schreurs et al. 1995; Bracha and Bloedel 1996; Steinmetz 1996). Examination of the more extensive of these models reveals multiple input and output pathways for most nuclei, and many feedback connections. The aINP's position as sole outflow of the cerebellar cortex gives it unusual status as a tight nexus in the system; destruction of this nexus probably affects the rest of the system to a degree that is difficult to match, even with large lesions elsewhere. On the basis of these data it could be suggested that some subset of the loops involving the cerebellar cortex to aINP connection underlies the production of timing in the eyeblink conditioning task. Perhaps feedback from the INP to the pons (Steinmetz and Sengelaub 1992), inferior olive (and the resultant looping of activity back through the cerebellar system), or even directly back to the cerebellar cortex (Buisseret-Delmas and Angaut 1989) has a role in the ability of the animal to precisely time a CR; perhaps eye-blink CR timing requires an intact loop between the cerebellum and hippocampus (Moyer et al. 1990). This data set offers no clue. What can be said, however, is that somehow the influence of the aINP on the cerebellar cortex assists in the shaping of normal eye-blink conditioning-related activity.

It should be stated that, although feedback loops seem to us to be the most reasonable explanation of the phenomena that we have observed in this experiment, it is not the only possible explanation. It is possible, for instance, that the aINP lesions changed the tonic level of excitation in the cerebellar cortex and in brain stem nuclei. It has been noted that the cerebellum exerts a tonic excitatory influence on the brain stem (Dow and Moruzzi 1958; Gilman et al. 1981), and certain authors have suggested that this influence has relevance to the impact that aINP lesions have on eye-blink conditioning (Bloedel et al. 1993; see also Steinmetz et al. 1992a). It is possible that the unusual disorganization observed in single-cell and population responses after aINP lesion had to do, not with feedback of currently cycling information, but rather with tonic changes in the activity of the brain stem.

We consider the existence of feedback connections, and of processing by recursive networks, to be a more likely explanation of the observed disorganization in the cerebellar cortex. These feedback connections have been shown to exist (see, e.g., Steinmetz and Sengelaub 1992; Schreurs et al. 1995; Bracha and Bloedel 1996), and in fact are a prominent part of the cerebellar system. In neural network theory, network simulations of other systems, and in experiment, these feedback connections are typically found to change system activity in major ways (Grossberg and Merrill 1992; Lisberger and Sejnowski 1992; Buonomano and Mauk 1994; Chrobak and Buzsáki 1996; Kirschfeld et al. 1996). Such may be the case in this system as well. This leads us to suggest the need for new theories that incorporate recursion as an integral part of the timing process.

The observation of excitatory conditioning-related firing patterns in neurons that also clearly received convergent information regarding the air puff in US-alone trials serves to intensify our belief that new theoretical constructs are needed. It is commonly assumed that off cells are of primary importance for the driving of eye blinks, and that on cells, which would be expected to inhibit the aINP, must serve some other, perhaps even nonessential function. We have consistently observed a 2:1 ratio of on to off cells and this is interesting in this regard, as is the fact that neurons receiving tone-air convergence may develop into on cells as a result of conditioning. Although it is possible that some facet of the microcircuitry either shunts the inhibitory influence away from the important areas

of the aINP or reverses the sign of the firing rate change (perhaps via inhibitory interneurons), the possibility that these firing patterns aid in the production of eye blinks must be considered.

Many avenues of inquiry remain open and even uncharted. It is possible that the explanation of eye blink conditioning rests not solely on the compelling across-trials average firing rate changes in single neurons; rather, the cooperation among many neurons in a single trial—their synchronization—may have a major role in the production of CRs. Synchronized populations of neurons could precisely and powerfully influence overall system activity, and synchronization is a phenomenon that short-latency feedback loops are known to cause (see, e.g., Buonomano and Mauk 1994; Chrobak and Buzsáki 1996). It is possible that the function of feedback in this system is to cause brief synchronization of neural populations in the cerebellar cortex, aINP, and brain stem. Such synchronization might be correlated with single-unit firing rate changes, but are neither isomorphic with nor entirely predicted by single-unit analysis (see, e.g., Chapin and Nicolelis 1995; Vaadia et al. 1995).

Even though our results suggest that cerebellar cortical neurons can maintain conditioning-related patterns of unit activity after removal of the deep nuclei, the functional significance of these cortical spiking patterns remain obscure. Future recording experiments that use multiple electrodes to simultaneously monitor a number of cerebellar neurons as well as experiments designed to study patterns of connectivity between cerebellar cortex and the aINP may be important for further delineating the relationship between cerebellar cortex and the deep nuclei during classical eye blink conditioning.

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References

Albus, J.S. 1971. A theory of cerebellar function. *Mathematical Biosci.* **10**: 25–61.

- Anderson, B.J. and J.E. Steinmetz. 1994. Cerebellar and brainstem circuits involved in classical eyeblink conditioning. *Rev. Neurosci.* **5**: 1–23.
- Berthier, N.E. and J.W. Moore. 1986. Cerebellar Purkinje cell activity related to the classically conditioned nictitating membrane response. *Exp. Brain Res.* **63**: 341–350.
- Bloedel, J.R. 1992. Functional heterogeneity with structural homogeneity: How does the cerebellum operate? *Behav. Brain Sci.* **15**: 666–678.
- Bloedel, J.R. and V. Bracha. 1995. On the cerebellum, cutaneomuscular reflexes, movement control and the elusive engrams of memory. *Behav. Brain res.* **68**: 1–44.
- Bloedel, J.R., V. Bracha, and P.S. Larson. 1993. Real time operations of the cerebellar cortex. *Can. J. Neurol. Sci.* **20** (Suppl. 3): S7–S18.
- Bower, J.M. and J. Kassel. 1990. Variability in tactile projection patterns to cerebella folia crus IIA of the Norway rat. *J. Compar. Neurol.* **302**: 768–778.
- Bracha, V. and J.R. Bloedel. 1996. The multiple-pathway model of circuits subserving the classical conditioning of withdrawal reflexes. In *Acquisition of motor behavior in vertebrates* (ed. J.R. Bloedel, T. Ebner, and S. Wise), pp. 175–204. MIT Press, Cambridge, MA.
- Bracha, V., M.L. Webster, N.K. Winters, K.B. Irwin, and J.R. Bloedel. 1994. Effects of muscimol inactivation of the cerebellar interposed-dentate nuclear complex on the performance of the nictitating membrane response in the rabbit. *Exp. Brain Res.* **100**: 453–468.
- Buisseret-Delmas, C. and P. Angaut. 1989. Anatomical mapping of the cerebellar nucleocortical projections in the rat: A retrograde labeling study. *J. Compar. Neurol.* **288**: 297–310.
- Buonomano, D.V. and M.D. Mauk. 1994. Neural network model of the cerebellum: Temporal discrimination and the timing of motor responses. *Neural Computat.* **6**: 38–55.
- Chapeau-Blondeau, F. and G. Chauvet. 1991. A neural network model of the cerebellar cortex performing dynamic associations. *Biol. Cybernetics* **65**: 267–279.
- Chapin, J.K. and M.A.L. Nicolelis. 1995. Beyond single unit recording: Characterizing neural information in networks of simultaneously recorded neurons. In *Scale in conscious experience: Is the brain too important to be left to specialists to study?* (ed. J. King and K.H. Pribram), pp. 135–153. Erlbaum Associates, Hillsdale, NJ.
- Chapman, P.F., J.E. Steinmetz, L.L. Sears, and R.F. Thompson. 1990. Effects of lidocaine injection in the interpositus nucleus and red nucleus on conditioned behavioral and neuronal responses. *Brain Res.* **537**: 149–156.
- Chrobak, J.J. and G. Buzsáki. 1996. High-frequency oscillations in the output networks of the hippocampal-entorhinal axis of the freely behaving rat. *J. Neurosci.* **16**: 3056–3066.
- Clark, R.E. and D.G. Lavond. 1996. Neural unit activity in the trigeminal complex with interpositus or red nucleus inactivation during classical eyeblink conditioning. *Behav. Neurosci.* **110**: 13–21.
- Clark, R.E., A.A. Zhang, and D.G. Lavond. 1992. Reversible lesions of the cerebellar interpositus nucleus during acquisition and retention of a classically conditioned behavior. *Behav. Neurosci.* **106**: 879–888.
- Cohen, J. 1992. A power primer. *Psycholog. Bull.* **112**: 155–159.
- Dow, R.S. and G. Moruzzi. 1958. *The physiology and pathology of the cerebellum* University of Minnesota Press, Minneapolis, MN.
- Gilman, S., J.R. Bloedel, and R. Lechtenberg. 1981. *Disorders of the cerebellum* F.A. Davis, Philadelphia, PA.
- Gould, T.J. and J.E. Steinmetz. 1996. Changes in rabbit cerebellar cortical and interpositus nucleus activity during acquisition, extinction and backward classical eyelid conditioning. *Neurobiol. Learn. Mem.* **65**: 17–34.
- Gould, T.J., L.L. Sears, and J. Steinmetz. 1993. Possible CS and US pathways for rabbit classical eyelid conditioning: Electrophysiological evidence for projections from the pontine nuclei and inferior olive to cerebellar cortex and nuclei. *Behav. Neural Biol.* **60**: 172–185.
- Grossberg, S. and J.W.L. Merrill. 1992. A neural network model of adaptively timed reinforcement learning and hippocampal dynamics. *Cognit. Brain Res.* **1**: 3–38.
- Gruart, A., P. Blazquez, and J.M. Delgado-Garcia. 1994. Kinematic analyses of classically-conditioned eyelid movements in the cat suggest a brain stem site for motor learning. *Neurosci. Lett.* **175**: 81–84.
- Hardiman, M.J. and C.H. Yeo. 1992. The effect of kainic acid lesions of the cerebellar cortex on the conditioned nictitating membrane response in the rabbit. *Eur. J. Neurosci.* **4**: 966–980.
- Ito, M. 1993. Movement and thought: Identical control mechanisms by the cerebellum. Reply to Leiner, Leiner and Dow. *Trends Neurosci.* **16**: 448–450.
- Kawato, M. and H. Gomi. 1992. The cerebellum and VOR/OKR learning models. *Trends Neurosci.* **15**: 445–453.
- Kirschfeld, K., R. Feiler, and F. Wolf-Oberhollenzer. 1996. Cortical oscillations and the origin of express saccades. *Proc. R. Soc. Lond. B. Sci.* **263**: 459–468.
- Krupa, D.J., J.K. Thompson, and R.F. Thompson. 1993. Localization of a memory trace in the mammalian brain. *Science* **260**: 989–991.
- Lavond, D.G. and J.E. Steinmetz. 1989. Acquisition of classical conditioning without cerebellar cortex. *Behav. Brain Res.* **33**: 113–164.
- Lavond, D.G., T.L. Hembree, and R.F. Thompson. 1985.

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- Effect of kainic acid lesions of the cerebellar interpositus nucleus on eyelid conditioning in the rabbit. *Brain Res.* **326**: 179–182.
- Lavond, D.G., C.G. Logan, J.H. Sohn, W.D.A. Garner, and S.A. Kanzawa. 1990. Lesions of the cerebellar interpositus nucleus abolish both nictitating membrane and eyelid EMG conditioned responses. *Brain Res.* **514**: 238–248.
- Lisberger, S.G. and T.J. Sejnowski. 1992. Motor learning in a recurrent network model based on the vestibulo-ocular reflex. *Nature* **360**: 159–161.
- Llinas, R. and J.P. Welsh. 1993. On the cerebellum and motor learning. *Curr. Opin. Neurobiol.* **3**: 958–965.
- Marr, D. 1969. A theory of cerebellar cortex. *J. Physiol.* **202**: 437–470.
- Moore, J.W. and D.E.J. Blazis. 1989. Simulation of a classically conditioned response: A cerebellar neural network implementation of the Sutton-Barto-Desmond model. In *Neural models of plasticity: Experimental and theoretical approaches* (ed. J.H. Byrne and W.O. Berry), pp. 18–207. Academic Press, San Diego, CA.
- Moyer, R.R., Jr., R.A. Deyo, and J.F. Disterhoft. 1990. Hippocampectomy disrupts trace eye-blink conditioning in rabbits. *Behav. Neurosci.* **104**: 243–252.
- Nordholm, A.F., J.K. Thompson, C. Dersarkissian, and R.F. Thompson. 1993. Lidocaine infusion in a critical region of cerebellum completely prevents learning of the conditioned eyeblink response. *Behav. Neurosci.* **107**: 882–886.
- Perrett, S.P., B.P. Ruiz, and M.D. Mauk. 1993. Cerebellar cortex lesions disrupt learning-dependent timing of conditioned eyelid responses. *J. Neurosci.* **13**: 1708–1718.
- Rosenthal, R. and R.L. Rosnow. 1991. *Essentials of behavioral research: Methods and data analysis* (2nd ed.) McGraw-Hill, New York, NY.
- Schmajuk, N.A. and J.W. Moore. 1985. Real-time attentional models for classical conditioning and the hippocampus. *Psychol. Psychol.* **13**: 278–290.
- Schreurs, B.G., M.M. Oh, C. Hirashima, and D.L. Alkon. 1995. Conditioning-specific modification of the rabbit's unconditioned nictitating membrane response. *Behav. Neurosci.* **109**: 24–33.
- Sears, L.L., S.F. Logue, and J.E. Steinmetz. 1996. Involvement of the ventrolateral thalamic nucleus in rabbit classical eyeblink conditioning. *Behav. Brain Res.* **74**: 105–117.
- Sears, L.L. and J.E. Steinmetz. 1990. Acquisition of classically conditioned-related activity in the hippocampus is affected by lesions of the cerebellar interpositus. *Behav. Neurosci.* **104**: 681–692.
- . 1991. Dorsal accessory inferior olive activity diminishes during acquisition of the rabbit classically conditioned eyelid response. *Brain Res.* **545**: 114–122.
- Steinmetz, J.E. 1996. The brain substrates of classical eyeblink conditioning in rabbits. In *Acquisition of motor behavior in vertebrates* (ed. J.R. Bloedel, T. Ebner, and S. Wise), pp. 91–114. MIT Press, Cambridge, MA.
- Steinmetz, J.E. and D.R. Sengelaub. 1992. Possible conditioned stimulus pathway for classical eyelid conditioning in rabbits. *Behav. Neural Biol.* **57**: 103–115.
- Steinmetz, J.E., L.L. Sears, M. Gabriel, Y. Kubota, A. Poremba, and E. Kang. 1991. Cerebellar interpositus nucleus lesions disrupt classical nictitating membrane conditioning but not discriminative avoidance learning in rabbits. *Behav. Brain Res.* **45**: 71–80.
- Steinmetz, J.E., D.G. Lavond, D. Ivkovich, C.G. Logan, and R.F. Thompson. 1992a. Disruption of classical eyelid conditioning after cerebellar lesions: Damage to a memory trace system or a simple performance deficit? *J. Neurosci.* **12**: 4403–4426.
- Steinmetz, J.E., S.F. Logue, and S.S. Steinmetz. 1992b. Rabbit classically conditioned eyelid responses do not reappear after interpositus nucleus lesion and extensive post-lesion training. *Behav. Brain Res.* **51**: 103–114.
- Thompson, R.F. 1986. The neurobiology of learning and memory. *Science* **233**: 941–947.
- . 1988. The neural basis of basic associative learning of discrete behavioral responses. *Trends Neurosci.* **11**: 152–155.
- Thompson, R.F. and J.E. Steinmetz. 1992. The essential memory trace circuit for a basic form of associative learning. In *Learning and memory: The behavioral and biological substrates* (ed. I. Gormezano and E.A. Wasserman), pp. 369–386. Lawrence Erlbaum Associates, Hillsdale, NJ.
- Thompson, L.T., J.R. Moyer Jr., E. Akase, and J.F. Disterhoft. 1994. A system for quantitative analysis of associative learning. Part 1. Hardware interfaces with cross-species applications. *J. Neurosci. Methods* **54**: 109–117.
- Vaadia, E., I. Haalman, M. Abeles, H. Bergman, Y. Prut, H. Slovin, and A. Aertsen. 1995. Dynamics of neuronal interactions in monkey cortex in relation to behavioural events. *Nature* **373**: 515–518.
- Van Ham, J.J. and C.H. Yeo. 1992. Somatosensory trigeminal projections to the inferior olive, cerebellum and other precerebellar nuclei in rabbits. *Eur. J. Neurosci.* **4**: 302–317.
- Welsh, J.P., E.J. Lang, I. Sugihara, and R. Llinas. 1995. Dynamic organization of motor control within the olivocerebellar system. *Nature* **374**: 453–457.
- Woodruff-Pak, D.S., D.G. Lavond, C.G. Logan, J.E. Steinmetz, and R.F. Thompson. 1993. Cerebellar cortical lesions and reacquisition in classical conditioning of the nictitating membrane response in rabbits. *Brain Res.* **608**: 67–77.

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Single-unit evidence for eye-blink conditioning in cerebellar cortex is altered, but not eliminated, by interpositus nucleus lesions.

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References

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