Brief Communication

fMRI of the Conscious Rabbit during Unilateral Classical Eyeblink Conditioning Reveals Bilateral Cerebellar Activation

Michael J. Miller,[1,2] Nan-kuei Chen,[2] Limin Li,[2,3] Brian Tom,[2] Craig Weiss,[4,5] John F. Disterhoft,[4,5] and Alice M. Wyrwicz[2,3,5]

[1]Biomedical Engineering Department, Northwestern University, Evanston, Illinois 60201, [2]Center for Basic Magnetic Resonance Research, Evanston Northwestern Healthcare Research Institute, Evanston, Illinois 60201, and Departments of [3]Radiology and [4]Physiology and [5]Institute for Neuroscience, Feinberg Medical School, Northwestern University, Chicago, Illinois 60611

The relative contributions of the ipsilateral and contralateral cerebellar cortex and deep nuclei to delay eyeblink conditioning have been debated and are difficult to survey entirely using typical electrophysiological and lesion techniques. To address these issues, we used single-event functional magnetic resonance imaging (fMRI) in the conscious rabbit to visualize the entire cerebellum simultaneously during eyeblink conditioning sessions. Examination of the blood oxygenation level-dependent (BOLD) response to a visual conditioning stimulus early in training revealed significant bilateral learning-related increases in the BOLD response in the anterior interpositus nucleus (IPA) and significant bilateral deactivation in hemispheric lobule VI (HVI) of the cerebellar cortex. Later in training, the BOLD response remained bilateral in the cortex and predominantly ipsilateral in the IPA. Conditioning stimulus-alone trials after conditioning revealed that both sides of HVI were affected similarly but that only the ipsilateral interpositus nucleus was activated. These results suggest that both sides of HVI normally influence the side of the IPA being conditioned and illustrate how fMRI can be used to examine multiple brain regions simultaneously in an awake, behaving animal to discover more rapidly the neural substrates of learning and memory.

Key words: **fMRI; eyeblink conditioning; cerebellum; rabbit; learning; memory; laterality**

Introduction

Classical conditioning of the rabbit eyeblink response has been used extensively to study associative learning (Gormezano et al., 1983). This task pairs a neutral conditioning stimulus (CS) with an aversive unconditioned stimulus (US) that evokes an eyeblink. After repeated presentations, the subject learns to associate the two stimuli and blinks in response to the CS, before onset of the US. Combined with selective lesions or electrophysiological recordings, eyeblink conditioning (EBC) provides a well controlled paradigm for investigating neurobiological mechanisms underlying learning and memory (Lavond et al., 1993; Thompson and Kim, 1996; Wu et al., 2002). However, these methods are invasive, examine restricted portions of the circuit at one time, and require many animals for a thorough analysis.

More recently, imaging techniques have enabled the simultaneous, noninvasive examination of the entire brain in humans. Positron emission tomography (PET) has been used to examine EBC but requires the use of radioactive tracers, which cannot be applied repeatedly, and suffers from low spatial and temporal resolution. Functional magnetic resonance imaging (fMRI), however, is suitable for repeated application and has superior spatial and temporal resolution (Ogawa et al., 1990; Buckner et al., 1996), provided the subject is immobile. We chose the rabbit as a model subject because of its tolerance for restraint. This characteristic offers a tremendous advantage for functional imaging experiments, which require immobilization of the subject. Previous fMRI studies in animals such as the rat (Hsu et al., 1998; Yang et al., 1998; Peeters et al., 1999; Preece et al., 2001) have generally required anesthesia to eliminate subject motion. Our initial study (Wyrwicz et al., 2000) examined the visual system in unanesthetized rabbits and obtained fMRI images free from motion artifacts that revealed contralateral activation of the lateral geniculate nucleus and primary visual cortex. That experiment indicated that our model was sufficiently robust to be extended to cognitive paradigms for examining the neuronal substrates of learning and memory.

In this study, single-event fMRI was used in parallel with behavioral measurements during delay EBC to examine the relative contributions of the cerebellar cortex and deep nuclei and of the ipsilateral and contralateral cerebellum. The cerebellum is particularly interesting because it is thought to be the essential site for processing the CS–US association in EBC (Yeo et al., 1985a,b;

Received June 4, 2003; revised Oct. 1, 2003; accepted Oct. 24, 2003.

This work was supported by National Institutes of Health Grants R01 MH58912 (A.M.W.) and R01 MH47340 (J.F.D.) and National Research Service Award Predoctoral Fellowship F31 MH65085 (M.J.M.).

Correspondence should be addressed to Alice M. Wyrwicz, Center for Basic Magnetic Resonance Research, 1033 University Place, Suite 150, Evanston, IL 60201. E-mail: a-wyrwicz@northwestern.edu.

N.-k. Chen's present address: Center for Neurological Imaging, Brigham and Women's Hospital, Boston, MA 02115.

 0270-6474/03/2311753-06$15.00/0

Berthier and Moore, 1986, 1990; Thompson, 1986) and should therefore exhibit significant changes in activity during EBC. The ipsilateral cerebellum would be expected to show activation because both the afferent input and efferent output cross the midline twice before reaching their targets. Contralateral cerebellar involvement would not be expected according to classical anatomy; however, several experiments using EBC have indicated that there is some degree of bilateral activation (McCormick et al., 1981; Lavond et al., 1994; Gruart and Yeo, 1995), suggesting that unilateral training affects both sides of the cerebellum. Our results provide more direct evidence for the bilateral activation of cerebellar cortex and the predominant ipsilateral activation of the interpositus nucleus in intact subjects. To our knowledge, the present findings represent the first fMRI study of EBC in an animal model system.

Materials and Methods

Animals. Four Dutch Belted female rabbits (2–3 kg) were used in this study. The rabbits were surgically prepared for the experiments, as described previously (Wyrwicz et al., 2000), which included securing four nylon restraining bolts to the skull with nylon skull screws and dental cement. The headbolt was stereotaxically implanted in the horizontal plane to position the skull with lambda 1.5 mm below bregma and was used to secure the radiofrequency (RF) coil and the rabbit's head in the same position to obtain a constant imaging angle and slice positioning among subjects. Each subject was habituated to the imaging environment before the experiments. All procedures were performed under National Institutes of Health, Evanston Northwestern Healthcare Research Institute, and Northwestern University Institutional Animal Care and Use Committee approved protocols.

fMRI data collection. All MR imaging experiments were performed using an Omega 4.7 T imaging spectrometer (GE/Bruker NMR Instruments, Fremont, CA) operating at a proton frequency of 200 MHz. This system is equipped with an Oxford horizontal magnet and an Acustar actively shielded gradient coil assembly with a clear bore of 26 cm. A flat, circular surface coil (40 mm diameter) was used for RF transmission and reception. A multi-slice, single-shot gradient echo, echo planar imaging (EPI) pulse sequence, with a repetition time (TR) of 1.1 sec, an echo time (TE) of 28 msec, and two acquisitions per image [number of acquisitions (NA), 2], was used to map brain activation. Coronal images in a plane perpendicular to the surface coil were collected from 12 slices (2.0 mm thickness) using a 56×56 matrix size and a 52.5×52.5 mm field of view (FOV), corresponding to an in-plane resolution of 940×940 μm. The most caudal slice was positioned tangential to the caudal edge of the cerebellar vermis as viewed in a midsagittal image of each subject on each day to ensure reproducibility between subjects. Thirty-two images were collected per trial. At the end of each session, multiple field maps were obtained using a phase-shifted EPI sequence to correct for ghost artifacts and geometrical distortion (Chen and Wyrwicz, 2001). High-resolution anatomic images (256×256 matrix; 42.5×42.5 mm FOV; 166×166 μm in-plane resolution) were obtained using a multi-slice spin echo sequence (2.0 mm slice thickness; TR of 1 sec; TE of 30 msec; NA of 2).

Stimulus generation and behavioral measurement. Reliable measurement of behavioral responses is vital to allow fMRI data to be analyzed at equivalent levels of learning across subjects that may learn at different rates. The eyelid of the stimulated eye was held open to measure movement of the nictitating membrane (NM) and to ensure activation by the visual CS; the nonstimulated eye was covered. Rabbits were provided with earplugs during the experiments to reduce noise from the gradients. The visual CS was delivered by a 2×2 array of green light-emitting diodes (2×2 mm, separated by 8 mm along each axis) flashing at 8 Hz. Although most EBC studies have used a tone CS, we chose a visual CS for this experiment to avoid potential complications attributable to the noise of the gradients. However, previous behavioral (Kehoe et al., 1984) and electrophysiological (Tracy et al., 2001) work has demonstrated that visual stimuli are effective for conditioning in the rabbit. The US consisted of a 3 psi air puff supplied by compressed air and controlled by a regulator and solenoid valve. NM movement was measured with an infrared reflectance sensor (OPR5005; Optek Technology, Carrollton, TX) positioned ~5 mm from the cornea. The sensor produced a voltage change proportional to the change in optical scattering caused by movement of the NM across the cornea. A conditioned response (CR) was defined as a change in the voltage from the infrared sensor that was 4 SD greater than the mean baseline amplitude and occurred at least 35 msec after onset of the CS but before the US. Eyeblink data were sampled at 300 Hz. Each subject received three sessions (30 trials per session) of pseudoconditioning, in which unpaired stimuli were presented in a random order. Subjects then received 10 training sessions in which paired, coterminating stimuli were presented. The durations of the CS and US were 900 and 100 msec, respectively. The intertrial interval was randomized between 90 and 120 sec. On the last day of training, the subjects also received 15 "memory" trials in which only the CS was presented.

Data analysis. All data were analyzed offline on a Sun Ultra 60 workstation (Sun Microsystems, Palo Alto, CA) using software written for IDL (Research Systems Inc., Boulder, CO). Behavioral responses were smoothed using a boxcar kernel with a width of 30 points. Data from all fMRI trials were averaged to generate a single response for each session. CS- and US-alone trials during pseudoconditioning were averaged separately. Before statistical analysis, EPI data were corrected for ghost artifacts and geometrical distortion using previously described methods (Chen and Wyrwicz, 2001). Activation maps were generated using a clustering algorithm (Tom et al., 1998), with seeding and clustering thresholds of 0.7 and 0.65, respectively. These two thresholds are correlation coefficients and govern the number and spatial extent of the clusters, respectively. In general, higher thresholds require a higher degree of similarity between neighboring voxels to assign them to the same cluster. Regions of interest (ROIs) within Larsell's hemispheric lobule VI (HVI) and the anterior interpositus nucleus (IPA) were selected, and the temporal responses of pixels within those areas were averaged to generate a mean response across time for each region. These responses were then averaged across subjects and were examined during four stages: pseudoconditioning, 40 and 80% CRs during training, and CS-alone memory trials after training. We chose a level of 40% CRs to examine early acquisition and a level of 80% CRs to examine brain activation when conditioning was well established.

Results

The rabbits adapted well to the restraint and imaging environment and showed no obvious sign of movement or struggling during the experiments. All animals reached the learning criterion of at least 80% CRs within 10 training sessions. Eyeblink responses from a representative animal are shown in Figure 1 for pseudoconditioning, training (40 and 80% CRs), and CS-alone memory trials. The baseline preceding CS onset is relatively flat, because rabbits tend to have few spontaneous eyeblinks. The small rectangular artifacts were caused by the pulsed field gradients of the EPI sequence. Animals responded only to the US during pseudoconditioning, as seen from the large voltage change that occurs after US onset. In contrast, after training (40 and 80% CRs), the animals began to blink in response to the CS, before US presentation, providing clear evidence of a learned behavioral response to the delay paradigm. Note the longer duration of the temporal responses present at 80% CRs and memory trials compared with 40% CRs. CRs were also evident in the memory trials of the final session, in which the well trained animals blinked when only the CS was presented.

Figure 2*a* shows functional images of the cerebellum from a single animal. The images reveal distinct differences in the evolution of the blood oxygenation level-dependent (BOLD) response between the cortex and deep nuclei. Neither region was active in response to either CS or US presentation during pseudoconditioning. At 40% CRs, the cortex showed significant bilateral BOLD decreases, whereas significant bilateral increases

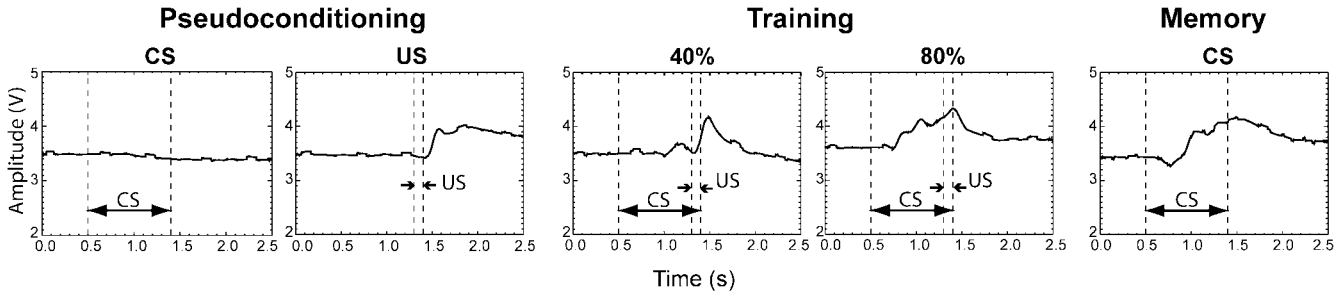

Figure 1. Behavioral response during eyeblink conditioning. Eyeblink responses are shown for a single animal during pseudoconditioning and training stages (40 and 80% CRs), as well as during CS-alone trials after training. Responses were recorded with an infrared reflectance sensor, with a voltage increase indicating a blink. Note the earlier onset of the responses as learning occurs. The dashed lines represent the timing of the stimuli.

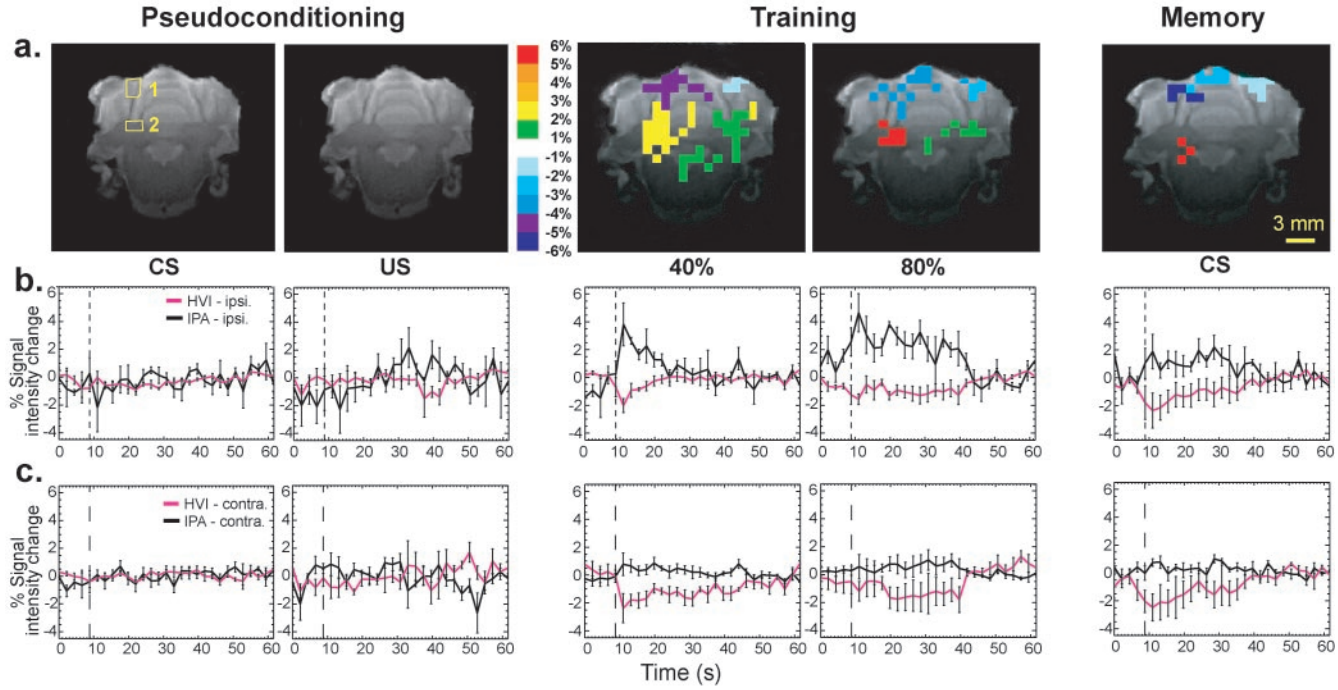

Figure 2. Functional activation in cerebellum during eyeblink conditioning. Functional maps are shown (*a*) for a representative animal during control and training stages, as well as during CS-alone memory trials after training. Colors denote the peak magnitude of the averaged response for each cluster. Selected anatomical regions corresponding to HVI (1) and IPA (2) were averaged across subjects ($n = 4$) to characterize the temporal response of each region on the ipsilateral (*b*) and contralateral (*c*) sides. The dashed line represents the onset of the stimuli; the error bars represent the SEM.

were seen in the deep nuclei. However, as the rabbit reached 80% CRs, the area of the response in the deep nuclei shrank visibly on both sides, and the magnitude of the ipsilateral side increased. In contrast, the ipsilateral and contralateral sides of the cortex were similar in the area and magnitude of the BOLD response at this stage. During CS-alone memory trials, the contralateral response in the deep nucleus was completely absent, whereas the cortex again displayed similar areas of response on both sides. Note the difference in BOLD response to the CS in pseudoconditioning versus memory trials. This difference presumably reflects brain activity required for the performance of the learned response. Thus, although both the ipsilateral and contralateral deep nuclei appear to be engaged early in the acquisition of the delay paradigm, albeit to a greater degree on the ipsilateral side, contralateral involvement diminishes greatly with training and is not necessary for memory trials immediately after training. In contrast, the cortex shows a consistent bilateral response throughout training and memory trials. We should also note that BOLD responses appeared in the vermis during training and may reflect learning-specific changes in the autonomic system (Supple and Kapp, 1993). The large signal intensity fluctuations that occur in the ventricles as a result of CSF pulsation resulted in an artifactual activation within the fourth ventricle at 40% CRs, as can be seen in Figure 2*a*.

A more comprehensive picture of the evolution of BOLD changes across the stages of training can be obtained by examining the temporal profiles of specific regions of interest averaged across all subjects. To quantify the temporal response of these changes, regions including Larsell's HVI and the IPA, outlined in yellow in Figure 2*a*, were selected in each animal. Figure 2*b* shows the time course profiles averaged across subjects ($n = 4$) for these two regions on the ipsilateral side. No activation appeared in either ROI during pseudoconditioning. At 40% CRs, however, the IPA showed a strong positive activation (3.8%), whereas HVI

showed a negative response (−2.5%) of similar duration. The responses in both regions lengthened in duration by 80% CRs, and the response in HVI decreased in amplitude. Temporal profiles in both regions were longer during memory trials, relative to 40% CRs. However, whereas the response amplitude in the IPA visibly decreased, the amplitude in HVI was relatively unchanged. Note the contrast between CS-alone trials performed during pseudoconditioning and those performed after training.

Figure 2*c* shows the responses of the contralateral HVI and IPA. As with the ipsilateral side, neither region is active during pseudoconditioning. The temporal profile from HVI at 40% CRs is similar in magnitude and duration to the ipsilateral side, and both decrease in magnitude and broaden in duration at 80% CRs. During memory trials, the temporal response in HVI is again longer, relative to the response at 40% CRs, and parallels the ipsilateral response in amplitude and duration. In contrast, the magnitude of the mean IPA response at 40% CRs is significantly lower (1.0 vs 3.8% on the ipsilateral side). Activity in the contralateral IPA diminishes even further at 80% CRs and is absent during memory trials.

Discussion

Our fMRI results from the cerebellum demonstrate a striking evolution of BOLD activity in both cortical and deep nuclear regions across the stages of the experiment in terms of response laterality, area, and time course and are consistent with a circuit hypothesized to mediate classical EBC, as described by Thompson (1986, 1990). In the proposed circuit, the CS and US pathways project via the pontine nuclei and rostral dorsal accessory olive, respectively, to both the ipsilateral HVI and IPA. Converging CS–US inputs in the cerebellar cortex are predicted to cause depression of Purkinje cell activity with a subsequent release of inhibition in the deep nuclei, which project to the motor circuitry to mediate CR expression. Findings by Gould and Steinmetz (1996) have suggested that depression of cortical Purkinje cell activity occurs together with increased activity in the IPA to produce CRs. The similarity of the BOLD response durations (Fig. 2*b*) of these regions suggests that HVI does not merely trigger activity in the IPA but that their activity occurs in parallel, indicating a consistent modulation of the IPA by HVI. Such a mechanism would account for the results reported by Yeo et al. (1985b) and Gruart and Yeo (1995), who showed that bilateral lesions of the cortex were necessary to abolish CRs. Interestingly, the duration of the responses in both regions appears to increase consistently over the course of training. A parallel increase in duration can be seen in the behavioral responses at 80% CRs and memory trials relative to 40% CRs.

We observed a consistent decrease in activated area in the deep nuclei across training. This pattern may suggest a refinement of the learned response as the strength of the association increases and the behavioral response converges from a more widespread response to that of the eyeblink alone. Such refinement is known to occur in well trained animals (McCormick et al., 1982), and therefore a corresponding modulation of the BOLD response over time should be expected. The BOLD response during memory trials was particularly striking when compared with CS-alone trials during pseudoconditioning. Although these two responses arose from identical CS stimulation, no activity was observed during the initial unpaired presentations of the CS during pseudoconditioning, whereas both cortical and deep nuclear regions were engaged during memory trials, indicating a change in the significance of the CS with training and the establishment of a learned BOLD response to the CS in the cerebellum.

Because of the double decussation of most cerebellar inputs and outputs, cerebellar cortical and nuclear activity related to EBC is expected to occur primarily ipsilateral to the stimulated side. However, involvement of the contralateral cerebellum in EBC is not unexpected in light of previous findings. Eyeblink CRs have been observed in the eye contralateral to the US, although they were small relative to the ipsilateral side (Disterhoft et al., 1977). McCormick et al. (1981) reported coordinated plasticity in the contralateral cerebellar system during EBC. These results were further supported by Lincoln et al. (1982), who demonstrated that training to one eye before ipsilateral cerebellar lesion facilitated subsequent learning in the contralateral eye. Lavond et al. (1994) showed that continued training of rabbits with IPA lesions facilitates acquisition when training is switched to the contralateral eye. Gruart and Yeo (1995) investigated the role of the contralateral cerebellar cortex in EBC by examining the effects of unilateral versus bilateral cortical lesions and found that bilateral lesions of HVI were necessary to prevent relearning of conditioned responses. Ivarsson et al. (1997) report bilateral disruption of CRs by unilateral cerebellar blockade, suggesting involvement of the contralateral cerebellum. Our observations that contralateral activity in HVI persisted throughout training and memory trials and that the contralateral IPA became active early in training but diminished over time agree well with these previous studies. By examining activity at different stages of learning, these results provide more direct evidence that the contralateral HVI and IPA are involved in EBC and exhibit significant evolution with learning. The pattern of this evolution indicates that the cerebellar cortex influences bilaterally the development of the CR and that, early in training, the deep nuclei are also engaged bilaterally, which suggests that both hemispheres are actively involved in unilateral expression of CRs. The reduction of the contralateral response in the IPA late in training and during memory trials, however, indicates that the nature of this involvement changes over time.

The concurrent deactivation in HVI and activation in IPA that we observed are consistent with the hypothesis that long-term depression of Purkinje cell activity may contribute to the formation of CRs. Such an effect has been shown to occur in response to pairing-specific stimulation of CS and US pathways and has therefore been proposed to influence the formation of the CS–US association during classical EBC (Schreurs et al., 1996). BOLD decreases in the cortex that we observed only in response to CS–US pairing and the increased activity in the deep nuclei are in agreement with the proposed circuit described above. Although we lack the spatial resolution to determine whether the decreased BOLD response in HVI is attributable to Purkinje cells specifically, our results agree with the proposed model, showing a large positive BOLD response in the IPA, accompanied by a negative response in HVI beginning in the early stage of training. In an electrophysiological study, Thompson (1990) reported that 87% of 77 Purkinje cells from conditioned rabbits decreased their activity in response to the CS, whereas only 31% showed an increase in activity to the CS. Other studies (Berthier and Moore, 1986; Gould and Steinmetz, 1996; Katz and Steinmetz, 1997) are less in agreement and report a 2:1 ratio of increases versus decreases in simple spike firing, although this disagreement may reflect differences in the paradigm [Berthier and Moore (1986) used discrimination conditioning] or sampling biases, because active Purkinje cells are more easily detected and specifically sampled for analysis (Gould and Steinmetz, 1996). The differences in excitation and inhibition of Purkinje cell responses may reflect the nature of the

conditioned response and the mechanism necessary to properly time the response at the onset of the US. This mechanism would require inhibition of the IPA to correctly delay the CR and excitation to initiate the CR via the red nucleus and motor neurons. Our results, using the more macroscopic technique of fMRI, suggest that the overall role of HVI during delay EBC is to release inhibition in the IPA to generate a CR, as predicted by Thompson (1986, 1990).

Several PET studies, as well as a single fMRI study in human subjects, have also shown significant cerebellar activity during delay EBC in human subjects. However, there is some inconsistency in the human data. Molchan et al. (1994) and Schreurs et al. (1997) reported bilateral regional cerebral blood flow (rCBF) decreases in the cerebellar cortex after conditioning, in agreement with our findings, as well as ipsilateral decreases during extinction. Blaxton et al. (1996) reported both increases and decreases in rCBF bilaterally, whereas Logan and Grafton (1995) observed ipsilateral increases in glucose metabolism in this region. Surprisingly, only Logan and Grafton (1995) observed activation in the ipsilateral deep nuclei. Using fMRI, Ramnani et al. (2000) showed primarily ipsilateral learning-related BOLD decreases in the cerebellar cortex, with a weaker contralateral component, and also observed contralateral BOLD decreases related to sensory prediction in this region. However, they detected no activity in the deep nuclei. One source of this inconsistency could be the weaker field strength of the magnet used in the human studies, which produces a weaker BOLD response. Furthermore, if the activated extent of these regions decreases with learning, as our results have indicated, the faster CR acquisition in human studies across a single session could obscure small responses after the averaging of several trials. The slower rate of learning in an animal model of EBC may more easily reveal with fMRI the progression of changes at different stages of learning.

In conclusion, our results illustrate the advantages of using fMRI to investigate EBC in the rabbit. We demonstrated striking differences in the activity of the cerebellar cortex and deep nuclei, which evolved across training in terms of laterality, area, and time course. Lesion and electrophysiological studies, as well as functional imaging studies in humans, strongly support the involvement of these regions in EBC. However, the animal model allows us to draw more directly on the extensive body of data obtained from rabbits over the last several decades, and the slower progression of acquisition in rabbits relative to humans has allowed us to study different stages of learning in greater detail. The ability of fMRI to visualize hemodynamic activity noninvasively and in multiple brain regions simultaneously in the conscious animal provides an important tool for understanding the circuitry underlying different stages of associative learning and memory consolidation.

References

Berthier NE, Moore JW (1986) Cerebellar Purkinje cell activity related to the classically conditioned nictitating membrane response. Exp Brain Res 63:341–350.

Berthier NE, Moore JW (1990) Activity of deep cerebellar nuclear cells during classical conditioning of nictitating membrane extension in rabbits. Exp Brain Res 83:44–54.

Blaxton TA, Zeffiro TA, Gabrieli JD, Bookheimer SY, Carrillo MC, Theodore WH, Disterhoft JF (1996) Functional mapping of human learning: a positron emission tomography study of eyeblink conditioning. J Neurosci 16:4032–4040.

Buckner RL, Bandettini PA, O'Craven KM, Savoy RL, Petersen SE, Raichle ME, Rosen BR (1996) Detection of cortical activation during averaged single trials of a cognitive task using functional magnetic resonance imaging. Proc Natl Acad Sci USA 93:14878–14883.

Chen N-K, Wyrwicz AM (2001) An optimized distortion correction technique for echo planar imaging. Magn Reson Med 45:525–528.

Disterhoft JF, Kwan HH, Lo WD (1977) Nictitating membrane conditioning to tone in the immobilized albino rabbit. Brain Res 137:127–143.

Gormezano I, Kehoe EJ, Marshall BS (1983) Twenty years of classical conditioning research with the rabbit. Prog Psychobiol Physiol Psychol 10:197–275.

Gould TJ, Steinmetz JE (1996) Changes in rabbit cerebellar cortical and interpositus nucleus activity during acquisition, extinction, and backward classical eyeblink conditioning. Neurobiol Learn Mem 65:17–34.

Gruart A, Yeo CH (1995) Cerebellar cortex and eyeblink conditioning: bilateral regulation of conditioned responses. Exp Brain Res 104:431–448.

Hsu EW, Hedlund LF, MacFall JR (1998) Functional MRI of the rat somatosensory cortex: effects of hyperventilation. Magn Reson Med 40:421–426.

Ivarsson M, Svensson P, Hesslow G (1997) Bilateral disruption of conditioned responses after unilateral blockade of cerebellar output in the decerebrate ferret. J Physiol (Lond) 502:189–201.

Katz DB, Steinmetz JE (1997) Single-unit evidence for eye-blink conditioning in cerebellar cortex is altered, but not eliminated, by interpositus nucleus lesions. Learn Mem 3:88–104.

Kehoe EJ, Morrow LD, Holt PE (1984) General transfer across sensory modalities survives reductions in the original conditioned reflex in the rabbit. Anim Learn Behav 12:129–136.

Lavond DG, Kim JJ, Thompson RF (1993) Mammalian brain substrates of aversive classical conditioning. Annu Rev Psychol 44:317–342.

Lavond DG, Kanzawa SA, Ivkovich D, Clark RE (1994) Transfer of learning but not memory after unilateral cerebellar lesion in rabbits. Behav Neurosci 108:284–293.

Lincoln JS, McCormick DA, Thompson RF (1982) Ipsilateral cerebellar lesions prevent learning of the classically conditioned nictitating membrane/eyelid response. Brain Res 242:190–193.

Logan CG, Grafton ST (1995) Functional anatomy of human eyeblink conditioning determined with regional cerebral glucose metabolism and positron-emission tomography. Proc Natl Acad Sci USA 92:7500–7504.

McCormick DA, Lavond DG, Clark GA, Kettner RE, Rising CE, Thompson RF (1981) The engram found? Role of the cerebellum in classical conditioning of nictitating membrane and eyelid responses. Bull Psychon Soc 18:103–105.

McCormick DA, Lavond DG, Thompson RF (1982) Concomitant classical conditioning of the rabbit nictitating membrane and eyelid responses: correlations and implications. Physiol Behav 28:769–775.

Molchan SE, Sunderland T, McIntosh AR, Herscovitch P, Schreurs BG (1994) A functional anatomical study of associative learning in humans. Proc Natl Acad Sci USA 91:8122–8126.

Ogawa S, Lee T-M, Kay AR, Tank DW (1990) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci USA 87:9868–9872.

Peeters RR, Verhoye M, Vos BP, Van Dyck D, Van Der Linden A, De Schutter E (1999) A patchy horizontal organization of the somatosensory activation of the rat cerebellum demonstrated by functional MRI. Eur J Neurosci 11:2720–2730.

Preece M, Mukherjee B, Huang CLH, Hall LD, Leslie RA, James MF (2001) Detection of pharmacologically mediated changes in cerebral activity by functional magnetic resonance imaging: the effects of sulpride in the brain of the anesthetized rat. Brain Res 916:107–114.

Ramnani N, Toni I, Ashburner J, Passingham RE (2000) Learning- and expectation-related changes in the human brain during motor learning. J Neurophysiol 84:3026–3035.

Schreurs BG, Oh MM, Alkon DL (1996) Pairing-specific long-term depression of Purkinje cell excitatory postsynaptic potentials results from a classical conditioning procedure in the rabbit cerebellar slice. J Neurophysiol 75:1051–1060.

Schreurs BG, McIntosh AR, Bahro M, Herscovitch P, Sunderland T, Molchan SE (1997) Lateralization and behavioral correlation of changes in regional cerebral blood flow with classical conditioning of the human eyeblink response. J Neurophysiol 77:2153–2163.

Supple WF, Kapp BS (1993) The anterior cerebellar vermis: essential involvement in classical conditioned bradycardia in the rabbit. J Neurosci 13:3705–3711.

Thompson RF (1986) The neurobiology of learning and memory. Science 233:941–947.

Thompson RF (1990) Neural mechanisms of classical conditioning in mammals. Phil Trans R Soc Lond B Biol Sci 329:161–170.

Thompson RF, Kim JJ (1996) Memory systems in the brain and localization of a memory. Proc Natl Acad Sci USA 93:13438–13444.

Tom B, Akhavein R, Wyrwicz AM (1998) Robust clustering of fMRI data by correlation. Proc Int Soc Magn Reson Med 6:250.

Tracy JA, Britton GB, Steinmetz JE (2001) Comparison of single unit responses to tone, light, and compound conditioned stimuli during rabbit classical eyeblink conditioning. Neurobiol Learn Mem 76:253–267.

Wu WW, Oh MM, Disterhoft JF (2002) Age-related alterations of hippocampal pyramidal neurons: implications for learning and memory. Ageing Res Rev 1:181–207.

Wyrwicz AM, Chen N-K, Li L, Weiss C, Disterhoft JF (2000) fMRI of visual system activation in the conscious rabbit. Magn Reson Med 44:474–478.

Yang XJ, Renken R, Hyder F, Siddeck M, Greer CA, Shepherd GM, Shulman RG (1998) Dynamic mapping of odor-elicited responses in rat olfactory bulb by functional MRI. Proc Natl Acad Sci USA 95:7715–7720.

Yeo CH, Hardimann MJ, Glickstein M (1985a) Classical conditioning of the nictitating membrane response of the rabbit. I. Lesions of the cerebellar nuclei. Exp Brain Res 60:87–98.

Yeo CH, Hardimann MJ, Glickstein M (1985b) Classical conditioning of the nictitating membrane response of the rabbit. II. Lesions of the cerebellar cortex. Exp Brain Res 60:99–113.