Learning- and Expectation-Related Changes in the Human Brain During Motor Learning

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Received 19 April 2000; accepted in final form 17 August 2000

Ramnani, N., I. Toni, O. Josephs, J. Ashburner, and R. E. Passingham. Learning- and expectation-related changes in the human brain during motor learning. J Neurophysiol 84: 3026–3035, 2000. We have studied a simple form of motor learning in the human brain so as to isolate activity related to motor learning and the prediction of sensory events. Whole-brain, event-related functional magnetic resonance imaging (fMRI) was used to record activity during classical discriminative delay eyelink conditioning. Auditory conditioned stimulus (CS+) trials were presented either with a corneal airpuff unconditioned stimulus (US, paired), or without a US (unpaired). Auditory CS− trials were never reinforced with a US. Trials were presented pseudorandomly, 66 times each. The subjects gradually produced conditioned responses to CS+ trials, while increasingly differentiating between CS+ and CS− trials. The increasing difference between hemodynamic responses for unpaired CS+ and for CS− trials evolved slowly during conditioning in the ipsilateral cerebellar cortex (Crus I/Lobule HV1), contralateral motor cortex and hippocampus. To localize changes that were related to sensory prediction, we compared trials on which the expected airpuff US failed to occur (Unpaired CS+) with trials on which it occurred as expected (Paired CS+). Error-related signals in the contralateral cerebellum and somatosensory cortex were seen to increase during learning as the sensory prediction became stronger. The changes seen in the ipsilateral cerebellar cortex may be due either to the violations of sensory predictions, or to learning-related increases in the excitability of cerebellar neurons to presentations of the CS+.

INTRODUCTION

Eyeblink conditioning can be used as simple model of associative motor learning in humans. In classical delay eyelink conditioning, an airpuff to the eye and the surrounding skin (unconditioned stimulus, US) unconditionally elicits an eyeblink reflex (unconditioned response, UR). If the airpuff is repeatedly paired with a preceding auditory tone (conditioned stimulus, CS), the initially neutral CS itself comes to elicit a well-timed, conditioned eyelink response (CR) after a sufficient number of pairings (Gormezano 1966; Yeo and Hesslow 1998). This process must depend on the ability of neuronal circuitry to undergo plastic changes. However, the issue of where such plasticity occurs in the brain is both controversial and unresolved (Yeo 1991). For brain areas to be identified as candidate sites, two important criteria must be met. The first is that learning should not progress if lesions or inactivations functionally compromise these areas. The second is that such areas must show learning-specific changes in activity as learning occurs.

The rabbit model has been particularly useful for identifying the pathways essential for the acquisition and expression of this conditioned reflex. In rabbits, movements of the external eyelid and the nictitating membrane (NM) are tightly coupled and highly correlated (McCormick et al. 1982). During conditioning, CRs and URs are therefore composed of compound movements of both the NM and external eyelid. Despite these behavioral similarities, the two movements are driven by separate muscle groups, which are innervated by different motor nuclei. The orbicularis oculi muscle is innervated by the seventh nerve from the facial nucleus and closes the external eyelid. The retractor bulbi muscle is innervated by the sixth nerve from the accessory abducens nucleus and causes nictitating membrane responses by eyeball retraction into the eye socket. It has been shown that the cerebellum is essential for the conditioning of both the external eyelid and the NM (Iverson et al. 2000; McCormick et al. 1981) together with its efferent connections with the red nucleus, the accessory abducens nucleus and the facial nucleus (Krupa et al. 1996; Rosenfield and Moore 1983, 1995; Rosenfield et al. 1985). Anatomically specific lesions have shown that the integrity of cerebellar cortical lobule HV1 and anterior parts of the interpositus nucleus are essential for NM conditioning (Yeo et al. 1985a–c, 1986). Although permanent lesions impair the expression of NM and eyelink CRs, it is an important finding that learning is prevented by reversible inactivations of the cerebellar nuclei during conditioning, irrespective of effects on the expression of NM and eyelink CRs (Krupa et al. 1993; Ramnani and Yeo 1996). It has been claimed that inactivation of the efferent fibers running in the brachium conjunctivum leaves learning intact (Krupa and Thompson 1995), and this finding has been used to suggest that plasticity for this form of motor learning may exist in cerebellar or precerebellar circuitry.

Areas in which there is plasticity for motor learning should show neuronal activity that changes as a function of learning. Functional brain imaging has the advantage that it records population activity for a whole region, and more recent studies have therefore used this method to study learning-related activity. There have been four positron emission tomography (PET) studies in which subjects were scanned during delay
conditioning of the external eyelid (Blaxton et al. 1996; Logan and Grafton 1995; Molchan et al. 1994; Schreurs et al. 1997). Typically, the subjects were first scanned during “no-learning” blocks and then during “learning” blocks in which CRs developed as a result of paired training. Stimulus presentations were matched in the two conditions. All of these PET studies have reported the presence of differential activity in the cerebellum and have therefore provided evidence that the human cerebellum becomes engaged during delay-eyelid conditioning. The precise nature of this engagement is not clear. It has recently been proposed that the learning of relationships between sensory events is an important component process in motor learning (Hikosaka et al. 1999). In line with recent theories, it is possible that the cerebellum is specifically engaged in learning the relationships between sensory events (Bower 1997). Such accounts are supported by findings that violations of sensory predictions activate the cerebellum (Blakemore et al. 1999; Tesch and Karhu 2000).

Event-related functional magnetic resonance imaging (fMRI) permits the analysis of activity at the level of a single trial, thus conferring specific advantages on classical conditioning experiments. It is possible, for example, to localize regions in which there are trial-by-trial changes in learning-related activity. Refinements in experimental design make it possible to interpolate experimental events with control events, such that control and baseline activity may be sampled simultaneously. This is particularly important for detecting learning-specific changes, where nonspecific changes must be discounted. It is also a strength of event-related fMRI that it is able to reveal activity that occurs during unpredicted “oddball” events (Josephs et al. 1997) such that which occurs when a US predicted by a preceding CS, fails to occur.

We have used whole-brain, event-related fMRI to scan during discriminatory delay eyelid conditioning. On one-third of the trials, one tone (CS+) was paired with an airpuff (US) delivered to the right eye (Paired CS+ trials). On one-third of the trials the same tone was presented, but the airpuff was omitted (Unpaired CS+ trials). On the remaining one-third of trials a different tone was presented but never paired with the airpuff US (CS− trials). Thus there was partial reinforcement for the CS+. The three trial types were randomly intermixed. The subjects increasingly learned to give conditioned eyelid responses (CRs) to the CS+, and the CR frequency on CS− trials decreased with conditioning. This design enabled us to analyze the data in two ways. First, we could look for changes over time when comparing Unpaired CS+ and CS− trials. No airpuff was given on either trial type, but as learning occurred, CRs were increasingly produced on the Unpaired CS+ trials. Second, we could look for changes over time which were related to sensory prediction. This could be done by comparing Unpaired CS+ trials with Paired CS+ trials. As learning occurred, the subjects increasingly predicted the occurrence of the US on both trial types, but on the Unpaired CS+ trials, there was an increasing mismatch between the predicted sensory outcome and the actual events.

METHODS

Subjects

The subjects were five, healthy right-handed male volunteers. Written informed consent was obtained prior to scanning.

Behavioral methods

STIMULI. The US was an airpuff (5 psi at source: 100 ms) delivered through tapered plastic tubing, the tip of which was positioned close to the cornea of the right eye. The CSs were auditory tones (700 ms, 85 dBA) that differed in frequency (600- and 1,400-Hz sine waves; see following text). These frequencies were clearly audible above the noise of the MRI scanner and are comparable with those reported in Moore (1964) in which differential human eyelid conditioning was characterized in detail.

APPARATUS. Each subject lay supine in the MRI scanner with the head immobilized by padded restraints. Sound was delivered directly into the subjects’ ears through MRI-compatible air-tubes. A self-fastening (Velcro) strap was wrapped securely around the forehead, on which a novel, light MRI compatible opto-mechanical low-torque transducer was affixed above the right eye. One arm of an articulated joint was coupled rigidly at a right angle to the freely rotating shaft of the transducer, and the other was a contacting arm, coupled to the base of the upper eyelid. This arrangement enabled eyelid movements to be transduced into voltage signal, without the need for restoring forces on the transducer. Eyelid movements were unimpeded by this arrangement. Stimulus delivery equipment was calibrated for accurate timing and intensity before the start of each conditioning session (see Data acquisition).

CONDITIONING. A differential conditioning procedure was used (Moore 1964). There were three trial types: Paired CS+ trials (the interstimulus interval between CS+ onset and US onset was 600 ms, and the US coterminated with the CS+); Unpaired CS+ trials (the CS+ trials occurred in the absence of the US); and CS− trials (the US occurred on its own).

Each trial was presented 66 times pseudorandomly. There were 198 trials (6 trials × 3 trial types × 11 blocks). The identity of the CS− and CS+ trials was counterbalanced across subjects, such that in three subjects, the CS+ was 1,400 Hz and in two subjects the CS+ was 600 Hz. The inter-trial interval (ITI) was calculated on the basis of the scanner repeat time (TR; see following text). The airpuff US was delivered to the right eye.

CONDITIONED RESPONSES. Eyelid responses were scored as CRs on the basis of amplitude and latency based on criteria established in other studies. The same criteria were applied to all subjects. Eyelid responses were measured in reference to a stable, 100 ms pre-CS baseline. Latency criterion. Short-latency “alpha” eyelid responses (onset latency <250 ms) sometimes occur unconditionally in response to auditory stimuli and are not associative (see Gormezano 1966). Some have reported that “voluntary” responses, as distinct from true CRs (Coleman and Webster 1988) also occur below this latency criterion. Responses occurring within 250 ms of CS onset were therefore not scored as CRs. On Paired CS+ trials, the US interrupted the execution of CRs, and so only the anticipatory component of the CR could be assessed. Responses were scored as CRs if their onsets occurred after the 250 ms criterion but before the US onset (600 ms after CS onset). We were able to assess the entire CS period for unpaired CS+ trials and CS− trials. Responses for these trial types were scored as CRs if they occurred after the 250 ms criterion but before 800 ms after CS onset. Onset times of responses were determined by finding the point in time at which the CR departed from the baseline.

Amplitude criterion. Some other studies have defined eyelid responses as CRs if they exceeded an absolute threshold of 0.5 mm (within a latency criterion window), regardless of individual subjects’ maximum eye aperture. This strategy is prone to biases such that subjects with larger eye apertures will exceed the 0.5 mm threshold more easily and quickly than subjects with smaller apertures, irrespective of the rate of learning. In the present study, we have elected to use a comparable but more conservative threshold that avoids this bias (see Clark and Squire 1998; Woodruff-Pak et al. 1996). Eyelid amplitude is expressed as a proportion of individual subjects’ maxi-
mum eye aperture. Maximum eyeblink closure was determined by finding the maximum UR-elicited deviation from the pre-CS baseline for each subject in Paired CS+ trials. Eyeblink amplitudes that exceeded 5% of maximum eyeblink closure were scored as CRs. This equates to a threshold of 0.5 mm (the standard criterion used by some others) for a subject with a maximum eyeblink amplitude of 10 mm. Our criterion may be more conservative than other studies for the detection of small CRs because in our study the probability of detecting a CR is not increased by the size of the eye aperture.

In our study, the differential CR frequency between Unpaired CS+ (in which the presence of CRs indicated learning) and CS− trials (in which the absence of CRs indicated learning) was our measure of learning. There could only be biases toward or away from learning if the latency and amplitude criteria for CRs in Unpaired CS+ trials were different from those in CS− trials. However, the criteria were the same for all trial types. Thus any effects that our criteria had on CS+ trials also affected CS− trials in the same way.

ACQUISITION OF BEHAVIORAL DATA. Behavioral data, scanner slice acquisition times, CS times, and US times were acquired simultaneously using an AD converter (1401 unit) and programmable signal amplifier (1902 unit) (Cambridge Electronic Design, Cambridge, UK). Eyelid movements were sampled at 1 kHz, and auditory stimuli were sampled at 3 kHz. US onset times and slice acquisition times were recorded as marker events. These data were acquired simultaneously from each subject during conditioning.

ANALYSIS OF BEHAVIORAL DATA. CR frequencies were expressed as percentages of CRs relative to the number of trials presented, for each block of trials, for each trial type. An ANOVA was conducted using SPSS for Windows 8.0 (SPSS) to determine the effect of BLOCK (11 levels, 6-trial blocks) and the effect of TRIAL TYPE (2 levels, unpaired CS+, CS−). The BLOCK × TRIAL TYPE interaction determined the statistical significance of differences between Unpaired CS+ and CS− trials that developed as a function of training. Comparisons were not made with Paired CS+ trials because the time interval for the assessment of CRs was different from the other trial types (see CONDITIONED RESPONSES).

MRI imaging

The imaging methods were similar to those reported in Buechel et al. (1998). Six hundred and fifteen EPI images were acquired continuously using a 2 Tesla Magnetom VISION whole body MRI system (Siemens, Erlangen, Germany) and a head volume coil. Images were T2*-weighted axial volumes (48 slices; TR, 4.73 s; TE = 40 ms;
voxel size, 3 mm$^3$; slice dimensions, 64 × 64 pixels). The volumes acquired covered the whole brain. After the experiment, structural images were acquired using a T1 MPRAGE sequence (TE = 5 ms; TR = 5.9 s; T1 = 600 ms; voxel size 1 × 1 × 1.5 mm). We sampled evoked hemodynamic responses (EHRs) at an effective frequency that was considerably higher than the TR (Josephs et al. 1997) by uniformly distributing random trial-to-trial variation in the interval between scan onset and trial onset [from 14.1 s (TR × 3) to 18.8 s (TR × 4)]. This determined the ITI.

### TABLE 1. Unpaired CS+$\tau$ > CS$-\tau$

<table>
<thead>
<tr>
<th>Area</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z Value</th>
</tr>
</thead>
<tbody>
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<td>-76</td>
<td>-30</td>
<td>3.29</td>
</tr>
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<tr>
<td>Left hippocampus</td>
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<td>-40</td>
<td>-2</td>
<td>3.10</td>
</tr>
<tr>
<td>Left extrastriate visual cortex</td>
<td>-8</td>
<td>-64</td>
<td>4</td>
<td>3.54</td>
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<tr>
<td>Left extrastriate visual cortex</td>
<td>-6</td>
<td>-94</td>
<td>14</td>
<td>3.46</td>
</tr>
</tbody>
</table>

**FIG. 2.** Effects of auditory and somatosensory stimulation. A: auditory CS+. Activity in primary auditory cortex (66, −42, 16; Z = 6.14). SPM(t) map are superimposed on coronal sections. Laterality of coronal sections indicated by L (left) and R (right). B: effect of Paired CS+ > Unpaired CS+: US/UR-related activity in ventral parts of the somatosensory cortex (56, 2, 0; Z = 4.30). Activations are rendered onto the typical canonical brain from the MNI series.

**FIG. 3.** Time-by-condition interactions. A: cerebellar time-by-condition interaction in Crus I/lobe HVI. SPM(t) map superimposed on a horizontal section. B: ventral motor/premotor cortex time-by-condition interactions. SPM(t) map rendered onto the typical canonical brain from the MNI series. * Insets: best fitting event-related hemodynamic response function models for cerebellar and motor cortical areas, each from a different subject.

### Image processing methods

Image processing and analysis methods were carried out in SPM97 (Friston et al. 1995b) and performed on Sparc computers (Sun Microsystems, Mountainview, CA). The following preprocessing methods were applied for data from each subject. The experiment began after four volumes were collected. These four volumes were then discarded to minimize T1 relaxation artifacts; 611 volumes were analyzed for each subject. After inspection of image quality, six head-motion parameters were estimated (3 translations and 3 rotations) from rigid body transformations that minimized the difference between each volume and the first (Friston et al. 1995b). The parameters were subsequently used to realign the time series. A mean T2*-weighted volume was computed from these functional volumes, and the structural T1 image was coregistered it. The functional and structural images were realigned and then spatially normalized into the reference system of Talairach and Tournoux (1988), using a representative reference brain from the Montreal Neurological Institute series (Evans et al. 1994) as a template. The functional images were subsampled to a voxel size of 2 mm$^3$, smoothed using an isotropic Gaussian kernel of 6 mm, and so conformed to the multivariate Gaussian assumptions of SPM97.
Image analysis method

MODELS. To model EHRs for each of the three trial types, three covariates were constructed to create a general linear model (GLM) that could be estimated in SPM97. Delta functions derived from trial onset times for each trial type were convolved with a synthetic “canonical” hemodynamic response function. The regressors (Paired CS+, Unpaired CS+, and CS−) were multiplied by a linear trend and mean corrected to form three more covariates that modeled linearly changing event-specific EHRs (Paired CS+, Unpaired CS+, and CS−). It should be noted that these regressors were completely orthogonal to each other.

CONTRASTS. Fixed effects analyses were performed using linear contrasts. The following contrasts were employed to generate SPM[t] maps: auditory (effect of unpaired CS+), Unpaired CS+; somatic and motor (effect of US and UR), Paired CS+ > Unpaired CS+; effects of learning (time-dependent effects), Unpaired CS+ > CS−; and effects of prediction error, Unpaired CS+ > Paired CS+.

A threshold of $P < 0.001$ was used to identify significant voxels for all analyses. It was also of interest to report trends that were present in the cerebellum below this threshold, and so activity in this region was reported at $P < 0.05$ and $P < 0.01$ where necessary.

RESULTS

Behavior

The subjects learned to produce well-timed CRs (Fig. 1B). The frequency of CRs given on Unpaired CS+ trials increased markedly with training, reaching an asymptote at the end of training (Fig. 1A). Initially, the frequency of CRs on CS− trials also increased, but as the subjects started to differentiate between CS+ and CS− events, the CR frequency for CS− trials declined to low levels.

There were two factors: CS TYPE (2 levels, Unpaired CS+ and CS−) and BLOCK (11 levels, blocks 1–11). There was a significant effect of BLOCK, indicating that there was an overall block-by-block change in CR frequency [ANOVA, $F(1,4) = 10.52; P < 0.05$]. There was also a significant effect of CS TYPE, indicating that overall, there were significantly more CRs for CS+ than for CS− [ANOVA, $F(1,4) = 159.85; P < 0.05$]. Most importantly, there was a significant

$F(1,4) = 55.76$, $P < 0.0001$. To ensure that between-subjects variation did not contribute significantly to this effect, the slopes were calculated for each subject individually, and a $t$-test was then performed on these values. The results of this analysis show that there was a linear trend in each subject and that the slopes for each subject were not significantly different from each other [1-sample $t$-test, $t(4) = 6.37, P < 0.005$]. The behavioral data confirm that our aims. Our behavioral results are consistent with other studies (Clark and Squire 1998; Moore 1964) and justify the use of linear models to assess the time-effects in the event-related bold signal.

fMRI results

EFFECTS OF AUDITORY CSs. The effects of presentation of the tones alone are shown by the effects for Unpaired CS+. There were robust activations in the primary auditory cortex (Fig. 2A) and auditory association cortex in the temporal lobes (see 1st contrast in Image analysis methods). In addition, activity was also present in the cerebellum bilaterally at the borders of Crus I and lobule HVI ($P < 0.05$; 26, −82, 24 and $24, 66, 22$).

EFFECTS OF US. The effects of presentation of the airpuff are shown by the main effect of Paired CS+ trials compared with Unpaired CS+ trials (2nd contrast). We did not present isolated US trials because this is known to significantly impair conditioning. There was an activation in the right ventral somatosensory cortex ipsilateral to the US (Fig. 2B; 56, 2, 0; $Z = 4.56$). This lay at a dorsoventral level corresponding to the face area of SI; however, we cannot exclude the possibility that the activation lay in the anterior part of SII. There was also a peak in a ventral motor area near the anterior bank of the left central sulcus (−58, 8, 2; $Z = 4.56$) contralateral to the US, which may relate to the execution of UR eyblinks.

TIME-DEPENDENT EFFECTS OF UNPAIRED CS+ COMPARED WITH CS−.

Unpaired CS+ > CS− This analysis looked for voxels at which there was a greater increase over time in event-related
bold activity for unpaired CS+ trials compared with CS− trials. Significant effects are reported in Table 1. There was a learning-related increase to CS+ in an area of the cerebellar cortex ipsilateral to the US, immediately above the horizontal fissure, but well below the primary fissure. The activation overlapped a medial portion of lobule HVI, but the maximally active voxel was located in an adjacent area of Crus I (Fig. 3A). As shown by the plotted best-fitting models (Fig. 3A, inset), hemodynamic responses for the peak voxel typically increased sharply for CS+ events in contrast to CS− events. No other cerebellar voxels were observed when the threshold was lowered to $P < 0.005$, but weaker activity was present in two small clusters in Lobule HVI of the contralateral cerebellar cortex when the threshold was lowered still further to $P < 0.01$ ($−14$, $−62$, $−22$ and $−24$, $−64$, $−26$).

Significant learning-related activity was also present in a ventral area of the precentral gyrus, just in front of the central sulcus (contralateral to the US airpuff; Fig. 3, B and inset). At such ventral levels, the distinction between area 4 (primary motor cortex) and adjacent area 6 (premotor cortex) is not clear and depends on the distribution of Betz cells in the region (see Wise 1985). It is not clear whether the peak lies in motor cortex or the adjacent ventral premotor cortex. However, Penfield and Boldrey (1937) have reported that electrical stimulation of tissue in this region elicited movements of facial musculature, including closure of the eyelids. The learning-related effects in the cerebellum and motor cortex are discussed in the following text.

**CS−r > Unpaired CS+r.** This analysis shows areas in which there was a relative decrease in activity for Unpaired CS+ trials over time. There was a relative decrease in activity in the amygdaloid complex ($38$, $0$, $−30$; $Z = 4.12$).

**EFFECTS OF UNPAIRED CS+ COMPARED WITH PAIRED CS+ (TIME DEPENDENT).** The final comparison reveals the effects of expectancy. As subjects learned, the predictive strength of the CS+ increased, and this prediction was confirmed on presentations of the Paired CS+ trials. However, on each Unpaired CS+ trial, the predicted airpuff US failed to occur as expected. We looked for responses to this error that became stronger with learning. EHRs to Unpaired CS+ compared with Paired CS+ trials increased over time in cerebellar lobule HVI contralateral to the US ($−22$, $−56$, $−22$; $Z = 3.63$; see Fig. 4 and Table 2). There was also an effect at a lower significance level ($Z = 3.07$) on the borders between lobule HVI and Crus I in the ipsilateral cerebellar cortex ($22$, $−78$, $26$). There was also an effect in somatosensory cortex ($64$, $0$, $4$, $Z = 3.63$). It is not clear if this lay within in the face area of SI or an anterior part of SII. Among other areas activated in this comparison were the right lateral orbitofrontal cortex, left frontal pole and the anterior cingulate cortex.

**DISCUSSION**

**Learning-related changes**

Subjects gradually developed well-timed CRs but were unaware that they had developed these adaptive responses to the CS+. The analysis revealing time-dependent changes therefore reflected implicit procedural learning. We looked for the areas involved in learning by comparing Unpaired CS+ trials with CS− trials. For both trial types, there were tones and for neither trial type was an airpuff received; but CRs were increasingly produced on Unpaired CS+ trials. We found learning-related increases in the ipsilateral cerebellum and the contralateral ventral motor/premotor cortex. A weaker effect was also found in the hippocampus.

**CEREBELLUM.** A learning-related change in activity was found in an area spanning medial Crus I and the neighboring lobule HVI of Larsell, ipsilateral to the US. This is consistent with the learning-related cerebellar activations of previous PET studies (Blaxton et al. 1996; Logan and Grafton 1995; Molchan et al. 1994; Schreurs et al. 1997). However, the present study differs from these in two crucial respects. The use of an event-related experimental design has allowed us to interpolate learning CS+ trials with no-learning CS− trials and thus control for nonspecific time effects much more stringently. Second, in the present study learning-related increases were revealed by comparing Unpaired CS+ trials with CS− trials. We have been able to use this event-related analysis to examine activity related to unexpected events (the interpretation of our cerebellar increase is discussed in the following text).

Unilateral lesions in lobule HVI have been reported to temporarily abolish conditioned responses of the nictitating membrane (Yeо et аl. 1985b), and bilateral lesions permanently abolish them (Gruart and Yeо 1995). The effects of isolated lesions of Crus I have not been tested, but it has been reported that combined excitotoxic lesions of Crus I and lobule HVI were more effective than similar lesions of lobule HVI alone (Hardiman and Yeо 1992). In none of these studies did the lesions impair the URs.

Anatomical data show that both areas receive afferent auditory and somatic information (Rosenfield and Moore 1995; Yeо et аl. 1985c). Both Crus I (Dietrichs and Walberg 1980) and lobule HVI (Yeо et аl. 1985c) project to common areas of the cerebellar nuclei and receive projections from a common region of the inferior olive (Kotchabakhdi et аl. 1978). Both of these areas are also essential for eyelid conditioning (Yeо et аl. 1985a, 1986). The motor cortex (Mihailoff et аl. 1985) and the primary and secondary somatosensory cortex project to the cerebellum via the pontine nuclei (Brodal and Steen 1983). Crus I of the cerebellar cortex is responsive not only to somatic facial stimulation in the rat (Chen et аl. 1996) but also shows CR-specific neuronal responses during eyelid conditioning in the rabbit (McCormick and Thompson 1984). Furthermore there are conditioning-specific increases in the expression of protein kinase C in both lobule HVI and Crus I in the rabbit cerebellum (Freeman et аl. 1998).

It has been reported that single cells in the region of the dentate and interpositus nuclei increased in activity during acquisition (McCormick and Thompson 1984) and that multiunit activity in the interpositus nucleus decreased during extinction (Gould and Steinmetz 1996). We failed to detect changes in the bold response in the cerebellar nuclei. It is not clear why this was so, but there are three possibilities. First, in the first study (McCormick and Thompson 1984), the comparison was with a baseline before the presentation of the CS, whereas in the present study there was a sensory baseline. Second, the present experiment used discriminatory conditioning whereas the animal experiments did not. Finally, the bold signal is not as sensitive as recording with an electrode.
What does activity in unpaired CS + trials reflect?

Our principle interest was the activity in Unpaired CS + that changed as a function of learning. The following explanations may account for activity in Unpaired CS + trials. First, it is possible that our predominantly ipsilateral cerebellar activity reflected the motor execution of CRs, irrespective of learning. Second, activity may reflect learning-related changes in excitability to CS + trials. Neurons may become increasingly responsive to presentations of CS + trials (such neurons have been found in the cerebellum and motor cortex in animals and are discussed in CEREBELLUM and MOTOR/PREMOTOR CORTEX). Third, it is possible that the changes in activity in Unpaired CS + trials reflected an error signal. As learning progressed, the subjects came increasingly to predict the US on CS + trials (since subjects increasingly produced CRs on CS + trials). However, on unpaired trials the US failed to occur. Thus with learning there was an increasing mismatch between the predicted occurrence of the US and its failure to occur. These three possibilities are discussed in the following text.

There has been a controversy about whether cerebellar lesions affect learning or simply the performance of learned motor responses (Llinas and Welsh 1993; Welsh and Harvey 1989). To try to resolve this issue, two groups have inactivated the anterior interpositus nucleus in rabbits during acquisition of CRs of the nictitating membrane (Hardiman et al. 1996; Krupa et al. 1993). When the animals were later tested without inactivation, they failed to show evidence of having learned CRs. These studies indicate that cerebellar lesions prevent the acquisition of CRs, irrespective of any effect on CR execution. In human eyeblink conditioning, the CRs are given bilaterally. Others have therefore argued that one would expect cerebellar activity to be bilateral if it reflected the motor execution of CRs (Logan and Grafton 1995). Thus it is more likely that the time-dependent changes in the present study reflect learning effects.

Our effects in the ipsilateral cerebellum and contralateral motor/premotor cortex were found by comparing the changes found during Unpaired CS + trials with changes found during CS − trials. In this comparison, the changes found in Unpaired CS + trials may reflect not only learning, but error signals associated with the failure of the increasingly predicted US. Both were present in Unpaired CS + trials, but neither was present in the CS − trials, and it is possible that one or both of these processes occurred in the ipsilateral cerebellar cortex.

Several groups have suggested that the olivo-cerebellar system is engaged in learning “inverse” (controller) and “forward” (predictive) models. The predictive models are likely to be engaged in detecting discrepancies between predicted and actual sensory feedback arising from movements (Miall 1998; Wolpert et al. 1998). Flamet et al. (1996) reported increases in the bold signal when subjects performed a visuomotor tracking task and the gain was altered, leading to a discrepancy between the predicted and actual movement of the cursor. Blakemore et al. (1999) have used imaging to show that there is a correlation between activity in the cerebellum and the size of the discrepancy between predicted and actual sensory feedback. Is it possible that such processes are present during eyeblink/NM conditioning?

During extinction learning, unpaired CS trials are repeatedly presented after subjects have acquired CRs. As the value of the CS as a predictor of the US declines with each presentation of the unpaired CS, the frequency and amplitude of CRs also declines. Extinction of NM CRs is prevented by reversible inactivations of the cerebellum (Ramnani and Yeo 1996), and sites within the cerebellum that are essential for CR acquisition in NM conditioning are also essential for their extinction.
(Hardiman et al. 1996). Although our experiment did not repeatedly present Unpaired CS+ trials in this way, it could be said that the patterns of activity in Unpaired CS+ trials may reflect the early stages of extinction learning in which there are initially violations of sensory predictions related to the CS and US. Changes in excitability to the CS+ were present in both Unpaired and Paired CS+ trials. However, only in the Unpaired CS+ trials were there error signals associated with the absence of an increasingly predicted US (this prediction was not violated in the Paired CS+ trial type). Comparing the activity in these trials would therefore reveal areas that became increasingly responsive to the absence of an increasingly predicted US, irrespective of excitability changes in to the CS+.

Such activity was found predominantly in the contralateral cerebellar cortex (Lobule HVI), although there was an effect ipsilaterally at a lower significance level. It is of note that there were also activations in somatosensory cortex and visual cortex. This BOLD response could reflect either activation or increased inhibition. However, Raji et al. (1997) have recorded in a sensory area with magnetoencephalography when a signal that is predicted fails to occur. They found that when predicted tones were omitted, there was activity in the supratemporal auditory cortices. We also found activity in the orbital and anterior cingulate cortex; and activity has been reported in the orbitofrontal (Nobre et al. 1999) and medial frontal cortex (Ploghaus et al. 1999) when there are breaches of expectation.

There is strong evidence that the contralateral cerebellar cortex plays an important role in eyelink conditioning. Anatomical evidence shows that the contralateral cerebellar cortex processes CS and US stimuli that support conditioning. Auditory information distributes to the cerebellum bilaterally through the pons from the auditory system and in our experiment, the CS tones were presented binaurally. Auditory information was therefore processed bilaterally. Although it is widely known that US somatic information from the face is conveyed from the contralateral inferior olive to ipsilateral lobule HVI, van Ham and Yeo (1992) have also shown that inputs to face areas of the inferior olive from the trigeminal system are bilateral. There are also direct mossy fiber projections from the trigeminal nuclei to lobule HVI, crus I, and crus II of the cerebellar cortex. Thus it is possible for ipsilateral somatic stimulation of the face to evoke activity in the contralateral cerebellar cortex. Indeed, Miles and Weisendanger (1975a,b) have recorded climbing fiber field potentials in both ipsilateral and contralateral lobule HVI and crus Ia during stimulation of the facial skin. Electrical stimulation of the facial areas of the primary somatosensory cortex also resulted in climbing fiber potentials in the same sites of the cerebellar cortex.

Lesion evidence provides direct and compelling evidence that the contralateral cerebellar cortex participates in eyelink conditioning. Animals with ipsilateral cerebellar lesions were initially impaired but reacquired CRs after extended retraining, but bilateral cerebellar lesions were effective in permanently abolishing CRs trained ipsilaterally (Yeo et al. 1997). This suggests that the contralateral cerebellum plays an important role in conditioning. To test this specifically, Ivarsson et al. (1997) unilaterally and reversibly blocked the outflow from the cerebellar cortex by micro-injections of lignocaine into the brachium conjunctivum. Both the ipsilateral and contralateral eyelink CRs were abolished. In summary, the contralateral cerebellar cortex controls ipsilateral and contralateral conditioned eyelink and NM responses.

Finally, other groups who have studied eyelink conditioning using PET functional imaging have also reported the involvement of the contralateral cerebellum in human eyelink conditioning (Blaxton et al. 1996; Logan and Grafton 1995). To this extent, their results are comparable with ours. As in our own study, Logan and Grafton (1995) reported strong contralateral activity in the cerebellar cortex during human eyelink conditioning. Neuroimaging data therefore also suggest that the contralateral cerebellar cortex plays an important role in human eyelink conditioning.

Conclusion

The results present a simple picture. We have shown learning-related changes in two areas (cerebellar Crus I/lobule HVI and ventral motor/premotor cortex) that have been shown in animal studies to be essential for conditioning of the external eyelid. These may reflect learning-related changes in neuronal excitability that may be essential for eyelink conditioning specifically and for motor learning more generally. We also found a learning related decrease in activity in the amygdaloid complex. In an event-related fMRI study with the same design (Buechel et al. 1998), there was also a decrease in activity in the amygdala during fear conditioning (unpaired CS+ trials). In that study, the US was a loud noise, whereas in our study the US was a mildly aversive airpuff.

Our event-related design also enabled us to look for changes in unpaired CS+ trials that could be related to the mismatch between sensory prediction and actual outcomes. The only difference between these trials and the comparison paired CS+ trials lay in violations of the sensory predictions on the unpaired trials. There was an effect in contralateral cerebellar lobule HVI for this comparison. This encourages the view that this change in activity may reflect the operation of the cerebellum in predicting sensory events.

We thank the referees for helpful comments. We also thank Drs. J. W. Moore, C. Evinger, and B. Scheurs for advice on behavioral methods and Prof. Karl Friston for comments on earlier versions of the manuscript. This work was supported by the Wellcome Trust.

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