Cerebellum and conditioned reflexes

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The central assumption of existing models of motor learning in the cerebellum is that cerebellar mossy fibres signal information about the context in which a movement is to be performed and climbing fibres signal in relation to a movement error. This leads to changes in the responsiveness of Purkinje cells, which on the next occasion will generate a corrected output in a given context. Support for this view has come mainly from work on adaptation of the vestibulo–ocular reflex. The discovery that classically conditioned eyeblink responses depend critically on the cerebellum offers the possibility to study the learning of a novel behaviour, rather than modification of an existing reflex. After repeated pairing of a neutral stimulus, such as a tone, with a blink-eliciting stimulus, the tone will acquire the ability to elicit a blink on its own. We review evidence from studies employing a wide variety of techniques that the cerebellum is critical in this type of learning as well as evidence that mossy and climbing fibres have roles assigned to them in cerebellar learning models.

Since the earliest studies by Flourens[1] and Luciani[2], it has become clear that cerebellar lesions disturb not only voluntary movements but also postural, proprioceptive, cutaneomuscular and ocular reflexes[3,4]. Although the cerebellum receives and organizes an extensive range of afferent inputs and distributes its outputs selectively to different efferent targets, the anatomical and physiological similarity of every cerebellar cortical region indicates a consistent type of information processing across the cerebellum. Any useful description of cerebellar function will embrace this common processing principle and explain the deficits in both voluntary and reflex movements seen after cerebellar lesions.

Many models suggest that this common cerebellar processing principle involves a mechanism for motor learning[5–9] and they all share one central proposition. The mossy fibre inputs to the Purkinje cell convey information about the context within which a movement is made and the climbing fibre input to the Purkinje cell instructs a change in efficacy of currently active, context-encoding parallel fibre/Purkinje cell synapses. So, when the context occurs again, the changed synaptic strengths alter the probability of firing of the Purkinje cell such that a correct movement is made. Marr's original theory[5] described how new voluntary movements might be controlled initially by the cerebral cortex which, at the same time, instructed olivary activity. So, with repeated practice, the new movement in its correct context would be learned by the cerebellum. Theories by Albus[6] and Ito[7] later freed the olive from a dependence upon cerebral cortical instruction and suggested that the olive coded an error signal which allowed the cerebellum to learn independently and thus participate both in the learning of voluntary movements and also in the calibration and maintenance of existing reflexes. There have been many adaptations of, and extensions to, these early models (see Houk *et al.*[10], Smith[11], Thach[12] for recent reviews).

Conjunctive activation of parallel fibre and climbing fibre inputs produces a long-lasting form of synaptic plasticity, the long-term depression (LTD) of parallel fibre to Purkinje-cell synapses that might be the mechanism underlying cerebellar learning[13]. However, it has proven difficult to establish a causal relationship between an identified cerebellar plasticity and the appearance of a specific learned movement. Initial single unit recording studies were encouraging. Gilbert and Thach[14] recorded Purkinje-cell activity consistent with Gilbert's models[8,9] by analysing complex spike activity (driven by the climbing fibre input) and simple spike activity (driven by the parallel fibre inputs) while learning a simple, wrist position compensation task. Neural mechanisms supporting the learning of more complex, multijoint movements are more difficult to analyse because the operation of limb premotor networks needs to be considered[10,15].

An alternative approach has been to examine the adaptation of simpler reflexes. Several studies of the role of the cerebellar flocculus in the modification of the vestibulo–ocular reflex (VOR) support the learning hypothesis (see Ito, pp. 313–321, this issue, and Ref. 7). A new contribution to the cerebellar learning debate was the important finding by Richard Thompson and his colleagues[16] that classical conditioning of the eyeblink/nictitating membrane response (NMR) of the rabbit – associative learning of a simple motor response – is critically dependent upon the cerebellum. This robust model learning system had previously been well-characterized behaviourally (see Box 1). It is less complex than some voluntary movement modification

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Box 1. Classical conditioning of the eyeblink/nictitating membrane response

Towards the end of the last century, Pavlov[a] introduced conditioning of motor and alimentary responses for analysing the neural basis of learning. The technique for Pavlovian, or classical conditioning is simple but powerful. A behaviourally neutral stimulus (the conditioned stimulus, CS) (see Fig.) is paired with another stimulus (the unconditioned stimulus, US) which reliably and unconditionally elicits a particular reflex response (the unconditioned response, UR). After a sufficient number of these stimulus pairings, the previously neutral CS now elicits a response (the conditioned response, CR). Procedurally, we may think of this as the simplest form of associative learning. Unlike more complex forms of learning, classical conditioning involves neural events which can be analysed in relation to external stimuli under stringent experimental control.

The earliest conditioning studies were of salivation but skeletal muscle responses such as leg flexion can also be conditioned. Much contemporary work on the neural mechanisms of conditioning has used the eyeblink and the nictitating membrane response – defensive reflexes elicited by tactile stimulation of areas of the face around the eye[b].

In rabbits, corneal or periocular stimulation elicits closure of the external eyelids – the eyeblink response – and a protective horizontal sweep of the third eyelid across the cornea – the nictitating membrane response (NMR). The complete eyeblink/NM response might be conditioned to an auditory, visual or somatosensory CS by pairing one of them with an unconditioned stimulus US such as an air puff to the cornea which reliably elicits an unconditioned reflex UR. After sufficient pairings, the visual or auditory CS comes to elicit a conditioned reflex (CR) blink. Because it has low levels of spontaneous activity and displays very little non-associative conditioning, the NMR has been considered to have particular advantages for analysis of the mechanisms underlying conditioning[c].

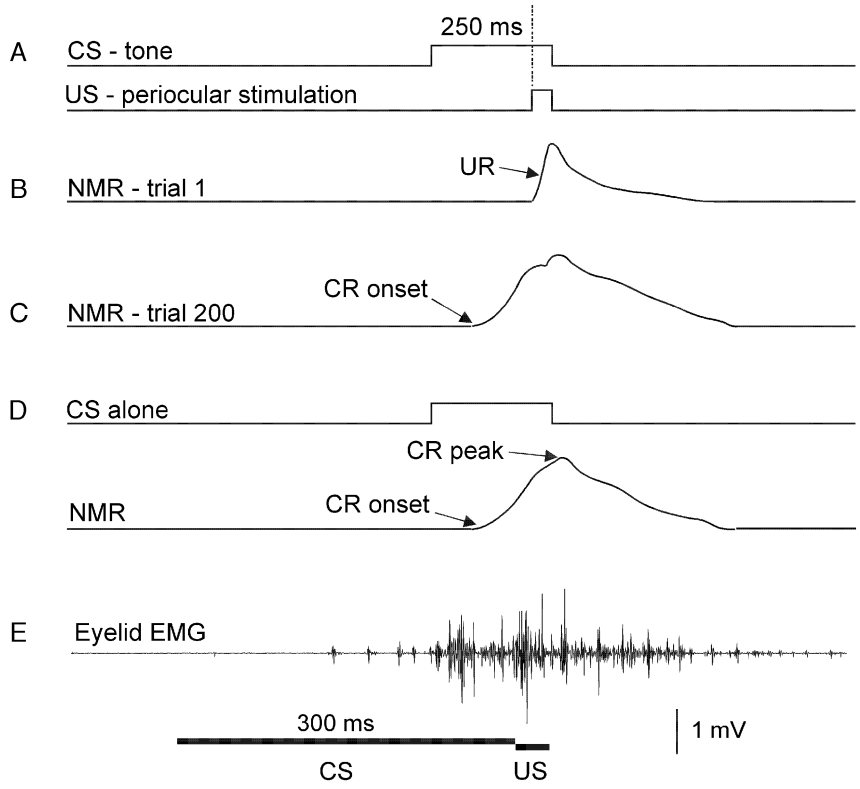

Fig. In a delay conditioning experiment, the onset of the US is after the onset of the CS and the timing of the CR develops accordingly. For NMR conditioning, this CS–US interval can be within the range 100–1000 ms. In this example **(A)** the interval is 250 ms. On the first trial, only the UR is seen **(B)** but after about 200 paired trials CRs have developed **(C)** and they are accurately timed so that their peak amplitude is reached as the US is delivered. The accurate timing of the CR is best seen in occasional presentations of the CS alone **(D)**. The timing has to be learned, since it is dependent upon the chosen CS–US interval and it indicates the skilled, adaptive quality even of this simple learned response. Eyeblink responses accompany the NMR in rabbits and they too become conditioned. Eyeblink responses have been conditioned in humans[d] and several other species. Because delay conditioning is not dependent upon forebrain mechanisms and survives precollicular decerebration[e], it has been studied in animal decerebrate models[f]. In example **(E)**, an electromyogram (EMG) from the upper eyelid of a decerebrate ferret shows a conditioned response produced by pairing a forelimb stimulation CS with a periocular stimulation US. The CS–US interval is 300 ms.

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paradigms and yet offers the possibility to study the role of the cerebellum in the learning of a new movement rather than the modification of an existing reflex.

Cerebellar lesions and eyeblink: NMR conditioning

In the original study[16], a unilateral lesion of the cerebellum abolished nictitating membrane (NM) conditioned responses (CRs) ipsilateral to the lesion and prevented their reacquisition. CRs developed normally contralateral to the lesion and the unconditioned reflex blink was intact. Subsequent studies refined and extended this observation culminating in a clear agreement that lesions of the anterior interpositus nucleus (AIP) are sufficient to abolish CRs but lesions of the fastigial, posterior interpositus or lateral dentate nuclei are not[17]. These studies have been reviewed extensively elsewhere[18–20].

It seemed likely that there might be areas critical for NMR conditioning within cerebellar cortical regions supplying the AIP[17,21]. Consistent with this proposal, unilateral lesions of lobulus simplex, or lobule HVI of Larsell[22], abolished ipsilateral CRs and prevented their reacquisition. Lesions of other cerebellar cortical areas had few effects upon CRs (Refs 23–26). Anatomical tracing of the connections of lobule HVI revealed a major output to AIP, climbing fibre inputs from the face representation area of the dorsal accessory olive (DAO) and rich projections from the

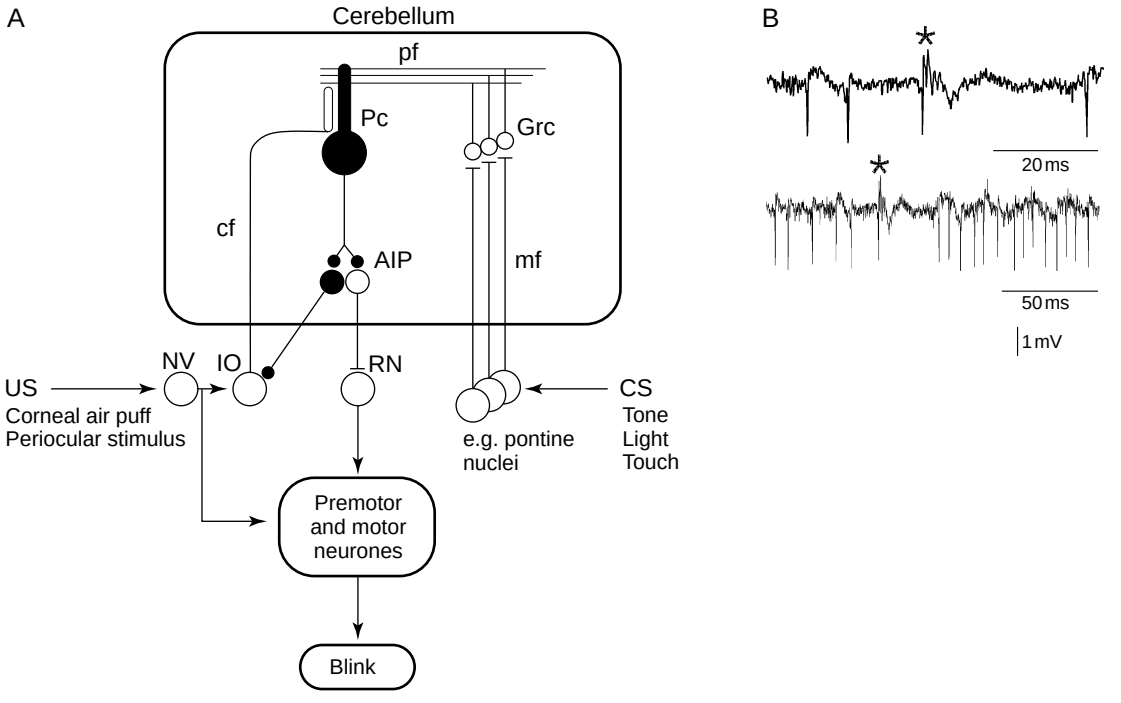

Fig. 1 The cerebellar cortical conditioning (CCC) model. (A) A simplified view of the crucial elements in the cerebellar cortical conditioning (CCC) model (many elements such as interneurones and collaterals of cerebellar afferents are omitted for clarity). The unconditioned reflex pathway is from the periorbital area or the cornea via the trigeminal nucleus (NV) to the premotor and motor neurones producing the blink[35,36].The US pathway is from the trigeminal nucleus (NV) to the inferior olive (IO) and via climbing fibres (cf) to the Purkinje cells (Pc) of eyeblink microzones of the cerebellar cortex. The CS can be one of a variety of sensory stimuli (tone, light, touch etc.). CS information is transmitted through mossy fibres (mf) from an appropriate precerebellar nucleus (e.g. pontine nuclei for a tone CS) to granule cells (Grc) and then through parallel fibres (pf) to the Purkinje cells. The inhibitory output from the Purkinje cells is via the anterior interpositus nucleus (AIP). From there, excitatory drive via the red nucleus (RN) to premotor elements and then motor neurones in the accessory abducens and the facial nuclei produce the nictitating membrane and eylid responses respectively. The AIP also sends an inhibitory projection to the IO. The parallel fibre and climbing fibre inputs to the Purkinje cells have quite different characteristics[37]. Each Purkinje cell receives as many as 250,000 (in man) synaptic contacts from the parallel fibres and ascending components of the granule cells. These inputs elicit normal action potentials, 'simple spikes', with frequencies that vary widely (commonly with the range 20–50 Hz). Climbing fibres, which normally fire at rates of 1–2 Hz, elicit 'complex spikes'. The CCC model asserts that the climbing fibre input changes the parallel fibre to Purkinje cell synapses. (**B**) Extracellular recordings (using different sweep speeds) of a Purkinje cell in a decerebrate ferret showing spontaneous simple spikes and a complex spike (*).

auditory and visual areas in the lateral and dorsolateral pontine nuclei[21]. This convergence of information permitted a simple implementation of cerebellar cortical learning based on earlier models[5–9]. We suggested that information related to an auditory or visual CS might be transmitted via pontine mossy fibres to parallel fibres synapses on lobule HVI Purkinje cells which can be modified by US-related instructions from climbing fibre inputs from the DAO[21]. This cerebellar cortical conditioning model (CCC model) derived from our HVI lesion and anatomical tracing studies and it forms the basis of many subsequent theories of cerebellar function in NMR conditioning (see Fig. 1).

Initially, others found little to support this learning model. Most studies found either no loss of CRs following extensive cortical lesions[30] or transitory deficits which recovered with further conditioning trials[31] especially if the subjects were highly overtrained before the lesion[32]. However, resistance to cortical damage could not relate only to levels of learning before the lesion since even acquisition from the naive state was demonstrated in rabbits with cortical lesions[33]. But a consistent feature of these studies was that CRs which developed or recovered after cortical lesions were of small amplitude and unusual topography.

Interpretation of cerebellar lesion effects and the performance hypothesis

The effects of cerebellar lesions upon conditioned eyeblink/NM responses can be summarized simply. Lesions of the AIP produce severe and sustained losses of CRs whereas lesions of the cerebellar cortex have produced effects ranging from abolition to mild impairments. How are these effects to be interpreted and why do the cortical lesion effects appear to vary so widely?

The simplest interpretation of the cerebellar lesion studies is that the neural circuitry supporting plasticity essential for conditioning is damaged and so the CCC model is supported. But an important alternative is that such lesions produce performance, rather than learning, deficits. This argument is consistent with the finding that lesions of the AIP mildly impaired UR rise-times to weak air puff stimuli[34]. Since the CR and UR share a common motor pathway, and AIP outputs are excitatory upon it, perhaps AIP lesions depress its excitability. The ability to express URs is impaired and the ability to express CRs (which might be inherently weaker responses) is lost completely. So the learning itself might be normal. This is a powerful argument that raised doubts about the CCC model and

provoked several more analyses of AIP lesion and inactivation effects upon the UR which variously supported or contradicted the original observation[35,36]. However, as the cerebellum, via the red nucleus efferent pathway, influences motor activity *and* regulates sensory transmission within the trigeminal system[37], deficits in the UR following AIP lesion could derive from a depression of excitability in the afferent limb of the reflex and need not indicate performance deficits of the CR at all. So-called performance deficits in the UR neither confirm nor invalidate cerebellar learning.

However, the arguments underlying the performance deficit hypothesis for AIP lesions are also valuable in analysis of the effects of other lesions. AIP lesions might damage learning and response performance, consistent with their devastating effects upon the CR. But the effects of cortical lesions should be quite different, since Purkinje cells are inhibitory upon their cerebellar nuclear targets and so cortical lesions might be expected to raise excitability in the eyeblink/NMR pathways by disinhibition of the AIP. We found this to be the case. Lesions of HVI increased UR amplitudes across a wide range of US intensities yet they abolished or impaired CR amplitudes[25,38]. Provided that these lesions do not exclusively alter excitability in the afferent limb of the UR, the dissociation of effects upon the CR and UR is entirely consistent with the CCC model.

The range of CR impairments seen after cerebellar cortical lesions depends largely upon the extents and locations of the lesions. Complete abolition of CRs would not be expected unless all of the eyeblink controlling areas were removed and whether this has been achieved is unknown because these areas have not been fully mapped in rabbits, as they have in cats (see Box 2). In some studies[39,40], the comparison of cortical lesion effects is made more difficult by inconsistent identification of lobules HVI (lobulus simplex) and HVII (within ansiform lobe) of Larsell[22]. An additional problem is that the reflex enhancing properties of cerebellar cortical lesions also contribute to the variable effects upon the CR. Unless the lesions affected all of the eyeblink controlling areas, the contribution of any residual areas to CR production would be greatly enhanced by the hyperexcitable reflex pathways.

Overtrained subjects can show recovery of CRs after unilateral cortical lesions[32] but even this additional plasticity is not necessarily outside the cerebellum because there is a small but significant contribution to conditioning from the contralateral cortex[38]. Bilateral lesions produced greater impairments than unilateral lesions and the CR frequency did not increase with further conditioning, in good agreement with physiological evidence that eyeblink responses are controlled by both sides of the cerebellar cortex[41,42]. The low level of residual CRs that survived bilateral cortical lesions might have been mediated by extracerebellar mechanisms but their timing was erratic and no longer adapted to the CS–US interval. This critical, learned feature of the CR was lost completely after cerebellar lesions.

Recording studies

Single and multiunit recordings have been made from many CNS structures during conditioning. In evaluating such evidence it must be recognized that correlation does not prove causation. For example, it is reported that unit activity in the hippocampus 'models' the CR but delay conditioning can proceed normally without the hippocampus. One way to address the correlation/causation problem is to look at lead times. If, for instance, activity in the AIP precedes the CR, it cannot be a consequence of it. However, this can be misleading, because activity which precedes the movement might not precede the command signal in the premotor neurones, which then sends feedback information to the cerebellum. Furthermore, several different responses, each with different onset latencies, can occur during conditioning. For instance, the animal might develop orientating reactions too weak to result in overt movements but, nonetheless, with neural activity in premotor elements. Hence, activity in the AIP which precedes the eyeblink could relate to feedback from another response learned at the same time.

Relationships between learned behaviour and neural activity can often be seen in multiple brain locations. For instance, correlates of the CR have been recorded in the AIP and also in the red nucleus and the facial nucleus – consistent with the view that they form parts of the efferent pathway for conditioned eyeblinks (see Fig. 1). But CR-related activity has also been observed in the pontine nuclei[43], which relay CS-related information to the cerebellum, and in the trigeminal complex[44], which relays US-related information to the cerebellum. It is possible to identify which of these activities is a primary correlate of the behaviour by blocking activity in one location while recording activity in another. For example, reversible inactivation of the AIP by cooling abolishes the CR-related activity in the pontine nuclei, but not the short-latency tone-evoked activity which is also present[43]. AIP cooling also abolishes CR-related activity, but not US-related activity in the trigeminal complex[44]. These findings indicate that the primary source of CR-related activity is within the cerebellum and they support the CCC model.

Another important concern with recording studies is the identification of the recorded cells. Four areas of the cerebellar cortex which are likely to control eyeblink have been identified in the cat, two of which are in lobule VI (see Box 2). These areas constitute only a small fraction of HVI cortex. Clearly, the probability of finding CCC model-related Purkinje cells when sampling the whole of lobule HVI is quite small. Even those cells which respond with complex spikes to the US are not necessarily related to eyeblink. Some Purkinje cells in the C2 zone, for instance, respond with complex spikes to facial stimulation, but they also respond to bilateral somatosensory stimulation of virtually the whole body, and also to auditory stimulation, so they are unlikely to be involved specifically in eyeblink conditioning (see Box 2).

Because parallel fibre and climbing fibre activations of Purkinje cells can be inferred from simple and complex spike recordings (see Fig. 1) we review two studies of NMR/eyeblink conditioning that have reported simple and complex spike activities in detail. The CCC model predicts that, with conditioning, CS-related simple spike firing of Purkinje cells should decrease. In the first study by Berthier and Moore[45], some Purkinje cells increased and some

Box 2. Eyeblink control areas of the cerebellar cortex

Running orthogonally through the anatomically distinct, transverse lobules of the cerebellar cortex is a series of longitudinal zones with highly specific input and output connections[a,b]. The zones have been studied in greatest detail in lobules IV and V of the anterior lobe of the cat – lobules that are particularly important for hindlimb and forelimb control (see Fig. A). Each zone receives climbing fibres from a distinct part of the olive and projects to a specific target cerebellar nucleus. For instance, one cell group in the dorsal accessory olive projects to the lateral C3 and the Y zones, another group to medial C3 and C1 and all three cortical zones project to the anterior interpositus nucleus. In contrast, the C2 zone receives its climbing fibre input from the medial accessory olive and projects to the posterior interpositus nucleus. Each zone may be subdivided further into small sagittal strips, called microzones. Each microzone controls a single muscle or muscle group and its olivary input has a specific somatosensory receptive field. The principles of this arrangement are illustrated in the schematic diagram of the C3 zone through lobules IV and V (Fig. B). It can be seen to contain orderly sets of microzones that control muscle groups within lower and upper body regions.

The muscles of the face and neck are mainly controlled from microzones in HVI and HVII (the hemispheral parts of lobules VI and VII of the posterior lobe). Although most face microzones have not been mapped in detail, eyeblink control areas in cats have been fully mapped and characterized using two defining characteristics. Firstly, eyeblink microzones have climbing fibre inputs that can be activated by periocular stimulation (see Fig. C) and, secondly, electrical stimulation of these areas of the cerebellar cortex causes a localized inhibition of eyeblink-controlling neurones in the AIP followed by a rebound excitation and indirectly elicits a long-latency blink[c] (see Fig. D). An additional finding is that stimulation of these eyeblink control areas also completely suppresses an ongoing conditioned eyeblink response[d] (see Fig. E). The locations of the cat eyeblink control areas are shown in Fig. F. Of these, the medial area in lobule HVI and the paramedian area are in the C3 zone. The area in lobule HVII is within the C1 zone but the zonal properties of the lateral area in HVI have yet to be clarified. It might be in a lateral part of C3 or the Y zone.

There are other areas receiving climbing fibre input activated by periocular stimulation in the B and C2 zones but they are unlikely to be involved in eyeblink control. The B zone projects to the lateral vestibular nucleus. The cells in the C2 zone which receive climbing fibre input from the face also receive bilateral input from hindlimbs and forelimbs as well as auditory input. Furthermore, electrical stimulation of these areas does not produce eyeblink responses. So, when interpreting electrophysiological recording studies of eyeblink/NMR conditioning, it must be borne in mind that climbing fibre responses activated by the US are not sufficient to identify those Purkinje cells which control eyeblinks and which are, therefore, directly implicated in the CCC model.

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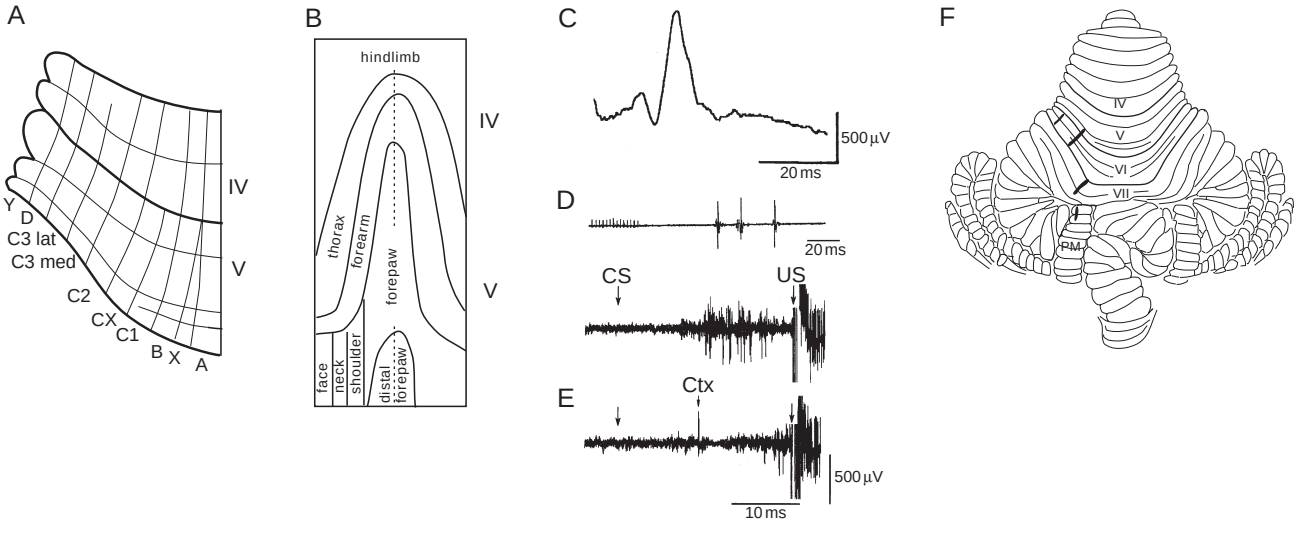

Fig. Cerebellar cortical zones. (A) Zones in lobules IV and V of the cat cerebellar cortex as defined by their different olivary inputs. Zone A is medial and zone Y is lateral. **(B)** Detailed somatotopical organization of climbing fibre input to the C3 zone in lobules IV and V of the cat. The lateral and medial parts (which have different climbing fibre input) are separated by a dashed line. Only a very small part of lobule V contains a face representation. **(C)** Surface recording from lobule VI of the C3 zone of cat. Averaged field potential showing characteristic mossy (first positive potential) and climbing fibre (second positive potential) responses to periocular stimulation. **(D)** EMG recording from a cat upper eyelid shows a long-latency blink response following a 40 ms train of stimuli to the site in cerebellar cortex from which the record in **(C)** was obtained. **(E)** Superimposed EMG records from the eyelid of a cat showing a conditioned response to a forelimb CS (upper trace). Lower trace shows the suppression of the CR by a brief electrical stimulus to the cerebellar cortex (Ctx) in the C3 eyeblink control area. **(F)** Diagram of the cerebellum of the cat with the cerebellar surface folded out in one plane, after Larsell[e]. Three eyeblink control areas, indicated in black, were found in the C1, C3 and Y zones of lobule HVI and HVII and one in the C3 zone in the paramedian lobe[c]. These areas in the cat all receive their climbing fibre input from the DAO and project to the AIP, in good agreement with the findings in our lesion and anatomy studies in rabbits.

decreased their simple spike firing rates in advance of the CR. Many of these neurones responded with complex spikes to the US and some also to the CS. Most of the recording sites were within HVI though it is uncertain from which zone these units were sampled. An interesting aspect of these recordings is that the subjects were conditioned to discriminate two different tone CSs, a CS^+ which was reinforced by US presentations and a CS^- which was not.

Sometimes, a CR was emitted, erroneously, on a CS^- trial and some of the cells increased or decreased their firing with the CR on these CS^- error trials. This is a critical finding because it confirms that some Purkinje cells have simple spike activity correlated specifically with the CR.

More recently, Hesslow and Ivarsson[46] recorded from Purkinje cells in an area of the C3 zone identified as controlling conditioned eyeblink responses in decerebrate ferrets (see Box 2). In untrained animals, the spontaneous activity of these cells was either completely unchanged, or they responded with weak (10%) increases or decreases in simple spike firing, during the CS–US period. After training most of the cells were unchanged, but up to 20% of the Purkinje cells responded with a very strong, often complete, suppression of simple spike firing during the later parts of the CS–US period. This suppression was sometimes preceded by a brief excitation. The latency of the suppression varied between 50 and 200 ms, which should be compared with 125–250 ms onset latencies of the CR. In contrast to the report by Berthier and Moore[45], no cells in these C3 eyeblink areas increased their firing towards the end of the CS–US period. When subjects were shifted from unpaired (CS only) to paired (CS–US) stimulation, simple spike firing decreases were apparent over 40 trials, in good correlation with typical CR development and suggesting that the simple spike suppression was a result of learning.

The data from both of these recording studies clearly demonstrate that there are learning-related changes in Purkinje cell simple spike activity and, together with the serial cooling studies outlined above, they are consistent with the CCC model. But they do not exclude that such activity reflects learning elsewhere, especially at early levels of CS processing. Additional experimental approaches have been used to analyse these possibilities.

Stimulation studies

A direct test of the CCC model is to replace the peripheral CS and US with direct stimulation of the putative central CS and US pathways, that is the mossy fibres and the climbing fibres. Such stimulus substitution experiments are logically attractive but they have severe methodological problems. It has been reported that electrical stimulation of the pontine nuclei as the CS can produce rapid conditioning[47,48]. However, stimulation in the brainstem ortho- and antidromically activates passing fibres and their collaterals that project to a variety of CNS targets, including the cerebral cortex, any of which could provide CS activation. Stimulation of the mossy fibres in the middle cerebellar peduncle (MCP) where it enters the cerebellum avoids fibres of passage[49] but still does not exclude antidromic activation of the pontine nuclei and their collaterals, so such studies are of limited value in testing the CCC model. AIP lesions abolish CRs to MCP stimulation CS (Ref. 47), a finding consistent with the CCC model but also with extracerebellar learning. Because the AIP lesion might have produced impairments of the CR through performance deficits similar to those discussed earlier, activation of other areas that might contribute to learning cannot be ruled out.

There are similar problems with inferior olive stimulation. Rabbits learned when the US was replaced by direct stimulation of the inferior olive[48,50] but this stimulation appears to have antidromically activated the trigeminal nucleus, because it caused eyeblinks, and so it might not differ crucially from a peripherally applied US.

It will be important to see whether the power of the stimulus substitution approach could be increased by combining with it the elegant inactivation techniques, described elsewhere in this review, to limit unwanted antidromic and orthodromic activation effects.

Role of inferior olive in conditioning

The CCC model of NMR conditioning gives the inferior olive/climbing fibre a reinforcing role by signalling the US and modifying CS-related parallel fibre synaptic efficacy. This proposal is one of the most clearly defined components of the model and testing it should provide the best evidence for, or against, the model. It might be thought that central interruption of the putative US pathway by an olive lesion would be equivalent to turning off the US peripherally, which would lead to behavioural extinction during continued paired presentations of the CS and US. However, olivary lesions are known to cause a rise in tonic firing of Purkinje cells and a virtual shutdown of cerebellar output equivalent to cerebellar inactivation[51,52]. One study using olivary lesions found extinction[53] but we found an immediate abolition of CRs[54]. Although the extinction result appears to confirm the CCC model, our findings are consistent with the physiology. The reasons for the difference in findings has not been resolved[55].

Another test of the role of the olive in conditioning could be based on the projection from the AIP to the dorsal accessory olive which is now known to be inhibitory[56–58]. We have proposed a negative feedback function for this pathway, such that when a CR of sufficient amplitude is generated in the AIP, olivary activity, and hence further learning, would be turned off. Hesslow and Ivarsson[59] found a strong negative correlation between the size of the CR and the size of a subsequent climbing fibre response to the ensuing US in the C3 zone. This olivary regulation could explain Kamin's blocking phenomenon[60] in classical conditioning. If a subject is first conditioned to CS A and then further conditioned to the compound CS A + B, then no conditioning will develop to the B component of the compond CS. It had been suggested that the AIP, when producing a response to A, also suppresses the olive so that no further climbing fibre response will be available to reinforce B during the compound conditioning[58]. A recent study using pharmacological blocking of the nucleo-olivary pathway[61] is consistent with this proposal.

Suppression of the DAO by nucleo-olivary inhibition during conditioning fits the CCC model very closely but does not rule out the possibility that the reinforcing mechanism in the cerebellum is unrelated to the climbing fibre input and that the observed blocking effects occur in parallel.

Reversible cerebellar inactivations and NMR conditioning

Permanent lesion studies alone cannot resolve whether the cerebellum actively contributes to the acquisition and storage of motor memories or whether the cerebellum only

regulates the performance of movements learned in other neural circuitry. Recent studies have addressed this problem using reversible lesion techniques. Conditioning trials can be given during a localized, functional inactivation of the cerebellum. If, after the inactivation is lifted, conditioned responses are present then they must have been formed during the inactivation and it can be concluded that the cerebellum is not essential for acquisition of the learning. If conditioned responses are not evident after the inactivation then the cerebellum is implicated in the acquisition of conditioning – there must be essential plasticity within the cerebellum itself or in circuitry supplied by cerebellar output.

Studies to date have employed localized inactivation of the AIP (Refs 62–67) which we presume disturbs function within all affected olivo-cortico-nuclear compartments and is thus a test of general cerebellar involvement[65,66]. The first study inactivated AIP with lidocaine and found that learning to a tone CS was not impaired if the subjects had previously learned to a light CS, apparently indicating that the cerebellum is not essential for conditioning. However, we have shown how this apparent learning might have been produced by general transfer effects during incomplete inactivation of the AIP[67]. All subsequent studies have shown that *de novo* conditioning to a tone CS is prevented using cold block, lidocaine or muscimol infusion techniques to inactivate AIP (Refs 63–67).

However, there are two reasons why failure to produce CRs after conditioning during AIP inactivation might not indicate a failure to condition. First, there might be a performance problem induced by long-term drug effects carrying over to the post-drug testing phase of the experiments. We tested this possibility and found that, in addition to acquisition learning, extinction learning is also completely prevented by AIP inactivation[65,66]. Because a failure to extinguish during the drug condition was indicated by high levels of CRs in the post-drug phase, we could be sure that there were no simple performance deficits after the inactivation either during acquisition or extinction learning. Secondly, there could be general, state-dependent learning (SDL) effects induced by diffusion of low levels of the drug into other brain areas directly or via the circulation. Hence, during the post-drug testing phase, the sensory properties of the CS and US will be different and, even if the subjects had learned during the drug state, no CRs would be produced. SDL effects were tested using drug infusions in a variety of sites around the AIP that would have allowed similar diffusions out of the cerebellum. Such infusions did not prevent conditioning, so SDL effects can be ruled out[65,67].

The AIP inactivation studies unequivocally provide the most powerful evidence that the cerebellum is essential for eyeblink/NMR conditioning. But they do not, in themselves, indicate that the critical plasticity is within the cerebellum. This final piece of evidence derives from the recent work by Krupa and Thompson[68] who showed that reversible inactivation with tetrodotoxin of cerebellar efferents in the brachium conjunctivum did *not* prevent NMR conditioning. If inactivation of the cerebellum prevents learning but inactivation of cerebellar efferents does not, we have the best evidence that the essential neural mechanisms of eyeblink/NMR conditioning are within the cerebellum. However, not all of the deep nuclear efferents run forward through the more rostral parts of the brachium conjunctivum. Some turn ventrally to brainstem targets. If any such efferents escaped inactivation, it will be important to determine whether there is important plasticity in CS and US processing circuitry afferent to the cerebellum but regulated by ventrally directed AIP efferents.

Conclusions

A wealth of lesion, inactivation, electrophysiological and stimulation studies provides evidence consistent with a cerebellar cortical learning model of classical conditioning but only a few of them support this model with the exclusion of other possibilities. Taken overall, reversible inactivation studies provide the best support for the critical dependence of this simple form of associative learning upon the cerebellum but the underlying mechanisms have not been identified. In our view the CCC model is the best candidate for testing because it contains clear predictions of the roles of the parallel and climbing fibres but the role of the inferior olive and its climbing fibre input to the cerebellar cortex remains the least understood aspect of the model. At present, there is no conclusive evidence that the climbing fibres provide the predicted reinforcing input via a conjunctive, LTD mechanism in NMR conditioning. Use-dependent, long-term potentiation (LTP)[69] and LTD[70] of parallel fibre to Purkinje cell synapses have both been demonstrated *in vitro* and neither require a reinforcing climbing fibre input. Either or both of these processes might contribute importantly to behavioural learning. The basic CCC model provides few insights into the timing of CRs, which are a crucial feature of this and other types of motor learning but there are useful extensions of the CCC model which address the timing of CRs in great detail[71,72]. We suggest that in order to evaluate these models, it is now of crucial

Outstanding questions

- The cerebellum is crucial for normal eyeblink conditioning but what is the underlying mechanism? There is clear evidence that CS information is transmitted by the mossy fibres, and US information by the climbing fibres, to the cerebellar cortex. But there is no direct evidence that these two inputs interact to produce conditioned responses. In particular, evidence is weak that the climbing fibres from the inferior olive are exclusively reinforcing.
- What is the nature of the interaction between the cerebellar cortex, the deep cerebellar nuclei and the brainstem reflex circuitry during learning? Are there synaptic changes at several levels?
- There are four or more microzones which control conditioned responses. Do they all contribute to conditioning? Are different aspects of the conditioned response controlled by different microzones? Are acquisition and extinction of conditioned responses controlled by plasticity at the same neurons?
- How general is the cerebellar conditioning mechanism? It has been investigated only for eyeblink but it is highly likely that it will turn out to be similar for other types of motor responses. The role of the cerebellum in conditioning of emotional, autonomic and other types of behaviour is much less clear.
- What are the synaptic mechanisms underlying conditioning? Cerebellar LTD and LTP are established phenomena that have been mainly studied *in vitro*. It is not yet clear how they could contribute to the temporal shaping of conditioned responses.

importance to understand the role of the climbing fibre input in NMR/eyeblink conditioning and whether it serves as a reinforcing input. More detailed electrophysiological studies of identified, eyeblink-control Purkinje cells are now needed.

This review has focused upon the most basic aspects of learning of a simple motor response but the same cerebellar mechanisms could, of course, support other forms of conditioning too. Indeed, there is good evidence that the cerebellum is also critically involved in heart rate conditioning – the adaptation of an autonomic response[73,74]. There is a highly controversial but strong scientific tradition with an extensive literature that treats cognitive functions as mainly covert behaviour controlled by essentially the same mechanisms as overt behaviour. This tradition (reviewed by Donahoe and Palmer[76]) tries to explain virtually all cognitive functions in terms of one basic conditioning mechanism in the cerebral cortex. While we do not endorse all of the implications of this view, we should point out that the sensory cortex provides rich sets of inputs to the cerebellum via pontine mossy fibres and the cerebellum projects back, not only to the primary motor cortex, but also to the prefrontal cortex (see Middleton and Strick, pp. 348–354, this issue). Through these connections the cerebral cortex could utilize the associative mechanisms of the cerebellum for processing that has no immediate motor outcome. This could enable the cerebellum to assist the forebrain in motor planning and other tasks that might be classified as 'cognitive'.

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