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REVIEW

THE ROLE OF THE CEREBELLUM IN CLASSICAL CONDITIONING OF DISCRETE BEHAVIORAL RESPONSES

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Abstract—The cerebellum and its associated circuitry constitutes the entire essential neuronal system for classical conditioning of eye-blink and other discrete responses (e.g. limb flexion) learned with an aversive unconditioned stimulus (US) using the standard delay paradigm where the conditioned stimulus (CS) and the US coterminate. Evidence reviewed here strongly supports the following conclusions. The CS pathway involves sensory relay nuclei projections to the pontine nuclei and its mossy fiber projections to the cerebellar cortex and nuclei. The US pathway involves activation of the inferior olive (dorsal accessory olive for eye blink) and its climbing fiber projections to the cerebellar cortex and nuclei. The conditioned response (CR) pathway involves the cerebellar interpositus nucleus, the superior cerebellar peduncle pathway to the magnocellular red nucleus and rubral projections to premotor and motor nuclei generating the behavioral response. Anatomical data, neuronal unit recordings, electrical stimulation, lesions and methods of reversible inactivation all strongly support the hypothesis that the essential memory trace for the learning of these discrete conditioned responses is formed and stored in the cerebellar interpositus nucleus. Neuronal/synaptic plasticity is also established in the cerebellar cortex in this form of learning but the role of the cortex is less clear. We argue that the cortex plays a key role in normal acquisition and adaptive timing of the conditioned response, under certain circumstances, but it remains unclear exactly what features of conditioning are being encoded in the cerebellar cortex in this basic form of associative learning and memory.

Key words: cerebellum, interpositus nucleus, inferior olive, classical conditioning, eye blink, limb flexion.

Contents

In this review, we treat the role of the cerebellum in acquisition and retention of the standard delay conditioned response (CR), where the conditioned stimulus (CS) precedes and coterminates with the unconditioned stimulus (US). Most of the data that have been collected over the years are from studies of eyeblink conditioning; hence we focus on that response system here. To the extent tested, the cerebellum is involved in the same way for all striated muscle responses learned to deal with an aversive US (e.g. forelimb and hind limb flexion, lever press, head turn). The neuronal substrates of the trace CR, where a period of no stimulation intervenes between CS offset and US onset, are more complex and less well understood than for the delay procedure, involving the hippocampus and other brain structures as well as the cerebellum and will not be reviewed here (see Woodruff-Pak and Disterhoft, 2008). By the same token, brain substrates of extinction of the eye-blink and other discrete responses are not yet well understood (Bouton, 2002; Medina et al., 2002; Robleto et al., 2004) and will not be treated here. We also note that

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Abbreviations: CR, conditioned response; CS, conditioned stimulus; DAO, dorsal accessory olive; EMG, electromyogram; IP, interpositus nucleus; ISI, interstimulus interval; LRN, lateral reticular nucleus; LTD, long-term depression; MATN, medial auditory thalamus nuclei; mcp, middle cerebellar peduncle; NM, nictitating membrane; scp, superior cerebellar peduncle; TTX, tetrodotoxin; UR, unconditioned response; US, unconditioned stimulus.

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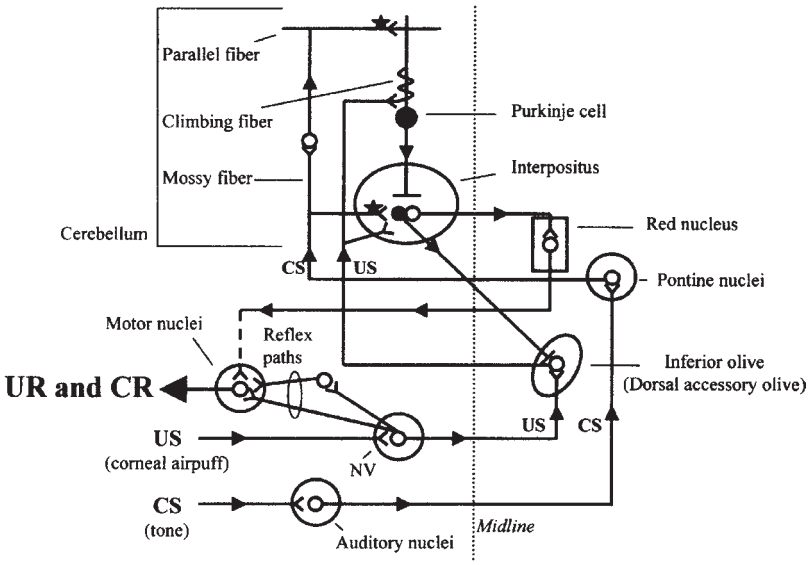

Fig. 1. Simplified schematic (most interneurons omitted) of the putative essential circuitry for delay classical conditioning of eyeblink (and other discrete responses) learned with an aversive US (the sensory and motor nuclei activated depend of course on the nature of the CS and US—the more central portions of the circuit appear to be general). The reflex US–UR pathway involves direct and indirect projections from the trigeminal nucleus to the motor nuclei (for the eyeblink UR and CR primarily accessories 6 and 7). The tone CS pathway projects from auditory nuclei to the pontine nuclei and to the cerebellum as mossy fibers. The US pathway includes projections from the trigeminal to the inferior olive and to the cerebellum as climbing fibers. The CR pathway projects from the interpositus to the red nucleus and on to premotor and motor nuclei. There is also a direct GABAergic inhibitory projection from the interpositus to the inferior olive. Solid cell bodies and bar terminals indicate inhibitory neurons, open cell bodies and fork terminals indicate excitatory neurons. Stars indicate sites of plasticity based on current evidence. See text for details. (Modified from Thompson, 1986.)

the relevant human literature was reviewed recently (Christian and Thompson, 2005; Woodruff-Pak and Steinmetz, 2000) and will not be treated here. To the extent tested, all findings described below for non-human animals apply equally to humans.

The cerebellum and its associated circuitry constitute the entire essential circuit for delay classical conditioning of eye-blink and other discrete responses. A schematic depicting the entire essential circuitry for eye-blink conditioning is shown in Fig. 1 (see Thompson, 1986). Fig. 2 shows coronal sections through a rabbit brain where some of the critical structures for conditioning have been identified. In this paper we focus on the cerebellar interpositus nucleus (IP) and the cerebellar cortex, with brief treatments of the essential CS, US and CR pathways as well. We first discovered the essential role of the cerebellum in classical conditioning of the eye-blink response in 1980–1981, using rabbits and the standard delay paradigm (a 350 ms tone CS and a coterminating 100 ms corneal air puff US), and recording extension of the nictitating membrane (NM) response (McCormick et al., 1981, 1982a, 1984a). In brief, using neuronal unit recordings we identified a critical region of the cerebellar deep nuclei showing an increase in frequency of dis-

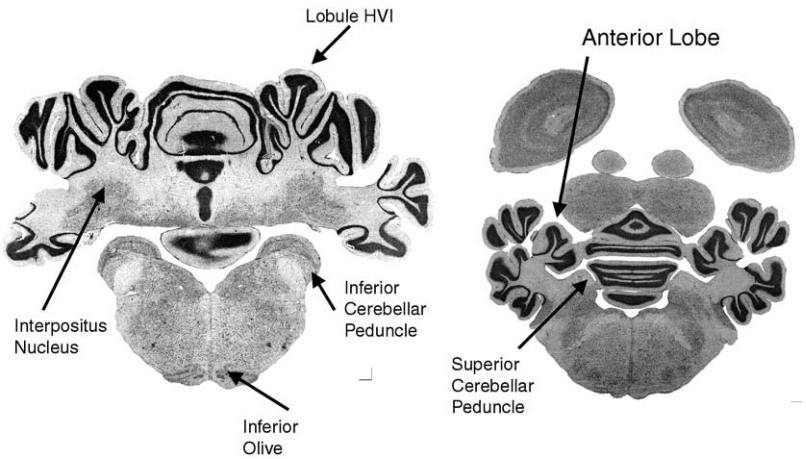

Fig. 2. Photomicrographs of two coronal sections of a rabbit brain showing key structures involved in eyeblink conditioning. The section on the left was taken 0.5 mm anterior to the lambda skull landmark while the section on the right was taken 3.0 mm anterior to lambda.

charge that preceded and predicted the occurrence and form of the behavior eye-blink CR. Further, lesions of the cerebellum, including this region, completely abolished the learned response with no effect on the reflex response (we treat these topics in detail below).

THE EYE-BLINK REFLEX RESPONSE

The eye-blink reflex in the rabbit is a coordinated response involving simultaneous and perfectly correlated external eyelid closure, eyeball retraction and resulting passive extension of the NM (e.g. Gormezano et al., 1962; McCormick et al., 1982c). It is but one adaptive response with several components. Initial studies of motor control of the reflex response focused on NM extension, actually eyeball retraction, controlled mostly (but not entirely) by the retractor bulbus muscle, which is innervated largely by axons of the sixth nerve originating on the accessory abducens (and to a lesser extent abducens) nuclei. However, the other extraocular muscles also contract synergistically with the retractor bulbus (Cegavske et al., 1976, 1979, 1987; Disterhoft et al., 1987; Berthier et al., 1987).

At the time there was much debate about the details of innervation of the retractor bulbus muscle (controlling NM extension), which in retrospect was somewhat irrelevant since the most prominent and sensitive component of the response is electromyogram (EMG) activity in the orbicularis oculus muscle controlling external eyelid closure (Lavond et al., 1990), innervated by the seventh nerve from the facial nucleus. Furthermore, in many mammalian species, including humans, the NM is vestigial. In terms of the reflex pathways, there are direct projections from neurons in regions of the trigeminal nucleus to the accessory abducens (and abducens) nuclei and to the facial nucleus (Cegavske et al., 1987; Berthier et al., 1987), as well as indirect projections relaying via the brainstem reticular formation, at least to the facial nucleus (Tamai et al., 1986).

THE EYE-BLINK CR

Gormezano et al. (1962) showed some years ago in separate studies of the rabbit that eyeball retraction, NM extension, and external eyelid closure all had essentially identical acquisition functions. Simultaneous recording of NM extension and external eyelid closure (EMG recordings from dorsal orbicularis oculi) during acquisition and extinction showed that they were, in essence, perfectly correlated, both within trials and over training (McCormick et al., 1982c; Lavond et al., 1990). Substantial learning-induced increases in neuronal unit activity that correlate very closely with the conditioned NM extension response have been reported in several motor nuclei: oculomotor, trochlear, motor trigeminal, abducens, accessory abducens, and facial (Berthier and Moore, 1983; Cegavske et al., 1979; Disterhoft et al., 1985; McCormick et al., 1983). These are all components of the same global CR involving, to the extent studied, essentially perfectly coordinated activity in a number of muscles and associated motor nuclei. The NM extension response is but one component of the CR. It has been suggested that different motor nuclei might somehow exhibit different CRs in the eye-blink conditioning paradigm (Delgado-Garcia et al., 1990). This possibility is not supported by the evidence cited above.

The CR and the unconditioned response (UR) are similar in eye-blink conditioning in the sense that to a large extent the same muscles and motor nuclei are engaged. However, the CR and the UR differ fundamentally in a number of respects. The minimum onset latency of the CR to a tone CS in well-trained rabbits, measured as NM extension, is about 90–100 ms; the minimum onset latency of the NM extension UR to a 3 psi corneal air puff US in the rabbit is about 25–40 ms. Perhaps most important, the variables that determine the topographies of the UR and CR are quite different. The topography of the UR is under the control of the properties of the US; for example, stimulus intensity, rise-time and duration. In marked contrast, the topography of the CR is substantially independent of the properties of the US and is determined primarily by the interstimulus interval (ISI) (the CS–US onset interval)—the CR peaking at about the onset of the US over a wide range of effective CS–US onset intervals (Coleman and Gormezano, 1971; Steinmetz, 1990a). This key property of the CR cannot be derived from the properties of the US or the UR. Another important difference is that the CR exhibits much greater plasticity in recovery from lesions of the motor nuclei that impair performance of the UR than does the UR itself (Disterhoft et al., 1985; Steinmetz et al., 1992a).

In sum, the conditioned eye-blink response involves highly coordinated activity in a number of motor nuclei and muscles; it is one global defensive response that is conditioned to a neutral stimulus as a result of associative training. The very small lesion of the IP, which is effective in completely and permanently abolishing the conditioned NM response (see below), also completely and permanently abolishes all other components of the CR that have been studied—eyeball retraction, external eyelid closure, orbicularis oculi EMG—without producing any impairment in performance of the reflex response (Steinmetz et al., 1992a).

THE IP

Recording studies

In our initial recording studies we identified the relevant region of the deep nuclei involved in eye-blink conditioning as the dentate–interpositus; the border between the two is difficult to determine in rabbit. In subsequent studies the critical nucleus region was shown to be anterior lateral IP (Thompson and Krupa, 1994).

As noted briefly above, recordings of neuronal unit activity from the IP during eye-blink conditioning revealed populations of cells in the critical region of the nucleus that, as a result of training, discharged prior to the execution of the learned eye-blink response and fired in a pattern of increased frequency of response that preceded and predicted the temporal form of the behavioral CR; that is, it formed a “neuronal model” of the CR (rabbits were used except where noted—Berthier and Moore, 1990; Foy et al.,

1984; Freeman and Nicholson, 2000 [rat]; Green and Arenos, 2007 [rat]; Green et al., 2002 [rat]; Gould and Steinmetz, 1996; King et al., 2001; McCormick and Thompson, 1984a; McCormick et al., 1981, 1982a, 1983; Rogers et al., 2001 [rat]; Stanton and Freeman, 2000 [rat]; Steinmetz, 1990b, 2000; Thompson, 1983, 1986; Tracy and Thompson, 1993; Tracy et al., 2001; Weninger and Thompson, 1997). In a most interesting series of studies, Freeman and associates (Freeman and Nicholson, 1999; Nicholson and Freeman, 2002) recorded neuronal activity from the IP of rats during both simple acquisition training (excitatory conditioning) and conditioned inhibition. In agreement with the literature cited above, acquisition training resulted in increased activity in the CS period but conditioned inhibition resulted in less activity.

Yang and Weisz (1992) recorded activity of single neurons in the dentate and interpositus nuclei, which had been identified as excitatory by antidromic activation from stimulation of the red nucleus, in response to the CS and US at the beginning of training. The great majority of cells showed increased discharge frequency to the US (substantial) and the CS (small but significant). Paired CS–US presentations resulted in marked enhancement of the response to the CS in cells in the IP and depression of response to the CS in cells in the dentate nucleus. In other animals, small lesions of the region of recording in the anterior IP completely prevented acquisition of the eyeblink CR but lesions of the dentate recording region had no effect at all on learning.

In a detailed single unit mapping study, Tracy (1995) analyzed response properties of single neurons in the anterior interpositus in both untrained (n=98) and trained (n=316) rabbits. In untrained animals, many neurons responded to auditory and somatosensory stimulation (half responded to both) with latencies that were longer than the reflex response (to somatosensory stimulation). In trained animals, a number of neurons exhibited the characteristic pattern: a neuronal model of the learned response that preceded and predicted the occurrence of the CR (in agreement with Berthier and Moore (1990) (see Fig. 3). In contrast to these reports, Gruart and Delgado-Garcia (1994) claimed that sensory response units in the interpositus were time-locked to the reflex responses. The studies cited above, however, do not support this claim.

An example of a single interpositus neuron that exhibits all the properties of the learned neuronal response is shown in Fig. 3A. This neuron was recorded from the critical eye-blink region of the dorsal anterior lateral IP. Note that the neuron shows an increased frequency of discharge following CS onset that precedes and predicts both the occurrence and form of the behavioral eye-blink CR. The US onset causes an immediate brief marked increase in discharge frequency, due presumably to direct projections of axons from the inferior olive to the IP (Kitai et al., 1977). Immediately thereafter, the IP unit is completely inhibited, due presumably to cortical Purkinje neuron inhibition (except on every 10th trial where no US is presented). After recording, a small marking lesion was made (see Fig. 1B). The current applied during the marking lesion elicited a full-blown eye-blink response. Following this, the CR was completely abolished with no effect at all on the reflex response. Here the neuronal region of the IP critical for the CR is considerably less than a cubic mm, consistent with our earlier kainic acid lesion result (Lavond et al., 1985 and see below).

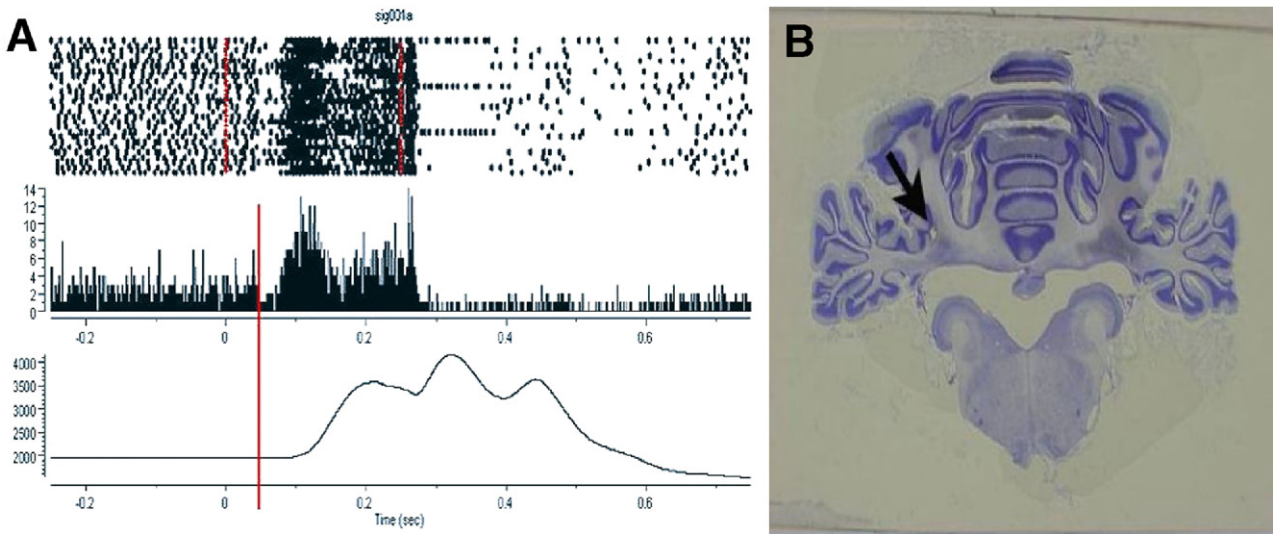

Fig. 3. (A) Response pattern of a single, isolated IP neuron in a well trained rabbit. Upper row, each dot in the raster is an action potential of the neuron. The first vertical red line is CS onset and the second is US onset. Middle row shows histogram of unit discharges. Lower row shows averaged extension of the NM response (eyeblink). The vertical red line in the lower two rows is the time of onset of changes in the temporal pattern of the neuron response, which precedes and "models" the conditioned NM response (but not the reflex response). Every 10th trial is CS alone. This response pattern is typical of neurons in this dorsal, lateral anterior region of the IP in trained animals. See text for details. (B) Location of the small lesion made to mark the location of the neuron in 1 A (50 μA for 10 s). This small current when applied elicited a complete eyeblink response (eyeball retraction, NM extension and external eyeblink closure). Following this lesion the eyeblink CR was completely and permanently abolished with no effect on the reflex response (J. Thompson, Christian, Robleto, Polous and R. Thompson, unpublished observations).

In an extraordinary example of an experimental non sequitur, Delgado-Garcia and colleagues recorded single neuron responses from the *posterior* IP (Jiménez-Díaz et al., 2002) and concluded (see also Delgado-Garcia and Gruart, 2002 and Gruart et al., 2000) that the IP is involved only in performance of the eye-blink CR and not in learning, which is in disagreement with all the literature cited above. They may be correct regarding the posterior IP, but this is irrelevant—the anterior interpositus is essential for eye-blink conditioning and there is no evidence that the posterior nucleus is critically involved. To be specific, the critical region has been defined as the dorsal, lateral region of the anterior interpositus, as noted above (see Tracy, 1995 and Fig. 3).

In an elegant study, Choi and Moore (2003) trained rabbits with two randomly alternating ISIs—the eye-blink CRs showed two distinctive peaks corresponding to the two ISIs and interpositus neurons similarly exhibited two peaks that were correlated with, and preceded, the eye-blink CRs. These data also argue strongly that neuronal activity in the anterior interpositus is causally related to production of behavioral CRs (see also Fig. 3). Engagement of the anterior interpositus in eye-blink conditioning has also been shown using fMRI in the conscious rabbit (Miller et al., 2003) and radiolabelled 2-DG in the rat (Plakke et al., 2007).

Lesions

The initial discovery of the key role of the cerebellum in eye-blink conditioning involved both large aspiration lesions including cerebellar cortex and nuclei and electrolytic lesions of the dentate-interpositus nuclear region (Clark et al., 1984; McCormick et al., 1981, 1982a). These lesions completely abolished the eye-blink CR with no effect on the reflex eye-blink response (UR). Stimulation of the critical nuclear region elicited the eye blink before training; the circuit is hard-wired from nuclei to behavior. In a subsequent series of studies it was found that the key nuclear region is the dorsal aspect of the anterior lateral IP ipsilateral to the trained eye, as noted above. Very large lesions of the cerebellar cortex that did not damage the IP impaired, but did not abolish, the behavioral CR, although CR latency was altered such that the eye closed and opened before the onset of the US—the timing of the behavioral response was no longer adaptive (see below and Logan, 1991; McCormick and Thompson, 1984a; Perrett et al., 1993).

In these initial studies it was shown that the interpositus lesion effect was ipsilateral—it abolished the eye-blink CR on the side of the lesion but did not impair eye-blink conditioning of the contralateral eye, providing a control for possible nonspecific or state variables. Furthermore, if the lesion was made before training, learning was completely prevented (Lincoln et al., 1982). These lesions had no effects at all on performance of the UR.

Yeo and associates (1985a) replicated the interpositus lesion result noted above, using light and white noise CSs and a periorbital shock US, thus extending the generality of the findings. A number of subsequent studies (using rabbit unless otherwise noted) showed identical results for interpositus lesions or inactivation, i.e. complete abolition of the CR in trained animals and complete prevention of learning in naïve animals (Bracha et al., 2001; Chen et al., 1996 [mouse]; Freeman et al., 1995 [rat]; Green et al., 2002 [rat]; 2006 [rat]; Katz and Steinmetz, 1997; Lavond et al., 1984a, 1987; Lee and Kim, 2004 [rat]; Neufeld and Mintz, 2001 [rat]; Nolan et al., 2002 [rat; infusing picrotoxin in the IP]; Polenchar et al., 1985; Sears and Steinmetz, 1990; Skelton, 1988 [rat]; Steinmetz et al., 1991, 1992b; Swain et al., 1999; Thompson, 1986; Wada et al., 2007 [mouse]; Weisz and Lo Turco, 1988; Wikgren and Korhonen, 2001 [rabbit; somatosensory CS]; Woodruff-Pak et al., 1985; Yang and Weisz, 1992). Interpositus lesioned animals have been trained daily for periods up to 12 months; no CRs ever developed after the lesion (Lavond et al., 1984b; Steinmetz et al., 1992b). The eye-blink CR is retained with no loss (100% savings) for 30 days and IP lesions made then still completely abolish the CR, regardless of whether or not reinstatement training is given (Christian and Thompson, 2005). The IP is essential for long-term retention of the CR. Kainic acid lesions of the interpositus as small as about 1 mm^3 in the anterior lateral region of the nucleus abolished the CR, indicating extreme localization of the critical region and ruling out the possibility that fibers of passage were involved (Lavond et al., 1984a and Fig. 1 above). Finally, lesions of the output of the cerebellar nuclei, the superior cerebellar peduncle (scp), abolished the behavioral CR with no effect on the UR (McCormick et al., 1982b; Rosenfield et al., 1985; Voneida, 2000 [limb flexion CR in cat]). It would appear there is universal agreement that appropriate lesions of the IP abolish the conditioned eye-blink response.

In an important series of studies, the IP was inactivated during acquisition training in initially naive animals, using reversible cooling (Clark et al., 1992); muscimol (Krupa et al., 1993; Krupa and Thompson, 1997; Freeman et al., 2005 [rat]); and lidocaine (Nordholm et al., 1993). In all cases, inactivation during training completely prevented learning, animals then learned as though completely naïve, i.e. there was no savings. Note that muscimol, at least, only inactivates neuronal soma-dendrites ($GABA_A$ receptor agonist) so all fiber projections to the cerebellar cortex (mossy and climbing fibers) are unaffected.

We note here the extremely important finding of Freeman and associates on conditioned inhibition and the IP in rat (see their recording studies noted above). In trained animals, infusion of the $GABA_A$ antagonist picrotoxin impaired performance of simple excitatory conditioning but not conditioned inhibition (Nolan et al., 2002). Infusion of muscimol in the IP in naïve rats completely prevented acquisition of the excitatory CR (in complete agreement with the above) but did not prevent acquisition of conditioned inhibition (Freeman et al., 2005).

Other conditioned skeletal muscle responses

Data to be discussed below suggest that any stimulus-elicited behavioral skeletal muscle response that can be conditioned requires the cerebellum/IP. The conditioned limb flexion response is a case in point. In elegant studies, Voneida (2000) showed that the conditioned forelimb flex-

ion response in the cat requires the scp, the efferent projection from the IP, as noted above. Interestingly, only the classically CR was abolished; the animals were not impaired in "voluntary" responses like batting a ball (see below and Voneida, 2000).

In a preliminary study we reported that the conditioned hind limb flexion response in the rabbit required the medial region of the anterior IP (Donegan et al., 1983). Recently, we trained rabbits in both eye-blink and hind limb flexion CRs and infused muscimol in the lateral and medial region of the IP (Mojtahedian et al., 2007). Results showed a clear double dissociation: muscimol infusion in the lateral anterior IP abolished the conditioned eye-blink response with no effect on the conditioned hind limb flexion response, whereas medial infusions abolished the conditioned hind limb flexion response but not the conditioned eye-blink response. These results are in clear agreement with the known somatotopic organization of the IP (Chambers and Sprague, 1955; Lockard, 1999) and disprove the claim by Bracha et al. (1999) that the representation of CRs in the IP does not exhibit somatotopic organization.

Issues

In the study of learned behaviors, a fundamental issue concerns performance. Do brain manipulations such as lesions act on learning, per se, or simply on performance of the learned response? A major reason we selected eye-blink conditioning as a model system is that performance of the reflex response (UR) can be measured separately and independently from performance of the learned response (CR). Interestingly, the same efferent system generates both the reflex and learned responses (see above). Hence if procedures that impair performance of the learned response have no effect on performance of the reflex response, effects on performance, per se, can be ruled out. In a challenge to our interpositus lesion result, Welsh and Harvey (1989) claimed that the lesion-induced abolition of the CR was secondary to the effects on the UR. However, they did not measure the effects of IP lesion on URs and CRs in the same animals. Actually, when intensity of the US is reduced so the UR is matched in amplitude and percent responses to the CR before lesion in the same animals, the IP lesion abolishes the CR with no persisting effect at all on the UR (Steinmetz et al., 1992a; Ivkovich et al., 1993). Indeed, reanalysis of Welsh and Harvey's own data does not support their argument (Steinmetz et al., 1992a; Thompson and Krupa, 1994). Hence, the "performance deficit" argument is not valid.

In only one study was it claimed that IP lesions did not abolish the eye-blink CR (Koekkoek et al., 2003 [mouse]), thus disagreeing with dozens of studies cited above showing IP lesion prevention/abolition of the CR in rabbit, rat and mouse. Unfortunately, this study is fatally flawed and their interpretation of the results can therefore be questioned. Koekkoek et al. used a 10 kHz tone as a CS, a stimulus that evokes startle responses in mice (Willott et al., 1984). The startle response is a short latency, innate, unlearned response that includes eye blink and is not dependent on the cerebellum (Davis, 1984). Indeed the "CR" Koekkoek et al. showed following IP lesion had an onset latency of about 20 ms, the latency of the startle response and much too short to be a CR (i.e. minimum of about 100 ms onset latency). It therefore appears that CRs were abolished by the interpositus lesions but not the startle responses.

THE CS, US AND CR PATHWAYS

The CS pathway

The pontine nuclei send axons as mossy fibers directly to the cerebellar cortex and IP, mostly contralaterally (Brodal, 1981; Mihailoff, 1993; Shinoda et al., 1992; Steinmetz and Sengelaub, 1992; Thompson et al., 1991). The pontine nuclei in turn receive projections from auditory, visual, somatosensory and association systems, both cortical and subcortical (Brodal, 1981; Glickstein et al., 1980; Schmahmann and Pandya, 1989, 1991, 1993). Appropriate lesions of the pontine nuclei can abolish the CR established to a tone CS but not a light CS, i.e. can be selective for CS modality. This can be contrasted with the effects of interpositus lesions, which abolish the CR to all modalities of CS (Steinmetz et al., 1987). Extensive lesions of the middle cerebellar peduncle (mcp), which conveys mossy fibers from the pontine nuclei and other sources to the cerebellum, abolish the CR to all modalities of CS (Lewis et al., 1987).

Electrical stimulation of the pontine nuclei serves as a "supernormal" CS, yielding more rapid learning than does a tone or light CS (Freeman et al., 2005; Steinmetz et al., 1986; Tracy et al., 1998). Stimulation of the mcp itself is an effective CS (Steinmetz, 1990a; Svensson and Ivarsson, 1999) and lesion of the IP abolishes the CR established with a pontine or middle peduncle stimulation CS (Steinmetz et al., 1986). When animals are trained using electrical stimulation of the pontine nuclei as a CS (corneal air puff US), some animals show immediate and complete transfer of the behavioral CR and of the learning-induced neural responses in the IP to a tone CS (Steinmetz, 1990b) and complete transfer from peripheral CSs to mossy fiber stimulation in the mcp (Hesslow et al., 1999). These results suggest that the pontine-mcp stimulus and tone must activate a large number of memory circuit elements (neurons) in common. In sum, the mossy fiber system, coming mostly from the pontine nuclei, is the CS activated pathway to the cerebellum (Thompson et al., 1997).

In a most important early series of studies, Doty and colleagues (e.g. Doty et al., 1956) used electrical stimulation of regions of the cerebral cortex as a CS in conditioning studies, a promising preparation for localization of memories. We replicated these observations using stimulation of the auditory field of neocortex as a CS in eye-blink conditioning in rabbit and showed that the necessary circuit was from this region of cerebral cortex to the pontine nuclei and the pontine mossy fiber projections to the cerebellum (Knowlton and Thompson, 1992; Knowlton et al., 1993).

Recent studies from Freeman's laboratory, using eye-blink conditioning in rat, have added important new infor-

mation concerning the essential auditory CS pathways to the cerebellum and their ontogenetic development in rat. Using an auditory CS, eye-blink conditioning emerges between days P17 and P24 (Freeman and Nicholson, 2004; Stanton et al., 1992). Stimulation of the pontine nucleus as a CS can establish eye-blink CRs in P12 rat groups, where no learning occurs to a peripheral auditory CS (Campolattaro and Freeman, 2008; Freeman et al., 2005). Importantly, lesion of the medial auditory thalamus nuclei (MATN—including the medial nucleus of the medial geniculate, the posterior intralaminar nucleus and suprageniculate nucleus contralateral to the trained eye), projecting to the pontine nucleus, severely impaired CR acquisition to an auditory CS (Halverson and Freeman, 2006), as do lesions of the regions of the inferior colliculus projecting to the MATN (Freeman et al., 2007). Furthermore, stimulation of the MATN was a very effective CS for conditioning (Campolattaro et al., 2007). When tested in those stimulation studies, inactivation of the IP nucleus completely abolished the CR.

Interestingly, neurons in a region of the pontine nuclei show the same pattern of learning-induced increases in discharge frequency in response to the CS as do neurons in the IP (Bao et al., 2000; McCormick et al., 1983). Hence this region might be considered an alternative site for the "memory trace." Reversible inactivation of the IP, however, abolishes this neuronal model in the pontine nuclei (Clark et al., 1997). But it also, of course, abolishes acquisition of the behavioral CR. However, inactivation of the red nucleus, an essential efferent CR relay from the IP (see below), does not prevent acquisition of the CR at all but does abolish the neuronal model in the pontine nuclei (Cartford et al., 1997), thus arguing strongly that the pontine neuronal model is simply relayed from the IP via red nucleus.

Electrical stimulation of the lateral reticular nucleus (LRN) is an effective CS for eye-blink conditioning, just as is stimulation of the pontine nuclei (Lavond et al., 1987). The neuronal model of the behavioral CR also develops in the pontine nuclei with LRN stimulation as a CS (Bao et al., 2000). Here too, inactivation of the IP abolishes the pontine neuronal model, but importantly, inactivation of the key pontine nuclear region showing the model now has no effect at all on the behavioral CR (Bao et al., 2000).

In a most interesting observation, Moore and associates reported the development of a learning-induced neuronal model of the behavioral CR in a region of the spinal trigeminal nucleus and adjacent reticular formation, raising the possibility that this might be a site of neuronal plasticity, e.g. a "memory trace" projecting to the cerebellum in eye-blink conditioning (Richards et al., 1991). However, reversible inactivation of the critical region of the red nucleus, which has no effect on acquisition of the behavioral CR, completely abolished the neuronal model of the CR in the trigeminal nuclear region (Clark and Lavond, 1996).

Consequently, these learning-induced neuronal models of the behavioral CR in structures afferent to the cerebellum, pontine nuclei and trigeminal nucleus, are not developed *in situ* but rather are induced via projections from the IP and red nucleus. Hence they are not sites of "memory trace" formation. But, what functions these phenomena serve is an important question for the future.

Issues

There appears to be agreement that the essential CS pathway for eye-blink conditioning is mossy fiber projections to the cerebellum, both cortex and IP, the predominant source of mossy fibers being the pontine nuclei, which receive projections from various sensory relay nuclei and other major brain regions.

The CR pathway

As noted earlier, electrical microstimulation of the critical region of the IP elicits an eye-blink response in both trained and untrained animals (McCormick and Thompson, 1984a). Output from the interpositus, both ascending and descending, is conveyed by the scp (Brodal, 1981). Lavond et al. (1981) first reported that lesions in the pontine reticular formation in the vicinity of the scp abolished the eye-blink CR. There was some uncertainty in this study whether the critical region was the scp. This was resolved in studies by McCormick et al. (1982b) and Rosenfield et al. (1985), showing that the scp ipsilateral to the cerebellum (and trained eye) is the essential descending efferent pathway for eye-blink conditioning. Further, the eye-blink response elicited by stimulation of the IP in naïve animals is abolished by lesion of the scp (McCormick and Thompson, 1984a).

The direct target of the descending fibers of the scp is the red nucleus contralateral to the cerebellar hemisphere (Brodal, 1981). The region of the contralateral magnocellular red nucleus that receives projections from the region of the anterior interpositus critical for eye-blink conditioning also exhibits a learning-induced pattern of increased unit activity in eye-blink conditioning very similar to that shown by interpositus neurons (Chapman et al., 1990). Microstimulation of this region of the red nucleus in naïve animals also elicits eye-blink responses. When this is used as a US, neither learning of the CR to a tone CS nor maintenance of a CR previously learned with tone-air puff training occurs. Further, there is no transfer from tone–red nucleus training to subsequent tone–air puff training (Chapman et al., 1988). If the red nucleus is reversibly inactivated in trained animals, the eye-blink CR is reversibly abolished but the learning-induced neuronal model of the CR in the IP is unaffected. In contrast, when the IP is reversibly inactivated, both the behavioral CR and the learning-induced neuronal model of the CR in the magnocellular red nucleus are completely abolished (Chapman et al., 1990; Clark et al., 1992; Clark and Lavond, 1993).

Small lesions of the appropriate region of the magnocellular red nucleus contralateral to the trained eye cause complete abolition of the CR with no effect on the UR (Chapman et al., 1988; Haley et al., 1983; Rosenfield et al., 1985; Rosenfield and Moore, 1983). This same lesion also abolishes the eye-blink response elicited in untrained animals by stimulating the IP. Microinfusions of nanomolar amounts of a neurotransmitter antagonist in a very local-

ized region of the magnocellular red nucleus reversibly abolished the conditioned eye-blink response with no effect on the UR (Haley et al., 1988). Krupa et al. (1993) showed convincingly that muscimol inactivation of the red nucleus blocked the CR without affecting the UR. Lesions of the red nucleus or descending rubral pathway abolished the conditioned limb flexion response in the cat with no effect on the reflex limb flexion response and no effect on normal behavioral movement control of the limb (Smith, 1970; Tsukahara et al., 1981; Voneida, 1990).

Issues

There appear to be no disagreements in the field concerning the fact that the efferent CR pathway projects from the IP via the scp to the contralateral red nucleus and then to ipsilateral motor nuclei critical for this eye-blink response. One aspect of the circuit not yet well defined is the details of descending innervations of motor and premotor nuclei critical for performance of the eye-blink response, not to mention the projection systems from red nucleus to the pontine nuclei or the trigeminal nucleus.

The US pathway

Neurons in the region of the inferior olive are essential for eye-blink conditioning. Specifically, these neurons originate in the dorsal accessory olive (DAO) and send climbing fiber projections directly to the cerebellar cortex, with collaterals projecting directly to neurons in the IP (Brodal, 1981; Groenewegen et al., 1979; Ito, 1984; Sugihara et al., 2001; Tracy et al., 1998). Physiologically, climbing fibers monosynaptically activate IP neurons with a 3 ms latency (Kitai et al., 1977; Nicholson and Freeman, 2000). The DAO receives predominantly somatosensory input relayed from appropriate cranial nuclei, including nociceptive input (Brodal, 1981). Lesions of the critical region of the inferior olive, the face representation in the DAO, completely prevent learning if made before training and result in extinction of the CR over a period of days with continued paired training when the lesion is made after training (McCormick et al., 1985; Mintz et al., 1994; Voneida et al., 1990 (limb flexion in cat); see also below and Yeo et al., 1986). Neurons in this critical DAO region do not respond to auditory stimuli (CS), respond only to US onset, show no learning-related activity, and the US evoked response decreases as animals learn (Sears and Steinmetz, 1991). All these data argue that the DAO–climbing fiber system is the essential US reinforcing pathway for the learning of discrete responses (Thompson, 1989; Thompson et al., 1998).

Electrical microstimulation of this region of the DAO elicits eye-blink responses before training; indeed, virtually any phasic behavioral response can be so elicited, depending on the locus of the stimulating electrode. When DAO stimulation is used as a US, the exact response elicited by DAO stimulation is learned as a CR to a tone CS (Mauk et al., 1986; Steinmetz et al., 1989). Control procedures showed that the effective stimulus activates the climbing fibers (Thompson, 1989). First, climbing fiber field potentials were recorded in cerebellar cortex during DAO stimulation when implanting the DAO electrodes. Second, interpositus lesions abolished both the CR- and DAO-elicited UR, thus ruling out the possibility of antidromic or current spread activation of reflex afferents (interpositus lesions do not affect reflex URs). Third, in some animals the 2-deoxy-glucose technique was used to map the regions of cerebellar cortex activated by the DAO-US—they corresponded to climbing fiber projections from the DAO. Fourth, electrodes just dorsal to the DAO in the reticular formation also elicited movements, but these could not be conditioned to a CS. We therefore conclude that the DAO–climbing fiber system is the essential US reinforcement or teaching pathway for classical conditioning of discrete responses.

Issues

Yeo et al. (1986) reported that their IO lesions immediately abolished the eye-blink CR rather than resulting in extinction. In our initial lesion study, done in two independent replications by David McCormick and Joseph Steinmetz, our lesions were small and targeted to the face representation in the DAO (McCormick et al., 1985). Voneida reported similar results for chemotaxic lesions of the IO with forelimb flexion conditioning in the cat (Voneida et al., 1990). The Yeo et al. lesions were larger. Similarly, large infusion of inactivating substances such as lidocaine (Welsh and Harvey, 1998), glutamate antagonists or muscimol (Zbarska et al., 2007; 2008) was reported to immediately abolish the eye-blink CR. [We note that Zbarska et al. (2007) did not cite the critically important study by Voneida et al., 1990.] Earlier studies showed that with substantial lesion or inactivation of the IO, the cerebellar circuitry is severely and transiently disrupted (Colin et al., 1980; Montarolo et al., 1982). In our studies and Voneida's study the fact that the CR extinguished over a period of days cannot be accounted for by transient disruption of cerebellar function.

Another criticism, raised by Deigo Contresas at a meeting at UCLA on May 8, 2008, is the fact that the effective US (corneal air puff or periorbital shock) likely activates mossy fiber projections to the cerebellum as well as climbing fiber projections. While it is true that the corneal air puff activates mossy fibers, such simultaneous activation of mossy fibers with climbing fibers is irrelevant for learning since the CS (mossy fiber activation) must *precede* the US (climbing fiber activation) by about 100 ms or more for learning to occur (see below).

CONJOINT ACTIVATION OF CS AND US PATHWAYS

If the above hypotheses concerning the identities of the CS and US pathways are correct, it should be possible to train behavioral responses by conjoint stimulation of these pathways. Steinmetz et al. (1989) stimulated the pontine nuclei–mossy fibers as a CS (below movement threshold) and DAO–climbing fibers as a US. Such US stimulus elicited movements included eye blink, head turn and limb flexion. The stimulation–US-evoked movements were learned to

the mossy fiber stimulation CS, just as is the case for peripheral CSs. Here the mossy fiber CS preceded the climbing fiber US by 250 ms. After training, the two stimuli were presented simultaneously and the CR extinguished. Just as with peripheral stimuli the mossy fiber CS must precede the climbing fiber US by at least 100 ms or so for learning to occur, as noted above.

The IP sends direct GABAergic projections to the DAO (Nelson and Mugnaini, 1989). Hence, as learning-induced increases in interpositus neuron activity develop, inhibition of the DAO neurons will increase (Hesslow and Ivarsson, 1996). This accounts for the fact that US-evoked activity in the DAO decreases as learning develops (Sears and Steinmetz, 1991). This also appears to serve as a part of the neural circuit essential for the behavioral learning phenomenon of "blocking," where prior training to one CS, e.g. tone, prevents subsequent learning to a light CS when it is then presented together with the tone in paired compound stimulus training (Kamin, 1969). Infusion of picrotoxin in the DAO to block the GABA inhibition from the interpositus during compound stimulus training completely prevents the development of behavioral blocking (Kim et al., 1998).

LOCALIZATION OF THE ESSENTIAL MEMORY TRACE

Evidence reviewed above is very consistent with the view that the essential memory trace for eye-blink conditioning is stored in the anterior lateral IP. Indeed, evidence indicates that the IP is also necessary for long-term, permanent memory storage of this CR (Lavond et al., 1984b; Christian and Thompson, 2005). Evidence presented above argues strongly against the possibility that the two major sources of afferents to the cerebellum, pontine nuclei and inferior olive, are sites of memory storage. However, localization of the essential memory trace to the IP is not proved beyond a reasonable doubt by all this evidence, at least for some.

Methods of reversible inactivation are a very powerful approach to localizing the site(s) of memory storage when entire essential neural circuit has been identified, as is the case here for standard delay eye-blink conditioning (Lavond et al., 1993; Thompson and Krupa, 1994). As noted above, reversible inactivation of the IP (in rabbit unless noted otherwise) using cooling (Clark et al., 1992), muscimol (Freeman et al., 2005 [rat]; Hardiman et al., 1996; Krupa et al., 1993; Krupa and Thompson, 1997), lidocaine (Nordholm et al., 1993) and baclofin (Ramirez et al., 1997) completely prevents learning. After removal of inactivation, animals learn as though completely naïve to the task. In dramatic contrast, reversible inactivation of the motor nuclei (Krupa et al., 1996; Zhang and Lavond, 1991) and red nucleus (Clark and Lavond, 1993; Krupa et al., 1993) although preventing performance of the CR, did not prevent learning at all. Finally, reversible inactivation of the efferent projection from the IP, the scp using tetrodotoxin (TTX) also prevented performance of the CR and did not prevent learning at all (Krupa and Thompson, 1995). A summary of the inactivation results is shown in Fig. 4.

Extremely important direct evidence for a strengthening of the mossy fiber–interpositus neuron synapses has been presented by Kleim et al. (2002), using eye-blink conditioning in the rat. They demonstrated a highly significant increase in the number of excitatory synapses in the IP but no change in inhibitory synapses following eye-blink conditioning, compared to unpaired stimulation control animals. Further, Weeks et al. (2007) found significant

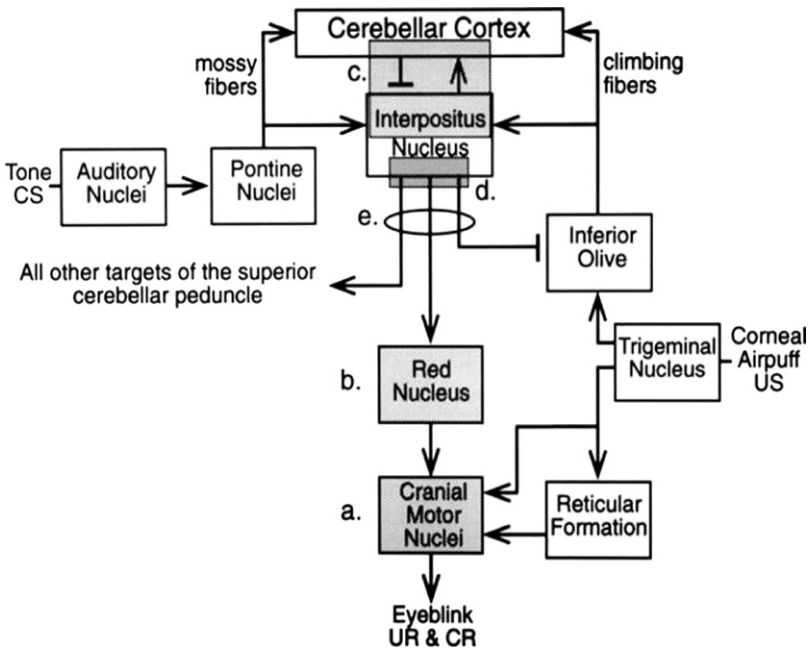

Fig. 4. Highly simplified schematic of the cerebellar memory circuit to indicate reversible inactivation during training experiments. (A) Motor nuclei; (B) red nucleus; (C) interpositus and some cortex; (D) only interpositus; (E) scp. See text for details. (Modified from Thompson and Krupa, 1994.)

changes in synaptic ultrastructure in IP during learning and Zhang et al. (2004) reported induction of LTP in the IP in an *in vitro* preparation. We note that some years earlier Racine et al. (1986) reported the development of LTP in interpositus following mossy fiber tetanus in the rat *in vivo*. Finally, using electrophysiology and Ca^{2+} imaging techniques, Pugh and Raman (2008) demonstrated how synaptic excitation and inhibition interact to generate a long-lasting synaptic plasticity in deep nuclear cells. In cerebellar slices they demonstrated that EPSCs are potentiated by high-frequency trains of presynaptic activity when applied coincidentally with postsynaptic hyperpolarization (i.e. a pattern that resembles mossy fiber activation and Purkinje cell–mediated inhibition). Further potentiation was most effective when the high-frequency trains of presynaptic activity preceded the postinhibitory rebound by around 400 ms. Interestingly, this asynchrony is similar to the optimal CS–US interval for eye-blink conditioning, which is generally considered to be in the range of 200–400 ms.

In terms of genetic substrates, protein synthesis in the IP is necessary for learning the conditioned eye-blink response (Bracha et al., 1998; Chen and Steinmetz, 2000; Gomi et al., 1999), as is true for other forms of long-term memory (e.g. Squire, 1987). Expression of one particular gene, coding for a cdc-related kinase, KKIAMRE (Gomi et al., 1999), is significantly and selectively increased in the IP ipsilateral to the trained eye in well-trained rabbits. This gene is normally involved in the cell division cycle but since adult neurons do not divide, it may play some other role in learning-induced neuronal/synaptic plasticity. Finally, in mouse IP, there is increased expression in a set of genes bilaterally early in training and increased expression of other genes ipsilateral to the trained eye in well-trained animals (Park et al. (2006).

The compelling conclusion from all this work is that the essential memory trace is stored in the IP. Training-induced neuronal plasticity also occurs in cerebellar cortex; the question is whether it is also essential or rather modulatory (see discussion of cerebellar cortex below).

Issues

Some years ago, Welsh and Harvey (1991) challenged the idea that the IP was critical for eye-blink classical conditioning. They trained animals to a light CS and then gave them tone transfer training during lidocaine infusion in the IP. They reported that animals learned to the tone during infusion training, although they, of course, did not express tone CRs until postinfusion training. However, control animals trained to tone after training to light, but with saline infused, showed marked savings in tone training, thus complicating the findings. More critical is the locus of infusion—some of their lidocaine infusions were at the ventral aspect of the IP (the dorsal aspect is critical for learning). Indeed, Nordholm et al. (1993) showed that lidocaine infusions at the dorsal aspect of the IP completely prevented learning but infusions at the ventral aspect of the IP, the origin of the scp fibers, although preventing performance of the CR, did not prevent learning at all. Lidocaine, like TTX, inactivates both soma-dendrites and axons. Here, results of both the Welsh and Harvey (1991) and Nordholm et al. (1993) studies support the Krupa and Thompson (1995) result.

Nilaweera et al. (2006) recently challenged the Krupa and Thompson (1995) result. They also infused TTX in the scp and reported that the infusion prevented both expression and learning of the eye-blink CR. However, there are several problems with this study. First, they injected substantially larger doses of TTX. Second they did not do the essential control of infusing the same amount (and effective concentration) of muscimol through their cannulae. Krupa and Thompson (1995) used this control. Since muscimol inactivates soma-dendrites but not axons, it is an ideal control to determine if TTX infusion spread to neurons of the IP. Muscimol had no effect at all on performance of the CR (Krupa and Thompson, 1995). This control, taken together with the results of Nordholm et al. (1993) argues strongly against the Nilaweera et al. (2006) report. Nilaweera et al. also did not control for the possibility that TTX may have acted on fibers of the mcp, conveying CS information to the IP. Indeed, it is difficult to see how the Krupa and Thompson (1995) result could possibly be explained by any result other than inactivation of the scp in the absence of any effect at all of TTX on acquisition of the CR (i.e. effect on the IP or fibers of the mcp).

Actually, the data and argument of Nilaweera et al. (2006) are somewhat irrelevant. We and Lavond et al. both showed earlier that inactivation of the magnocellular red nucleus region receiving the direct projections from the IP via the scp during training does not prevent learning of the eye-blink CR at all, although it does prevent expression of the CR during inactivation (Krupa et al., 1993; Clark and Lavond, 1993). And, as discussed above, Chapman et al. (1990) clearly showed that inactivation of the red nucleus did not abolish learning-related activity in the IP. Thus, Nilaweera et al. must conclude that the memory trace is formed along the axons of the scp between IP and red nucleus (or, more likely, that they inactivated critical regions of the IP or mcp).

Finally, the remote possibility that in the Krupa and Thompson study, they may have missed inactivating ascending fibers from the IP is ruled out by the fact that decerebration rostral to the red nucleus does not abolish the eye-blink CR (Mauk and Thompson, 1987).

In conclusion, we argue the evidence is overwhelming that the essential memory trace for standard delay eye-blink conditioning is formed, stored and retained in the IP. We now turn our attention to the role of the cerebellar cortex in eye-blink classical conditioning.

CEREBELLAR CORTEX

Several models of cerebellar plasticity have been proposed that are based on excitability changes at the parallel fiber–Purkinje cell synapse as a result of co-activation of mossy fibers and climbing fibers, the two sensory input systems that project information into the cerebellum (e.g. Albus, 1971; Marr, 1969). In general, these models pro-

pose that when these fiber systems are co-activated, a long-term inhibition of Purkinje cells occurs that in turn causes a disinhibition of deep nuclear neuronal activity (because all Purkinje cells inhibit deep nuclear cells). The long-term decrease in Purkinje cell excitability due to the conjunctive activation of mossy fibers and climbing fibers is called "long-term depression" (LTD) (Ito and Kano, 1982). Several studies have demonstrated an LTD effect at the parallel fiber–Purkinje cells synapses with conjunctive stimulation (Ekerot and Kano, 1985; Ito, 1984, 1989; Linden and Conner, 1991). Because mossy fiber (CS) and climbing fiber (US) co-activation appeared to be involved in classical eye-blink conditioning, these models of cerebellar plasticity seem to be applicable to this type of learning. Long-lasting excitatory plasticity has also been demonstrated in cerebellar cortex (e.g. Hirano, 1990; Jorntell and Ekerot, 2002) and possible potentiation processes at cerebellar synapses have been considered in models of cerebellar function (e.g. Attwell et al., 2001; Medina and Mauk, 2000). The natural questions that emerge from these observations are, is plasticity in cerebellar cortex necessary for eye-blink classical conditioning? What role does the cerebellar cortex play in conditioning?

LOBULE HVI AND EYE-BLINK CLASSICAL CONDITIONING

Figs. 1 and 2 provide a summary of the basic cerebellar and brainstem circuitry that is critical for eye-blink conditioning. As detailed above, the CS during eye blink conditioning is projected to the cerebellum along mossy fibers that originate in the basilar pontine nuclei. The US is projected to the cerebellum via climbing fibers that originate in the inferior olivary complex. One region of cerebellar cortex that is known to receive overlapping CS and US inputs is Larsell's lobule HVI. Lobule HVI contains Purkinje cells that send axons to the critical region of the IP that is involved in acquisition and performance of CRs.

Anatomical connectivity

There is a solid anatomical basis for overlapping CS and US inputs in lobule HVI. Early studies explored patterns of retrograde degeneration of axonal connections to many areas of cerebellar cortex, including lobule HVI, after brainstem lesions (e.g. Brodal et al., 1975). Yeo et al. (1985c) studied anterograde and retrograde transport of wheat germ–agglutinated horseradish peroxidase after it was injected into lobule HVI. Strong anterograde transport to the IP was observed along with evidence of retrograde transport to many brainstem areas including the pontine nuclei and the inferior olivary complex. In another study, Steinmetz and Sengelaub (1992) infused cholera toxin–conjugated HRP into the IP and observed anterograde transport to the pontine nuclei and inferior olive as well as strong retrograde labeling to lobule HVI.

Issues

There are virtually no disagreements in the field as to whether or not lobule HVI is an area of cerebellar cortex that receives convergent input from pontine mossy fibers and olivary climbing fibers that are thought to carry information about the CS and US during eye-blink conditioning. Together the anatomical studies cited above provide strong support for the idea that the pontine nuclei and inferior olive send projections to the IP and lobule HVI, and that lobule HVI projects axons to the IP—this region therefore qualifies as a potentially important region for encoding eye-blink classical conditioning.

Lesions

Electrolytic or ablation techniques have been used to establish the involvement of lobule HVI in conditioning. McCormick and Thompson (1984a) first observed that lesions of much of the ipsilateral hemisphere of the cerebellar cortex (including HVI) did not abolish the response if the deep cerebellar nuclei were spared. They reported, however, that the cortical lesions affected the timing of the CR in some animals. Conversely, Yeo et al. (1985b) lesioned area HVI after 5 days of training, allowed the animals to recover, then gave them 5 days of additional paired training. They reported that small lesions of HVI abolished or severely disrupted the CRs and that examination of the lesion site revealed sparing of the deep cerebellar nuclei. Animals that showed no post-lesion CR deficits had considerable sparing of area HVI. Based on these data they concluded that any sparing of HVI, especially at the base of the lobule and just above the dentate and interpositus nuclei, left the CR intact. These data suggested that a rather specific region of HVI was important to eye-blink conditioning.

Lavond et al. (1987) trained rabbits using the same conditioning parameters as Yeo et al. (1985a), but extended the number of post-lesion training days from 5 to 10. Under these conditions, a temporary abolition of conditioned responding was observed. A more rapid recovery of CRs was seen when an auditory CS was used than when a light CS was used. And, complete recovery of CRs to both CSs was seen by the tenth day of post-lesion training. Lavond et al. suggested that the additional complexity intrinsic to Yeo's conditioning parameters (e.g. longer ISIs, two CS types, and more trials per training session) may have made the learning more vulnerable to lesion effects. This study was followed by another in which lobule HVI lesions were given before training (Lavond and Steinmetz, 1989). While significant slower acquisition rates were observed with CR amplitudes greatly reduced, almost all rabbits showed the development of CRs when paired CS and US trials were presented.

The involvement of cerebellar cortex during trace conditioning has also been assessed. Trace conditioning is different than standard delay conditioning procedures because a temporal gap (trace) between the termination of the CS and the onset of the US is introduced. Trace conditioning is generally considered to be relatively more complex for the animal than delay conditioning as it is assumed that the animal must use some sort of internal memory mechanism or timing mechanism to successfully perform the task. Woodruff-Pak et al. (1985) trained rabbits

using trace conditioning procedures then lesioned the deep nuclei, the deep nuclei and the cortex (including HVI), or the cortex only (including lobules HVI and HVII). They showed that damage to the IP abolished CRs. Animals that sustained only cortical damage showed a reduction in CRs on the first day of post-lesion training, but CRs reemerged with additional training thus suggesting that an intact cerebellar cortex was not required for the reacquisition of the trace CR.

Issues

Lesions of cerebellar cortex have resulted in a variety of reported effects on eye-blink classical conditioning that range from complete CR abolition to partial effects to little or no effect. It remains a possibility that task complexity and choice of training parameters may determine whether or not cerebellar cortex is important for eye-blink conditioning (e.g. see Lavond and Kanzawa, 2001). For example, the basic paradigm used by Yeo and colleagues involved two CSs (a tone and a light), up to 200 trials a day and relatively long ISIs. CR amplitude instead of percent CRs was used as the dependent measure to characterize conditioning. Other cortical aspiration studies have used single CSs, relatively short ISIs and have usually reported percent CRs as the dependent measure. Also, the ablation lesion method itself could contribute to differences in observed results. Relative to the rather discrete IP, lobule HVI is quite large and documenting the extent and completeness of the lesion, as determined from post hoc histological reconstructions, is quite difficult. Also, lobule HVI is only a few millimeters above the IP, a site that when damaged can produce a loss of conditioned responding. Indeed, placement of a recording electrode alone into the IP has been known to produce enough damage to severely impair CR production. Many of the fibers that enter the IP from brainstem regions do so from above the nucleus and it is therefore possible that damage to the white matter above the nucleus could decrease or eliminate CS and US input to the nucleus and impair learning. Any or all of these factors could contribute to differences in results that have been reported concerning the role of lobule HVI in eye-blink conditioning. At this point, this issue has not been satisfactorily resolved even though a number of other studied have been conducted (Gruart and Yeo, 1995; Harvey et al., 1993; Lavond and Steinmetz, 1989; Woodruff-Pak et al., 1993; Yeo and Hardiman, 1992).

Chemical lesion and inactivation

Several pharmacological agents are available to permanently eliminate neurons in a given brain area (e.g. kainic acid and ibotenic acid) or temporarily inactivate an area (e.g. lidocaine, muscimol, and picrotoxin), and these methods have been used to study the involvement of cerebellar cortex in conditioning. In addition, brain cooling techniques, such as those used by Lavond and his colleagues (Clark et al., 1992; Zhang et al., 1986) have proven useful for temporarily eliminating neuronal activity in a brain region and have also been used. There are some advantages to using these infusion methods. Whereas, permanent chemical lesions destroy neurons in the area of infusion and do little damage to surrounding fibers, ablation methods do not discriminate between cell body and fiber damage. The chemical lesions can be applied selectively to distinct populations of neurons and areas of the brain can be inactivated during the time of the infusion and then reactivated at a different time. Ablation lesions do not discriminate between cells types and are permanent. There are some disadvantages in using chemical inactivation techniques—infusing chemicals into the brain can be difficult to control and it is difficult to measure the extent of spread of the chemicals to ascertain the area that was affected by the infusion.

Infusions of CNQX and muscimol into cerebellar cortex have been used to disturb Purkinje cell activity in lobule HVI. CNQX infusions block excitatory AMPA/kainate receptors, which are numerous on the dendrites of Purkinje cells. It has been reported that CNQX infusions before paired CS–US training prevents learning of eye-blink CRs (Attwell et al., 2001). CNQX administered to well-trained animals reportedly affected CR expression for 10–60 min post-infusion in a dose-dependent manner (Attwell et al., 1999). The region identified by the authors as critical to the learning and performance of the eye-blink CR was the medial portion of rostral lobule HVI, which was the same area of lobule HVI that was reported important for previous aspiration lesion studies. Desensitization of AMPA receptors at parallel fiber–Purkinje cell synapses has been reported to be one cellular mechanism that may be important for some forms of cerebellar LTD. These data would seem to suggest that these receptors (and perhaps the LTD mechanism) may be involved in the acquisition of eye-blink CRs. Alternatively, there are data that indicate that eliminating all excitatory input to Purkinje cells results in a large and sustained increase in Purkinje cell discharge, which would inhibit interpositus activity (e.g. Hausser and Clark, 1997). This could explain the post-trial disruption of conditioning that was observed.

In another study, Yeo and his colleagues reported that lobule HVI may play a role in post-training memory consolidation processes. They infused muscimol into lobule HVI immediately after five daily training sessions ended and observed that eye-blink CRs did not form (Attwell et al., 2002). Post-training muscimol infusions into the deep cerebellar nuclei did not produce this effect. This might not be surprising given differences in the net effect of muscimol in the two brain areas. Muscimol infusions into the deep nuclei prevent or dampen activation of interpositus neurons. Muscimol infusions into the cerebellar cortex prevent or dampen Purkinje cell activation (presumably by activating inhibitory GABAergic basket and stellate cell synapses onto the Purkinje neurons). The loss of the normal tonic inhibitory Purkinje cell influence on deep nuclear cells may result in a hyper-excitable nucleus, which may in some fashion contribute to the disruption of consolidation.

Also, at least one attempt at replicating the Atwell et al. results was not successful. As part of her doctoral dissertation, Christian (2004) trained three groups of rabbits. Two groups received either muscimol or saline infusions

into lobule HVI immediately after each of five training session. The third group received muscimol infusions into the IP immediately after each of five training session. All groups received 5 days of training with no post-session infusions and then a single day of training where muscimol or saline was infused prior to training. She observed that post-training infusions of muscimol into either the cerebellar cortex or the IP failed to cause differential rates of acquisition or differential asymptotic levels of conditioning on infusion or non-infusions days. Muscimol before training blocked CR expression only when infused into the IP. That is, no consolidation effects were observed as reported by Attwell et al.

It should be noted also that other cerebellar cortical cellular mechanisms have been identified as candidates for the consolidation process. For example, Bickford and her colleagues (Cartford et al., 2004) have completed an elegant series of experiments that suggest cerebellar cortical norepinephrine may be important for post-training consolidation of eye-blink conditioning.

Brain recording

Unit recordings have also been used to establish a role for cerebellar cortex in eye-blink conditioning. Berthier and Moore (1986) recorded from lobule HVI Purkinje cells after acquisition of the classically conditioned eye-blink response. Their procedures employed discrimination training, where two CSs were used, a CS+ that was followed by a US and a CS– that was not followed by a US. The most frequent pattern of Purkinje cell discharge that they saw was an increase in firing in anticipation of the CR on CS+ trials. Some Purkinje cells showed decreases in activity during the CS–US interval. The observation of both excitatory and inhibitory units is interesting. As we described above, Purkinje cells only inhibit neurons in the deep cerebellar nuclei. Cells that increased their firing rate in relation to the CR actually inhibit nuclear neurons while cells that decreased their firing rate can potentially increase the excitability of the IP neurons through phasic disinhibition. That is, the inhibitory HVI Purkinje cells would promote CR-related interpositus activity while excitatory lobule HVI Purkinje cells would depress CR-related interpositus activity. Berthier and Moore recorded more cells in lobule HVI that appeared to be involved in CR suppression than in CR promotion.

Other studies have described spiking patterns of Purkinje cells in area HVI during eye-blink conditioning. Gould and Steinmetz (1996) gathered both single- and multiple-unit recording data from cells in area HVI and the anterior IP while animals received forward-paired training, backward-paired training, explicitly unpaired training, or CS-alone presentations. Interpositus cells showed learning-related activity only when forward CS–US pairings were presented. Changes in Purkinje cell activity were much less specific and could be seen after forward pairing, backward pairing, and surprisingly even after unpaired CS–US presentations, although responsiveness seemed significantly greater after forward pairing than after backward pairing. During CS alone extinction training, neurons in HVI continued to show evidence of learning-related activity during extinction that lasted long after interpositus cells had ceased responding to the CS-alone presentations. These data suggest that the cells in these two cerebellar regions do not encode CS and US information in the same manner—while the IP seems to reflect behavioral responding rather precisely, neurons in lobule HVI seem to change their firing rates in response to a variety of stimulus arrangements. In her doctoral dissertation, Christian (2004) managed to hold and record from identified Purkinje cells over the entire course of acquisition of conditioning. Consistent with the recording studies detailed above, she reported that some cells decreased simple spiking as CRs emerged, while other cells showed increased simple spiking during learning. Interestingly, some cells showed biphasic patterns of responses—an increase in simple spiking followed by a decrease—a firing pattern that would promote CR performance in the IP.

Finally, Hesslow and colleagues (Hesslow and Ivarsson, 1994; Jirenhed et al., 2007) have recorded Purkinje cell activity from the C3 zone during classical conditioning in decerebrate ferrets. They observed the gradual acquisition of inhibitory responses in Purkinje cell simple spike firing as paired conditioning trials were given. Further, the inhibition occurred in later portions of the CS period as would be predicted if Purkinje cells are involved in conditioning. They also observed that Purkinje cell response latencies changed as ISIs were altered and that the inhibition eventually decreased with extinction training.

Schreurs and his associates (1991) have used intracellular recording techniques to investigate plasticity mechanisms of lobule HVI Purkinje cells. Schreurs and colleagues (1991) trained rabbits using a tone CS and periorbital shock US. About 24 h after training, they took slices of lobule HVI and made intracellular recordings from Purkinje cell dendrites. They observed a conditioning-specific increase in the excitability of Purkinje cell dendrites indicating that the firing properties of these neurons had changed after training.

Schreurs and Alkon (1993) developed a more reduced preparation and made intradendritic recordings from Purkinje cells obtained from rabbit cerebellar slices. Direct stimulations of parallel fibers and climbing fibers were used to conjunctively activate the Purkinje cells. They observed that stimulation of climbing fibers followed by stimulation of parallel fibers produced significant decreases in Purkinje cell responsiveness as has been reported by many other experimenters who have studied LTD in cerebellar cortex (e.g. Ekerot and Kano, 1985; Ito, 1989; Linden and Conner, 1991). It should be noted that this arrangement of stimulation is essentially a backward conditioning arrangement for eye-blink conditioning (assuming that the US activates climbing fibers and the CS activates parallel fibers via mossy fiber inputs). In other cerebellar slices, relatively low frequency stimulation of mossy fibers was presented before climbing fiber stimulation (which mimics forward CS–US pairing during eye-blink conditioning) and this stimulus arrangement produced a short-term depression effect. Higher frequency stimulation of mossy fibers

produced a more lasting depression. Schreurs and Alkon (1993) concluded that although plasticity at the parallel fiber–Purkinje cell synapse could be obtained, the LTD-like effect was not specific to forward CS–US because depression could also be seen with backward and unpaired CS–US presentations, a result that was similar to what Gould and Steinmetz (1996) observed with extracellular recording techniques. Also, similar to the extracellular recordings of Berthier and Moore (1986) and Gould and Steinmetz (1996), Schreurs and colleagues observed both increases and decreases in the dendritic excitability of Purkinje cells and increases in Purkinje cell excitability were much more prevalent than decreases in excitability.

It should be noted that in the LTD studies by Schreurs and colleagues, GABA activity was blocked by the addition of bicuculline to the bath. Chen and Thompson (1995) studied differences in LTD production in a cerebellar slice preparation conditions when parallel fiber activation preceded climbing fiber activation and when the parallel fiber and climbing fiber systems were activated simultaneously. They showed that under physiological conditions, good LTD was obtained when parallel fiber stimulation preceded climbing fiber stimulation by 250 ms but not when the two fiber systems were activated simultaneously. When GABA was blocked by adding bicuculline to the bath, good LTD was obtained with the simultaneous stimulation.

In total, the extracellular and intracellular recording studies provide solid evidence that neurons in lobule HVI change their firing patterns as a result of CS and US presentations, these changes do not appear to be limited to stimulus conditions that promote CR formation and there appear to be more Purkinje cells that show learning-related increases in firing than decreases in firing.

Cortical–nuclear interactions

Fundamental for advancing our understanding of the role of cerebellar cortex in eye-blink conditioning is knowing how the cerebellar cortex and IP interact during training to produce conditioned responding. Katz and Steinmetz (1997) attempted to explore this issue using a combined infusion and recording approach. Rabbits were conditioned to a criterion before they received infusions of either kainic acid or vehicle into the IP. Single-unit recordings were then taken from Purkinje cells in lobule HVI of the lesioned and control rabbits. Learning-related excitatory and inhibitory patterns of action potentials were still evident in lobule HVI despite the fact that CRs had been abolished by the kainic acid lesion of the IP. At some recording sites, lobule HVI activity in the interpositus-lesioned rabbits appeared to be disorganized in that the firing patterns were mistimed and more units with mixed excitatory and inhibitory patterns of discharge were found. These data demonstrated that the learning-induced plasticity that developed in lobule HVI survived permanent interpositus damage thus suggesting that conditioning-related plasticity in cerebellar cortex is at least maintained somewhat independently of the deep cerebellar nuclei.

Using a similar strategy, Steinmetz and colleagues examined the effects of temporarily inactivating the IP on conditioning-related activity in lobule HVI. In an unpublished study, Steinmetz and colleagues used temporary inactivation methods in an attempt to replicate the findings of the Katz and Steinmetz (1997) study (Baker et al., 2002). Rabbits were trained for 5 days then given an additional 5 days of training during which either muscimol or saline was infused in the IP while lobule HVI recordings were taken. The activity of approximately 140 neurons was examined during acquisition and retention training. During muscimol infusion when CR production was severely impaired, the pattern of unit responses of muscimol-infused rabbits was very similar to the control rabbits and thus similar to what Katz and Steinmetz (1997) reported after permanent kainic acid lesions.

In another study, acquisition of unit responses in lobule HVI was examined while the IP was inactivated with muscimol (Villarreal and Steinmetz, 2005). In this study, rabbits were given training with either muscimol or saline infusions into the IP and then training with no infusions. As expected and consistent with the Krupa et al. (1993) results, no eye-blink CRs were evident in the muscimol animals during the first 5 days of training while the saline animals acquired robust CRs. During the second 5 days of training CR acquisition was seen in the muscimol group while the saline group maintained their asymptotic level of conditioning. Recordings from the control rabbits replicated earlier lobule HVI Purkinje cell recording experiments—largely excitatory learning-related pattern of spiking was seen. In muscimol rabbits, a significant number of Purkinje cells developed CS-period excitation over training even though no CRs were present. Again, the data suggest that eye-blink conditioning-related plasticity in lobule HVI can be established independently of activity in the IP.

In an elegant study, Wada et al. (2007) used genetic techniques to reversibly inactivate the functioning of granule neurons in cerebellar cortex during eye-blink conditioning in mouse. Such inactivation prevented expression of the CR during and after training. These results are in close agreement with earlier studies of *stargazer* and *waggler* mutant mice where absence of BDNF and functional AMPA receptors in cerebellar granule cells resulted in marked impairment of eye-blink conditioning (Qiao et al., 1998; Bao et al., 1998, 1999; Chen et al., 1999). However, in Wada et al. (2007), following removal of granule neuron inactivation, CRs were normal. Lesion of the interpositus in this preparation post-training completely abolished the eye-blink CR. Consequently, interpositus mediated learning of the eye-blink CR can occur with inactivation of cortical granule neurons such that no performance of the CR occurs during inactivation. This result agrees closely with the reversible inactivation studies in rabbit noted above and suggests that the essential eye-blink CR memory trace can be established in the IP even in the absence of functioning of cerebellar cortex.

Issues

The lobule HVI recording experiments have consistently demonstrated that Purkinje cells alter their firing patterns during eye-blink conditioning. However, these studies also

show that the majority of neurons in lobule HVI increase their simple spiking rates as conditioning proceeds, which is opposite of what one would expect if this region is contributing to IP plasticity and CR acquisition and performance; a decrease (or cerebellar LTD) would be expected. It is possible that the learning-related excitatory Purkinje cells are important for within-trial "sculpting" of the CR or in inhibiting other behavioral responses to create the discrete, well-timed eye-blink CR that is normally observed.

Microstimulation

Electrical stimulation delivered to lobule HVI has also been used to study its involvement in conditioning. In a series of studies, Thompson and his colleagues delivered stimulation in the region of HVI in place of peripheral CSs and/or USs. For example, Swain et al. (1992) implanted stimulating electrodes in the white matter beneath lobule HVI and observed movements of facial and neck muscles when electrical stimulation was delivered. Importantly, these movements could be conditioned by pairing a tone CS with the cortical stimulation US. Conditioned movements were also observed when stimulation was delivered to two regions of cerebellar cortex, parallel fibers as a CS and white matter as a US, demonstrating that activation of cortical sites in the region of lobule HVI could produce conditioning (Shinkman et al., 1996). Lesions of the IP abolished the conditioning produced by the tone CS—cerebellar–cortical-stimulation US pairings thus demonstrating the cerebellar dependency of the conditioning (Swain et al., 1999).

ANTERIOR LOBE AND EYE-BLINK CONDITIONING

The majority of work to date on the role of cerebellar cortex in eye-blink classical conditioning has concentrated on the involvement of Larsell's lobule HVI. Relatively recently, another cortical area has received some attention—the anterior lobe. In one of the initial papers on the effects of cortical lesions on eye-blink conditioning, McCormick and Thompson (1984a) reported that damage that included ansiform and paramedian lobules resulted in alteration of the timing and magnitude of the CR. This result was confirmed and extended in a study in which widespread cortical damage decreased CR onset and peak latency (Logan, 1991). Logan reported a graded effect of lesion size on the number if CRs observed but that only the largest lesions disrupted the timing of the CR. The extensive lesions that included the anterior lobe produced smaller-amplitude CRs and reduced onset latencies and peak latencies.

Mauk and colleagues (Perrett et al., 1993) attempted to target the anterior lobe more exclusively and showed a correlation between reductions in CR onset latency, peak latency, and amplitude, and the degree of damage to the anterior lobe. They have since examined the anterior lobe as a principal cortical region involved in the acquisition and performance (including timing) of CRs and have studied acquisition and extinction of CRs to make a case that the anterior lobe is centrally involved in the learning (Perrett and Mauk, 1995; Garcia et al., 1999; Mauk and Buonomano, 2004). Among their findings, they have reported that anterior lobe lesions alter CR timing (i.e. to produce short-latency CRs) and that the lesions could also disrupt post-lesion acquisition to a novel CS (Garcia et al., 1999).

A recent recording study supports the idea that Purkinje cells in the anterior lobe of the cerebellum are critically involved in the acquisition and expression of eye-blink CRs (Green and Steinmetz, 2005). In this study, an ISI discrimination procedure was used to explore the firing patterns of anterior lobe Purkinje cells. The ISI discrimination procedure involves the presentation of high and low frequency tones as two different CSs. The CSs are followed by an air puff US after either a short (250 ms) or long (750 ms) ISI elapses. In this manner, rabbits learn to execute well-timed CRs at two different ISIs that are signaled by two different CSs. After training, recordings from isolated Purkinje cells were made during further paired training with the two CSs. Similar to lobule HVI recordings, CS-, US- and CR-related neurons were found. A variety of firing patterns was seen: some neurons responded selectively on either long- or short-ISI trials while other neurons responded equally to both CSs. Importantly, and different from recordings made in lobule HVI, many more inhibitory Purkinje cells were found than excitatory—about two-thirds of the neurons showed decreases in spiking during the CS–US interval. Further, the excitatory neurons tended to fire early in the CS period while the inhibitory neurons tended to fire later in the CS period, thus generating the within-trial firing pattern that would be expected if this region were somehow modulating the IP during CR expression on a given trial (see also Christian et al., 2004). That is, Purkinje cells appeared to inhibit the nucleus early in the trial period and disinhibit the nucleus later in the trial period. Fig. 5 shows examples of anterior lobe Purkinje cell firings during ISI discrimination training. This pattern of Purkinje cell activity is compatible with the anterior lobe lesion and picrotoxin infusion data where short-latency responses were seen—the absence of Purkinje cell inhibition on the IP early in the trial period could produce short-latencies CRs (e.g. Garcia et al., 1999). These findings suggest that the anterior lobe of the cerebellum is involved in the timing of the eye-blink CR.

GLOBAL DISRUPTION OF CEREBELLAR CORTICAL FUNCTION

The mouse in eye-blink conditioning

Because of the extraordinary advances in genetics, the mouse has become a favored preparation in biology. In the case of basic associative learning, particularly eye-blink conditioning, mutant and transgenic mice have added greatly to our knowledge. Most of the focus in this work has been on the role of the cerebellar cortex, particularly LTD at parallel fiber–Purkinje neuron synapses.

The standard mouse and rat preparations involve recording the EMG from the dorsal aspect of the orbicularis oculi muscles, the muscle controlling closure of the external eyelids (Skelton, 1988; Stanton et al., 1992). Recently,

Fig. 5. Examples of Purkinje cell spiking during training in a rabbit with two different signaled ISIs. (A) Eyeblink (top) and Purkinje cell activity (bottom) recorded on a trial with a 250 ms ISI. (B) Eyeblink (top) and Purkinje cell activity (bottom) recorded on a trial with a 750 ms ISI. The inset shows a Purkinje cell discharge from the spike train with the characteristic complex spike discharge. The horizontal bar under each spike train indicates when the tone CS was on. (Adapted from Green and Steinmetz, 2005.)

confusion has been introduced in the field in a paper by Koekkoek et al. (2002) claiming that the EMG measure of eye blink had problems. They reported that their EMG measure (ventral to the orbit) was contaminated by other muscle actions, e.g. those controlling the upper lip and vibrissa. However, in his original study, Skelton (1988 and personal communication) examined various recording loci from the orbicularis oculi muscle and found that recordings from regions other than dorsal to the orbit (e.g. ventral) were contaminated by adjacent muscle action due to sniffing and jaw movements. Freeman (personal communication) found that when stimulating the cerebellar nuclei, EMG responses dorsal to the orbit occurred only when lid movements were elicited and not during nose twitches, vibrissae, jaw movements, head turns, etc. In short, dorsal recordings are not contaminated at all by other muscle actions (e.g. false positives) but ventral recordings are. The Koekkoek et al. objection to EMG recordings applies only their ventral recordings, not to dorsal recording. The correlation between the dorsal EMG measure and lid (NM) movement ranges from 0.98 to 0.99 (rabbit—McCormick et al., 1982c). Further, concomitant recordings of dorsal orbicularis oculi EMG and NM extension (rabbit) showed that interpositus lesions effective in completely abolishing the conditioned NM response also completely abolished the conditioned EMG response (Lavond et al., 1990).

In a number of genetically altered mouse preparations, cerebellar cortical LTD and standard delay eye-blink conditioning performance have been shown to be correlated—impaired LTD is associated with impaired eye-blink conditioning and normal LTD with normal learning (Aiba et al., 1994; Chen and Thompson, 1995; Ichise et al., 2000; Kishimoto et al., 2001a, 2001b, 2001c; Miyata et al., 2001; Kishimoto and Kano, 2006; Shibuki et al., 1996; Woodruff-Pak et al., 2006). (Interestingly, in several of these studies where LTP and delay eye-blink conditioning were both impaired, trace eye-blink conditioning was not impaired.) In contrast to all the above, Welsh et al. (2005) reported that systemic treatment with a drug that prevents cerebellar cortical LTD (Kimura et al., 2005) does not impair eye-blink conditioning in rat. Actually, they did not record LTD in the animals that were trained. Also, their animals showed very high levels of non-associative EMG response in their unpaired control animals, probably because they used a 10 kHz tone.

Mutant mice have been used to explore the role of cerebellar cortex in eye-blink conditioning. Chen et al. (1996), for example, studied the acquisition of eye-blink conditioning in mutant *pcd* mice, which show degeneration of Purkinje cells about 2 weeks postnatally. In essence, elimination of the Purkinje cells, which are the sole output neurons of the cerebellar cortex, eliminates the cerebellar cortex itself. The *pcd* mice showed significant deficits in both the rate of acquisition and the asymptotic level of conditioning, but did express some levels of learning and extinction in the absence of cortical input to the interpositus. The peak latencies of the CRs in the mutants were somewhat decreased compared to wild-type mice. In a later study, bilateral lesions of the IP in *pcd* mice were shown to block CR acquisition thus demonstrating the critical role for the deep nuclei in learning (Chen et al., 1999).

Pharmacological manipulations

Other studies have explored the effects of more global disruption of cerebellar cortex on eye-blink conditioning using pharmacological manipulations. Nolan and Freeman

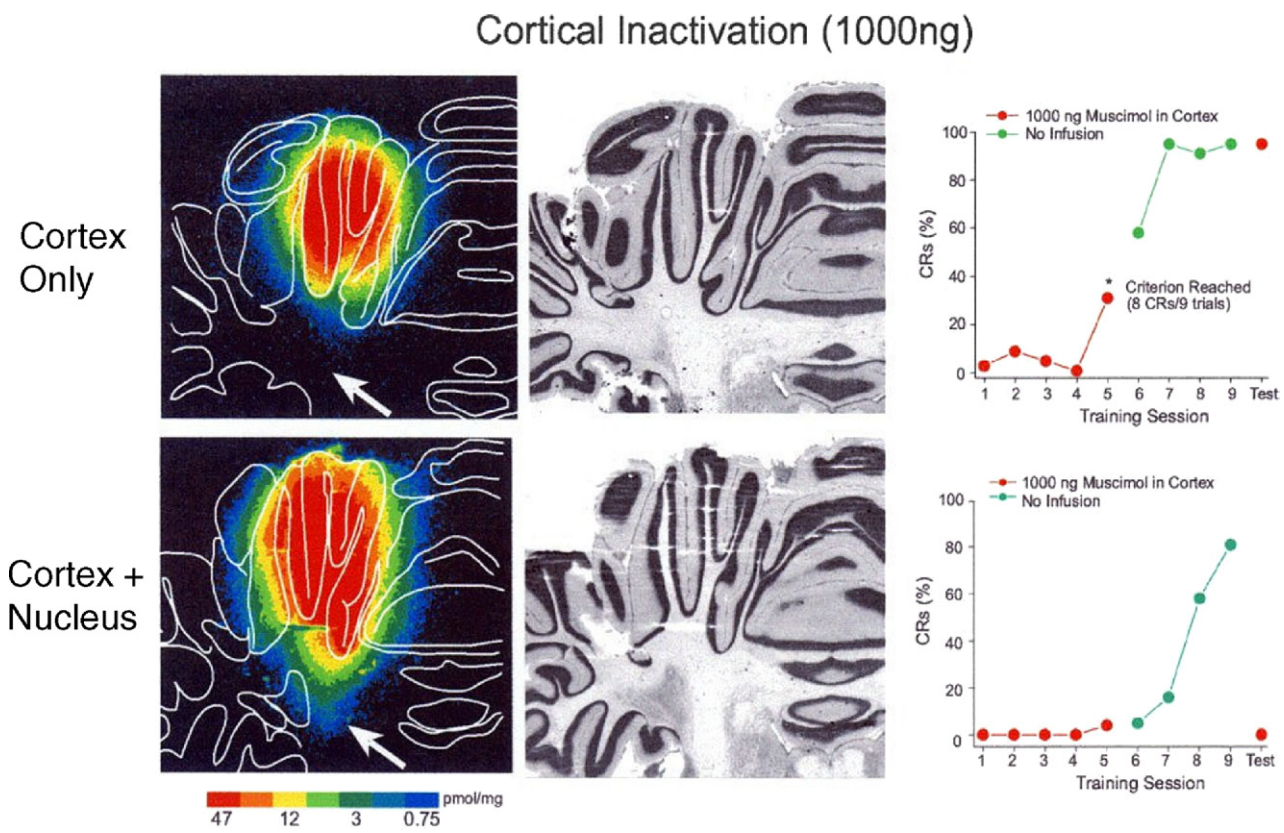

Fig. 6. The panels on the right of the diagram show conditioning functions for a rabbit that received muscimol infusions during training that were largely restricted to the cerebellar cortex (top) and for a rabbit that received muscimol infusions during training that affected the IP as well as cerebellar cortex (bottom). Infusions that did not include the IP caused a significant slowing of learning (sessions 1–5), but CRs were acquired rapidly when muscimol infusions were discontinued (session 6–9). Infusions that included both the IP and cerebellar cortex completely prevented learning (sessions 1–5) and no savings of training was seen in this animal when infusions were discontinued and additional paired training was given (sessions 6–9). The panel on the left shows the extent of the infusions as revealed by radiolabeled muscimol infusions for animals whose behavioral data are shown in the right panel. The middle panels show coronal brain sections that are represented by the radiolabeled schematics in the left panels (adapted from Krupa, 1993, unpublished dissertation).

(2005) intraventricularly infused the immunotoxin OX7-saporin after rats had acquired the conditioned eye-blink response. The OX7-saporin selectively destroys Purkinje cells in cerebellar cortex. They showed that while the reacquisition of the CR was significantly impaired, the rats could show learned inhibitory responses when conditioned inhibition training was given after infusion thus suggesting differential roles for cerebellar cortex in excitatory and inhibitory learning.

As part of his doctoral dissertation, Krupa (1993) examined the effects of muscimol infusions into cerebellar cortex. The results of these experiments are summarized in Fig. 6. He showed that when muscimol infusions were confined mainly to cerebellar cortex, eye-blink conditioning was significantly slowed, but not abolished. When the muscimol infusions invaded the IP as well as cerebellar cortex, CR acquisition was prevented. These data are in agreement with several studies cited above that indicate a major but not essential role for cerebellar cortex in CR acquisition and performance and again provides support for the essential role of the IP in eye-blink conditioning.

Infusions of picrotoxin, a GABA antagonist, into the deep cerebellar nuclei have also been used to study the involvement of cortex in conditioning. Garcia and Mauk (1998) reported that picrotoxin injections into the anterior IP resulted in short-latency, reduced-amplitude CRs that occurred as frequently as control animals. Other studies have reported that picrotoxin infusions into the interpositus block the expression of CRs completely (Mamounas et al., 1987; Bao et al., 2002). Bao et al. (2002) used sequential infusions of muscimol and picrotoxin coupled with conditioning. In this procedure, muscimol is used first to block the synaptic transmission from the cortex and the baseline level of excitability in the interpositus neurons is restored through the subsequent application of picrotoxin (Bao et al., 2002). After this procedure, onset and peak latencies were decreased in well-trained animals, but CR amplitudes were increased. Finally, the global involvement of cerebellar cortex in eye-blink conditioning has also been studied during an ISI switching task during picrotoxin infusions (Vogel et al., 2009). In this study, rabbits were initially trained with either a short (250 ms) or long (750 ms) ISI after picrotoxin or saline infusions into the IP. After initial training, the ISI was switched and training under picrotoxin or saline continued. The results showed little if any disruption of behavioral responding during training with the short

ISI. Picrotoxin disrupted conditioning, however, when the long ISI was used thus suggesting that cerebellar cortex is increasingly engaged and important when non-optimal ISI are used (i.e. ISI of greater than 200–500 ms). However, response timing did not seem to be affected as the animals switch ISI. Short-latency CRs similar to those reported by Mauk and colleagues were seen in a few rabbits, but most showed normally timed CRs when they were executed.

Issues

Compared to studies of the involvement of lobule HVI in eye-blink conditioning, relatively few studies have examined the role of the anterior lobe in eye-blink conditioning. Nonetheless, this region appears to receive convergent CS and US input and to project to the anterior IP (Green and Steinmetz, 2005). That is, the region contains the anatomical substrates necessary for its involvement in eye-blink conditioning. Purkinje cells in the anterior lobe show learning-related discharges and the observed firing patterns are consistent with what is expected—most Purkinje cells show inhibition of simple spiking and when considered as a population seem to excite and inhibit in a manner that would suggest they are involved in CR production and timing. Further studies will be needed to further define the role of the anterior lobe in conditioning and how it might be related to IP function and lobule HVI function.

Overall, in contrast to what we know about the role of the IP in eye-blink conditioning, it appears that much more work needs to be done to establish a clear role for the cerebellar cortex in eye-blink conditioning. Existing data clearly show that the structure is somewhat widely engaged. It is unclear, however, what features of conditioning are being encoded during this engagement.

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