Annu. Rev. Neurosci. 1994. 17:519–549

ORGANIZATION OF MEMORY TRACES IN THE MAMMALIAN BRAIN

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KEY WORDS: cerebellum, learning and memory, eyeblink conditioning, memory localization, classical conditioning

INTRODUCTION

Perhaps the most fundamental issue in the broad field of neuronal substrates of learning and memory concerns the physical/biological mechanisms underlying long-term memory formation, storage, and retrieval in the mammalian brain. Considerable progress has been made in elucidating memory storage mechanisms in simpler invertebrate systems (Abrams et al 1991, Bergold et al 1990, Clark & Schuman 1991, Crow & Forrester 1990, Nelson & Alkon 1990), but similar advances have yet to be made in understanding the mammalian brain. As Lashley (1929) stressed so many years ago, the overriding problem for understanding memory mechanisms in the mammalian brain is localization of memory storage. Mechanisms of memory storage cannot be analyzed until the memory storage sites, whether localized or distributed, have been identified. Given the nature of the vertebrate brain, memories should, to some degree, be distributed over ensembles of neurons, but whether this is within a localized region, over several regions in a given structure, or over several structures, is not yet known for most forms of memory.

Once the locus, or loci, of a given form of long-term memory has been determined, we should be able to analyze the mechanisms of memory storage. These will likely involve chemical/structural changes at synapses and possibly long-lasting changes in gene expression in the relevant neurons. Determining these mechanisms, however, will not inform us of what the memory is; the content of a neuronal memory store can only be determined by a detailed characterization of the neural networks that subserve the memory.

We now know that different forms or aspects of memory critically involve different neural systems in the brain. The hippocampus plays a key role in relational, contextual, spatial, and olfactory learning in lower mammals (Becker et al 1980, Berger & Orr 1983, Eichenbaum et al 1986, Lynch 1986, Moyer et al 1990, O'Keefe & Nadel 1978, Ross et al 1984, Solomon et al 1986b, Squire 1992). In monkeys, the hippocampus and adjacent cortex, particularly the perirhinal, parahippocampal, and entorhinal areas, are critical for delayed non-matching to sample and for establishing (and/or retrieving) long-term visual memories, which are often termed declarative or working, but do not appear to be the site of long-term storage (Meunier et al 1993, Squire 1992, Zola-Morgan & Squire 1990). The cerebral cortex is the proposed repository of long-term declarative memories, but conclusive evidence supporting this belief is lacking. The amygdala plays a key role in initial learning of conditioned heart rate and blood pressure, conditioned potentiation of startle, conditioned freezing to tone, and instrumental avoidance (Hitchcock & Davis 1986, Iwata et al 1986, Fanselow et al 1991, Liang et al 1982); however, it may not be the site of long-term storage (see Lavond et al 1993, McGaugh 1989). [Interestingly, the hippocampus is critical for initial learned freezing to context (Kim & Fanselow 1992).] Recent evidence suggests that the basal ganglia may play an important role in some aspects of instrumental learning (Packard et al 1989). A region of the cerebellum is necessary for basic delay classical conditioning of discrete motor responses (see below). On the other hand, it is possible that all these memory systems become engaged, to varying degrees, in multiple aspects of learning, with each system playing its separate role (see Lavond et al 1993, Wagner & Brandon 1989).

With the possible exception of declarative or working memory, all these aspects of learning exhibit similar basic parametric features (e.g. massed vs spaced practice, temporal relations of stimuli, stimulus salience, etc), which suggests that at some level common mechanisms are involved. As Rescorla stressed (1988a,b), basic associative learning, which results from exposure to relations among events in the world, is the way organisms, including humans, learn about causal relationships in the world. For both modern Pavlovian and cognitive views of learning and memory, the individual learns a representation of the causal structure of the world and, as a result of experience, then adjusts this representation to bring it in tune with the real causal structure of the world, thus striving to reduce any discrepancies or errors between its internal representation and external reality (see also Dudai 1989, Squire 1987).

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THE ISSUE OF LOCALIZATION

Permanent lesions that abolish a learned behavior (i.e. the behavioral expression of the memory) but do not interfere with the ability of the organism to otherwise perform the behavior can serve to identify the brain structures necessary for a given form of memory but cannot illuminate precisely what roles these structures play in memory storage. Recording of neuronal activity that changes in tight correlation with learning can identify structures involved in or influenced by formation of the memory. Such evidence, per se, cannot localize the site(s) of memory formation. But at the very least the locus of memory storage must in some way receive information about the conditioned or signal stimulus and the unconditioned or reinforcing stimulus and/or response and must exhibit learning-related changes in activity. Hence, electrophysiological evidence can serve to both identify putative sites of storage and rule out possible storage sites. Electrical microstimulation, substituting for peripheral stimulation, can help to identify the neuronal circuitry sufficient for a given form of learning. Anatomical and biochemical changes, [in number or microstructure of synapses, in receptor number or affinity, in gene expression, in activation (e.g. cytochrome oxidase), etc] can indicate persisting and localized changes. However, unlike electrophysiological activity, these changes cannot be directly related temporally to learning-induced changes in behavior. Further, unless the locus of the synaptic plasticity coding a memory trace has been determined, as in certain invertebrate preparations, it is difficult to distinguish between changes in neuron activity, per se, and memory storage. Collectively, these methods can serve to identify an essential (necessary and sufficient) memory circuit, but they cannot provide definitive evidence to identify the locus of memory storage. Note that identification of the neural circuitry essential for a given form of learning and memory is also necessary to characterize the content of the memory store.

The methods of reversible inactivation offer promise of localizing sites of memory storage. The logic is straightforward, given that the essential circuit for a given form of memory has been identified. If naive animals are trained during reversible inactivation of a structure or region that is part of the essential circuit, the animals will, by definition, show no expression of learning during inactivation training. If they show no evidence of having learned in post-inactivation training, and learn with no savings as though naive, then the inactivated region is either the site of memory storage or a mandatory afferent to the site of memory storage. If, on the other hand, the animals show asymptotic learned performance from the beginning of postinactivation training, the inactivated region cannot be the site of the memory storage and is efferent from the site. The same logic would seem to apply if the memory trace is distributed over several regions. Degree of savings in post-inactivation training as a function of regions inactivated, compared to non-inactivated controls, can assess the relative contribution of each component of a distributed memory representation.

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CLASSICAL CONDITIONING OF DISCRETE RESPONSES

The specific focus of this review is on identification of the essential memory trace circuit and localization of the memory trace within that circuit for a basic form of long-term associative memory, basic delay classical conditioning of discrete behavioral responses. Eyeblink conditioning is the most widely used procedure, but classical conditioning of other discrete responses, particularly limb flexion, provides additional evidence. Perhaps the simplest descriptor of these types of basic associative learning is sensory-motor memory. We will argue that the evidence now strongly favors the hypothesis that the essential memory trace circuit includes the cerebellum and its associated brain stem circuitry and that the memory traces are formed and stored in the cerebellum. For reasons that are not entirely clear, this has been an extremely contentious field. Several researchers appear to have an unshakable a priori belief that associative memory traces cannot possibly be formed and stored in the cerebellum (Bloedel 1992, Welsh & Harvey 1989).[1]

Dating from the classic papers of Brindley (1964), Marr (1969), and Albus (1971), the cerebellum has long been a favored structure for modeling a neuronal learning system. Recent empirical studies reviewed below have been guided by these models and the related views of Eccles et al (1967), Eccles (1977), and Ito (1972, 1984), and results to date constitute a remarkable verification of the spirit of these earlier theories. The highly simplified schematic block diagram of Figure 1 summarizes results to date and may be considered a qualitative model of the role of the cerebellum in classical conditioning of discrete behavioral responses (see also reviews by Lavond et al 1993, Steinmetz et al 1992, Thach et al 1992, Thompson 1986, 1990). Earlier Russian research indicated that removal of the cerebellum impaired or abolished classical conditioning of the leg-flexion response (Karamian et al 1969, see also discussion in Clark et al 1984 and Thompson et al 1983); however, these observations were not pursued.

Based on recent anatomical findings, patterns of neural discharge related to specific behaviors, and the effects of focal lesions, Thach et al (1992) have recently proposed a model of cerebellar function that suggests that the job of

[1]For an interesting example of how extreme this bias can be, see the recent chapter by Welsh & Harvey (1992) in which most of the directly relevant evidence is not considered or even cited.

the cerebellum is, among other things, to coordinate elements of movement in its downstream targets and to adjust old movement synergies while learning new ones. Although an in-depth discussion of this literature is beyond the scope of this review, a brief mention of this work is warranted, especially as it pertains to motor learning. The model was inspired, in part, by recent findings that there is a somatotopic mapping of body parts and modes of motor control within the cerebellar deep nuclei (Asanuma et al 1983, Kane et al 1988, Thach et al 1982). This fact, coupled with recent assessments of a much longer length for the cerebellar parallel fibers than previously thought existed (Brand et al 1976, Mugnaini 1983), suggested that beams of Purkinje cells connected by the longer parallel fibers could link actions of different body parts represented within each nucleus and exert control across nuclei into coordinated multijointed movements. This hypothesis is supported, in part, by the finding that reversible or permanent lesions of deep cerebellar nuclei selectively and severely disrupt tasks requiring coordinated activation of multi-jointed movements while having little or no effect upon previously trained single-joint movements (Kane et al 1988, 1989). Although these lesion studies suggest only a minor role for the cerebellum in single-jointed movements, recordings from the deep nuclei during these tasks find marked changes in neural discharge correlated with the parameters of movement (Scheiber & Thach 1985, Thach 1968, 1970). It appears, however, that while performing the single-joint movements, the monkeys also moved many more muscles than those minimally required for the task, and the recorded-unit activity may have been better correlated with the covert multijointed movements than with the single-jointed task (Thach et al 1992, p. 420).

The model Thach et al proposed suggests the cerebellum is involved not only in coordinating multijointed tasks, but that it also learns new tasks through activity-dependent modification of parallel-fiber Purkinje cell synapses (Thach et al 1992, Gilbert & Thach 1977). Evidence in support of this hypothesis includes Purkinje cell recordings, which reveal patterns of activity consistent with a causal involvement in the modification of the coordination between eye position and hand/arm movement (Keating & Thach 1990) and studies in which focal lesions by microinjection of muscimol severely impair adaptation of hand/eye coordination without affecting the performance of the task (Keating & Thach 1991). The implications of this model for eyeblink conditioning are intriguing since, as noted below, the conditioned response is a highly coordinated activation of several muscle groups.

The cerebellum is believed to be critically involved in a number of other learned behaviors. Supple & Leaton (1990a,b) have recently shown that the cerebellar vermis is necessary for classical conditioning of heart rate in both restrained and freely moving rats. A growing body of literature, which is beyond the scope of this review, implicates the cerebellum as critical for a range of instrumental tasks in animals: avoidance learning of the eyeblink in rabbits (Polenchar et al 1985); avoidance but not appetitive learning of bar press in rats (Steinmetz et al 1993); and Morris water maze (invisible platform, i.e. place learning) in mutant pcd (Purkinje cell degeneration) mice (Goodlett et al 1992) and in mutant staggerer mice (Purkinje, granule and inferior olive neuron degeneration) (Lalonde 1987) (see Lalonde & Botez 1990 for review). There is also an increasing amount of research on the human cerebellum that indicates critical cerebellar involvement in complex learning/cognitive tasks (Akshoomoff & Courchesne 1992, Canavan & Homberg 1991, Daum et al 1991, Fiez et al 1992, Grafman et al 1992, Ingvar 1990, Petersen et al 1989; see Leiner et al 1989, Schmahmann 1991, for reviews).

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The Nature of the Eyeblink Conditioned Response

Gormezano et al (1962) showed some years ago in separate studies of the rabbit that eyeball retraction, nictitating membrane (NM) extension, and external eyelid closure all had essentially identical acquisition functions (Deaux & Gormezano 1963, Schneiderman et al 1962). Simultaneous recording of NM extension and external eyelid closure (EMG recordings from *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). Furthermore, some degree of conditioned contraction of facial and neck musculature also developed and was also strongly correlated with NM extension. These observations led to characterization of the conditioned response (CR) as a "synchronous facial 'flinch' centered about closure of the eyelids and extension of the NM" (McCormick et al 1982c, p. 773). 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 & Moore 1983, Cegavske et al 1979, Disterhoft et al 1985, McCormick et al 1983). These are all components of the same global conditioned response 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 conditioned response. The suggestion that different motor nuclei might somehow exhibit different conditioned responses in the eyeblink conditioning paradigm (Delgado-Garcia et al 1990) is not supported by evidence.

The CR and the unconditioned response (UR) are similar in eyeblink 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 conditioning stimulus (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 airpuff unconditioned stimulus (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 (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 & Gormezano 1971, Millenson et al 1977, Schneiderman 1966, Steinmetz 1990a). This key property of the CR cannot be derived from the properties of the US or the UR. The CR and the UR also differ in that certain components of the UR can be elicited separately by appropriate peripheral stimuli but the CR always occurs as a global coordinated response (McCormick et al 1982c). 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 1992).

In sum, the conditioned eyeblink 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 interpositus nucleus, which is effective in completely and permanently abolishing the conditioned NM extension response (see below), also completely and permanently abolishes all other components of the conditioned response 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 1992).

Interpositus Nucleus and the CR Pathway

Recordings of neuronal unit activity from the interpositus nucleus during eyeblink conditioning revealed populations of cells that discharged when the conditioning stimuli (CS and US) were presented, and, more importantly, populations of cells that, as a result of training, discharged just prior to execution of the classically conditioned response (Berthier & Moore 1990, Foy et al 1984, McCormick & Thompson 1984a,b; McCormick et al 1981, 1982a). These cells fired in a pattern that was similar to the learned behavioral response, i.e. they formed an amplitude-time course "model" of the learned response that preceded and predicted the occurrence of the behavioral conditioned response within trials and over the trials of training. Recently, Yang & 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 in 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 US in cells in the interpositus nucleus and depression of response to the US in cells in the dentate nucleus. In other animals, small lesions of the region of recording in the anterior interpositus nucleus completely prevented acquisition of the eyeblink CR but lesions of the dentate recording region had no effect at all on learning (see below).

McCormick et al (1981), using a tone CS and corneal airpuff US in the basic delay paradigm (CS 350 ms; US 100 ms; coterminating), initially reported that lesions of the cerebellum ipsilateral to the trained eye (large aspirations and electrolytic lesions of the interpositus nucleus) abolished the eyeblink conditioned response (CR) completely and selectively, i.e. the CR was completely abolished, but the lesion had no effect on the unconditioned response (see also McCormick et al 1982a). The lesions did not prevent learning in the contralateral eye. If the lesion was made before training, animals were completely unable to learn any CRs at all with the eye ipsilateral to the lesion (Lincoln et al 1982). In other studies the same results were obtained with lesions of the superior cerebellar peduncle, the efferent pathway from interpositus to red nucleus (Lavond et al 1981, McCormick et al 1982b, Rosenfield et al 1985).

Electrolytic lesions of the interpositus nucleus ipsilateral to the trained eye again demonstrated that if the lesions completely destroyed the critical region of the interpositus nucleus the CR was abolished, with no effect on the UR (Clark et al 1984). Importantly, if the lesions were incomplete, there was a marked decrease in the amplitude and frequency of occurrence of the CR and a marked increase in CR onset latency that did not recover with post-operative training (see also Welsh & Harvey 1989). Since electrolytic lesions of the interpositus cause retrograde degeneration in the inferior olive, kainic acid lesions of the critical region of the interpositus were made, with identical results, i.e. complete and selective abolition of the CR (Lavond et al 1985). Yeo and associates (1985a) replicated the interpositus lesion result, using light and white noise conditioned stimuli (CSs) and a periorbital shock US, thus extending the generality of the findings. A number of subsequent studies showed identical effects of interpositus lesions, i.e. complete and selective abolition of the ipsilateral eyeblink CR with no effect on the UR (Lavond et al 1984a,b; Lavond et al 1987, McCormick & Thompson 1984a, Polenchar et al 1985, Sears & Steinmetz 1990, Steinmetz et al 1991, Weisz & LoTurco 1988, Woodruff-Pak et al 1985). This effect was extremely localized. Electrolytic interpositus lesioned animals were periodically trained for periods up to 8 months; no CRs ever developed on the side of the lesion (Lavond et al 1984b). Reversible inactivation by microinfusion of nanomolar amounts of

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neurotransmitter antagonists in the critical region of the interpositus completely and reversibly abolished the CR, in a dose-dependent fashion, with no effect at all on the UR (Mamounas et al 1987).

Recently, the interpositus lesion abolition of the CR was studied in great detail (Steinmetz et al 1992). Appropriate interpositus lesions completely and permanently prevent acquisition and completely and permanently abolish retention of the eyeblink CR, over all conditions of training and measurements that have been used, and have no persisting effects on performance of the UR, regardless of the amount of pre- or postoperative training (see also Steinmetz & Steinmetz 1991). Finally, there are now several human studies showing that appropriate cerebellar damage completely prevents learning of the eyeblink CR (Daum et al 1993, Lye et al 1988, Solomon et al 1989).

Electrical microstimulation of the critical region of the anterior interpositus nucleus evokes an eyeblink response in naive animals, and lesion of the superior cerebellar peduncle abolishes this response; the eyeblink circuit is hard-wired from interpositus nucleus to behavior (McCormick & Thompson 1984a). If the stimulus intensity is set to elicit an eyeblink comparable in amplitude to that elicited by the standard 3 psi corneal airpuff and this interpositus stimulus is used as a US, neither learning of the CR to a tone CS nor maintenance of a CR previously learned to tone with a corneal airpuff US occurs. However, animals given tone-interpositus stimulation training show marked transfer in subsequent tone-airpuff training (Chapman et al 1988).

The region of the contralateral magnocellular red nucleus that receives projection from the region of the anterior interpositus critical for eyeblink conditioning also exhibits a learning-induced pattern of increased unit activity in eyeblink conditioning very similar to that shown by interpositus neurons (Chapman et al 1990). Microstimulation of this region of the red nucleus in naive animals also elicits eyeblink 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-airpuff training occurs. Further, there is no transfer from tone-red nucleus training to subsequent tone-airpuff training (Chapman et al 1988). If the red nucleus is reversibly inactivated in trained animals, the eyeblink CR is reversibly abolished but the learning-induced neuronal model of the CR in the interpositus nucleus is unaffected. In contrast, when the anterior interpositus nucleus 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 & 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 & Moore 1983). This same lesion also abolishes the eyeblink response elicited in untrained animals by stimulating the interpositus nucleus. Microinfusions of nanomolar amounts of neurotransmitter antagonists in a very localized region of the magnocellular red nucleus reversibly abolished the conditioned eyeblink response with no effect on the UR (Haley et al 1988). Identical results were obtained for the conditioned limb flexion response; appropriate interpositus lesions abolished the conditioned hindlimb flexion response with no effect on reflex flexion in the rabbit (Donegan et al 1983). 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).

The Issue of Performance

One of the advantages of classical conditioning for neurobiological analysis is that performance of the behavioral response, per se (the UR), can be measured independently of the performance of the learned response (the CR). A consistent finding of the many studies showing interpositus lesion abolition of the CR is that the lesion has no effect on the UR (see above). Welsh & Harvey (1989) claimed that interpositus lesions did affect the UR. However, they did not in fact measure the effects of interpositus lesions on reflex responses in the same animals; instead they compared only some of their postlesion animals separated post hoc into different groups. Importantly, they did not find any effect of interpositus lesions on amplitude of the UR at any US intensity. They reported only very small lesion effects on UR topography at low US intensities. Many factors can influence the UR and there are extreme individual animal differences in properties of the UR. In order to demonstrate effects of lesions on the UR it is essential to compare URs in the same animals before and after lesion. Given the fact that the evidence necessary to demonstrate interpositus lesion effects on performance of the UR does not exist, it is somewhat surprising that the performance argument could have been taken seriously. In any event, possible effects of interpositus lesions (that abolished the CR) on the UR to US alone stimuli were examined in detail over a wide range of US intensities, comparing pre- vs postlesion URs in the same animals. No persisting effects of the lesions were found on any property of the UR (Steinmetz et al 1992).

Welsh & Harvey (1989) asserted that, "when one attempts to equate the CS and the UCS [unconditioned stimulus] as response-eliciting stimuli, the deficits in the CR and the UCR [unconditioned response] become more alike" (p. 309). The only way to evaluate this statement is to equate the CS and the US in terms of response elicitation prior to lesion and then determine the effect of the lesion on the two responses that were "psychophysically

equivalent" prior to lesion. Welsh & Harvey did not make this comparison and did not provide any information on the properties of the URs prior to lesion in their animals. When this is done, e.g. when the intensity of the US is reduced so that the UR (US alone trials) is matched in amplitude and percent response to the CR before lesion, the interpositus lesion abolishes the CR and has no effect at all on the prelesion equivalent UR (Steinmetz et al 1992). All these studies used a standard US intensity for training (e.g. $\geq$3 psi), which typically yields a UR (US alone trials) larger in amplitude than the CR. Recently, animals were trained with a low intensity US just suprathreshold to establish learning (Ivkovich et al 1993). Under these conditions the CR (CS alone trials) and the UR (US alone trials) are equivalent in amplitude (the CR is numerically but not statistically larger than the UR). Interpositus lesions completely abolished the CR and had no effect at all on the UR.

There is in fact a double dissociation in terms of various brain lesion effects on the CR and the UR in eyeblink conditioning. Appropriate partial lesions of the motor nuclei involved in generating the CR and the UR cause immediate abolition of both the CR and the UR; however, with post-operative training the CR recovers almost to the pre-operative level but the UR shows little recovery (Disterhoft et al 1985, Steinmetz et al 1992). Large lesions of appropriate regions of cerebellar cortex that markedly impair or abolish performance of the CR (see below) result in an increase in the amplitude of the UR (Logan 1991, Yeo 1991).

Collectively, this evidence decisively rules out the "performance" argument that interpositus lesion abolition of the CR is somehow due to lesion effects on the UR.

The CS Pathway

The pontine nuclei receive projections from auditory, visual, and somatosensory systems (Brodal 1981, Mower et al 1979). Several regions of the pontine nuclei exhibit short latency evoked unit responses to auditory stimuli (Aitkin & Boyd 1978, Steinmetz et al 1987). 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 (interpositus lesions abolish the CR to all modalities of CS) (Steinmetz et al 1987). Lesions of the regions of the pons receiving projections from the auditory cortex abolish the CR established with electrical stimulation of auditory cortex as a CS (Knowlton & Thompson 1992). Infusion of lidocaine into the pontine nuclei in trained animals reversibly attenuates the CR (tone CS) (Knowlton & Thompson 1988). 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 (Steinmetz et al 1986). With a pontine stimulation CS, lesion of the middle cerebellar peduncle abolishes the CR, thus ruling out the possibility that the pontine CS is activating noncerebellar pathways, e.g. by stimulation of fibers of passage or antidromic activation of sensory afferents (Solomon et al 1986a). Stimulation of the middle cerebellar peduncle itself is an effective CS and lesion of the interpositus nucleus 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 airpuff us), some animals show immediate and complete transfer of the behavioral eyeblink CR and the learning-induced neuronal model of the behavioral CR in the interpositus nucleus to a tone CS. These results suggest that the pontine stimulus and tone must activate a large number of memory circuit elements (neurons) in common (Steinmetz 1990b).

Electrical stimulation of the pontine nuclei can elicit a variety of movements, depending on electrode location, but at rather high thresholds. When such stimulation well below movement threshold is used as a CS (corneal airpuff us), the eyeblink CR develops to the CS and the threshold CS to elicit the eyeblink CR becomes very low, orders of magnitude lower than the threshold for the movement (e.g. head turn) elicited before training (Tracy et al 1992). This is true regardless of the nature of the high threshold pontine-elicited movement before training. Extinction training results in a return to the high threshold movement. The eyeblink threshold to stimulation of the critical region of the interpositus nucleus does not change at all over the course of such training and extinction. Very small lesions via the interpositus stimulating electrode abolish the CR to the pontine stimulation CS. Thus, learning results in a marked increase in synaptic efficacy in the cerebellar circuit between the pontine and interpositus electrodes.

These results have interesting implications for the readout from higher brain structures to specific learned behaviors. Stimulation of any region of the pontine nuclei can serve as an effective CS for eyeblink conditioning. The pontine nuclei receive input from virtually all areas of the cerebral cortex (Brodal 1981) and from the hippocampus via the retrosplenial cortex (Berger & Bassett 1992). Thus, stimulation of the auditory cortex as a CS in eyeblink conditioning relays through the pontine nuclei to the cerebellum (Knowlton & Thompson 1992; 1993). The organization of the pontine-mossy fiber projections to the cerebellum is such that activation of virtually any subset of pontine neurons (so long as a minimum number are activated) can become associated with any specific response to be learned. The cerebellum appears able to translate distributed input via the pontine-mossy fibers into a behavioral response that is completely specific to the local sign of the US-UR. The latter is determined, we and others have suggested (see below), by the US-activated

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climbing fiber projections to the cerebellum (see Marr 1969). This view implies that the convergence of mossy-parallel fibers and climbing fibers is such that virtually any subset of mossy fibers can converge to virtually any subset of climbing fibers, i.e. those activated by a particular aversive US. This convergence may be due in part to the complex, patchy fractured sensory representation in cerebellar cortex (Welker 1987).

Collectively, these data strongly support the view that the essential CS pathway includes the pontine nuclei and mossy fiber projections to the cerebellum.

The US Pathway

Neuronal unit activity recorded in the critical region of the dorsal accessory olive (DAO, see below) exhibits no responses at all to the tone CS and a clear evoked increase in unit activity to the onset of the corneal airpuff US prior to training, which supports the argument that the memory trace is not in the DAO. Interestingly, this US-evoked neuronal activity decreases as animals learn and perform the CR but is still fully present on US alone trials (Sears & Steinmetz 1991), as is true for complex spikes evoked in Purkinje neurons by US onset (see below).

Electrolytic or chemical lesions of the critical region of the inferior olive, which is the face representation in the dorsal accessory olive (DAO), completely prevented learning of the conditioned eyeblink response if made before training and yielded extinction with continued training and hence abolition of the CR if made after training (McCormick et al 1985, Mintz et al 1988; see also Yeo et al 1986). The lesion had no effect at all on the UR. Voneida et al (1990) made chemical lesions of the DAO in cats trained in limb flexion conditioning and found extinction of the CR with continued training. All studies agree that the DAO is necessary for both learning and retention of the CR.

Electrical microstimulation of this region of the DAO elicits eyeblink 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 is to 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 (2DG) 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. Fifth, DAO stimulus intensities below movement elicitation threshold can serve as an effective US. Sixth, extensive lesions of the reticular formation just dorsal to the DAO (where low threshold movements can be elicited by stimulation, but such stimulation is not an effective US) have no effect on the CR (Swain 1992). Finally, movements (URs) elicited by electrical stimulation of cerebellar cortex and white matter (US) can also be conditioned to a tone CS, and interpositus lesions abolish both the CR and the cerebellar elicited UR (Swain et al 1992, Swain 1992). So far as can be determined, the DAO-climbing fiber-cerebellar circuit is the only system other than reflex afferents in the brain where discrete specific movements elicited by stimulation can be conditioned to neutral stimuli. These data suggest that this is a system specialized for the learning of discrete movements.

It should be noted that when peripheral stimuli are presented, e.g. tone CS and corneal airpuff US, the CS may evoke some climbing fiber activity and the US will certainly evoke converging climbing fiber and mossy fiber activity to localized regions of the cerebellum. This converging activity may play an important role in learning with peripheral USs but does not appear essential, i.e. with direct stimulation of climbing fibers as a US. Indeed, normal behavioral learning occurs when electrical stimulation of pontine nuclei, which are mossy fibers, is used as a CS and electrical stimulation of the DAO–climbing fibers is used as a US; whatever behavioral response is evoked by the DAO stimulation US, this response is learned to the pontine nuclei CS, which itself does not elicit movements before training (Steinmetz et al 1989).

Collectively, this evidence strongly supports the hypothesis that the DAO and its climbing fiber projections to the cerebellum forms the essential US, or reinforcing, pathway for the learning of discrete responses. This evidence also rules out the possibility that the memory trace is localized to the DAO.

Cerebellar Cortex

Using extracellular single unit recording, it has been found that many Purkinje neurons, particularly in lobule HVI, are responsive to the tone CS and the corneal airpuff US in naive animals. Before training, the majority of Purkinje neurons that are responsive to the tone show variable increases in simple spike discharge frequency in the CS period (Foy & Thompson 1986). After training, the majority show learning-induced decreases in simple spike frequency in the CS period that correlate closely in onset latency with the behavioral CR; however, a significant number show the opposite effect (Berthier & Moore 1986, Donegan et al 1985, Foy et al 1992). These results are consistent with the possibility that a process of long-term depression (LTD) may be a

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mechanism of synaptic plasticity in cerebellar cortex in eyeblink conditioning (Chen & Thompson 1992, Ito 1989, Schreurs & Alkon 1992) (see below).

Before training, Purkinje neurons that are influenced by the corneal airpuff consistently show an evoked complex spike to US onset. In trained animals, this US evoked complex spike is virtually absent on paired CS-US trials when the animal gives a CR but present and normal on US alone test trials (Foy & Thompson 1986, Krupa et al 1991). This learning-induced reduction in US-evoked complex spikes is consistent with, and accounted for, by the DAO unit recording study noted above (Sears & Steinmetz 1991). These results led to the hypothesis that the DAO-climbing fiber system, a necessary part of the US pathway, could function as the error-correcting algorithm in classical conditioning (Rescorla & Wagner 1972) by way of the direct GABAergic descending pathway from the interpositus to the inferior olive (Nelson & Mugnaini 1989) (see Figure 1) (Donegan et al 1989, Gluck et al 1990, Thompson 1990). Current evidence supports this view: when animals are trained in the "blocking" paradigm, infusion of the GABA antagonist picrotoxin into the DAO during the compound stimulus training phase completely prevents blocking, as revealed in subsequent training to the novel stimulus of the compound (Kim et al 1992).

In initial studies, there appeared to be some disagreement about effects of cerebellar cortical lesions on eyeblink conditioning (McCormick & Thompson 1984a, Yeo et al 1985b). However, there now appears to be a growing consensus. In trained animals, lesions limited largely to HVI cause variable degrees of impairment in the conditioned response but with substantial or complete recovery (Lavond et al 1987, Yeo & Hardiman 1992). Larger lesions can cause greater impairments, and very large lesions (HVI, Crus I, Crus II, paramedian, and anterior lobes) can cause substantial and, in some animals but not others, persisting impairments (Lavond et al 1987, Perrett et al 1993, Yeo 1990, Yeo & Hardiman 1992). If lesions were made before training, lesions limited to HVI slowed acquisition somewhat; larger lesions (HVI, Crus I, Crus II, paramedian lobule and in one study the flocculus and paraflocculus as well) markedly impaired acquisition (Lavond & Steinmetz 1989, Logan 1991); and very large lesions, including some anterior lobe, markedly impaired and in a few cases, but not others, prevented acquisition (Logan 1991). Animals with these very large lesions that do learn and/or retain CRs exhibit disruption of adaptive timing of the CR (Logan 1991, Perrett et al 1993). Such lesions also prevent acquisition of conditioned inhibition (Logan 1991). Experimentally, it is extremely difficult to remove a very large extent of cerebellar cortex without damaging the cerebellar nuclei. It seems clear that cerebellar cortex plays a critically important role in normal learning of the eyeblink CR; whether or not it is essential remains unresolved.

Latency measures are consistent with the cerebellar hypothesis. Under standard training conditions (85 dB tone CS, 3 psi corneal airpuff, 250 ms CS-US onset interval) the mean minimum onset latency of the NM extension CR is about 90–100 ms. The onset of learning-induced increase in unit activity in the interpositus nucleus varies somewhat from animal to animal; it can precede onset of the learned NM response by as much as 60–70 ms and a typical value is about 50 ms. Learning-induced decreases in Purkinje neuron simple spikes can precede the onset of the learned NM response by as much as 60–80 ms (Foy & Thompson 1986). The latency of activation of the cerebellum by peripheral somatosensory stimuli is about 20 ms (Ekerot et al 1987). Finally, onset of the NM extension response to electrical stimulation of the interpositus nucleus is about 50 ms (McCormick & Thompson 1984a). These time delays account for the otherwise puzzling fact that the minimum onset latency of the conditioned NM response is about 90–100 ms, substantially longer than the minimum onset latency of the reflex NM response to corneal airpuff (25 ms) and the even more puzzling fact that no learning occurs if the CS-US onset interval (the interstimulus interval used in training) is shorter than about 80 ms (Gormezano et al 1983, Steinmetz 1990a).

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Decerebration

If normal rabbits are trained in eyeblink conditioning and then acutely decerebrated, they exhibit retention of the CR, compared with control animals, if, and only if, the transection is sufficiently rostral not to damage the red nucleus (Mauk & Thompson 1987). Bloedel and associates (Kelly et al 1990) claimed to have established conditioned eyeblink responses in the acute decerebrate, decerebellate rabbit. However, their training procedure was extreme massed practice (9 s intertrial interval); they used an idiosyncratic definition of the CR; they did not measure or control the excitability of their preparations; they did not run any control groups for alpha conditioning, sensitization, or pseudoconditioning; and they reported results from only a very few of the animals they ran. Replication of their procedures in intact rabbits showed that no learning at all occurred (Nordholm et al 1991). Yeo (1991) and Yeo & Hardiman (1992) reported that they could establish eyeblink CRs in the acute decerebrate rabbit, using more standard training procedures. Subsequent removal of the cerebellum in their preparations completely abolished the eyeblink CR. It seems very likely that the eyeblink responses reported by Bloedel and associates (Kelly et al 1990) in the acute decerebrate, decerebellate rabbit were due to nonassociative processes (see discussion in Nordholm et al 1991).

For the sake of argument, let us assume that there is an associative component to the eyeblink responses of the decerebrate-decerebellate preparation, i.e. that with appropriate controls and training conditions and sufficient training, an associative conditioned eyeblink response could be established.

As with any new preparation for the study of learning, a number of control procedures must be done in order to define and characterize the phenomenon (Cohen 1984, Gormezano et al 1962, Rescorla 1988a,b; Schneiderman et al 1962, Carew et al 1983). A case in point is work on classical conditioning of the hindlimb flexion reflex in the acute spinal mammal (Beggs et al 1983, Durkovic 1975, Fitzgerald & Thompson 1967, Patterson et al 1973). Necessary control procedures demonstrate that there is indeed an associative component but that the conditioned increase in flexion reflex differs in certain of its properties from hindlimb flexion conditioning in the intact animal. Indeed, it is clear that the spinal cord alone does not contain the circuits that are necessary and sufficient for limb flexion conditioning in the intact animal. Thus, in the otherwise intact animal, lesions in the cerebellar nuclei, red nucleus, or rubrospinal tract produce complete and specific abolition of the conditioned limb flexion response with no effect on the reflex limb flexion response (Donegan et al 1983, Smith 1970, Voneida 1990). Further, normal animals that undergo leg flexion training prior to spinal transection show no retention or savings of conditioned responses following transection (J Steinmetz, personal communication). The isolated spinal cord is thus capable of mediating a kind of associative neuronal plasticity but does not subserve classical conditioning of the limb flexion response in the intact animal, whereas the cerebellar circuitry does. Spinal conditioning is a useful model to study basic associative plasticity in a simplified neuronal network, but it does not tell us where or how such memories are formed in the intact animal.

The same may well be true for the decerebrate-decerebellate preparation. Once the necessary control procedures have been run, it may prove to be a simplified brainstem model of associative plasticity but with different properties than associative learning in the intact, behaving animal. Indeed, there is some evidence suggesting that it may be possible to establish eyeblink CRs or at least eyeblink responses to tone in the brainstem preparation. Yeo & Hardiman (1992) reported that with extended training following decerebellation in their decerebrate preparations, some small eyeblink responses developed to the CS. However, as they note, they did not run controls for nonassociative processes. An earlier study by Norman et al (1977) with long-term chronic decerebrate cats reported that eyeblink responses to tone could be developed within sessions in some animals, although performance was quite variable. In some of their animals, the transections appeared to be below the level of the red nucleus. No separate control groups were run to evaluate nonassociative processes. In any event this says nothing about where and how the memory traces for classical conditioning of the eyeblink response are formed in the intact animal (see discussion in Thompson et al 1983).

The only way to demonstrate that a significant component of the memory trace for eyelid conditioning in the normal mammal is established in the brainstem would be to demonstrate that animals trained in the normal state show significant retention following decerebration and decerebellation, compared to appropriate control groups for nonassociative processes. Indeed, as noted above, in our study of retention of the conditioned eyeblink response following decerebration, there was retention of the CR if the decerebration was sufficiently high that it did not damage the red nucleus, but there was no retention if the red nucleus (a part of the essential CR pathway from the cerebellum) was damaged (Mauk & Thompson 1987).

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Reversible inactivation

The diagram of Figure 1 shows in highly simplified schematic form the essential memory trace circuit for classical conditioning of discrete responses, based on the lesion, recording, and stimulation evidence described above. Interneuron circuits are not shown, only net excitatory or inhibitory actions of projection pathways. Other pathways, known and unknown, may also of course be involved. Many uncertainties still exist, e.g. concerning details of sensory-specific patterns of projection to pontine nuclei and cerebellum (CS pathways), details of red nucleus projections to premotor and motor nuclei (CR pathway), the relative roles of the cerebellar cortex and interpositus nucleus, and the possible roles of recurrent circuits.

Several parts of the circuit have been reversibly inactivated for the duration of training (eyeblink conditioning) in naive animals, indicated by shadings

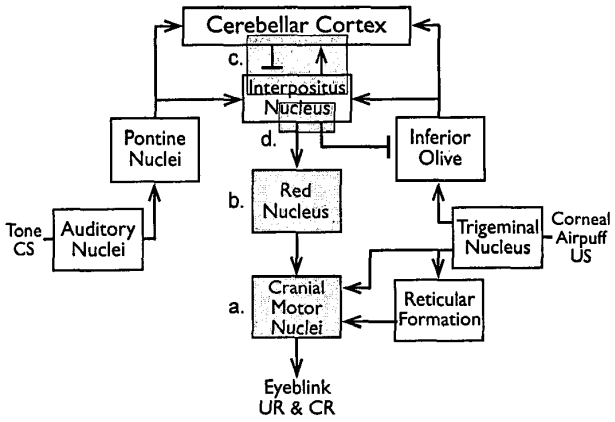

Figure 1 Simplified schematic of the essential brain circuitry involved in eyeblink conditioning. Shadowed boxes represent areas that have been reversibly inactivated during training. (*a*) Inactivation of motor nuclei including facial (seventh) and accessory (sixth). (*b*) Inactivation of magnocellular red nucleus. (*c*) Inactivation of dorsal aspects of the interpositus nucleus and overlying cerebellar cortex. (*d*) Inactivation of white matter ventral to the interpositus.

labeled a, b, c, d in Figure 1. The motor nuclei essential for generating the UR & CR (primarily seventh and accessory sixth) were inactivated by infusion of muscimol (six days) or cooling (five days) during standard tone-airpuff training (*a* in Figure 1) (Thompson et al 1993, Zhang & Lavond 1991). The animals showed no CRs and no URs during this inactivation training; indeed they showed no behavior at all; performance was completely abolished. However, the animals exhibited asymptotic CR performance and normal UR performance from the very beginning of postinactivation training. Thus, performance of the CR and UR are completely unnecessary for normal learning and the motor nuclei make no contribution to formation of the memory trace; they are efferent from the trace.

Inactivation of the magnocellular red nucleus is indicated by *b* in Figure 1. Inactivation by low doses of muscimol for six days of training had no effect on the UR but completely prevented expression of the CR (Krupa et al 1993). Yet animals showed asymptotic learned performance of the CR from the beginning of postinactivation training. Training during cooling of the magnocellular red nucleus gave identical results—animals learned during cooling, as evidenced in postinactivation training, but did not express CRs at all during inactivation training (Clark & Lavond 1993). However, cooling did impair performance of the UR (but the animals learned normally), yet another line of evidence against the performance argument. Consequently, the red nucleus must be efferent from the memory trace.

Inactivation of the dorsal anterior interpositus and overlying cortex (*c* in Figure 1) by low doses of muscimol (six days), by lidocaine (three and six days), and by cooling (five days) resulted in no expression of CRs during inactivation training and no evidence of any learning at all having occurred during inactivation training (Clark et al 1992, Krupa et al 1993, Nordholm et al 1993). In subsequent postinactivation training, animals learned normally as though completely naive; they showed no savings at all relative to noninactivated control animals. None of the methods of inactivation had any effect at all on performance of the UR in US alone trials. In one study (Nordholm et al 1993), cerebellar lidocaine infusions effective in abolishing the CR and preventing learning were subsequently tested on US alone trials over a wide range of US intensities and had no effect on performance of the UR; indeed URs were numerically larger with lidocaine inactivation of CRs than with saline control infusions that had no effect on CRs. The distribution of [^{3}H]-muscimol completely effective in preventing learning included the anterior dorsal interpositus and overlying cortex of lobule HVI, a volume approximately 2% of the total volume of the cerebellum (Krupa et al 1993). The region of the cerebellum essential for learning this task is extremely localized.

Welsh & Harvey (1991) gave rabbits extensive training to a light CS; then gave them transfer training to a tone CS with interspersed light CSs (one session) with lidocaine infusion in the anterior interpositus; and then trained them to the tone without infusion. Control animals were treated identically except given saline infusions during the transfer training session. Welsh & Harvey reported that CRs to the light CS were prevented during lidocaine infusion but that the animals exhibited virtually asymptotic performance to the tone CS in post-infusion training. As noted above, if naive animals are given tone CS training during lidocaine infusion (three or six days) they do not learn at all during infusion and subsequently learn with no savings in postinfusion training. The simplest explanation of the Welsh & Harvey (1991) results is that substantial transfer of training occurred; indeed, their control animals showed very substantial transfer compared to naive animals (see also Schreurs & Kehoe 1987). Cannula location may also be a factor. When lidocaine was infused in the white matter ventral to the interpositus to inactivate the efferent projections from the interpositus (see below and *d* in Figure 1), normal learning occurred although no CRs were expressed during infusion training (Nordholm et al 1993), a result analogous to the result reported by Welsh & Harvey (1991). In any event, all studies in which naive animals were trained during inactivation of the anterior interpositus and overlying cortex (cooling, muscimol, lidocaine) agree in showing that no learning at all occurs during inactivation training.

The white matter ventral to the interpositus nucleus includes the efferent projections conveying information from this portion of the cerebellum to other brain structures. Animals were trained (three days) during lidocaine inactivation of this fiber region (*d* in Figure 1) (Nordholm et al 1993). They showed no evidence of CRs during inactivation training but exhibited asymptotic CR performance from the beginning of postinactivation training. These ventral infusions had no effect on performance of the UR. This result argues that interpositus projections to other brain regions play no essential role in this learning and memory. The only alternative is that efferent fibers from the interpositus project to structure X, which is critical for formation of the memory trace, and X in turn projects back to the cerebellum. The only way this could occur is if the lidocaine infusion ventral to the interpositus does not inactivate these efferent fibers, since the animals learn. However, this hypothetical pathway can play no role in expression of the CR since the ventral infusion, although not preventing learning, completely prevents expression of the CR. Although this seems most unlikely, it is possible. This is not to say, however, that such recurrent feedback circuits play no role in learning (see p. 533). A recent connectionist-level computational model of this circuit (Gluck et al 1993) suggests that feedback to the cerebellum from the output of the interpositus may also play a key role in adaptive timing of the CR.

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Over a wide CS-US onset interval range, the peak of the CR occurs at about the time of onset of the US.

Collectively, these data strongly support the hypothesis that the memory trace is formed and stored in a localized region of the cerebellum (anterior interpositus and overlying cortex). Inactivation of this region (Figure 1*c*) during training completely prevents learning but inactivation of the output pathway from the region (Figure 1*d*) and its necessary (for the CR) efferent target, the red nucleus (Figure 1*b*) do not prevent learning at all. In no case do the drug inactivations have any effect at all on performance of the reflex response on US alone trials. If even a part of the essential memory trace were formed prior to the cerebellum in the essential circuit, then following cerebellar inactivation training, the animals would have to show savings, and they show none at all. Similarly, if a part of the essential memory trace were formed in the red nucleus or other efferent targets of the interpositus, then following red nucleus (Figure 1*b*) or interpositus efferent (Figure 1*d*) inactivation training, animals could not show asymptotic CR performance but they do.

These results would seem conclusively to rule out the possibility, as hypothesized by Bloedel (1992) and Welsh & Harvey (1989, 1991), that the essential memory trace is formed and stored in the brainstem reflex pathways. The brainstem hypothesis has not been elaborated much beyond the assertion that the memory trace occurs in the brainstem and there is at present no evidence to support it. There are perhaps two alternative possibilities: (*a*) the trace is established in the brainstem largely independent of the cerebellum, but excitatory drive from the cerebellum is necessary for its behavioral expression, or (*b*) the trace is established in the brainstem at least in part as a result of the actions of the cerebellum on the brainstem circuitry. Insofar as expression of the CR is concerned, lesions of the interpositus nucleus, its efferent projection to the red nucleus via the superior cerebellar peduncle, the target region of the magnocellular red nucleus, or the descending rubral pathway projecting to the brainstem and motor nuclei completely prevent and abolish expression of the CR with no effect on the UR (see above). Consequently, the cerebellum must exert its actions on the brainstem and motor nuclei, regarding the eyeblink CR, via its efferent projection to the red nucleus and rubral projections to the brainstem. So if the cerebellum–red nucleus circuit simply facilitates expression of the brainstem CR, then inactivation of the critical region of the cerebellum during training could not possibly prevent learning, yet it does. On the other hand, if the cerebellum–red nucleus circuit actually establishes the brainstem CR, reversible inactivation of either structure, each of which completely prevents expression of the CR, must have the same effect on learning of the CR in naive animals. However, as noted, inactivation of the critical cerebellar region completely prevents learning, but inactivation of the red nucleus region does not prevent learning at all, even though in both cases the necessary cerebellar actions on the brainstem are completely prevented.

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PUTATIVE MECHANISMS

The evidence for localization of memory traces for classical conditioning of discrete responses to the cerebellum is now sufficiently compelling that a focus on cerebellar cellular mechanisms of plasticity is warranted. Indeed, since much of the essential circuitry has been identified, this system may provide the first instance in the mammalian brain where the entire circuitry from memory trace to learned behavior, the read out of memory, and the neuronal content of the memory store, can be analyzed.

Although the question of whether or not the cerebellar cortex is essential has not yet been resolved, it is clear that it is critically important for normal learning (see above). The fact that the majority of Purkinje neurons exhibiting learning-related changes show decreases in simple spike responses in the CS period, which would of course result in disinhibition of interpositus neurons, is consistent with a mechanism of long-term depression (LTD). A possible counter-example was reported by Schreurs et al (1991). They took slices of cerebellar cortex from trained and untrained rabbits and reported that the majority of Purkinje neurons from trained animals showed decreases in afterhyperpolarizations to intracellular depolarization relative to controls, implying an increase in excitability. (This of course does not indicate possible changes in synaptic efficacy.) However, there is a sampling problem; many Purkinje neurons in HVI, which is the tissue they used, do not show any learning-related changes and a significant number show increases in simple spike discharges in the CS period (see above). It is possible that the two types of learning-induced changes are from different microzones in cerebellar cortex (see Ito 1984). Indeed, microstimulation through the recording microelectrode evokes small eyelid closure movements when the recording is from Purkinje neurons that show decreases during the CS period, but no such responses are evoked by stimulation when recording is from Purkinje neurons that do not exhibit this pattern of simple spike response (DJ Krupa & RF Thompson, unpublished observations).

Mechanisms of LTD have been reviewed elsewhere in this volume and earlier (Ito 1989). In brief, conjoint activation of mossy-parallel fiber and climbing fiber synapses on Purkinje neurons produces a prolonged depression of the parallel fiber-Purkinje dendrite synapses (AMPA receptors). Current evidence suggests that glutamate activation of AMPA and metabotropic receptors together with increased intracellular calcium, which is produced by

climbing fiber activation, yields the persisting decrease in AMPA receptor function (see also Ito & Karachot 1990, Linden & Connor 1991). LTD has been proposed as the mechanism underlying synaptic plasticity in the flocculus that subserves adaptation of the vestibulo-ocular reflex (Ito 1989).

In behavioral studies, classical conditioning of discrete responses, for example, eyeblink and head turn, evoked by stimulation of the DAO-climbing fibers occurs when paired with stimulation of the mossy fibers as a CS (Steinmetz et al 1989), the exact procedure used in the initial studies of LTD (Ito et al 1982). However, the temporal properties of LTD and classical conditioning appear to differ: most studies of LTD have used near-simultaneous activation of parallel and climbing fibers, but in classical conditioning, mossy-parallel–fiber stimulation must precede climbing fiber stimulation by about 100 ms and best learning occurs with an interval of about 250 ms; simultaneous activation results in extinction of the CR so induced (Steinmetz et al 1989). Virtually all current studies of LTD have used the cerebellar slice or tissue culture and have used GABA blockers. In recent work, parallel fiber-Purkinje cell field potentials in cerebellar slice were used to assess the temporal properties of LTD (Chen & Thompson 1992, Chen 1993). Simultaneous stimulation of parallel fibers and climbing fibers (100 pairings) yielded LTD in the presence of bicuculline, as in other studies, but did not yield LTD in the absence of bicuculline; however if parallel fiber stimulation preceded climbing fiber stimulation by 250 ms, robust LTD developed in the absence of bicuculline, suggesting that GABA, i.e. inhibitory interneurons, may play a key role in determining the temporal properties of the synaptic plasticity underlying LTD. This possibility is not inconsistent with current views of the mechanisms of LTD, e.g. G protein activation of intracellular cascades (Ito 1994, Ito & Karachot 1990, Linden & Connor 1991). It is perhaps relevant that very large cerebellar cortical lesions severely disrupt adaptive timing of the conditioned eyeblink response (see above; Logan 1991, Perret et al 1993). Nitric oxide (NO) has also been implicated in the establishment of LTD in cerebellar cortex (Ito & Karachot 1990) but there are complications; for example, there is apparently no NO synthase in Purkinje neurons (see Ito 1994). Interestingly, Chapman et al (1992) reported that systemic injection of an NO synthase inhibitor impaired acquisition of the conditioned eyeblink response.

The possibility of learning-induced synaptic plasticity in the interpositus nucleus has not been much explored. One study (in vivo anesthetized rat) reported that tetanus of white matter yielded long-term potentiation of the stimulus-evoked field potential in the interpositus (Racine et al 1986). Given the critical importance of the anterior interpositus nucleus for classical conditioning of discrete responses, more work must be done on possible processes of synaptic plasticity in this structure.

Insofar as classical conditioning is concerned, and granting that the memory trace is formed and stored in the cerebellum, evidence at present precludes exclusion of either cerebellar cortex or interpositus as the site of memory storage. The most conservative hypothesis is that there are multiple sites of storage in cortex and interpositus (Thompson 1986). [Interestingly, recent modeling of the VOR circuitry (Lisberger & Sejnowski 1992a,b) suggests that adaptation of the VOR involves synaptic plasticity both in cerebellar cortex and vestibular nuclei, the latter analogous to the interpositus nucleus.] Because the neurons in the interpositus nucleus send substantial direct projections to cerebellar cortex (Batini et al 1989, Buisseret-Delmas & Angaut 1989, Chan-Palay 1977), inactivation limited to the critical region of the interpositus during training that completely prevents learning would still not rule out involvement of the cerebellar cortex since such inactivation would also of course inactivate these nucleo-cortical projections. The *experimentum crucium* would seem to be complete inactivation of the ipsilateral cerebellar cortex but not the interpositus during training; this experiment has not yet been achieved.

ACKNOWLEDGMENT

This paper was supported by the National Institute on Aging grant AF05142, the Office of Naval Research contract N00014-91-J-0112, the National Science Foundation (IBN 9215069), and Sankyo.

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Annual Review of Neurosci.
Volume 17, 1994

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