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Developmental Changes in the Neural Mechanisms of Eyeblink Conditioning

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Abstract

Eyeblink conditioning has been used as a model system for examining the ontogeny of associative learning and its neural basis in rodents. Associative eyeblink conditioning emerges between postnatal days (P) 17 and 24 in rats. Neurophysiological studies in infant rats during eyeblink conditioning revealed developmental changes in the activity of cerebellar neurons that correspond to the ontogenetic emergence of eyeblink conditioning. The developmental changes in cerebellar neuronal activity suggest that the ontogeny of eyeblink conditioning is related to changes in learning mechanisms rather than motor performance mechanisms. Additional neurophysiological and neuroanatomical studies demonstrated that the developmental changes in neuronal activity in the cerebellum are due to developmental changes in interactions between the cerebellum and its inputs, the inferior olive and pontine nuclei. Developmental changes in cerebellar inputs and regulation of its inputs affect the induction of learning-related plasticity, thereby affecting the rate and magnitude of conditioning.

Keywords

learning; classical conditioning; ontogeny; cerebellum; eyelid

The eyeblink conditioning paradigm is a useful model system for examining the ontogeny of associative learning and developmental changes in the neural mechanisms of learning and memory (Stanton & Freeman, 2000). Eyeblink conditioning is ideal for neurobiologically based studies of the ontogeny of learning because the behavioral and neural mechanisms of this form of conditioning have been studied extensively in adult mammals (Christian & Thompson, 2003). Classical conditioning of the eyeblink response is established in humans and animals by presenting a conditioned stimulus (CS) such as a tone or light paired with an unconditioned stimulus (US) such as a brief shock to the periorbital region of the face or a puff of air to the cornea (see Figure 1a; Gormezano, 1966). The eyeblink response is recorded by measuring eyelid movement, eyelid muscle activity, or movement of the nictitating membrane. At the start of conditioning, the US elicits a reflexive eyeblink response, but no response occurs during the CS (see Figure 1b). Continued pairing of the CS and US will yield an eyeblink response that occurs during the CS, the conditioned response (CR; see Figures 1c and 1d). The maximum amplitude of the eyeblink CR typically occurs at the onset time of the US. The behavioral laws of associative learning were, to a large extent, determined by detailed studies of the parameters that influence the rate and magnitude of eyeblink conditioning in adult humans and rabbits (e.g., Gormezano, Kehoe, & Marshall, 1983; Grant & Schneider, 1948; Spence, 1953). As with other classical conditioning paradigms, associative learning, sensitization, and performance can be assessed independently using eyeblink conditioning, making it possible to dissociate developmental changes in learning from developmental

changes in other processes. Perhaps the most significant advantage of using eyeblink conditioning in developmental studies of learning is that the neural circuitry underlying acquisition and retention of the conditioned response has been identified in adult mammals (see Figure 2; Christian & Thompson, 2003; Thompson, 2000; Thompson & Krupa, 1994; Woodruff-Pak & Steinmetz, 2000; Yeo & Hesslow, 1998). The adult neural circuitry underlying eyeblink conditioning provides a neural roadmap for identifying developmental changes in the mechanisms of associative learning (Freeman & Nicholson, 2001).

CEREBELLAR LEARNING MECHANISMS

The intermediate cerebellum including the interpositus nucleus and cortical lobules V and HVI ipsilateral to the conditioned eye is necessary for the acquisition and retention of the eyeblink CR in adults of various species (Lincoln, McCormick, & Thompson, 1982; McCormick, Clark, Lavond, & Thompson, 1982; McCormick & Thompson, 1984; Skelton, 1988). Learning-specific neural changes occur in both the interpositus nucleus and the cortical areas (see Figure 2), although the functions of the two neural systems are thought to differ. Changes that occur in the interpositus nucleus are essential for acquisition and retention of the eyeblink CR, whereas modifications in cortical areas are necessary for the normal amplitude and timing of the CR (Bao, Chen, & Thompson, 2002; Christian & Thompson, 2003; Garcia & Mauk, 1998; Mauk, 1997; McCormick & Thompson, 1984; Medina et al., 2000). The neural plasticity underlying eyeblink conditioning is thought to occur in synaptic connections between the conditioned stimulus (CS) pathway and neurons in the cerebellum (see Figure 2). The CS pathway includes mossy fiber axons from the pontine nuclei, which have collaterals that form excitatory synapses in the cerebellar nuclei and in the granule cell layer of the cerebellar cortex (see Figure 2; Mihailoff, 1993). The granule cells give rise to the parallel fibers, which form excitatory synapses on Purkinje cells (see Figure 2). The US pathway is the climbing fiber projection from the inferior olive to the cerebellar nuclei and Purkinje cells (see Figure 2; Sugihara, Wu, & Shinoda, 1996, 2001; Van der Want, Wiklund, Guegan, Ruigrok, & Voogd, 1989). The synaptic mechanisms underlying eyeblink conditioning are thought to include depression of synaptic efficacy at parallel fiber synapses with Purkinje cells (long-term depression [LTD]) and enhancement of synaptic efficacy at mossy fiber synapses with neurons in the interpositus nucleus (long-term potentiation [LTP]; Mauk & Donegan, 1997; Medina et al., 2000; Thompson, 1986). The depression of Purkinje cells releases the interpositus nucleus from inhibition resulting in an increase in activity that helps drive the eye blink response. Cortical LTD may also be necessary for the induction of nuclear LTP by disinhibiting the interpositus nucleus, allowing its neurons to be strongly activated by synaptic input from mossy fiber collaterals (Mauk & Donegan, 1997; Medina et al., 2000). LTD in the cerebellar cortex is thought to result from conjunctive stimulation of parallel and climbing fiber inputs to Purkinje cells. LTP in the interpositus nucleus may arise from conjunctive stimulation of mossy and climbing fiber collaterals or by pairing mossy fiber activation with the disinhibition produced by Purkinje cell LTD (Medina et al., 2000). The comprehensive models of the cerebellar mechanisms underlying eyeblink conditioning in adult mammals provide a detailed framework for generating and testing hypotheses about specific ontogenetic changes in the neural mechanisms of cerebellar learning.

It could be argued that the neural circuitry underlying associative learning might change substantially between infancy and adulthood, rendering the results from experiments using the neural roadmap approach ambiguous (Goldman-Rakic, Isseroff, Schwartz, & Bugbee, 1983; Moriceau & Sullivan, 2004). With this general concern in mind, Stanton and colleagues began the neurobiological analysis of the ontogeny of eyeblink conditioning by examining whether the essential components of the eyeblink conditioning circuitry in adults are necessary for the ontogenetic emergence of conditioning (Stanton & Freeman, 2000). Two experimental approaches were taken to examine the role of the cerebellum in the ontogeny of eyeblink

conditioning. First, aspiration lesions of the cerebellum were made in infant rats prior to acquisition training (Freeman, Carter, & Stanton, 1995). Lesions that included the interpositus nucleus ipsilateral to the conditioned eye completely abolished acquisition in all ages tested. The results of the lesion study indicate clearly that the cerebellum is necessary for eyeblink conditioning in infant rats. The second approach was to examine the effect of impairing cerebellar neurogenesis early in the postnatal period with the antimitotic methylazoxymethanol (MAM) on the ontogeny of eyeblink conditioning (Freeman, Barone, & Stanton, 1995). The postnatal MAM treatment severely disrupted the cellular development of the cerebellum, primarily by preventing mitosis of granule cells, the source of parallel fibers in the cerebellar cortex (see Figure 2). Acquisition of eyeblink conditioning was impaired at all of the ages tested, indicating that normal cerebellar development is necessary for the ontogenetic emergence of eyeblink conditioning. The lesion and MAM treatment studies showed that the cerebellum is essential for the ontogeny of eyeblink conditioning but provided little information about the specific neurobiological processes underlying the ontogeny of learning.

DEVELOPMENT OF CEREBELLAR LEARNING MECHANISMS

One approach for assessing specific developmental changes in neural processes is to use electrophysiological recording techniques to monitor neuronal activity during conditioning. A technique for recording neuronal activity in moving rat pups (and mice) was developed by Freeman and Nicholson (2000) to examine developmental changes in neural processes within the eyeblink conditioning neural circuitry. Bundles of 4 to 8 microwire electrodes (25 μ m in diameter) were used to record ensemble unit activity. The electrodes were connected to miniature connectors and a lightweight preamplifier. The miniaturized recording equipment made it possible to record unit activity in unrestrained rat pups. The first neurophysiological studies examined the activity of neurons in the cerebellum during eyeblink conditioning in developing rats. The anterior interpositus nucleus was the first part of the circuitry examined neurophysiologically because its neurons show increased activity in response to the CS and US, develop learning-specific activity profiles, and the output of the interpositus nucleus activates the red nucleus and facial motor nucleus, which generates the conditioned blink response (see Figure 2). The neurophysiological analysis of neuronal activity in the interpositus nucleus was, therefore, able to assess the strength of stimulus inputs and induction of learning-specific plasticity. This study was also able to help determine whether the ontogenetic emergence of eyeblink conditioning is due to the development of learning mechanisms or motor performance mechanisms. A developmental change in learning-specific activity in the interpositus nucleus would be consistent with a developmental change in learning, whereas the presence of learning-specific neuronal activity in the interpositus nucleus of younger rats (nonlearners, P17) would suggest that there is a developmental change in motor performance mechanisms.

The magnitude of neuronal activity following CS and US onset increased with postnatal age in parallel with the development of the CR (see Figure 3; Freeman & Nicholson, 2000). In addition, fewer neurons in the younger rats (P17) developed learning-specific modifications of neuronal activity, and the neurons that did develop learning-specific activity showed minimal changes in activity relative to the older rats (P24; see Figure 3). The developmental trend in the induction of learning-specific neuronal activity suggests that developmental changes in eyeblink conditioning are due to the development of learning mechanisms rather than the development of motor performance mechanisms. In addition, electrical stimulation of the interpositus nucleus elicited eyeblinks in P17 pups at the same current levels as used in the P24 pups (and adults), suggesting that maturation of the output pathways necessary for performance of the CR cannot account for the ontogenetic emergence of eyeblink conditioning (Freeman & Nicholson, 2000).

Developmental changes in Purkinje cell activity in cerebellar cortical lobule HVI were also examined. Simple spikes were recorded using the same experimental techniques as the Freeman and Nicholson (2000) study. Stimulus-evoked and learning-related modulation of Purkinje cell simple spike activity from eye regions within lobule HVI emerged ontogenetically in parallel with eyeblink conditioning (Nicholson & Freeman, 2004). During paired CS-US training, the proportion of simple spikes that showed learning-specific increases or decreases in activity increased with age. Moreover, as seen in the interpositus nucleus (see Figure 3), the learning-specific changes in cerebellar cortical neuronal activity in the older rats (P24) were greater in magnitude than the learning-specific changes observed in the younger rats (P17).

US PATHWAY DEVELOPMENT

In addition to developmental changes in the frequency and magnitude of learning-specific neuronal activity in the cerebellum, there was a substantial developmental increase in the magnitude of neuronal responses to the CS and US (Freeman & Nicholson, 2000; Nicholson & Freeman, 2004). The ontogenetic increase in the cerebellar response to the US suggests that the neural pathway that conducts US stimulation to the cerebellum or the synaptic connections between the US pathway and cerebellar neurons undergoes substantial developmental change postnatally. The US pathway in eyeblink conditioning includes the inferior olive and its synaptic projection to the cerebellum, the climbing fibers (see Figure 2; Mauk, Steinmetz, & Thompson, 1986; McCormick, Steinmetz, & Thompson, 1985; Steinmetz, Lavond, & Thompson, 1989). Our initial investigation of developmental changes in the US input to the cerebellum examined the neuronal response to the US in the inferior olive and climbing fiber activity in the cerebellum. The expected outcome of these studies was that there would be no evidence of a developmental change in the inferior olive or that P17 rats would exhibit weaker inferior olive responses to the US relative to P24 rats. Much to our surprise, the neuronal response to the US was greater at P17. This finding initially appeared to be inconsistent with the developmental change in learning in that greater US-related activity should promote stronger conditioning. However, we considered the possibility that a developmental change in inhibitory feedback from the cerebellum to the inferior olive contributes to the robust US response in younger animals. The inhibitory cerebello-olivary feedback pathway regulates the US inputs to the cerebellum in two important ways. First, as learning related plasticity develops in the cerebellum during training; interpositus neurons increase activity during the CS, driving the response. The increased interpositus activity also increases the magnitude of inhibitory feedback on the inferior olive (Hesslow & Ivarsson, 1996; Sears & Steinmetz, 1991; Kim, Krupa, & Thompson, 1998). The increase in inhibitory feedback is thought to result in a decrease in the ability of the US to support new learning, a process analogous to the decrease in US efficacy posited in the Rescorla and Wagner model of associative learning (Rescorla & Wagner, 1972; Thompson, Thompson, Kim, Krupa, & Shinkman, 1998). The regulation of US input prevents the acquisition of redundant and potentially distracting associations (Kim et al., 1998; Medina, Nores, & Mauk, 2002). Second, the inhibitory regulation of the US pathway maintains the spontaneous rate of inferior olive activity, and its climbing fiber responses in the cerebellum, at an “equilibrium” level that helps to maintain learning-specific plasticity in cerebellar neurons during periods in which the CS and US are not presented, which includes the intertrial and intersession intervals (Medina et al., 2002). If the level of spontaneous climbing fiber activity becomes too high, Purkinje cell synapses will saturate with LTD and eventually, the learning-specific synaptic plasticity established during conditioning trials will be erased. In contrast, if the level of spontaneous climbing fiber activity is too low, learning-specific LTD established during conditioning trials will be reversed and Purkinje cell synapses will become potentiated, eventually saturating the system with LTP. A developmental change in cerebellar inhibitory regulation of the inferior olive would, therefore, produce weaker learning-specific plasticity that decays rapidly between trials and between sessions in younger rats (Nicholson & Freeman, 2003a).

The first evidence of a developmental change in cerebellar feedback to the inferior olive was found in a trial by trial analysis of activity in the inferior olive and climbing fiber activity, comparing trials with CRs versus trials without CRs (Nicholson & Freeman, 2000, 2003a). Previous studies found that neuronal responses to the US are suppressed by cerebellar feedback on conditioning trials in which a CR is produced (Hesslow & Ivarsson, 1996; Kimetal., 1998; Sears & Steinmetz, 1991). The neuronal responses of US pathway neurons were suppressed during CRs in the older rats (P24) that learned well, but no CR-related modulation of US pathway activity was seen in the younger rats (P17) that did not show robust conditioning. A more detailed neurophysiological analysis of ontogenetic changes in the US pathway examined developmental changes in spontaneous and evoked climbing fiber activity in anesthetized rat pups (Nicholson & Freeman, 2003b). Previous studies using adult animals demonstrated that cutaneous stimulation similar to the US used in eyeblink conditioning elicits two distinct patterns of climbing fiber activity (Bloedel & Ebner 1984; Llinas & Sasaki 1989). One response pattern includes a long-latency (> 50 ms) rhythmic climbing fiber discharge with no short-latency spike. The other response pattern includes a short-latency (< 50 ms) climbing fiber spike with no long-latency rhythmic discharge. A rhythmic discharge is defined as two or more complex spikes occurring at approximately 10 Hz, shortly after brief sensory stimulation. The rhythmic discharge in long-latency climbing fiber responses is produced by oscillations in the membrane voltage of electrically coupled inferior olivary neurons. Segregation of response patterns following stimulation and the synchronized rhythmicity among multiple olivary neurons are regulated by inhibitory feedback from the cerebellum (Bloedel & Ebner, 1984; Lang, Sugihara, & Llinas, 1996; Llinas, 1974; Llinas, Baker, & Sotelo, 1974; Llinas & Sasaki, 1989; Llinas & Yarom, 1981a, 1981b, 1986). Climbing fiber activity in developing rats was monitored to determine whether inhibition from cerebellar feedback and excitation from sensory input within the inferior olive exhibit ontogenetic changes that correspond to the developmental time course of eyeblink conditioning. Recordings of climbing fiber activity showed that the segregation of short- and long-latency climbing fiber responses emerges ontogenetically in parallel with eyeblink conditioning (see Figure 4). The role of cerebellar inhibition in the developmental change in climbing fiber response segregation was assessed directly by infusing picrotoxin, a GABA antagonist, into the inferior olive. Blocking inhibitory activity in the inferior olive abolished response pattern segregation in the P24 rats, producing an evoked pattern of climbing fiber activity that was identical to the pattern seen in P17 rats (see Figure 4; Nicholson & Freeman, 2003b). The findings of the neurophysiology studies support the view that cerebellar inhibitory feedback to the inferior olive undergoes substantial changes that parallel the ontogenetic emergence of eyeblink conditioning.

A neuroanatomical basis for the developmental change in inhibitory feedback from the cerebellum to the inferior olive was then identified using quantitative electron microscopy (Nicholson & Freeman, 2003a). More than 90% of the inhibitory synapses in the inferior olive come from the cerebellum (De Zeeuw, van Alphen, Hawkins, & Ruigrok, 1997). We were therefore able to assess the development of cerebellar inhibitory feedback to the US pathway by examining developmental changes in the total number of inhibitory synapses within the olive. The physical disector and systematic random sampling were used to obtain unbiased estimates of the total number of excitatory axodendritic, excitatory axospinous, inhibitory axodendritic, and inhibitory axospinous synapses in the division of the inferior olive that projects to the intermediate cerebellum and provides the necessary and sufficient US information to support eyeblink conditioning (Mauk et al., 1986), the dorsal accessory olive (DAO). There was an ontogenetic increase in the overall number of synapses in the DAO (see Figure 5). However, the younger rats had disproportionately fewer inhibitory synapses than the older rats (see Figure 5). The most substantial developmental difference was an age-related increase in the number of inhibitory axospinous synapses (see Figure 5). Between P17 and P24, there was a threefold increase in the number of inhibitory axospinous synapses in the DAO. The quantitative neuroanatomical study identified a specific mechanism underlying the

developmental change in inhibitory feedback from the cerebellum to the inferior olive. The findings of this study also indicate that the younger rats are not able to regulate the US pathway, even when they produce occasional eyeblink CRs.

The chronic lack of inhibitory regulation of the inferior olive in younger rats leaves climbing fiber activity unable to filter out redundant, and potentially interfering sources of plasticity in the cerebellum (see Figure 6). The climbing fiber activity in younger pups is also unable to attain a state of equilibrium, impairing the maintenance of cerebellar plasticity during the intertrial and intersession intervals (see Figure 6). The gradual and incremental plasticity established on a trial to trial basis in older rats cannot be induced in the younger rats because each increment in cerebellar plasticity evaporates before the next increment can be added. In principle, the younger rats should be able to learn as well as rats with mature cerebello-olivary inhibition if the lack of inhibition in the inferior olive could be overcome, allowing the climbing fiber activity to attain a state of equilibrium.

CS PATHWAY DEVELOPMENT

The identified developmental changes in interactions between the cerebellum and inferior olive are compelling, but do not completely account for the ontogenetic emergence of eye blink conditioning. There are also developmental changes in interactions between the CS pathway and the cerebellum. The CS pathway for an auditory stimulus includes projections from the cochlear nuclei to the pontine nuclei and from the pontine nuclei to the cerebellum via mossy fibers (Gould, Sears, & Steinmetz, 1993; Steinmetz et al., 1987, 1989; Steinmetz, Lavond, & Thompson, 1985; Steinmetz, Rosen, Chapman, Lavond, & Thompson, 1986; Steinmetz & Sengelaub, 1992; Tracy, Thompson, Krupa, & Thompson, 1998). The cerebellum sends excitatory feedback connections to the pontine nuclei (Bao, Chen, & Thompson, 2000; Clark, Gohl, & Lavond, 1997). The excitatory cerebellar feedback results in an enhancement of pontine neuronal activity during the CS that emerges as CRs develop. The enhancement of pontine activity during training is due to feedback from the cerebellar nuclei (Bao et al., 2000; Clark et al., 1997).

The first direct assessment of ontogenetic changes in the CS pathway examined developmental changes in neuronal activity in the pontine nuclei during eyeblink conditioning (Freeman & Muckler, 2003). The experiment was designed to determine whether the developmental change in CS-elicited activity in the cerebellum described above is due to developmental changes in pontine responses to the CS. An additional goal of this study was to determine whether there are developmental changes in excitatory feedback from the cerebellum to the pontine nuclei. Developmental changes in stimulus-elicited and learning-specific neuronal activity in the pontine nuclei correspond ontogenetically to the emergence of eyeblink conditioning. The neuronal activity profiles of units in the pontine nuclei are heterogeneous. Three general categories of unit activity have been identified in developing rats: (a) phasic units show a short latency (less than 100 ms) increase in activity following the onset of the CS, which decreases to the pre-CS baseline within the next 100 ms interval; (b) sustained units show short latency increases in activity that are sustained for at least 200 ms; and (c) late units exhibit a significant increase in activity during the CS relative to the pre-CS baseline, more than 100 ms after the onset of the CS (see Figure 7). An ontogenetic increase in baseline and stimulus-elicited activity was observed for the phasic and sustained response units during a pretraining session (see Figure 7). After paired training, the older rats (P24) had significantly more sustained and late units than the younger rats (P17). The magnitude of baseline and stimulus elicited activity of sustained response units was also greater in the older rats after training. In addition, trial-by-trial analyses revealed an ontogenetic increase in the number of units showing CR-related activity as a result of training. The developmental change in the number of neurons showing

CR-related modulation suggests that there may be developmental changes in excitatory feedback from the cerebellum to the pontine nuclei (Freeman & Muckier, 2003).

The ontogenetic changes in pontine neuronal activity suggest that the developmental differences in cerebellar responses to the CS (Freeman & Nicholson 2000; Nicholson & Freeman 2003a) are at least partially due to changes in the responsiveness of pontine neurons, rather than solely due to changes in the efficacy of the mossy fiber projection to the cerebellum (see Figure 8). The development of CS inputs to the cerebellum could play a critical role in the induction of learning-related plasticity during conditioning. Weaker CS inputs in younger rats would lead to weaker learning-specific plasticity in the cerebellum and weaker conditioning (see Figure 8). An ongoing experiment is examining whether the developmental limitations in CS pathway responsiveness to the auditory stimulus can be bypassed by using electrical stimulation of the mossy fiber pathway as a CS in developing rats. Preliminary findings indicate that stimulation of the mossy fiber pathway results in robust conditioning in rats that are too young to show robust conditioning to a peripheral CS such as a light or tone. The preliminary findings of the stimulation study combined with the observed developmental changes in pontine neuronal activity indicate that the development of CS pathway input to the cerebellum plays a significant role in the ontogeny of eyeblink conditioning.

Developmental changes in cerebellar feedback to the pontine nuclei might also contribute to the ontogeny of eyeblink conditioning by influencing the induction of cerebellar plasticity during training. The role of cerebellar excitatory feedback to the pontine nuclei in conditioning is thought to be a mechanism for enhancing CS efficacy or salience (Bao et al. 2000; Clark et al. 1997). If the enhancement of CS salience enhances learning, then the development of excitatory feedback from the cerebellum to the pontine nuclei could contribute to the development of eyeblink conditioning.

CONCLUSIONS

Cerebellar learning depends on the temporal orchestration of input stimulation, learning-specific plasticity mechanisms, output mechanisms, and feedback to the input pathways (Bao et al., 2002; Medina & Mauk, 2000; Medina et al., 2002; Thompson, 1986; Yeo & Hesslow, 1998). The synaptic connections between the cerebellum and brainstem form functional loops that are continuously active and are therefore continuously interacting. Interactions between the cerebellum and brainstem produce learning-specific synaptic plasticity during conditioning trials and maintain the synaptic plasticity between conditioning trials and training sessions (Medina & Mauk, 2000; Medina et al., 2002). The pontine nuclei, their mossy fiber projection to the cerebellum, and feedback connections from the cerebellum form a functional loop that is regulated by positive feedback, which enhances CS input during learning. The inferior olive, its climbing fiber projection to the cerebellum, and feedback connections from the cerebellum form a functional loop that is regulated by negative feedback, which reduces US input during conditioning trials after learning and maintains climbing fiber activity in a state of equilibrium between conditioning trials and conditioning sessions. Perturbations of inputs or feedback mechanisms in the CS or US pathway loops can disrupt cerebellar plasticity mechanisms and thereby disrupt learning (Medina & Mauk, 2000; Medina et al., 2002; Yeo & Hesslow, 1998).

The developmental studies outlined in this article demonstrate substantial developmental changes in both the CS and US pathway loops (see Figure 9). The primary developmental change in the US loop is an increase in feedback from the cerebellum to the inferior olive (see Figures 6 and 9). The developmental change in inhibitory cerebellar feedback to the inferior olive changes the negative feedback mechanism and influences the ability of the US loop to maintain climbing fiber activity in a state of equilibrium. The inability to maintain climbing

fiber activity in a state of equilibrium between trials and sessions in young rats results in less robust learning because the increments in cerebellar plasticity that are produced during conditioning trials fade with time. Interpositus neurons in younger rats, therefore, do not develop and maintain the learning-specific increase in CS-elicited activity that drives the production of CRs, as seen in more mature rats.

The developmental changes in the CS loop include a change in the magnitude of input to the cerebellum and a change in positive feedback from the cerebellum to the pontine nuclei (see Figures 8 and 9). The developmental changes in the CS loop might have two significant effects on the induction of learning-specific plasticity in the cerebellum. First, the weaker CS input to the cerebellum in younger rats is less effective at stimulating cerebellar neurons, probably resulting in weaker synaptic plasticity in the interpositus nucleus and cerebellar cortex. The weaker synaptic plasticity is further diminished by the unregulated climbing fiber activity, resulting in little or no plasticity in the cerebellum after training. Second, the developmental change in positive feedback from the cerebellum to the pontine nuclei limits the induction of cerebellar plasticity by determining the magnitude or salience of the input part of the CS loop, which would further influence the strength of input activation of cerebellar neurons.

Our current view of the developmental processes underlying the ontogeny of eyeblink conditioning does not specify a role for developmental changes in the cellular mechanisms of plasticity in the cerebellum. Developmental changes in the mechanisms underlying synaptic plasticity in cerebellar neurons would have significant effects on learning. However, there have been many demonstrations of synaptic plasticity and changes in excitability in developing cerebellar neurons in vitro (Aizenman & Linden, 2000; Hansel, Linden, & D'Angelo, 2001). Current studies are examining the possibility of ontogenetic changes in the cellular mechanisms of cerebellar learning, including synaptic plasticity and excitability. For the moment, however, the ontogeny of learning appears to be primarily due to developmental changes in the synaptic organization of and network activity within the brainstem-cerebellum eyeblink neural circuit.

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Figure 1. Eyeblink Conditioning in Rodents

NOTE: The figures are arranged as follows: (a) temporal relationship between the conditioned stimulus (CS) and unconditioned stimulus (US); (b) eyelid muscle (orbicularis oculi) activity on a conditioning trial with an unconditioned response (UR) but no conditioned response (CR); (c) eyelid muscle activity on a conditioning trial with a CR, and the gap in muscle activity during the US is due to gating the input of the amplifier to eliminate the stimulus artifact; and (d) eyelid muscle activity on a CS-alone test trial with a CR.

Figure 2. Simplified Schematic of the Neural Circuitry Underlying Eyeblink Conditioning

NOTE: The cerebellar interpositus nucleus (IPN) and Purkinje cells in the cerebellar cortex (PKJ) receive convergent input from the conditioned stimulus (CS) and unconditioned stimulus (US) neural pathways. The CS pathway includes inputs to the pontine nuclei (PN), the mossy fiber (mf) projection to the IPN and cortical granule cells (gr), and the parallel fiber (pf) projection to PKJs. The US pathway includes inputs to the dorsal accessory division of the inferior olive (DAO) and the climbing fiber (cf) projection to the IPN and PKJs. The pathway for performance of the conditioned response (CR) includes the IPN projection to the magnocellular red nucleus (RN) and its projection to brainstem motor nuclei (VI, VII) that activate eyeball retraction and eyelid closure. The unconditioned response (UR) is elicited by activation of the trigeminal nuclei (V), which then activate VI and VII. Filled triangles indicate excitatory synapses and open triangles indicate inhibitory synapses. Gray ellipses indicate the sites of synaptic plasticity underlying retention of eyeblink conditioning.

Figure 3. Mean Firing Rate (Spikes/s) for Single Units Recorded From the Infant Rat Cerebellar Interpositus Nucleus During Eyeblink Conditioning

SOURCE: Adapted from Freeman and Nicholson (2000). NOTE: The firing frequencies of the units recorded from rats trained on postnatal days (P) 17 (left) and P24 (right) are displayed. The upper histograms display activity from trials with paired presentations of the conditioned stimulus (CS) and unconditioned stimulus (US). The lower histograms display activity from CS-alone test trials. Arrows indicate the onset times of the CS and US. The gap in unit activity during the US is due to the stimulus artifact, which precludes recording unit activity. Note that the unit activity is greater on P24 relative to P17 during the CS and after the US.

Figure 4. Histograms of the Activity of Representative Purkinje Cell Complex Spikes From a Postnatal Day (P) 17 Rat (left column), a P24 Rat (middle column), and a P24 Rat Given Picrotoxin in the Inferior Olive (PTX; right column) for Trials With (a) Short-Latency Spikes, (b) Long-Latency Spikes, and (c) Both Together

SOURCE: Adapted from Nicholson and Freeman (2003b). NOTE: In 4a, note the differences in activity between the P17 complex spike (left) and the P24 complex spike (middle), and the similarities between the activity of the P17 complex spike (left) and the P24 complex spike after picrotoxin infusion in the inferior olive (right).

Figure 5. Estimated Mean Total Number (+SEM) of Excitatory Axodendritic (D+) and Axospinous (S+) Synapses: Inhibitory Axodendritic (D-) and Axospinous (S-) Synapses in the Dorsal Accessory Inferior Olive on Postnatal Days (P) 17 and 24

SOURCE: Adapted from Nicholson and Freeman (2003a). NOTE: There is an age-related increase in S+, D-, and S- synapses.

Figure 6. Simplified Schematic of the Unconditioned Stimulus (US) Pathway

NOTE: The gray ellipse (1) indicates that the primary site of developmental change in the US pathway is an ontogenetic increase in the inhibitory projection from the cerebellar nuclei to the dorsal accessory inferior olive (DAO). The ontogenetic increase in cerebellar inhibition of the DAO results in a decrease in spontaneous and stimulus-elicited climbing fiber (cf) activity, which influences the induction and maintenance of synaptic plasticity in the cerebellum. Filled triangles indicate excitatory synapses and open triangles indicate inhibitory synapses. Abbreviations are the same as in Figure 2.

Figure 7. Mean Firing Rate (spikes/s) of Single Units Recorded From the Pontine Nuclei in Infant Rats During Presentations of a Tone Conditioned Stimulus

NOTE: The firing frequencies of phasic (A), sustained (B), and late (C) units recorded from rats trained on postnatal day (P) 17 (left column) and P24 (right column) are displayed. The gray lines indicate the onset time of the conditioned stimulus. Note the age-related increase in the activity of phasic and sustained units, especially just after the onset time of the stimulus (from Freeman & Muckier, 2003).

Figure 8. Simplified Schematic of the Conditioned Stimulus (CS) Pathway

NOTE: The gray ellipse around the pontine nuclei (2) indicates that the primary site of ontogenetic change in the CS pathway is an increase in the responsiveness of pontine neurons to auditory stimuli, which might be influenced by a developmental change in the excitatory feedback projection from the interpositus nucleus (IPN). The other gray ellipses indicate the sites of ontogenetic increases in the strength of CS stimulation in the cerebellar cortex (3) and nuclei (4). Filled triangles indicate excitatory synapses and open triangles indicate inhibitory synapses. Abbreviations are the same as in Figure 2.

Figure 9. Developmental Mechanisms Underlying the Ontogeny of Eyeblink Conditioning

NOTE: Gray ellipses indicate sites of developmental change that affect the ontogeny of eyeblink conditioning. 1 = inhibitory feedback regulation of the dorsal accessory inferior olive (DAO) increases with age—the ontogenetic increase in cerebellar inhibition of the DAO results in a decrease in spontaneous and stimulus-elicited climbing fiber (cf) activity, which alters the induction and maintenance of synaptic plasticity in the cerebellum; 2 = pontine neuronal responsiveness to the CS increases with age; 3 = parallel fiber (pf) input to Purkinje cells (PKJ) increases with age; and 4 = mossy fiber (mf) input to the interpositus nucleus (IPN) increases with age. Note that 3 and 4 are affected by 2. Weaker CS pathway inputs combined with an unregulated DAO leads to weaker synaptic plasticity in the cerebellum (IPN and PKJ), and weaker conditioning in younger rats. Abbreviations are the same as in Figure 2.