Developmental Changes in Evoked Purkinje Cell Complex Spike Responses

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INTRODUCTION

The interconnections between the cerebellum and brain stem allow cerebellar neurons to influence their own afferent input (Andersson et al. 1988). Elucidating this unique property of brain-stem-cerebellum circuitry may provide insight into the organization and production of certain movements and learning-related changes in cerebellar neuronal activity (Albus 1971; Eccles et al. 1967; Ito 1984; Kim and Thompson 1997; Llinas 1974, 1981; Marr 1969; Mauk and Donegan 1997; Thach et al. 1992; Thompson 1986; Thompson and Krupa 1994). In addition, characterizing changes in brain-stem-cerebellum interactions during developmental time windows in which certain behaviors emerge may provide insight into their function (Freeman and Nicholson 2001; Nicholson and Freeman 2003a).

There are two stages of output within the cerebellum: the cerebellar cortex and the deep cerebellar nuclei (DCN) (Eccles et al. 1967; Llinas 1981). Purkinje cells are the only output neurons of the cerebellar cortex and form inhibitory synapses onto DCN neurons, which provide the only output from the entire cerebellum (except in the flocculonodular lobe). The two main afferent systems to the cerebellum are the mossy fibers and the climbing fibers. The pontine nuclei are a major source of mossy fibers in the cerebellum and receive predominantly excitatory feedback from neurons in the DCN (Schwarz and Schmitz 1997). In contrast, DCN feedback to the inferior olive, the only source of climbing fibers, is exclusively inhibitory (De Zeeuw et al. 1989, 1997).

The regulation of climbing-fiber activity by cerebellar inhibition may be important for the production (Lang et al. 1996; Llinas 1974; Welsh et al. 1995) and acquisition (Ito 1984; Kim and Thompson 1997; Mauk and Donegan 1997; Thompson 1986) of learned movements. Specifically, changes in synaptic strength between mossy fiber inputs and the cerebellum may be regulated by cerebellar inhibition (Medina and Mauk 1999). Mossy fibers drive the activity of parallel fibers in the cerebellar cortex. Parallel fiber activity in the absence of climbing-fiber activity leads to a long-term potentiation (LTP) of those active parallel fiber–Purkinje cell synapses (Lev-Ram et al. 2002; Sakurai 1987). Parallel fiber activity in the presence of climbing-fiber activity induces a long-term depression (LTD) of those active parallel fiber–Purkinje cell synapses (Chen and Thompson 1995; Ito et al. 1982; Linden and Connor 1995; Schreurs and Alkon 1993). Therefore climbing-fiber activity influences whether activity-dependent increases or decreases occur at parallel fiber–Purkinje cell synapse, and possibly mossy fiber–DCN synapses (Kim and Thompson 1997; Mauk and Donegan 1997; Medina and Mauk 1999). The inhibitory feedback from the DCN to the inferior olive may therefore play a critical role in influencing Purkinje cell activity, which in turn may influence activity and plasticity in the DCN (Aizenman and Linden 1999, Aizenman et al. 2000, 2003; Eccles et al. 1967; Kim and Thompson 1997; Mauk and Donegan 1997; Medina and Mauk 1999). It is likely then that developmental changes in DCN feedback to the inferior olive have consequences on the activity of neurons in the inferior olive, Purkinje cells, and the DCN neurons themselves.

Neurons in the inferior olive are very responsive to somatosensory stimulation (Armstrong 1974; Gellman et al. 1983, 1985; Llinas and Sasaki 1989; Murphy and Sabah 1971; Nicholson and Freeman 2000, 2003a; Thach 1967; Weiss et al. 1993). The climbing fibers from the inferior olive produce all-or-none complex spikes in Purkinje cells (Eccles et al. 1966, 1967; Thach 1967). Therefore complex spike activity exclusively reflects activity within the inferior olive. In addi-
tion, somatosensory stimulation elicits two nearly mutually exclusive patterns of complex spike activity: a single short-latency complex spike with no long-latency rhythmic discharge and a long-latency rhythmic discharge with no short-latency complex spike (Bloedel and Ebner 1984; Llinas and Sasaki 1989). Rhythmicity in complex spikes is characterized by two or more complex spikes occurring 60–200 ms apart, shortly after brief somatosensory stimulation. The rhythmic discharge in long-latency evoked complex spike activity is produced by rhythmic oscillations (≈8–12 Hz) in the membrane voltage of electrically connected aggregates of inferior olivary neurons, which are regulated by cerebellar feedback (Andersson et al. 1988; Lang et al. 1996; Llinas 1974; Llinas and Sasaki 1989; Llinas and Yarom 1981a,b, 1986; Llinas et al. 1974). Moreover, the segregation between the two evoked complex spike response patterns is regulated by inhibitory feedback, likely originating in the DCN (Lang et al. 1996; Llinas and Sasaki 1989). Accordingly, monitoring evoked complex spike activity in postnatal day 17 (PND17) and PND24 rats may provide a method for determining whether inhibition (e.g., cerebellar feedback) and excitation (e.g., afferent sensory input) within the inferior olive exhibit changes during the developmental time window in which a simple form of motor learning (eye-blink conditioning) emerges (Stanton et al. 1992).

Previous studies presented evidence that cerebellar inhibition within the inferior olive exhibits substantial developmental changes between PND17 and PND24 in rats (Nicholson and Freeman 2000, 2003a). However, the short period of time after onset of sensory stimulation (150 ms) and movement artifacts precluded a comprehensive analysis of the rhythmic firing properties of complex spikes at PND17 and PND24. Moreover, an analysis of the pharmacological influences on rhythmicity was not possible in the previous studies. The present experiment examined complex spike response patterns of single Purkinje cells in PND17 and PND24 rats after peri-orbital stimulation. If the cerebellum regulates climbing-fiber activity at PND17, then patterns of evoked complex spike activity at both ages should be segregated into trials with short-latency complex spikes and trials with long-latency rhythmic complex spikes but rarely both after a single stimulus (Bloedel and Ebner 1984). In addition, if cerebellar inhibition influences the segregation of these two complex spike response patterns, infusions of picrotoxin into the inferior olive should desegregate these response patterns and increase the number of trials on which short-latency complex spikes are followed by long-latency rhythmic spiking after a single peri-orbital stimulus (Llinas and Sasaki 1989).

METHODS

Subjects

The subjects were 33 PND17–18 and 31 PND24–25 Long-Evans rat pups. Sex was balanced. Mothers and their litters were housed in standard laboratory cages (48 × 20 × 26 cm) in the animal colony of Spence Laboratories of Psychology at the University of Iowa. Food and water were available ad libitum. Pups were weaned from the litter on P21. The animal colony was maintained on a 12:12 h light/dark schedule (lights on at 0700).

Surgery

Rats were anesthetized with an intraperitoneal injection of urethan (1.4 g/kg) and given atropine (2.5 mg/kg) to reduce excess respiratory tract secretions during anesthesia. Proper hydration was maintained with an initial prophylactic injection of 0.3–0.5 ml of lactated Ringer solution that was supplemented every hour with 0.1–0.2 ml injections. Rectal temperature was kept within 35–37°C with a heating pad or a heat lamp. Using these procedures, anesthesia could be maintained for ≤17 h.

On onset of anesthesia, an incision was made down the midline of the head, the tissue was deflected, and the skull was cleaned with cotton tipped applicators saturated with hydrogen peroxide. The muscle tissue overlying the first and second cervical vertebrae was deflected to expose the foramen magnum for visualization of obex and to allow dorsal access to the contralateral inferior olive for picrotoxin infusions. This area was kept moist with warm mineral oil. Rats were then placed on a stereotaxic headholder with their head oriented such that yaw was 0°, roll was −20–30° ipsilateral ear up, and pitch varied between 20° nose-up and 45° nose-down. A stainless steel skull hook was affixed to the skull with dental acrylic –1 mm anterior to lambda. A dental acrylic dam (~5 mm diam) was then constructed over the left half of the cerebellum such that vermal and hemispheric portions of the paramedian lobule, crus I and crus II of the ansiform lobule, and lobules VI and V could be accessed after skull removal. The acrylic dam served as a reservoir for warm mineral oil. Portions of the occipital bone and bony tentorium were removed with a drill to expose the dura (periosteum) overlying the cerebellar cortex. Access windows drilled in the skull were then cleaned with infusions of warm saline and kept moist by filling the acrylic reservoir with warm mineral oil. Access windows >2 mm were covered with gel foam soaked in warm mineral oil to reduce tissue pulsations. Immediately before single unit recording, the dura was deflected carefully with dissection tweezers and the sharp tip of a 23-gauge needle to visualize cerebellar arteries under a dissection stereo. Occasionally a blood vessel would rupture and bleed, which was stopped with gel foam. The probability of finding active Purkinje cells, however, was sometimes reduced drastically in areas proximal to ruptured blood vessels.

Infusion cannula

To examine the effects of blocking cerebellar regulation of climbing fibers, a 33 g infusion cannula was implanted in the contralateral inferior olive of four PND17 and five PND24 rats under stereotaxic guidance before preparation of the recording area. For these experiments, the rat’s head was placed in a stereotaxic head holder and aligned in three planes to bring it into orientation required for placement of the infusion cannula. The infusion cannula was then affixed to the skull and a stainless steel skull hook, after the dental acrylic hardened, the general surgical procedures outlined in the preceding text were followed. Picrotoxin, a γ-aminobutyric acid type A receptor (GABA A) blocker (Sigma; 0.5–3.0 ml; 0.25–1.0 mM), was infused into the inferior olive through the cannula at a rate of 6 ml/h (Harvard Apparatus; Holliston, MA). Two of the PND24 rats received infusions through an infusion cannula inserted dorsally into the caudal brain stem through the foramen magnum. After conclusion of the infusion experiments, a marking lesion was made in the inferior olive with a small wire inserted through the cannula (DC current, 1.0 mA, 5 s).

Recording

All data were acquired using an Xcell-3+ Isolated Microelectrode Amplifier customized with an impedance check module, audio-
ior, adjustable stimulus artifact suppressor, and DC output (FHC, Bowdoinham, ME). Activity was amplified (gain = 10,000), band-pass filtered 100–5,000 or 500–5,000 Hz, digitized, written to computer disk, and analyzed off-line using pClamp6 (Axon Instruments, Foster City, CA). Glass microelectrodes (resistance: 3–6 MΩ, filled with 2.0 M NaCl) were fabricated by pulling glass pipettes (1.0 OD; A-M Systems, Carlsborg, WA) with a Flaming/Brown micropipette puller (Model P-97; Sutter Instruments, Novato, CA).

Electrodes were lowered slowly into the surface layers of cerebellar folia with a micromanipulator (World Precision Instruments, Sarasota, FL) under a dissection stereoscope, taking special care to avoid cerebellar blood vessels. Purkinje cells were identified by the presence of spontaneous complex spike activity (Fig. 1) (Eccles et al. 1967). Once a Purkinje cell complex spike was identified, spontaneous activity was monitored for 3 min, after which its responsiveness to periorbital stimulation was assessed.

**Stimulation procedures**

Stimulation in all experiments was a single 200-ms cathodal pulse (range = 150–700 μA; Iso-Flex Stimulator, AMPI) delivered through two miniature test clips, separated by 1 mm, attached to the bottom left eyelid. A programmable stimulator (Master-8, AMPI) delivered stimuli at a rate of 0.25 Hz. For a Purkinje cell to be considered responsive to periorbital stimulation, complex spikes had to be elicited consistently with a latency of <20 ms for PND24 rats and <25 ms for PND17 rats (Figs. 2, A–C). The difference in the latency criterion is necessary in light of the ongoing myelination of climbing fibers in younger rats (Lang and Rosenbluth 2003). To ensure that a particular complex spike waveform originated from a single Purkinje cell, the waveforms of an individual isolated unit were overlaid on a digital oscilloscope (Tektronix, Beaverton, OR) during repeated stimulus presentations before data acquisition. In addition, waveforms were also scrutinized off-line in pClamp6 by “zooming in” to small time scales to compare the individual complex spike waveform to complex spike waveforms on other trials or at other time points on the same trial. A complex spike was considered to originate from a single Purkinje cell when it exhibited the same waveform across the stimulus presentation trials. All data in the present study derive from complex spikes that satisfied this waveform criterion. Once a complex spike satisfied the waveform criterion and was determined to be responsive to periorbital stimulation (e.g., Fig. 2, A–C), a sequence of 200 stimulation trials was presented.

Data were acquired for 500 ms after stimulus onset at a rate of 10,000 Hz. Unit histograms were then generated for each single Purkinje cell with a bin width of 10 ms. For the purposes of categorization, trials on which a Purkinje cell exhibited a complex spike within 50 ms of stimulus-onset were considered to be short-latency complex spikes (Bloedel and Ebner 1984). Minor variations (±20 ms) in this temporal criterion had no effect on the outcome of the study. All other trials were considered to have long-latency complex spikes. Complex spike activity under control conditions and complex spike activity during infusions of picrotoxin were investigated in separate animals.

**Data analysis**

Spontaneous complex spike activity was examined for all Purkinje cells for a period of 3 min. The total number of complex spikes was divided by 180 s to obtain the average firing rate (in Hz) for each neuron. Onset latency was determined as the earliest time point following stimulation that ≥10 complex spikes could be reliably elicited (range = ~5 ms) elicited (Fig. 2, A–C). Data from each Purkinje cell were analyzed in the form of events per 10-ms bin. To examine rhythmic firing properties of Purkinje cells in rats at each age, autocorrelations were performed on the binned neuronal activity collapsed across all 200 trials. Histograms were also generated for trials with only short-latency and only long-latency complex spikes separately. A complex spike was considered to display rhythmicity when there were clearly separated peaks in the individual unit histograms; and there were significant autocorrelations with time lags of 60–200 ms (Nicholson and Freeman 2003a).

**R E S U L T S**

A total of 114 complex spikes at PND17 and 122 complex spikes at PND24 were recorded. Picrotoxin was infused for 39 (34%) of the units at PND17 and 44 (36%) of the units at PND24. The average spontaneous complex spike firing rates under control conditions and after picrotoxin infusion in PND17 rats were 1.24 and 1.38 Hz, respectively. The average spontaneous firing rates for complex spikes under control conditions and after picrotoxin infusion in PND24 rats were 0.96 and 1.25 Hz respectively. An ANOVA on the spontaneous firing rate yielded significant main effects of age [$F(1,232) = 2.66, P < 0.01$] and infusion [$F(1,232) = 2.78, P < 0.01$], but no interaction.

The average onset latencies of stimulus-evoked complex spikes in PND17 rats under control conditions and after olivary picrotoxin infusion were 21.9 and 23.2 ms, respectively. The average onset latencies of stimulus-evoked complex spikes in
PND24 rats under control conditions and after olivary picrotoxin infusion were 14.5 and 15.3 ms, respectively. An ANOVA yielded a significant main effect of age \([F(1,232) = 9.6, P < 0.01]\), attributable to shorter onset latencies in older rats. Picrotoxin infusions did not affect onset latencies. Consistent with previous studies, complex spikes at PND17 exhibited a more synchronous response to periorbital stimulation (Fig. 3, top) (Nicholson and Freeman 2000, 2003a). In addition, picrotoxin increased complex spike responsivity in older rats and increased rhythmic firing in the mean neuronal activity (Fig. 3, bottom) (Llinas and Sasaki 1989).

**Complex spike rhythmicity**

Complex spike rhythmicity (i.e., significant autocorrelations at time lags of 60–200 ms) was observed in 48% of the Purkinje cells at PND17 under control conditions and 64.2% after picrotoxin infusion (Fig. 4A). In PND24 rats, complex spike rhythmicity was observed in 52% of the Purkinje cells during control conditions and 75% after picrotoxin infusion (Fig. 4A). As can be seen in Fig. 4, B and C, the significant peaks in the autocorrelograms tended to occur at time lags between 60 and 150 ms (i.e., ~6–16 Hz). If maturation of the membrane voltage oscillations in the inferior olive contributed to any of the age-related differences in complex spike rhythmicity, then age-related differences in the distributions of significant autocorrelation time lags might be expected. Comparisons of the distributions of significant time lags between the two ages, however, failed to find any reliable difference.

If blocking cerebellar feedback to the inferior olive significantly enhances rhythmicity, as reported in adult rats (Lang et al. 1996; Llinas and Sasaki 1989), then a concentration of the distribution of significant autocorrelation time lags, compared with control conditions, centered ~10 Hz might be expected after infusion of picrotoxin. There were no reliable differences in the distributions of significant autocorrelation time lags between control conditions and after picrotoxin infusion in PND17 rats. There was, however, a reliable difference between the distributions of significant autocorrelation time lags between control conditions and after picrotoxin infusion in PND24 rats \((\chi^2 = 86.454, P < 0.01)\).

Bloedel and Ebner (1984) reported that rhythm in complex spike activity tended to occur on trials with long-latency (>50 ms) responses but only rarely on trials with short-latency responses in decerebrate adult cats. Llinas and Sasaki (1989) provided evidence that this segregation of complex spike response patterns into trials that have rhythmic complex spike activity and trials that have only short-latency responses requires GABAergic regulation within the inferior olive in rats. If cerebellar feedback to the inferior olive is immature at PND17 and is an important component in regulating complex spike response patterns and rhythmicity (e.g., ensuring that short-latency complex spikes are not followed by nonsynaptically evoked rhythmic complex spikes), then the segregation of different complex spike response patterns (e.g., short- and long-latency responses) may exhibit age-related changes between PND17 and PND24.

To test this, complex spike patterns for each individual Purkinje cell were categorized as characteristic of one of three patterns. In the first response pattern, trials with short latency complex spikes were completely segregated from trials with long-latency complex spikes >30% of the time (Fig. 5). In the second response pattern, short-latency com-

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**FIG. 3.** Mean complex spike activity under control conditions and after olivary picrotoxin infusion. Histograms of the mean complex spike activity recorded from Purkinje cells at PND17 (left) and PND24 (right) under control conditions (top) and after infusion of picrotoxin into the inferior olive (bottom). Note the age difference in the magnitude of the short-latency complex spike response and the increased long-latency activity after picrotoxin infusion in older rats.
plex spikes and long-latency rhythmic complex spikes occurred on the same trials. In the third response pattern, short- and long-latency complex spikes occurred on the same trials on 31–59% of the time. Importantly, varying the criterion percentages had no effect on statistical analyses.

Figures 7, A–D, depicts the complex spike data of two representative Purkinje cells that illustrate the age-related differences in the segregation of complex spike response patterns. As can be seen, trials with rhythmic long-latency complex spikes were completely segregated from trials with nonrhythmic short-latency complex spikes at PND24 (Fig. 7, right). In contrast, rhythmic long-latency complex spikes were present on trials with and without short-latency complex spikes at PND17 (Fig. 7, left). When complex spikes exhibiting significant rhythmicity were categorized according the criteria outlined in the preceding text, there was a striking age-related difference in the distributions of the three types of response patterns (Fig. 4D), which was statistically significant ($\chi^2 = 214.74, P < 0.01$). Specifically, complex spike response patterns of Purkinje cells from PND17 rats were grouped overwhelmingly into the pattern that exhibited long-latency rhythmic discharge on the same
trials as short-latency complex spikes (Fig. 4D, S). In contrast, the short- and long-latency complex spike responses of Purkinje cells from PND24 rats occurred primarily on different trials (Fig. 4D, D), which replicated previous studies in adult rats (Llinas and Sasaki 1989) and adult decerebrate cats (Bloedel and Ebner 1984).

If the failure to separate short-latency complex spike response patterns from rhythmic long-latency complex spike response patterns is attributable to an inability to regulate climbing-fiber activity through cerebellar inhibition at PND17 (Nicholson and Freeman 2003a), then picrotoxin infusion into the inferior olive of PND24 rats may be expected to transform complex spike response patterns of older rats into patterns more characteristic of younger rats.

Figure 8 shows a representative example of the complex spike response pattern in PND24 rats after infusion of picrotoxin into the inferior olive. Note how the histograms are very similar to the unit histograms for the Purkinje cell in the PND17 rat in Fig. 7, A–D. When the distribution of complex spike response patterns in PND24 rats under control conditions was compared with the distribution of complex spike patterns after picrotoxin infusion, there was a dramatic shift to a distribution more characteristic of PND17 rats (Fig. 4D: \( \chi^2 = 201.76, P < 0.01 \)). That is, after infusion of picrotoxin into the inferior olive of PND24 rats, short- and long-latency rhythmic complex spikes predominantly occurred on the same trials (Fig. 4D). In contrast, picrotoxin infusion had no reliable effect on the distribution of complex spike response patterns in PND17 rats.
DISCUSSION

The ability of neurons in the DCN to influence the brain stem afferent inputs (e.g., mossy and climbing fibers) to the cerebellum is a unique property that may have implications for the production and preservation of learned, coordinated movements (Ito 1984; Kim and Thompson 1997; Mauk and Donegan 1997; Medina and Mauk 1999; Thompson 1986; Welsh et al. 1995). Feedback from neurons in the DCN reflects computations not only within the DCN neurons themselves but also information processing within the Purkinje cells in the cerebellar cortex, which are exclusively inhibitory. It is important then to characterize the changes in cerebellar feedback that occur in parallel with the developmental emergence of certain forms of motor learning (e.g., eyelink conditioning). Unit-recording studies may help elucidate how network interactions between the cerebellum and brain stem organize developmentally to allow learning in the cerebellum to occur. The results of the present experiment suggest that cerebellar feedback to the inferior olive exhibits substantial developmental changes between PND17 and PND24.

Purkinje cell complex spikes are produced solely by the climbing fibers from the inferior olive (Eccles et al. 1967). The dendrites of neurons in the inferior olive are electrically coupled through gap junctions (Llinas et al. 1974; Sotelo et al. 1974). GABAergic input to the inferior olive regulates the strength of this coupling (Lang et al. 1996; Llinas 1974; Llinas and Sasaki 1989). In addition, gap junctions allow membrane voltage oscillations in single olivary neurons to influence the oscillations in other, electrically coupled, neurons, producing neuronal aggregates with synchronous oscillatory fluctuations in excitability and spiking. The organization of these aggregates can be disrupted by blocking GABAergic synapses with picrotoxin or by lesioning the deep cerebellar nuclei (Lang et al. 1996), which provide >90% of the GABAergic input to the inferior olive (De Zeeuw et al. 1989; Fredette and Mugnaini 1991). The present study examined whether developmental differences existed in cerebellar feedback to the inferior olive to further examine the notion that olivocerebellar interactions may play a critical role in behaviors that emerge early in postweanling development (Freeman and Nicholson 2001; Nicholson and Freeman 2000, 2003a).

By infusing picrotoxin into the inferior olive, which blocks GABAergic synapses, the complex spike response patterns of individual Purkinje cells in PND24 rats were transformed into patterns more characteristic of PND17 rats. This pattern shift in older rats indicates that complex spike response patterns in PND17 rats may be analogous to complex spike response patterns in PND24 rats under blockade of GABAergic synapses. The distributions of the time lags at which complex spike activity exhibited significant autocorrelations did not show any reliable age-related differences, which suggests that the membrane properties that produce olivary oscillations are intact and mature by PND17 (Benardo and Foster 1986; Bleasel and Pettigrew 1992; Pettigrew et al. 1988). These points indicate that a very localized developmental change in the olivocerebellar system (i.e., inhibitory feedback from the cerebellum to the inferior olive) could have consequences on at least two additional synapses.

The inability to regulate climbing-fiber activity in younger rats renders the cerebellar deep nuclei unable to influence the inhibitory input from Purkinje cells. This breakdown in olivocerebellar regulation may have the consequence of changing the balance between excitation and inhibition that characterizes the eyblink neural circuit in general, and the olivocerebellar system in particular (Kim and Thompson 1997; Mauk and Donegan 1997). What detrimental effects could short-latency complex spikes followed by rhythmic long-latency activity have during eyelink conditioning? Most behavioral and neural network models of eyelink conditioning stress the importance of the temporal relationship between the conditioned stimulus.
(CS) and the unconditioned stimulus (US) (Bartha et al. 1991; Bullock et al. 1994; Gluck et al. 1995; Mauk and Donegan 1997; Moore and Choi 1997; Raymond et al. 1996). The all-or-none nature of olivary neuronal activity allows specific temporal information about the onset of the US to be transmitted to the cerebellum through the climbing fibers. The inability of PND17 rats to partition complex spike patterns into short-latency and rhythmic long-latency patterns may have the effect of transmitting imprecise temporal information about the onset of the US. In addition, because cerebellar regulation of climbing-fiber activity controls the precise somatotopic organization of the olivocerebellar system (Lang et al. 1996; Llinas 1974; Welsh 2002), unregulated olivary activity in PND17 rats likely transmits somatotopically imprecise information about the US. That is, the inability to regulate climbing-fiber activity through cerebellar inhibition probably disrupts the discrete somatotopic organization within the cerebellar cortex, such that stimulation of the periorbital region may elicit complex spikes in eye, face, cheek, or nose regions of the cerebellar cortex in younger rats (Llinas and Sasaki 1989). The lack of spatially and temporally accurate information about the onset of the US during eyeblink conditioning in PND17 rats may obstruct the emergence of discrete learning-related cerebellar plasticity.

A series of recent studies have provided evidence that cerebellar neurons do not inhibit inferior olive neuronal activity in younger rats (Nicholson and Freeman 2000, 2003a). In addition, the number of inhibitory synapses within the dorsal accessory olive, which is critical for acquiring the eyeblink conditioned response (McCormick et al. 1985; Mintz et al. 1994; Yeo et al. 1986), exhibits a dramatic increase between PND17 and PND24 (Nicholson and Freeman 2003a). Therefore, the cerebellum in younger rats may be chronically unable to regulate climbing-fiber activity. Recent models suggest that the inability of younger rats to regulate climbing-fiber activity through cerebellar inhibition of the inferior olive, combined with spatially and temporally inaccurate information about the onset of the US during eyeblink conditioning, may obstruct the induction and preservation of discrete cerebellar plasticity (Medina and Mauk 1999; Medina et al. 2002).

Although the present study was conducted in urethan-anesthetized rats, two points indicate that the findings accurately reflect brain-stem-cerebellum interactions as they occur in unanesthetized rats. First, analysis of neuronal activity from the inferior olive (Nicholson and Freeman 2000) and complex spikes in lobule HVI (Nicholson and Freeman 2003a) of unanesthetized rats during presentations of a periorbital shock US given before eyeblink conditioning indicated that urethan had a minimal effect, if any, on cerebellar control of complex spike response patterns. Specifically, applying the same criteria for characterizing complex spike patterns used in the present study to these archival data revealed the same (within 10% in all categories) developmental differences in response patterns (data not shown). Second, the spontaneous firing rate of complex spikes under control conditions in the present study was similar to the spontaneous firing rate of complex spikes recorded in unanesthetized rats in a previous study (Nicholson and Freeman 2003a). This latter result would be unexpected if urethan significantly affected the rhythmic firing properties of the olivocerebellar system. Together these points indicate that the results of the present study are relevant for the behaving animal and that developmental differences in the interactions between the cerebellum and inferior olive are relatively unaffected by urethan anesthesia. These points are also consistent with previous work indicating that urethan is suitable for maintaining anesthesia during electrophysiological studies (Hara and Harris 2002; Koblin 2002; Krasowski and Harrison 1999; Maggi and Meli 1986).

In sum, the balance of excitation and inhibition within the olivocerebellar system is not mature until at least PND24 in rats. Without cerebellar regulation of climbing-fiber activity, temporally and somatotopically imprecise information may obstruct the induction of discrete cerebellar plasticity during eyeblink conditioning. The weak cerebellar plasticity induced in younger rats (Freeman and Nicholson 2000; Nicholson and Freeman 2003b) may be further compromised by their inability to regulate climbing-fiber activity through cerebellar inhibition within the inferior olive (Medina and Mauk 1999; Nicholson and Freeman 2003a). These two age-related changes combined offer a possible explanation for the ontogenetic emergence of eyeblink conditioning in rats.

DISCLOSURES

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