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Spontaneous and Harmaline-Stimulated Purkinje Cell Activity in Rats with a Genetic Movement Disorder

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The genetically dystonic rat (*dt*) displays a complex movement disorder in the absence of morphological defects in the nervous system. This mutant is also insensitive to the tremorogenic effects of harmaline. Because harmaline is known to act on the cells of the inferior olive to induce activity at the tremor frequency in the olivocerebellobulbar pathway, this pathway has been investigated as a possible site of a defect in the *dt* rat. Biochemical studies suggested the presence of abnormalities at the level of the Purkinje cell or its afferent input. Thus, the present study investigated the harmaline response of Purkinje cells in *dt* rats and unaffected littermate controls with extracellular single-unit recording techniques. The spontaneous, simple spike and complex spike firing rates of *dt* rats were significantly lower than those of normal littermate controls. In normal rats, 2 responses to systemic harmaline injection were seen. Simple spikes were either completely suppressed for periods of 30–180 min, or were intermittently suppressed, pausing repeatedly for periods of 1–18 sec. Cells that showed complete suppression of simple spike activity also showed increased frequency and rhythmicity of complex spikes. In *dt* rats, intermittent simple spike responses were seen in a proportion (41%) similar to that in normal rats (53%). However, the proportion of cells showing high-frequency, rhythmic, complex spikes and complete suppression of simple spikes was low in the *dt* rats in comparison with littermate controls (18 versus 47%). In addition, 41% of the cells from *dt* rats displayed no change, or an anomalous change, in firing patterns in response to harmaline. Since the rhythmic activation of olivary neurons that results in the rhythmic, complex spike discharge of Purkinje cells is assumed to be responsible for the appearance of harmaline tremor, the failure of the *dt* rat to display tremor is most likely due to a failure at the olivocerebellar level, rather than at a site efferent to the cerebellum.

Systemic administration of harmaline produces a fine motor tremor with a frequency of 8–14 Hz in a variety of mammalian species (Fuentes and Longo, 1971; DeMontigny and Lamarre, 1973; Mariani and Delhaye-Bouchaud, 1978; Busby and Lamarre, 1980; Barragan et al., 1985). This effect of harmaline has been attributed to the action of the drug on the inferior olive climbing fiber system (Lamarre and Mercier, 1971; Lamarre et al., 1971; Biscoe et al., 1973; DeMontigny and Lamarre, 1973; Lamarre and Puil, 1974). Harmaline induces an increase in the frequency and rhythmicity of olivary neuronal activity that is reflected in the complex spike activity of the Purkinje cells of the cerebellar cortex (DeMontigny and Lamarre, 1973; Llinás and Volkind, 1973). The olivary climbing fibers provide one of the 2 major afferent inputs to the Purkinje cells and are responsible for the appearance of the complex spike waveform in extracellular recordings from the Purkinje cells. The complex spike waveform can be distinguished from that of simple spikes that result from activity in the other major afferent input, the mossy fiber–parallel fiber pathway. In the vermal and paravermal cortex, Purkinje cells generally show increased complex spike activity and decreased simple spike activity after harmaline administration (DeMontigny and Lamarre, 1973; Bernard et al., 1984). Interruption of the olivocerebellobulbar circuit at any point is sufficient to block the appearance of tremor, but not the synchronous activity of the olivary neurons (DeMontigny and Lamarre, 1973; Llinás and Volkind, 1973; Simantov et al., 1976; Bardin et al., 1983). Thus, the cells of the inferior olive are thought to be the pacemaker cells for the tremor.

In the course of studies on the dystonic rat (*dt*), a mutant with a motor syndrome of central origin, we observed that rats homozygous for this autosomal recessive mutation failed to display harmaline tremor (Lorden et al., 1985). This was true even at high doses that produced a severe and prolonged tremor in phenotypically normal rats. Another tremorogenic drug with a different mechanism of action, oxotremorine (Kaelber and Hamel, 1960, 1961), was effective in the *dt* rats. This suggested that the defect responsible for the behavioral insensitivity to harmaline was located at a supraspinal level.

In the absence of any discernible morphological lesion in the *dt* rat (Lorden et al., 1984; McKeon et al., 1984), we have used biochemical techniques to identify potential sites for a neural defect that could account for the dystonic rat's behavioral insensitivity to harmaline. These measures have uncovered evidence of cerebellar abnormalities in the *dt* rat. Basal levels of cerebellar 3′,5′-cGMP are low in the *dt* rat, and harmaline, which stimulates cGMP production in the cerebellum, increases cGMP levels in *dt* rats only to that of untreated normal rats (Lorden et al., 1985). In addition, there is a significant and selective increase in glutamic acid decarboxylase (GAD) activity

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in the deep cerebellar nuclei of *dt* rats in comparison with normal littermates (Oltmans et al., 1984, 1986). GAD is the synthetic enzyme for GABA, one of the major Purkinje cell neurotransmitters (Chan-Palay, 1982). The deep cerebellar nuclei are the primary projection sites of the Purkinje cells, and, within the deep nuclei, GAD is thought to be localized principally, although not exclusively, in Purkinje cell terminals (Fonnum et al., 1970; Fonnum and Walberg, 1973).

Other studies have shown that in normal animals, lesions of the climbing fiber system produced with the neurotoxin 3-acetylpyridine (3AP) can increase GAD activity in the deep nuclei (Oltmans et al., 1985). Other parallels between *dt* rats and rats treated with 3AP include insensitivity to harmaline tremor (Simantov et al., 1976; Bernard et al., 1984), reduced cerebellar cGMP, and reduced cGMP response to harmaline (Guidotti et al., 1975). The similarities between 3AP-treated rats with inferior olive lesions and *dt* rats with an apparently intact olivocerebellar projection extend to behavior. 3AP treatment produces a motor syndrome that includes pivoting, ataxia, axial twisting, and abnormal limb placement (Sukin et al., 1987). Rats affected by the *dt* mutation show abnormal locomotor development that is detectable at about postnatal day 10, when normal rat pups begin coordinated crawling (Lorden et al., 1984). Abnormal movements include excessive pivoting, hyperextension of the limbs, and frequent falls. Limb placement is poor and the mutant pups show twisting of the limbs and axial musculature. Although there is no evidence of a lesion in the inferior olive in the *dt* rat, the similarities between the features of the *dt* and 3AP syndromes suggest a dysfunction of the olivocerebellar pathway in the *dt* rat.

The current electrophysiological investigation was designed to examine the functional properties of cerebellar inputs and Purkinje cell responsiveness in *dt* rats. Since the effects of lesions of the inferior olive on Purkinje cell activity have previously been described (Batini and Billard, 1985; Batini et al., 1985), the status of the olivocerebellar system in the *dt* rat can be compared directly with that of animals lacking a climbing fiber pathway. We recorded single-unit extracellular Purkinje cell activity in *dt* rats and phenotypically normal littermates both before and after harmaline administration to assess the response of the olivocerebellar pathway of the *dt*.

A preliminary report of these data was presented at the Second International Conference on Dystonia, New York, April 1985.

Materials and Methods

Mutant (*dt*) and phenotypically normal rat pups were obtained from the dystonic animal colony maintained at the University of Alabama at Birmingham. Litters were culled to 8 or fewer pups at 10 d of age. Recordings were made at 20–28 d of age. At this age the motor syndrome of the *dt* rat is well-established but the pups are otherwise in good health.

Before the recording sessions, all animals were anesthetized with intraperitoneal injections of urethane (1.9 gm/kg). This dose was sufficient to maintain a surgical level of anesthesia throughout the recording session. Silastic jugular catheters were implanted in all animals to permit drug administration during the recording sessions. Body temperature was monitored with a rectal thermometer and maintained with Deltaphase heating pads (Braintree Scientific).

Parylene-coated tungsten microelectrodes (Microprobe) (impedance, 1.5–2.5 MΩ) were used to record extracellular single-unit activity of Purkinje cells in the cerebellar cortex. A 1–2 mm hole was drilled in the cranium over the cerebellum, through which electrodes were lowered with a Kopf hydraulic microdrive. To minimize drying and movement of the brain, the dura was punctured only enough to allow the passage of the electrode. Single-unit activity was amplified by a BAK A-1 high-impedance amplifier and monitored on an oscilloscope and an audio monitor. The signal was digitized and stored on VHS videotape for off-line computer processing.

Purkinje cells were identified by the presence of spontaneous, discriminable complex spikes and simple spikes. Confirmation of a unitary source for both complex and simple spikes was based on spike amplitude. Recordings of spontaneous activity from each cell were made for 10–20 min prior to drug injection. Bolus injections of harmaline (10 mg/kg) were then given through the jugular catheter while maintaining isolation of the unit. With one exception, postdrug activity was recorded from each cell for a minimum of 30 min. When possible, recording continued until the cell's activity returned to the predrug level. In some cases it was possible to maintain well-isolated single units for as long as 3½ hr. Each rat was given only one harmaline injection, and data from each cell are from a different animal. While this approach limited the amount of data that could be obtained from each animal, it allowed for a within-cell comparison of pre- and postdrug responses in normal and *dt* rats. This approach also provided an extended profile of the response of each cell to the drug.

Unit activity was played back into 2 amplitude and time window discriminators (DIS-1; BAK Electronics) to separate complex spike and simple spike activity. Photographs were taken of the complex and simple spike waveforms accepted by the discriminators. The discriminator outputs were stored on magnetic tape with a 0.1 msec resolution by a PDP 11/73 computer. Autocorrelation histograms (autocorrelograms) were computed from higher-order interspike interval densities according to a renewal process. This method has been described by others (Gerstein and Kiang, 1960; Perkel et al., 1967). The higher-order interspike interval densities were used to generate spike counts, which display the temporal probability of spikes within a given spike train. The counts are converted to an impulse rate by dividing the count in a given bin by the product of bin width and the total number of spikes in the original spike train. This is displayed on the ordinate of the autocorrelogram (Knox, 1981). Autocorrelograms of complex spike and simple spike activity for each cell were computed with a 1 msec bin width from 5 min recording samples immediately before the drug injection and after each cell reached a stable response following the drug injection. Owing to differences in firing frequency among cells, the 5 min samples contain between 600 and 22,500 spikes. The conversion to impulse rate normalizes the peak of the autocorrelogram according to the total number of spikes. An index of spike rhythmicity (rhythmicity index value, R) was computed from the autocorrelograms by dividing the number of counts at the peak of the autocorrelogram by the total number of counts and multiplying by 1000. This measure was devised to normalize the peak height according to the average impulse rate on which the peak is superimposed. Each R value is directly proportional to the number of counts at the peak of the autocorrelogram, and reflects the regularity of spike activity within the train. As a result, larger R values reflect more rhythmic complex spike or simple spike activity.

Results

Single-unit recordings were made from 33 normal and 41 *dt* rats. Only those cells in which simple and complex spikes could be distinguished with the 2 window discriminators were retained. Spontaneous predrug activity was analyzed in 17 cells in normal rats and 19 cells in *dt* rats. Harmaline was injected in 17 normal and 17 *dt* rats. Recordings were made from a variety of depths and sagittal locations in lobules V–VIIb, as indicated in Tables 1 and 2. The cell numbers in these tables are those used in the text and figures.

Spontaneous activity

The average simple spike and complex spike frequencies for each cell were computed from 5 min samples of activity taken immediately prior to the drug injection, and are listed in Tables 1 and 2. The average spontaneous simple spike frequency (mean ± SE) seen in normal rats (35 ± 5.1 spikes/sec) was significantly higher than the 12 ± 2.1 spikes/sec rate seen in *dt* rats ($F(1,34) = 18.7$, $p < 0.001$). The average complex spike rate was also significantly higher for normal rats than for *dt* rats (1.3 ± 0.18

Table 1. Cell locations and spike analyses for Purkinje cells from normal rats

Cell	Location[a]	Predrug Frequency (spikes/sec)				Postdrug Frequency (spikes/sec)			
		SS	R_{ss}[b]	CS	R_{cs}[b]	SS	R_{ss}[b]	CS	R_{cs}[b]
N1	p, cr II	65	110	0.7	10	0	—	3.0	20
N2	v, lob. V	41	70	0.8	10	0	—	4.7	30
N3	v, lob. VIc	34	80	1.5	10	0	—	4.4	40
N4	p, l. pmd. a	17	10	2.2	10	0	—	3.9	20
N5	v, lob. VIc	59	90	3.2	10	0	—	6.0	40
N6	v, lob. VIc	16	40	1.4	10	0	—	5.6	70
N7	p, cr II	14	20	1.2	0	0	—	6.0	30
N8	v, lob. VIa	40	160	1.2	0	0	—	4.8	50
N9	v, lob. VIIa	15	40	2.0	10	15	30	1.9	10
N10	lh, cr II	28	40	0.8	10	18	40	1.9	10
N11	v, lob. VIb	24	40	1.6	10	13	50	2.1	10
N12	p, cr II	31	100	0.8	10	20	50	1.2	10
N13	mlh, cr II	75	120	2.3	10	32	100	0.5	10
N14	mlh, cr I	16	40	1.1	10	4	60	0.3	10
N15	p, lob. simplex	60	100	0.5	10	41	100	1.5	10
N16	mlh, cr II	8	20	0.3	0	20	80	0.8	10
N17	mlh, cr II	50	110	1.1	10	21	40	1.0	0

Frequencies and R values are based on 5 min samples of spike activity for each cell, taken before and after drug injection. SS, simple spikes; CS, complex spikes.

[a] Cell locations are identified by sagittal, followed by anterior–posterior, position. Abbreviations: p, paravermis; cr, crus; v, vermis; lob., lobule; l. pmd. a, paramedian lobule; lh, lateral hemisphere; mlh, medial lateral hemisphere.

[b] R_{ss}, rhythmicity index for simple spike activity; R_{cs}, rhythmicity index for complete spike activity. See text for definition.

Table 2. Cell locations and spike analyses for Purkinje cells from *dt* rats

Cell	Location[a]	Predrug Frequency (spikes/sec)				Postdrug Frequency (spikes/sec)			
		SS	R_{ss}[b]	CS	R_{cs}[b]	SS	R_{ss}[b]	CS	R_{cs}[b]
Dt1	v, lob. VIb	4	10	0.8	0	0	—	5.6	40
Dt2	mlh, cr II	8	10	0.4	0	0	—	3.9	20
Dt3	mlh, cr II	19	30	0.4	10	0	—	3.7	20
Dt4	v, lob. VIb	11	30	0.4	0	6	20	0.5	0
Dt5	mlh, cr I	19	40	0.4	0	12	40	0.7	0
Dt6	mlh, cr II	12	50	1.1	0	12	70	1.2	0
Dt7	v, lob. VIb	4	10	0.3	0	3	20	0.5	0
Dt8	v, lob. VIb	10	30	0.4	0	3	10	0.7	10
Dt9	v, lob. VIb	8	10	0.5	0	4	20	0.7	0
Dt10	mlh, cr II	2	10	1.2	10	6	20	0.1	0
Dt11	v, lob. VIIb	11	40	0.9	10	0	—	3.0	10
Dt12	v, lob. VIIb	2	0	0.3	0	0	—	0.7	0
Dt13	v, lob. VIIa	6	10	1.5	10	4	10	4.5	20
Dt14	p, lob. simplex	24	70	0.1	0	19	40	0.1	0
Dt15	v, lob. VIIb	18	20	0.4	0	17	20	0.5	0
Dt16	mlh, cr II	39	60	0.8	30	21	30	0.4	10
Dt17	v, lob. VIb	7	20	1.2	10	12	20	1.0	10
Dt18	v, lob. VIb	10	40	0.6	10				
Dt19	v, lob. VIc	19	80	1.8	20				

Frequencies and R values are based on 5 min samples of spike activity for each cell, taken before and after drug injection. SS, simple spikes; CS, complex spikes.

[a] Cell locations are identified by sagittal, followed by anterior–posterior, position. Abbreviations: v, vermis; lob., lobule; mlh, medial lateral hemisphere; cr, crus; p, paravermis.

[b] *R_{ss}, rhythmicity index for simple spike activity; R_{cs}, rhythmicity index for complete spike activity. See text for definition.*

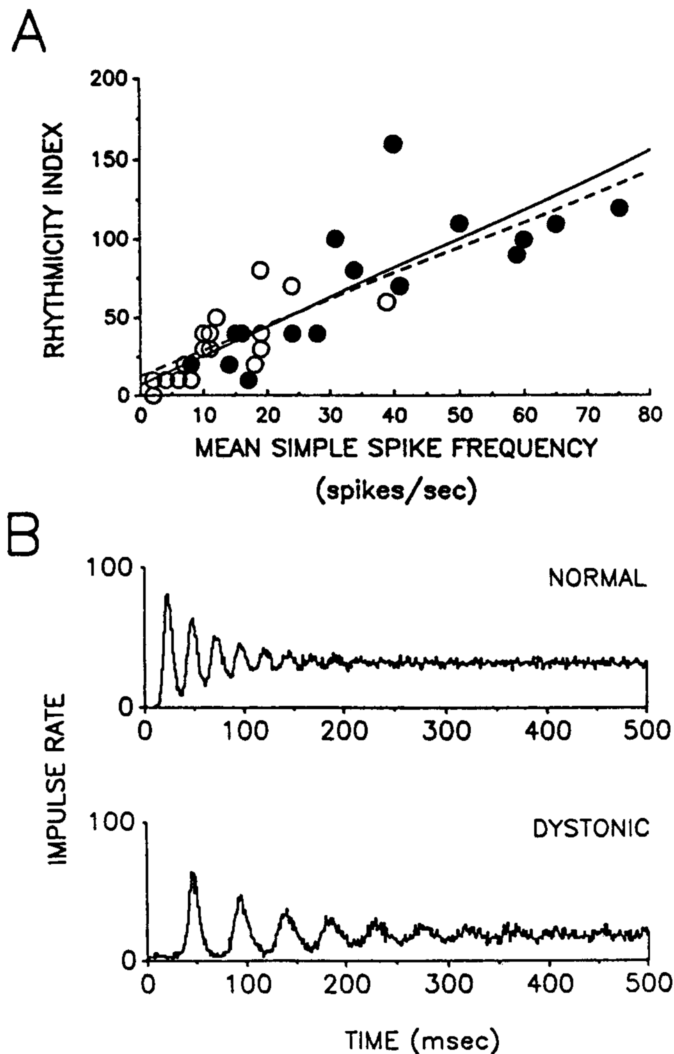

Figure 1. *A,* Predrug rhythmicity index values are plotted as a function of mean predrug simple spike frequency for each cell in Tables 1 and 2. The slopes of the regression lines are not significantly different for the 2 phenotypes. Cells from *dt* rats are shown as *open circles* with a solid regression line, and those from normal rats as *filled circles* around a dashed line. *B,* Autocorrelograms from 5 min periods of spontaneous activity of cells from (*top*) a normal rat (N3) and (*bottom*) a *dt* rat (Dt8). The *R* values are 80 and 50, respectively. Bin width for this and all subsequent autocorrelograms is 1 msec, unless specifically noted. Cell numbers in all figures correspond to those in Tables 1 and 2.

spikes/sec versus 0.7 ± 0.1 spikes/sec; $F(1,34) = 10.3$, $p < 0.01$).

Prior to drug administration, differences were also seen in the regularity of simple spike activity between normal and mutant rats. The mean *R* values (Tables 1 and 2) for the normal rats (mean = 70, SE = 10.4) were significantly greater than those for *dt* rats (mean = 30 ± 5.2; $F(1,34) = 12.5$, $p < 0.001$). A significant positive correlation ($r = 0.84$, $p < 0.001$) was found between Purkinje cell simple spike firing rate and rhythmicity for all cells. In Figure 1*A*, the relationship between mean simple spike frequency and the corresponding *R* value for each cell is plotted for both normal and *dt* rats. Although *dt* rats tended to have lower values, the slopes of the regression lines for the 2 phenotypes were not significantly different ($p < 0.5$). Examples of the simple spike autocorrelograms on which these plots were based are shown in Figure 1*B* for cell N3 from a normal rat and cell Dt8 from a mutant. The predrug *R* values for the complex spikes were low and did not differ significantly between the normal and mutant rats (mean = 8.24, SE = 0.95 for normal rats; 5.78 ± 1.92 for *dt* rats).

Responses to harmaline in normal rats

Complete simple spike suppression. In normal rats, approximately half the cells (8/17) showed an increase in the frequency and rhythmicity of complex spikes, accompanied by sustained suppression of simple spikes within 3 min of the harmaline injection. These 8 cells were located in the vermal and paravermal regions of the cerebellar cortex. In 5 cases in which we were able to continue recording until simple spike rate returned to normal, the duration of simple spike suppression varied between 30 and 180 min. Frequency plots of complex spike and simple spike activity of a cell from a normal rat (N8), showing total simple spike suppression with recovery to predrug levels, are shown in Figure 2*A*. After harmaline administration ($t = 0$), complex spike activity increased from 1 spike/sec to 5 spikes/sec, while simple spike activity was completely suppressed. The reciprocal relationship between complex and simple spike activity is evident in the postdrug response.

Autocorrelograms of pre- and postdrug complex spike activity for cell N8 are shown in Figure 2*B*. Prior to drug administration, no rhythmicity was evident in the complex spikes. The development of a rhythmic, complex spike firing pattern following the drug can be seen in comparisons of the predrug and 10 min postdrug autocorrelograms. Approximately 65 min after harmaline administration, cell N8 returned to its predrug complex spike frequency of about 1 spike/sec for about 20 min. This episode is illustrated in the autocorrelograms in Figure 2*B*. The simple spike *R* value for both this and the predrug period was 160, which suggests a similarity in firing pattern. Eighty-five minutes after the injection, the complex spike frequency again increased and simple spikes were suppressed. Although there was increased variability in the complex spike frequency at this time, in comparison with the immediate predrug period, the pattern of complex spike activity was still rhythmic (Fig. 2*B*, bottom panel). Thus, even though the drug effect may have been declining at this point, the response of the cell was similar to that seen earlier. Increases in complex spike frequency and rhythmicity were consistently associated with the complete suppression of simple spike activity in normal rats. For cells N1–N8, mean complex spike frequency increased from 1.5 (SE = 0.29) to 4.8 ± 0.37, and the mean *R* values from 7.5 ± 1.6 to 37.5 ± 5.89; $p < 0.01$ for both.

Intermittent simple spike suppression. In normal rats, 9 of the 17 cells showed a periodic, rather than a complete, suppression of simple spike activity in response to harmaline. Intermittent simple spike suppression was accompanied by small increases in complex spike frequencies in about half of the cells. The simple spike activity during this response was intermittently interrupted by periods of simple spike suppression, lasting from 1 to 18 sec. This response appeared with the same latency as the complete simple spike suppression response and was equally persistent. However, as indicated by the complex spike *R* values in Table 1, no cells in this category developed rhythmic complex spike activity. Neither of these 2 response types—complete suppression and intermittent suppression—could reliably be predicted based on predrug complex spike or simple spike frequencies or firing patterns. The cells showing intermittent suppression were found more frequently in the paravermian

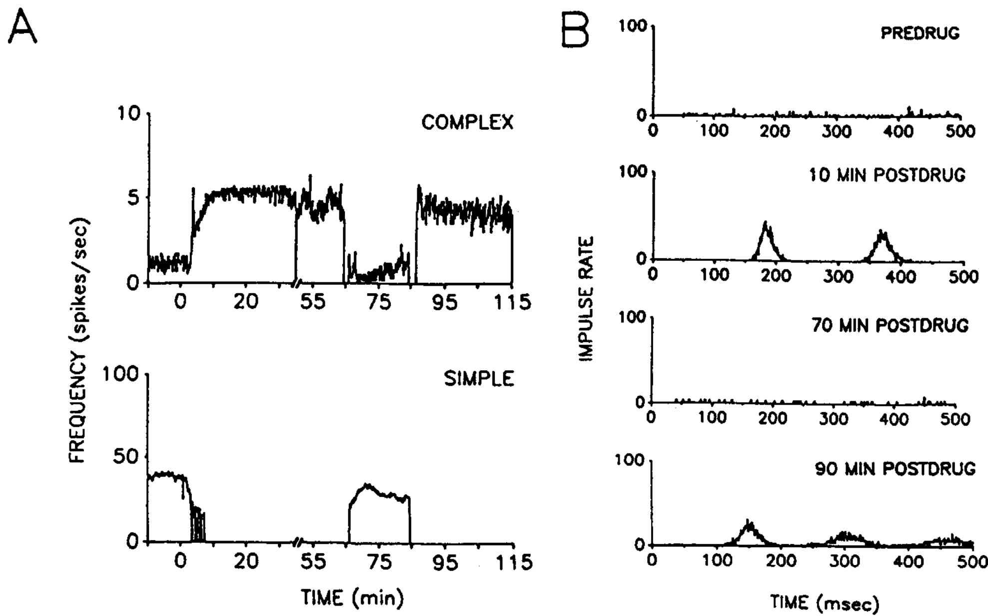

Figure 2. *A,* Frequency plots of complex spike activity (*top*) and simple spike activity (*bottom*) from cell N8 illustrate complete suppression of simple spike activity in response to harmaline. Harmaline was injected at time 0. A reciprocal relationship between complex spike activity and simple spike suppression is evident. Bin widths are 6 sec for both complex spike and simple spike activity in this and all other frequency plots, unless otherwise specified. *B,* Complex spike (*top*) autocorrelogram from the period 5–10 min before harmaline administration for cell N8. The complex spike autocorrelograms for 3 postdrug periods, 10, 70, and 90 min after harmaline injection, are shown below. The *R* values for the complex spikes were 0, 50, 10, and 30 during the predrug and 3 postdrug periods, indicating that the complex spikes increased in rhythmicity as well as frequency during the periods of simple spike suppression.

regions and medial parts of the lateral hemispheres than in the vermis.

The frequency plots of complex spike and simple spike activity of a representative cell from a normal rat (N15) are shown in Figure 3*A*. The postdrug activity is presented on an expanded time base in Figure 3*B* to illustrate the periodic cessations in simple spike firing following harmaline. This shows the abrupt change in firing rate between periods of activity and inactivity observed for cells producing this type of response. The reciprocal relationship between complex and simple spike activity demonstrated in Figure 2 for a complete suppression cell was not evident here. Although the complex spike rate for this cell increased after harmaline, we were unable to detect reliable increases in the complex spike rate during pauses in simple spike firing, compared to the complex spike rate during an equal period of time after simple spike activity had resumed (paired $t = 1.68$, $df = 78$; $p = 0.1$).

The cell (N15) shown in Figure 3, *A, B,* retained some of its predrug simple spike rhythmicity, despite the intermittent nature of this activity and the overall decrease in the frequency. This was typical of cells that gave an intermittent response to harmaline, and is evident in a comparison of the pre- and postdrug simple spike *R* values for cells in this response category (N9–N17) in Table 1. The mean predrug *R* value for this group of cells was 67.8 (SE = 12.9) and the postdrug value, 61.1 ± 8.7.

Harmaline responses in dt *rats*

The principal difference between normal and *dt* rats' responses to harmaline was in the distribution of cells in the response categories. All cells recorded from normal rats could be placed in either the complete or intermittent simple spike suppression categories. In the *dt* rats, 3 cells (Dt1–3, Table 2) showed simple spike suppression coupled with an increase in the frequency and rhythmicity of complex spikes, and 7 cells (Dt4–10) showed intermittent suppression. However, the next 7 cells (Dt11–17) did not meet the criteria for inclusion into either of these categories. A chi-square analysis indicated a significant difference between the normal and *dt* distributions ($\chi^2 = 9.52$; $p < 0.01$).

The complex spike and simple spike frequency plots of a cell

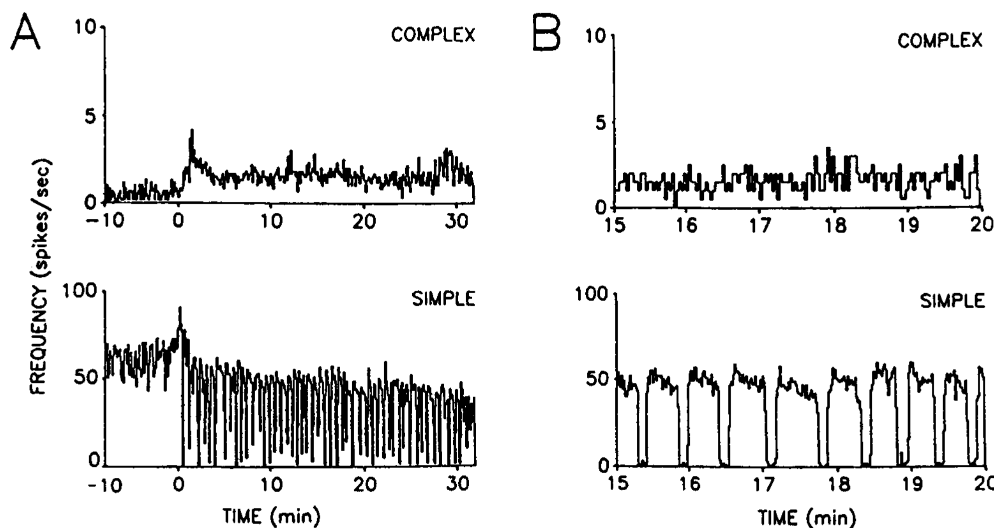

Figure 3. *A,* Frequency plots of complex spike (*top*) and simple spike (*bottom*) activity from cell N15, showing intermittent suppression of simple spike activity following harmaline administration at time 0. *B,* Frequency plots of complex spike (*top*) and simple spike (*bottom*) activity for the period 15–20 min postdrug for cell N15, showing the abrupt changes in simple spike activity. No change is evident in the instantaneous complex spike frequency during periods of simple spike suppression. Bin widths are 2 sec for the complex spike activity and 0.5 sec for the simple spike activity.

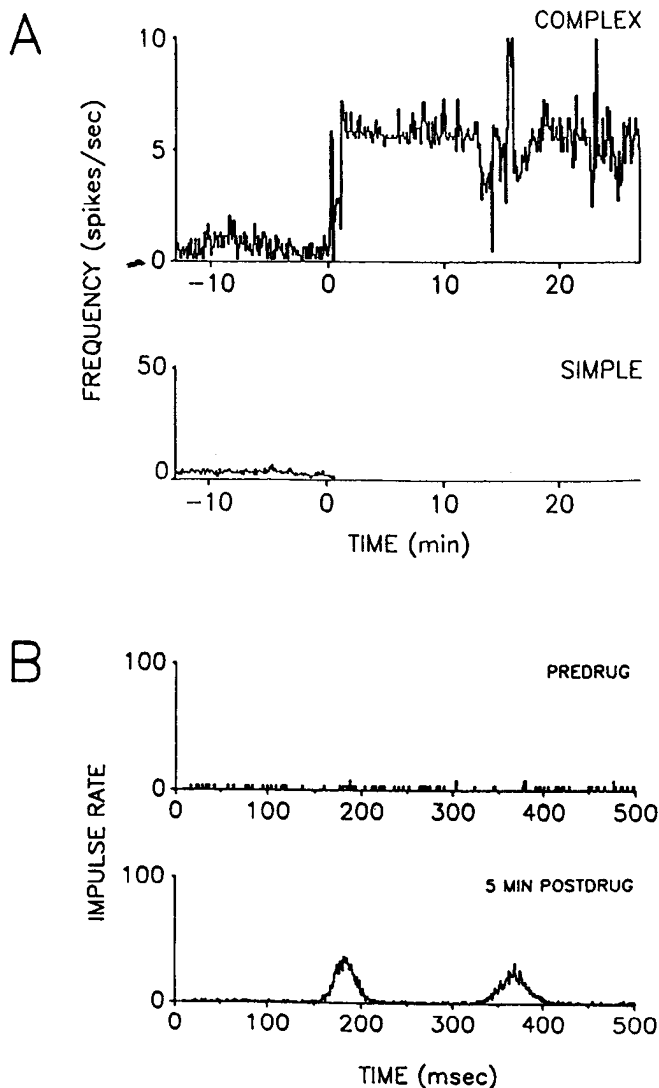

Figure 4. *A,* Frequency plots of (*top*) complex spike activity and (*bottom*) simple spike activity from cell Dt1, illustrating complete suppression of simple spike activity following the harmaline injection at time 0. *B,* Complex spike autocorrelograms for 5 min periods before (*top*) harmaline and after (*bottom*) harmaline for cell Dt1. The predrug *R* value was 10 and the postdrug value, 40, demonstrating the increase in complex spike regularity following harmaline.

from a mutant rat (Dt1) that showed a complete suppression response similar to that observed in normal animals is illustrated in Figure 4*A*. The predrug simple spike firing rate for this cell was low: 4 spikes/sec. However, postdrug suppression of simple spike activity appears to occur over a wide range of predrug firing rates in both normal and *dt* rats, as shown in Tables 1 and 2. The complex spike autocorrelograms for the periods immediately before and after harmaline administration (Fig. 4*B*) reflect the increase in complex spike rhythmicity following harmaline administration. The average complex spike frequency for cells Dt1–Dt3 increased from 0.5 to 4.4 spikes/sec following drug administration. Although the spontaneous complex spike firing rate of these cells was low in comparison with that of normal rats, the mean postdrug frequency was similar to that recorded in cells N1–N8 (4.8 spikes/sec). The mean complex spike *R* values also increased from 3.3 to 26.7 for these 3 cells.

Two of the cells in *dt* rats that showed sustained suppression of simple spikes (Dt11 and Dt12) showed no increase in the rhythmicity of complex spikes after harmaline, although both had an increase in complex spike rate. In cell Dt13, harmaline produced an increase in the frequency and rhythmicity of complex spike activity similar to that seen in cells showing complete suppression. However, neither complete nor intermittent suppression was observed. Three cells (Dt15–Dt17) did not show any discernible response to harmaline. Thus, overall, the *dt* sample showed more variability in response to harmaline than did the normal sample.

Discussion

We have examined the response to harmaline of Purkinje cells in the *dt* rat in an effort to determine the cause of the *dt* rat's failure to display harmaline tremor. It is generally assumed that harmaline tremor results from the synchronous firing of cells in the inferior olive (DeMontigny and Lamarre, 1973). In the cerebellar cortex, this synchronous activity is reflected in regular 6–12 spikes/sec complex spikes in the Purkinje cells in the vermis and paravermis and in a suppression of simple spikes in these neurons (DeMontigny and Lamarre, 1973). Rhythmic bursting activity at the complex spike frequency has been recorded simultaneously in the fastigial, bulbar reticular, and vestibular nuclei in the cat (DeMontigny and Lamarre, 1973). Lesions efferent to the olive can block the appearance of the tremor, but do not prevent the expression of rhythmic activity in the olive (DeMontigny and Lamarre, 1973; Llinás and Volkind, 1973).

The data presented here suggest that the insensitivity of the *dt* rat to harmaline is not exclusively the result of a lesion in a pathway efferent to the cerebellum. Rather, there are quantitative changes in the properties of the olivocerebellar pathway that may account for this finding. Purkinje cells of *dt* rats have discriminable simple and complex spikes and many show responses to harmaline that are similar to those seen in unaffected littermates. However, normal and mutant rats could be distinguished on the basis of (1) the firing rate of the Purkinje cells, and (2) the distribution of response types following harmaline administration. The results suggest that the *dt* rat's lack of response to harmaline is a consequence of a failure to produce sufficient rhythmic activation of the olivocerebellar pathway to produce a tremor.

Spontaneous activity

In our sample of normal rats, the average simple spike firing rate was 35 spikes/sec, and the average complex spike rate, 1.3 spikes/sec. These values are within the range of those reported by others. From recordings in adult rats, estimates of average simple spike frequencies range from 36 spikes/sec in locally anesthetized, awake rats (Savio and Tempia, 1985) to 23–46 spikes/sec in barbiturate-anesthetized rats (Montarolo et al., 1982; Benedetti et al., 1984; Batini and Billard, 1985) and 16 spikes/sec in urethane-anesthetized rats (Savio and Tempia, 1985). Beginning at about 16 d of age and continuing into adulthood, the spontaneous frequency of complex spikes can be expected to vary between 0.5 and 2 spikes/sec (Montarolo et al., 1982; Savio and Tempia,1985).

In *dt* rats there was a significant difference in spontaneous simple spike frequencies (−66%) as compared with normal littermates. In our sample, as well as others, there was a wide range (8–75 spikes/sec) in simple spike firing rates in normal

rats. The reduction in spontaneous firing rate seen in the Purkinje cells of the *dt* rats was associated with a reduction in the range of firing rates (2–39 spikes/sec). Complex spike discharges are known to inactivate Purkinje cells and might be expected to reduce the overall simple spike rate (Granit and Phillips, 1956; Murphy and Sabah, 1970; Bloedel and Roberts, 1971). However, this does not appear to explain the change in firing rate of the Purkinje cells of the *dt* rat, as the complex spike rate was also 50% lower than normal in the mutant.

The spontaneous simple spike activity of Purkinje cells in the *dt* rat was less regular than normal. A relationship between firing rate and Purkinje cell simple spike regularity has been observed by others in a variety of species (McCarley and Hobson, 1972; Belcari et al., 1977; Colin et al., 1980; Montarolo et al., 1982; Demer et al., 1985; Savio and Tempia, 1985), and is found in both the presence and the absence of complex spikes. In the dystonic rats, the relationship between firing rate and rhythmicity did not differ from normal. Thus, the decrease in the regularity of simple spike discharge in the *dt* rat may be accounted for by the difference in firing rate between normal and mutant rats.

Harmaline responses

Two distinct types of Purkinje cell responses to harmaline treatment were observed in normal weanling rats. In approximately 50% of the cells, harmaline produced rhythmic activation of complex spikes, accompanied by complete suppression of simple spikes. In the remaining cells, although there was no increase in the rhythmicity of the complex spike activity, a pattern of intermittent suppression of simple spike activity developed in response to the drug. The 2 cell types did not differ in initial firing rate. In other experiments in normal rats, we have found similar distributions of cells into these categories (Lorden et al., in press). Both of these response types were also seen in the *dt* rats, although fewer cells were found that showed rhythmic complex spikes and complete suppression of simple spike discharge, and 41% of the cells did not fall into either category. In all the cells studied here, the response pattern, whether complete or intermittent suppression, appeared to be a stable property of the cell. No conversion from one response to the other was observed over the course of postdrug recording periods. This was true for both normal and *dt* rats. Thus, there do not appear to be any differences in the duration or time of onset of the harmaline response between normal and *dt* rats that would account for our identifying fewer cells of a specific type in the mutant rats.

Since the tremorogenic response to harmaline develops postnatally (Knowles and Phillips, 1980), a developmental delay could account for the insensitivity of the *dt* rat to harmaline. Although climbing fiber-induced responses in Purkinje cells can be evoked by olivary stimulation as early as day 3, maturational processes continue until about day 22, with rapid changes occurring between days 10 and 20 (Crepel, 1971, 1972; Woodward et al., 1971; Shimono et al., 1976; Puro and Woodward, 1977). It does not appear, however, that harmaline tremor depends on these late-occurring maturational changes, as the tremor can be seen as early as day 9 in normal rats, when bursting complex spike activity in response to harmaline appears (Knowles and Phillips, 1980). Examination of the dendritic arbors of Purkinje cells in the *dt* rat has not revealed any of the morphological changes associated with climbing fiber loss in early development (Sotelo and Arsenio-Nunes, 1976; Lorden et al., 1985; Anderson and Flumerfelt, 1986). Thus, the failure of the *dt* rat to respond to harmaline is not accounted for by a failure to develop climbing fiber responses.

Cells showing different responses to harmaline had different spatial distributions. As expected, on the basis of earlier work (DeMontigny and Lamarre, 1973; Bernard et al., 1984), the cells in which simple spike discharges were completely suppressed tended to be located in the vermis or paravermis. In our sample of normal rats, 8 of the 12 cells recorded in the vermis or paravermis displayed this response. Other studies have reported that 60–90% of the cells in these areas show a similar response (Bernard et al., 1984; Lorden et al., in press). In the *dt* rats, however, only one of the 11 cells recorded in the vermis or paravermis showed rhythmic activation of the climbing fibers and prolonged suppression of simple spikes. In both normal and *dt* rats, the cells showing intermittent suppression of simple spikes were about equally divided between vermal and hemispheric regions. Thus, the difference between normal and *dt* rats appears to be a change in the number of cells showing a specific response profile, and not a change in the spatial distribution of cell types.

The work of Busby and Lamarre (1980) suggests that harmaline tremor occurs in conjunction with rhythmic bursts of activity in the cells of the deep cerebellar nuclei. This activity is paced by rhythmic inhibitory input to these cells from complex spikes generated in Purkinje cells by olivary input. With simple spike activity effectively silenced in Purkinje cells, the deep nuclei cells receive only the rhythmic inhibitory input from the complex spike activity. This idea is supported by other types of evidence in the rat. Using horseradish peroxidase injections at vermal sites at which cells show a rhythmic complex spike rate of 5–10 spikes/sec in response to harmaline, Bernard et al. (1984) labeled cells in the caudal medial accessory olive (CMAO). In addition, following harmaline, olivary metabolic activity, measured with the 2-deoxyglucose technique, was found to increase only in the CMAO (Bardin et al., 1983; Bernard et al., 1984). These results have been interpreted as indicating that the action of harmaline on a specific part of the olivocerebellar pathway originating in the CMAO is responsible for harmaline tremor. This suggests that the tremor pathway involves specifically those Purkinje cells that show rhythmic complex spike activation and that those cells receive their input from the CMAO. In the *dt* rat, the paucity of Purkinje cells with this type of response to harmaline may adequately account for the failure of this animal to develop harmaline tremor. This change in the distribution of response types in *dt* rats is similar to that observed in normal rats made tolerant to the tremorogenic effects of harmaline by repeated administration of the drug. In harmaline-tolerant animals, fewer Purkinje cells can be found in the vermis that show rhythmic activation of complex spikes and suppression of simple spikes in response to acute systemic injections of harmaline (Lorden et al., in press).

The reduction in both spontaneous complex and simple spike rates in the *dt* rat suggests the presence of an underlying abnormality common to both the climbing fiber and the parallel fiber inputs. This would tend to argue for a defect in the processing of excitatory information by the Purkinje cells. However, in normal animals, the complex spike activity of the Purkinje cell has frequently been used as an index of olivary activity. The rhythmicity of complex spike activity is thought to reflect an intrinsic property of olivary neurons. Neurons of the mammalian inferior olive have been found to show spontaneous oscillatory properties that can be enhanced by harmaline administration (Armstrong et al., 1968; Llinás and Yarom, 1981a, b;

1986) and electrotonic coupling between olivary neurons that is thought to account for the synchronous activation of aggregates of olivary neurons (Llinás et al., 1974; Sotelo et al., 1974; Llinás and Yarom, 1981a; Benardo and Foster, 1986). Thus, the apparent scarcity of cells in the *dt* rat that show rhythmic activation of complex spikes in response to harmaline may also point to a defect at the level of the olive.

Relationship of electrophysiological and biochemical abnormalities in the dt *rat*

Previous biochemical study of the *dt* rat revealed decreased cGMP levels in the cerebella of the mutant rats (Lorden et al., 1985). Accumulations of cGMP in the cerebellum are known to result from stimulation of excitatory afferents or the application of excitatory amino acids (Ferrendelli et al., 1974; Mao et al., 1974; Biggio et al., 1977; Rubin and Ferrendelli, 1977; Novelli et al., 1987). Thus, chronic reductions in the activation of the mossy fiber and climbing fiber systems, as suggested by the low spontaneous firing rate of Purkinje cells in the *dt* rat, might be expected to reduce basal levels of cGMP. The effects of harmaline on cGMP levels in the *dt* rat also mirror the drug response of the Purkinje cells in this study. Harmaline increased cGMP levels in the *dt* rat, but only to a level similar to that seen in phenotypically normal saline control rats (Lorden et al., 1985).

The decreased spontaneous firing rate of the Purkinje cells of the *dt* rat may also explain the observation that GAD activity is elevated in the deep cerebellar nuclei of this mutant (Oltmans et al., 1984, 1986). An increase in GAD activity in the GABAergic cells of the deep nuclei could result from decreased inhibition of the nuclear cells by the Purkinje cells. This may be important in understanding the insensitivity of the *dt* rat to the tremorogenic effects of harmaline. The GAD-containing neurons of the cerebellar nuclei appear to project to the inferior olive (Nelson et al., 1986), and GABAergic processes in the olive are well placed to modulate the coupling and firing patterns of olivary neurons (Sotelo et al., 1986).

GAD is also localized in the terminals of the Purkinje cells (Fonnum et al., 1970; Fonnum and Walberg, 1973). If the increase in GAD activity in the deep cerebellar nuclei of the *dt* rat is localized primarily in the terminals of the GABAergic Purkinje cells, one would expect higher, rather than lower than normal, Purkinje cell firing rates in the *dt* rat. However, there is evidence that a dissociation between GAD activity in the deep cerebellar nuclei and Purkinje cell firing rate occurs in rats treated with the toxin 3AP. Following olivary destruction with 3AP, a 100% increase in simple spike activity has been reported to occur within 24 hr (Batini and Billard, 1985; Batini et al., 1985; Savio and Tempia, 1985). The initial increase in simple spike rate is followed by a gradual, but steady, decline to prelesion rates over a 2–3 week period. Within 2 d of the lesion, GAD activity in the deep nuclei increases significantly, and the magnitude of this increase grows steadily, peaking at about 14 d postlesion in adults, but continuing to increase for at least 28 d in 16-d-old animals (Oltmans et al., 1985; Sukin et al., 1987). Thus, while GAD activity in the deep nuclei appears to be related to Purkinje cell firing rate, there may be a lag between changes in the firing rate of cells and changes in the activity of enzymes for neurotransmitter synthesis following 3AP lesions. It is possible that a mismatch between Purkinje cell firing rate and deep nuclei GAD activity in the *dt* rat could be the result of an event that occurs earlier in the course of the disease.

Relationship of the harmaline response to the motor syndrome of the dt *rat*

We have used harmaline as a probe to search for probable sites of abnormality in the *dt* rat. Although we have not identified the mechanism underlying the *dt* rat's insensitivity to harmaline, the data presented here suggest that the failure to tremor may be due to a defect in the inferior olive. The function of the inferior olive in motor control is still the subject of investigation, but the oscillatory tendencies of olivary neurons lend themselves to the generation of rhythmic patterns needed for timing processes, error correction, or responses to unexpected events (Ito, 1984; Gellman et al., 1985; Benardo and Foster, 1986; Llinás and Yarom, 1986). In the developing rat, bursting responses to harmaline can first be evoked in the olive at the same time that harmaline tremor can be produced (Knowles and Phillips, 1980). This is the time at which normal rats begin to acquire adult patterns of locomotion and when *dt* rats can first be distinguished from their unaffected littermates. This coincidence of events suggests that the *dt* rat's motor syndrome and its failure to respond to harmaline are related. However, recent observations show that the oscillations of the olive can be damped by stimulation extrinsic to the olive (Benardo and Foster, 1986). This raises the possibility that if the abnormal movements of the *dt* rat are not caused by an olivary defect, the rhythmic activity of the olive may still be disturbed by abnormal input from another site, perhaps as a consequence of the movements.

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