EMG Analysis of Harmaline-Induced Tremor in Normal and Three Strains of Mutant Mice with Purkinje Cell Degeneration and the Role of the Inferior Olive

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SUMMARY AND CONCLUSIONS

1. The effects of intraperitoneal injections of 10 mg/kg harmaline were tested in normal mice and three strains of cerebellar mutant mice with Purkinje cell degeneration. Ten normal (wild-type) mice (+/+), as well as five lurcher (lc/+) mice (nr/nr), and eight Purkinje cell degeneration (pcd/pcd) mutants were implanted with chronic electromyogram (EMG) electrodes in the hamstring and quadriceps muscle groups of the right hindlimb.

2. EMGs were recorded in each of the mice during spontaneous activity before and after intraperitoneal injections of 0.3 ml harmaline (10 mg/kg). Spectral analysis was used to quantify the amplitude and frequency of tremor found in the EMGs after harmaline administration. Normal mice responded to harmaline with strong, continuous 1- to 14-Hz tremor. Mutants from the pcd/pcd strain also reacted with continuous tremor, but of lower amplitude and frequency. In contrast, nr/nr mutants exhibited intermittent paroxysmal tremor lasting for only a few seconds, and lc/+ mutants showed no evidence of tremor whatsoever.

3. In order to detect covert tremor that was possibly not revealed by focal intramuscular EMG recordings, several mutant and normal mice were also tested on a suspended platform to which an accelerometer was attached. The results confirmed the findings from EMG recording.

4. An incidental observation made during the course of this study was that harmaline tremor disappeared from the normal mouse during swimming and reappeared when the animal was withdrawn from the water.

5. Although Purkinje cells appeared to increase both the depth of modulation and the frequency of tremor, the inhibitory action of the cerebellar cortex does not seem to be essential for the generation of tremor.

6. Parasagittal cerebellar sections of the normal, wild-type mice and the three strains of cerebellar mutant mice of various ages were stained with cresyl violet and examined for Purkinje cell degeneration. Purkinje cell degeneration was found to be complete in the pcd/pcd and lc/+ strains. Although an initial examination of parasagittal sections of the nr/nr strain failed to find any surviving Purkinje cells, further examination of sections cut in the coronal plane revealed small clusters of Purkinje cells in the vermal area of the posterior lobe.

7. The retrograde transport of wheat-germ-agglutinin-conjugated horseradish peroxidase (WGA-HRP) pressure-injected into the cerebellar cortex was used to study the olivocerebellar projections in the wild-type mice and the three strains of cerebellar mutant mice.

8. The volume of cerebellar cortex injected with WGA-HRP varied from ~50% in the least case to close to 90% in the greatest case, and some diffusion into the cerebellar nuclei occurred in all four groups of mice. The degree of retrograde labeling differed markedly among the strains. In the wild-type mouse, all olivary nuclei appeared heavily labeled, whereas retrograde transport produced moderate labeling in the pcd/pcd strain and only very faint marking in the nr/nr strain. At low magnification, no labeling was seen in the lc/+ mouse, although a few faintly marked cells were identified at higher (x400) magnification. Together these observations suggest that differences in the functional integrity of inferior olivary neurons among the three mutant strains is a plausible explanation for the disparate reaction to the tremor-inducing drug harmaline.

INTRODUCTION

The systemic injection of harmaline, a beta-carboline derivative, produces a high-frequency 8- to 14-Hz tremor in mice (Agarwal and Bose 1967; Ahmed and Taylor 1959), rats (Busby and Lamarre 1980), cats (DeMontigny and Lamarre 1973, 1975; Lamarre and Weiss 1973), and monkeys (Batista et al. 1970; Poirier et al. 1966). Although the precise mechanism of the tremorogenic effect of harmaline is unknown, several possible explanations have been proposed. Harmaline has a hyperpolarizing effect on neurons of the inferior olive as well as a direct facilitation of low-threshold calcium conductance in these cells (Llinas 1991). In addition, harmaline may act on y-aminobutyric acid (GABA) receptors controlling the electrotonic coupling among oligodendrocytes (Llinas and Sasaki 1989; Sotelo et al. 1986; Stratton and Lorden 1991), and thereby enhancing the synchronous rhythmic activity of populations of inferior olivary neurons (DeMontigny and Lamarre 1973; Llinas and Volkind 1973). This synchronous rhythmic olivary activity would then be transmitted to the cerebellar Purkinje cells by the climbing fiber afferents (DeMontigny and Lamarre 1973, 1975; Llinas and Volkind 1973). The enhanced complex spike activity in Purkinje cells in response to harmaline is usually accompanied by an attenuation of simple spike discharge (Lamarre et al. 1971).

It has yet to be established whether the periodic inhibition from rhythmically discharging Purkinje cells is essential for the induction of electromyographic (EMG) tremor after harmaline administration. Climbing fibers are known to send collaterals to the deep cerebellar nuclei, which would convey rhythmic excitation from the inferior olive to cerebellar different nuclear cells directly. In fact, Llinas and Volkind
(1973) reported that cooling of the cerebellar cortex produced a definite desynchronization of the rhythmic activity in motoneurons induced by harmaline, but that the basic 10-Hz firing could still be observed. The tentative conclusion derived from this study was that the rhythmic Purkinje cell inhibition of the neurons of the deep cerebellar nuclei shaped and reinforced the harmaline tremor, but that nuclear cell disinhibition was not the essential engine driving the tremor.

To determine the importance of sculpturing inhibition in the generation and appearance of the tremor, we studied three strains of mutant mice with Purkinje cell degeneration for their reaction to systemically injected harmaline. Purkinje cell degeneration appeared to be complete in the heterozygous dominant lurcher (lc+/+) and the homozygous recessive Purkinje cell degeneration (pcd/pcd) strains, thus providing a completely decorticated cerebellum for physiological study. In the homozygous recessive nervous (nr/nr) strain, however, a small degree of Purkinje cell sparing was found in the vermal region, and this observation is documented in the previous one. This produced a total of 30 spectra, which were then averaged.

**METHODS**

**Experiment 1**

This study was carried out on 10 normal mice and 5 lc/+, 6 nr/ nr, and 8 pcd/pcd cerebellar mutants. The mutants were bred from stock originally obtained from the Jackson Laboratory. Because early identification of the lurcher genotype was not important in this study, the lc gene was segregated from the associated microwhite gene mutation, from which it was originally discovered (Phillips 1960), and was transferred from the agouti C3H background to the generally more robust C57BL/6 strain.

In the first set of experiments, intramuscular EMG electrodes were chronically implanted in the hamstrings and quadriceps muscle groups of the right hindlimb. The electrodes consisted of bipolar pairs of enamel-insulated, single-stranded, 50-µm stainless steel wire from which ~5 mm of insulation had been stripped at one end.

The mice were anesthetized with an 0.2- to 0.3-ml intraperitoneal injection of 6.5 mg/ml pentobarbital sodium equivalent to ~45-65 mg/kg. Using a cosmetic depilatory, hair was removed from the right leg and the head. Incisions were made on the thigh for inserting the EMG electrodes and in the scalp for mounting a miniature connector strip. Each electrode wire was inserted into the cannula of a 30-gauge hypodermic needle and folded back to form a hook. The hypodermic needle was then inserted into the belly of the muscle and withdrawn, leaving one end of the wire within the muscle. Four wires were implanted (two in each muscle group) and led under the skin to the head, where they emerged through a small incision in the skin. The incision in the leg was then sutured.

The free ends of the wires were soldered to small gold-plated pins that were snapped into a supporting miniature strip connector along with a ground lead placed next to the skull. Epoxy was allowed to harden around the connector and sutured to the skin. The mouse was then placed in a box under a heat lamp to recover. The EMGs were again recorded for a period of 24 h after surgery.

During a recording session, EMGs were recorded for several minutes before harmaline injection while the mouse was allowed to roam freely about the cage. A volume of 0.3 ml harmaline (10 mg/kg) was then injected into the peritoneum. When visible signs of tremor appeared (normally in ~5 min after harmaline injection), the EMGs were again recorded for a period of several minutes. In the case of some mutant mice, tremor was often intermittent or even absent. Consequently, each mouse was observed for a period of ~20 min after harmaline injection and further recording was performed if tremor occurred. EMG signals were amplified with a 5-K gain, filtered from 10 to 3,000 Hz, and recorded with an FM tape recorder having a bandwidth from 0 to 5 kHz. These signals were later digitized at 4 kHz for analysis.

In a second series of observations, the tremor was quantified by analyzing the acceleration of a suspended platform on which the mouse was free to move. The platform consisted of a light rigid box, suspended at its four corners with taut thread, with an accelerometer glued to the underside. During a recording session the mouse was placed in the box and the acceleration was recorded for several minutes before harmaline injection while the mouse was allowed to move about the box freely. A volume of 0.3 ml harmaline was then injected into the peritoneum, and as before, recording began either when tremor became visible or at regular intervals from 10 to 30 min after injection. The acceleration signal was amplified and low-pass filtered at 1.0 Hz before it was recorded on tape.

The acceleration signals were later digitized from the tape recordings at 1 kHz for spectral analysis. Sixteen-second data sequences were used. Spectra consisting of 512 points were obtained by analyzing consecutive 1,024-ms records within the 16-s sequence. Each record was shifted by 512 ms with respect to the previous one. This produced a total of 30 spectra, which were then averaged.

**Experiment 2**

These experiments were carried out on the same wild type, C57BL/6, and the same three mutant strains as used in experiment 1. lc/+, nr/nr, and pcd/pcd. In the first investigation, 13 lc/+ mice (ages 13-145 days), 15 nr/nr mice (ages 12-263 days), 13 pcd/pcd mice (ages 23-306 days), and 6 wild-type mice (ages 12-227 days) were killed with a lethal dose of pentobarbital sodium and perfused with Bouin's fixative. Frozen parasagittal sections of the cerebella were stained with cresyl violet, and the medial, middle, and lateral thirds were examined for Purkinje cell degeneration.

In the second investigation, a total of 16 adult mice (including 4 wild type, 5 lc/+, 3 nr/nr, and 4 pcd/pcd) of ~100 days of age were anesthetized with an 0.2- to 0.3-ml intraperitoneal injection of 6.5 mg/ml solution of pentobarbital sodium. The head was fixed in a stereotaxic frame adapted for mice, and the calvarium posterior to the tentorium was removed to expose the cerebellum. Three or four injections of 0.04 µl each of 2.5% wheat-germ-agglutinin-conjugated horseradish peroxidase (WGA-HRP) were placed mediolaterally in the cerebellar cortex using a stereo dissecting microscope for visual guidance. The injections were made slowly with a 1.0-µl Hamilton syringe attached to a glass micropipette. The pipette with a beveled tip diameter of 20-50 µm penetrated the relatively thin dura without significant indentation. After the final injection the skin incision was sutured and the animals were allowed to recover from the anesthesia.

After a survival period of 48 h, the animals were reanesthetized with pentobarbital sodium and subjected to intracardial perfusion with 0.9% normal saline followed by a 0.5% paraformaldehyde-1.25% glutaraldehyde solution. The cerebella were then removed and postfixed for ~24 h in 30% phosphate-buffered sucrose (pH 7.4) fixative solution. The cerebellum and brain stem were removed together and cut as a single block into 40 frozen coronal sections. The sections were stained for WGA-HRP using the tetramethyl benzidine method (Mesulam 1978).
RESULTS

It was clear from the outset, both from visual inspection as well as from EMG records and acceleration traces, that the harmaline-induced tremor was much stronger in normal than in the cerebellar mutant mice. Most notably, the lc/+ mutants did not demonstrate any effects of harmaline that could be observed either overtly, in the EMG, or on the accelerometer platform. The EMGs after harmaline injection in the normal mouse, the nr/nr mutant strain, and the pcd/pcd mutant strain are shown in Fig. 1. The EMGs of the normal mouse consisted of strong bursts of activity in both the hamstring and quadriceps muscle groups that tended to be synchronous rather than reciprocal. The tremor frequency measured from the spectral analysis for this particular mouse was ~11 Hz. This very regular tremor was present both at rest and when the mouse was moving about the cage.

Although overtly the pcd/pcd mice showed evidence of continuous tremor, the EMGs revealed only periodic bursts of activity at ~9 Hz. However, as seen in Fig. 1, the EMG bursts were less clearly distinguished from the background activity than in the normal mouse. The nr/nr mouse showed only intermittent overt signs of tremor, but as Fig. 1 illustrates, the tremor, when present, was as strong as that seen in the pcd/pcd mutants. The synchronous bursts of EMG activity occurred at a somewhat lower frequency (8 Hz) than in the normal mouse and were observed at sporadic, widely separated intervals and only for 2 or 3 s.

There was no evidence of periodic bursts of muscle activity at a frequency of ~8 Hz in either leg muscle of the lc/+ mutant. Whatever periodic activity could be observed was similar before and after harmaline injection and did not exceed a frequency of 5 Hz. In an attempt to determine whether harmaline tremor was absent in the lc/+ mutants at all ages, young mice were injected with harmaline beginning at postnatal day 12. Harmaline-induced tremor was visible in lc/+ mutant until postnatal day 16, when it disappeared and failed to reappear subsequently at any age.

An earlier study showed that lc/+ mutant mice have a highly ataxic terrestrial gait, but nonetheless showed nearly normal rhythmic swimming movements (Fortier et al. 1987). We therefore decided to examine the effect of harmaline tremor on swimming as well as terrestrial locomotion. A normal mouse was injected with harmaline and placed in a water-filled tank after the onset of tremor. The EMG just before and after placing the mouse in the tank is shown in Fig. 2. All evidence of the harmaline-induced tremor disappeared as soon as the animal was released in water and began swimming. The EMG bursts associated with the tremor were replaced by rhythmic activity at the frequency of the swimming movements only. The harmaline-induced tremor disappeared immediately when the mouse was placed in water but required 30–60 s to be visually apparent and gradually reemerge electromyographically after the mouse had been taken out of the water.

The tremor frequencies recorded using the platform-mounted accelerometer were similar to the EMG records. Slight differences occurred in the tremor frequencies because the data were derived from another group of mice. In Fig. 3 the power spectra of the acceleration, computed before harmaline injection and after the injection of harmaline, were compared for the four strains of mice.

In the case of the normal mouse, almost all of the platform acceleration occurred at ~14 Hz after the onset of tremor. This spectral peak at 14 Hz was totally absent without harmaline. In fact, there was very little acceleration of any kind before harmaline injection and the activity concentrated at
lower frequencies (centered at 3–4 Hz) was probably associated with locomotion. The spectral power peaks seen in the normal mouse and the pcd/pcd mouse were similar and were almost three times greater than the peak observed in the nr/nr mouse.

Similarly, before harmaline injection, the platform acceleration produced by the pcd/pcd mutant occurred principally at 4 Hz. This low-frequency 4-Hz acceleration also persisted after the onset of tremor. The most striking feature of the postharmaline pcd/pcd spectrum was the appearance of the tremor frequency occurring at 9–10 Hz, which was nearly as powerful as that observed in the normal mouse.

In the nr/nr mutant, there was also a 4- to 5-Hz acceleration peak both before and after harmaline injection that was, in fact, even more pronounced after the onset of tremor than before. The 4- to 5-Hz acceleration peak was also accompanied by a high-frequency peak at 13–14 Hz, although neither peak approached the power of the harmaline tremor peaks found either in the normal or pcd/pcd mice. The fact that this peak was less salient was probably because the episodes of tremor occurred both intermittently and rather infrequently, as noted previously.

No tremor whatsoever could be observed in the platform acceleration spectrum of the lc/+ mutant mouse. The only difference in the spectrum before and after harmaline injection was a reduction in the low-frequency peak (2–3 Hz), which could explained by greater movement about the cage during the period before harmaline injection.

Experiment 1 raised the puzzling question as to why the three mutant strains without Purkinje cells demonstrated such varied responses to the tremorogenic compound harmaline. The tremor was totally absent in the lc/+ strain, whereas in the pcd/pcd and nr/nr strains it was merely reduced in amplitude, frequency, and duration. Among the potential explanations was the possibility that some Purkinje cells might have survived in the pcd/pcd and nr/nr strains compared with the lc/+ strain. Another alternative was that the secondary degeneration of inferior olivary cells might have been different among the three mutant strains.

To answer these questions we first examined an extensive sample of parasagittal sections of the cerebella of adult mutants to assess the possibility of selective or regional survival of Purkinje cells. Subsequently, in a different group of animals, we injected as much of the cerebellar cortex with WGA-HRP as possible in order to retrogradely label the inferior olive nuclei as completely as possible in the normal and mutant strains.

Degeneration of Purkinje cells

Representative parasagittal sections from the medial, middle, and lateral thirds of the cerebellum were examined for the presence of surviving Purkinje cells. Using a camera lucida, the separation of the granule cell layer from the molecular layer was traced, and when present, Purkinje cells were indicated on the drawing. This procedure allowed a quantitative estimate of Purkinje cell density per unit length in animals where the degeneration was not yet complete. The Purkinje cell mortality rates were slightly different for each of the three strains, and although surviving Purkinje neurons were very rarely observed after 60 days of age, occasional cells were found scattered within the cerebella of all three strains. For all practical purposes, no Purkinje cells remained after 90 days in either the lc/+ or the pcd/
NORMAL

NERVOUS

PCD

LURCHER

![Power spectra of a platform mounted accelerometer, computed before (---) and after (-----) harmaline injection, comparing the 4 strains of mice. Note that the scaling of the Y-axis is different in each of the 4 images.](image)

Fig. 3. Power spectra of a platform mounted accelerometer, computed before (---) and after (-----) harmaline injection, comparing the 4 strains of mice. Note that the scaling of the Y-axis is different in each of the 4 images.

pcd strains. Figure 4 shows typical sagittal sections of the cerebellar cortex of both the wild-type mouse (Fig. 4A) and the three mutant strains (Fig. 4, B–D). Although devoid of Purkinje cells, the cortex of the pcd/pcd strain has molecular and granular cell layers that are of nearly normal thickness (Fig. 4C). In contrast, Fig. 4B shows the moderate molecular layer atrophy of the nr/nr mutant and the severe atrophy of both the molecular and granular cell layer in the lc/+ mouse (Fig. 4D). Initially, we did not find any surviving Purkinje cells in the parasagittal cerebellar sections of nr/nr mutants (see Fig. 4B). However, we then examined the sections of three additional nr/nr mutants killed at an age of ≥7 mo cut in the coronal plane. In these specimens a few surviving Purkinje cells could still be found in the vermal regions of the cortex, and an example is shown in Fig. 4E. These surviving Purkinje cells were the only ones found in this serially sectioned cerebellum. In all three cases of coronally sectioned nr/nr mutants, the Purkinje cells were virtually absent except for the isolated aggregates in the vermal region. The surviving Purkinje cells tended to be grouped together adjacent to one another, as described by Wassef et al. (1987). However, we were unable to find any of the paravermal or lateral Purkinje cell clusters shown in that paper.

WGA-HRP injections in cerebellar cortex

The objective of this experiment was to inject the cerebellar cortex as completely as possible with WGA-HRP to maximize the labeling of the climbing fibers afferents from olivary neurons. However, as shown in Fig. 5, the injections frequently failed to label the most lateral cerebellar regions. Although Fig. 5 also shows that the injections sometimes spread across the midline to label the cortex near the midline...
FIG. 4. Cresyl-violet-stained sections of the mouse cerebellar cortex. A: wild type with conspicuous Purkinje cells. B: sagittal section of n/n cerebellar cortex with thinner molecular and granular layers and no Purkinje cells. C: pcd/pcd cerebellar cortex with near normal appearance except for the complete absence of Purkinje cells. D: lc/+ cerebellar cortex with severe atrophy of both granular and molecular layers in addition to complete Purkinje cell degeneration. E: coronal section of n/n vermal cerebellar cortex at higher magnification in which a small cluster of Purkinje cells with visible nuclei are indicated by arrows.
FIG. 5. Typical injection of wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) into cerebellar cortex is shown for a single example from the wild-type, pcd/pcd, nr/nr, and lc/+ mice. Each pair of transverse sections shows both cerebellum and olive. Diagonal shading: injection area. Black shading: area of retrograde labeling in the contralateral olive.

bilaterally, the retrograde labeling was only evaluated in the contralateral inferior olive. The labeling of inferior olivary neurons was strongest in the normal mouse and weakest in the lc/+ mutant, as illustrated with dark field photomicrographs in Fig. 6. It is very unlikely that this very weak transport was due to a smaller injection area because the atrophied size of the lc/+ cerebellum made it the most completely labeled cerebellar cortex of the four strains (see Fig. 5). Moreover, alternate sections counterstained with neutral red indicated the presence of unlabeled olivary neurons in the lc/+ mutant. Although at the low magnification shown in Fig. 6, no marked cells can be seen, at X400 magnification a few lightly labeled neurons were visible. The inferior olive of the pcd/pcd strain was less heavily labeled than that of the normal mouse but was still more profusely labeled than that of the nr/nr mouse. There did not appear to be any preferential labeling of particular subnuclear regions of the inferior olive in any of the three mutant strains. The labeled
neurons were found randomly and sparsely distributed throughout the rostrocaudal extent of the inferior olive and evenly disseminated among the subnuclei.

DISCUSSION

Experiment 1

CHARACTERISTICS OF HARMALINE TREMOR. The initial question that inspired this study was whether functional Purkinje cells are necessary for the appearance of harmaline tremor, and the answer appears to be more complicated than originally expected. The results revealed a surprising range of reactions to harmaline among the three mutant strains with Purkinje cell degeneration. In the pcd/pcd strain, the tremor, although continually present after the harmaline injection, was of a lower amplitude and frequency than that observed in normal mice. In contrast, the nr/nr mutant mouse displayed only intermittent tremor, and no evidence of tremor whatsoever was observed in the lc/+ mouse.

The relatively low-frequency peaks (3–5 Hz) seen in the spectra of the acceleration records are thought to represent periods of locomotor activity. Although intense tremor was invariably observed in normal mice at rest, in cerebellar mutant mice the tremor was generally more pronounced during periods of locomotor or exploratory activity. For this reason, the spectrum of data records chosen for analysis in the case of the cerebellar mutants was likely to have a greater low-frequency content than that of normal mice, which tended to be relatively immobile after harmaline injection.

The disappearance of harmaline tremor in the normal mouse during swimming was a surprising and unexpected observation. The harmaline-induced tremor frequency was considerably higher than that of locomotion, ranging from ~10–14 Hz, whereas the frequency of EMG bursts during swimming was found to be near the high end of locomotor rhythms (i.e., more equivalent to running at ~5 Hz). In the case of the normal mouse, this high-frequency harmaline tremor was clearly visible whether the mouse was moving about or at rest. However, as soon as the mouse was placed in water and began swimming, the high-frequency tremor disappeared immediately. Moreover, it only reappeared gradually in ~1 min of the time the mouse was taken from the water and placed on a dry surface. Whether the water exerts the critical cutaneous stimulus that can interrupt and override the rhythmic excitation from supraspinal descend-
ing pathways, or whether some other variable is at work, is unknown. Further research is needed to elucidate this inhibitory effect of swimming on harmaline-induced tremor.

DIFFERENCES AMONG THE CEREBELLAR MUTANT MICE. In this study we initially assumed that the three cerebellar mutants all had identical Purkinje cell loss and that no other potential differences would affect their sensitivity to harmaline. However, only two of the three mutant strains in the present study appeared to have complete Purkinje cell atrophy. Although the degeneration in the nr/nr mouse was found to be incomplete, the number of surviving Purkinje cells in our specimens of the nr/nr mutants was even less than that reported by Wassef et al. (1987). The small patches of surviving Purkinje cells in the nr/nr mutants were thought to be too few in number to have sustained harmaline tremor. Furthermore, the surviving Purkinje cells in the nr/nr mutant offer no explanation as to why in the two remaining strains, pcd/pcd and lc/+ , one demonstrated a harmaline-induced tremor and the other did not.

In the lc/+ mouse the severe loss of granule cells causes a significant shrinkage of the cerebellar cortex, which explains why it is more severely atrophied than that of either the nr/nr or pcd/pcd mouse (Wetts and Herrup 1982b). Although the nuclear cell volume and cell density in lc/+ mice were originally thought to be normal (Caddy and Biscoe 1979), according to a more recent study by Heckroth (1994), lc/+ mice may have significant nuclear cell atrophy, particularly among the small GABA-containing neurons projecting to the inferior olive. In addition, a substantial degeneration of the inferior olive in lc/+ mice has been noted by several investigators (Caddy and Biscoe 1979; Heckroth and Eisenman 1991) and shown by Wetts and Herrup (1982a) to be secondary to the lack of Purkinje cells.

PURKINJEE CELL SURVIVAL. An examination of cresyl-violet-stained parasagittal sections of the cerebellar cortex confirmed the complete Purkinje cell degeneration in adult lc/+ and pcd/pcd strains of mice (Mullen et al. 1976; Siddman and Green 1970; Swisher and Wilson 1977; Wassef et al. 1987). The few aggregates of surviving Purkinje cells found in the vermal region of nr/nr mutants confirmed the observation of Wassef et al. (1987) that Purkinje cell degeneration is incomplete in this strain. However, the failure to find any surviving Purkinje cells in the paravermal or lateral cerebellum in these nr/nr mutants was puzzling. Beyond 3 months of age, none of the nr/nr mutants exhibited the “checkerboard” appearance clearly shown by Wassef et al. (1987). Although anti-Purkinje cell antibodies were not used in our study, identifying Purkinje cells in Nissl-stained sections did not seem to be a problem and therefore is not considered a likely explanation for the discrepancy. Age differences among the animals or greater genetic penetrance are possible alternative explanations for the differences. Whatever the reason, the few clusters of surviving Purkinje cells amounted to far less than 1% of the normal complement of Purkinje cells, and their presence cannot account for the intermittent harmaline tremor. Instead, the complete Purkinje cell degeneration in pcd/pcd mutants that showed clear harmaline tremor indicates that the inhibitory action of the cerebellar cortex is not essential for the generation of tremor.

INFERIOR OLIVARY DEGENERATION. The differences in the inferior olivary labeling among the three strains were striking. Although the present study did not attempt to evaluate precisely the area of cerebellar cortex injected with WGA-HRP among the four strains, it is unlikely that the results obtained could be accounted for by differences in the volumes of the labeled areas. The cerebellar cortex of the adult lc/+ mouse in particular is severely atrophied, and, as a result of this small size, a greater portion of the total cerebellar cortex was labeled in these mice. Although a secondary degeneration of the inferior olive of the lc/+ mouse has been reported, several investigators have described labeling of ~30% of inferior olivary neurons with HRP (Caddy and Biscoe 1976; Heckroth and Eisenman 1991; Wetts and Herrup 1982a, b). In contrast, the quantity of labeled olivary cells found in the present study was ~<1%, much less than the 30% one would have expected, and we have no reasonable explanation for the difference. Neurons of the inferior olive were clearly visible in sections counterstained with neutral red, and in agreement with Heckroth and Eisenman (1991), the surviving neurons were scattered both evenly and randomly throughout the subnuclei of the inferior olive. Where present, the labeling of the inferior olivary neurons in lc/+ mice was very faint and only visible at very high magnification. It appears that a rather large portion of the surviving neurons in the inferior olive in our lc/+ mice was simply incapable of the retrograde transport of WGA-HRP to the same degree as either the wild type or the other mutants with Purkinje cell degeneration.

The genetically dystonic rat also fails to display any harmaline-induced tremor without any apparent cerebellar or olivary atrophy (Lorden et al. 1985; Stratton and Lorden 1991). In this case it was thought that the absence of tremor must have been due to a failure of olivocerebellar synaptic transmission.

Heckroth (1994) has reported that in lc/+ mice there is a partial atrophy of the small neurons of the dentate and interpositus nuclei, which are thought to contain GABA (Nelson and Mognain 1989) and project exclusively to the inferior olivary nucleus (Legendre and Courville 1987). This pathway may be responsible for the electrotonic coupling of olivary neurons under the influence of harmaline (Llinás and Sasaki 1989; Sotelo et al. 1986; Stratton and Lorden 1991), implying that the surviving olivary neurons in the lc/+ mouse are not as strongly coupled as in the other mutants. However, a similar although perhaps less severe degeneration of GABA-containing cerebellar nuclear cells has also been reported in pcd/pcd mutants (Triarhou et al. 1987; Wassef et al. 1986), and this does not appear to have greatly affected their sensitivity to harmaline.

In general, the transport of WGA-HRP indicated a close association between the number of retrogradely labeled neurons in the inferior olivary nuclei and the intensity of harmaline tremor, confirming the suggestion that harmaline tremor is driven by an olivonuclear reverberating circuit (DeMontigny and Lamarre 1973). The results of the present study support the suggestion by Llinás and Volkind (1973) that removing Purkinje cell inhibition does not abolish the harmaline-induced rhythmic EMG activity, whereas dysfunction or atrophy of the inferior olive does.
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