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Behavioral and biochemical effects of L-tryptophan and buspirone in a model of cerebellar atrophy

N. Le Marec[a,b,c,*], A.R. Ase[a], T. Botez-Marquard[b], L. Marchand[b], T.A. Reader[a,b,1], R. Lalonde[a,b,1]

[a]*Centre de Recherche en Sciences Neurologiques, Faculté de Médecine, Université de Montréal, Montreal, Quebec, Canada*
[b]*Unité de Neurologie du Comportement, Neurobiologie et Neuropsychologie Centre Hospitalier de l'Université de Montréal, Campus Hôtel-Dieu de Montréal, Montreal, Quebec, Canada*
[c]*Centre de Recherche de l'Hôpital du Sacré-Coeur, Montreal, Quebec, Canada*

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Abstract

The *Lurcher* mutant mouse can be considered an adequate model of autosomal dominant spinocerebellar atrophy because of the severe degeneration of its cerebellar cortex and inferior olive. The purpose of this study was to determine whether the motor coordination deficits of *Lurcher* mutants could be improved after chronic administration of the serotonin (5-hydroxytryptamine; 5-HT) precursor, L-tryptophan, or of the 5-HT_{1A} agonist, buspirone. During these treatments, the mice were submitted to behavioral evaluations using the *coat hanger* and the rotorod tests, as well as an inclined screen and a vertical grid test. At the end of treatments, 5-HT and 5-hydroxindole-3-acetic acid (5-HIAA) were measured in six brain regions. On the coat hanger test, administration of L-tryptophan accelerated movements along the horizontal bar by 44%, while buspirone increased the time spent on the apparatus by 11%. Neither drug had an effect on climbing ability or on the time spent on a rotating beam. Administration of L-tryptophan increased 5-HIAA levels in frontal cortex, neostriatum, thalamus, brainstem, cerebellum and spinal cord, but elevated 5-HT only in neostriatum, brainstem and cerebellum. In contrast, buspirone led to 5-HT increases in cerebellum and augmented 5-HIAA in the spinal cord. The modest test-specific improvements are consistent with some of the clinical data concerning 5-HT pharmacotherapy in patients suffering from cerebellar atrophy.

Keywords: *Lurcher* mutant mice; Cerebellum; Behavioural testing; Serotonin; HPLC; Ataxia

1. Introduction

The heredodegenerative ataxias comprise various genetic abnormalities, but with a common neuropathology, namely, the degeneration of the cerebellum and some of its associated brainstem regions (Hammans, 1996; Koeppen, 1999). Improvements of motor functions have been observed in cerebellar ataxia patients (Botez et al., 1991, 1996, 1999; Peterson et al., 1988) and in cerebellar animals (Lalonde et al., 1993) after treatment with amantadine, a dopamine-releasing agent that also exhibits antagonist properties at *N*-methyl-D-aspartate receptors. For approximately 10 years, improvements of motor functions have been reported following administration of agents capable of modifying serotonin (5-hydroxytryptamine; 5-HT) neurotransmission; the drugs used included the immediate precursor, 5-hydroxytryptophan (5-HTP) (Trouillas et al., 1995, 1998), as well as the 5-HT_{1A} agonist, buspirone (Lou et al., 1995; Trouillas et al., 1997). However, the interpretation of some of the experimental data from clinical trials has differed, and conflicting reports have appeared (Currier, 1995; Currier et al., 1995; Wessel et al., 1995) that revealed no improvements on motor coordination in cerebellar patients after 5-HTP administration. It is thus relevant to examine further this issue in animal models of cerebellar ataxia, as well as to continue to carry on research on substitutive pharmacological therapies for ataxic patients, since putative genetic

* Corresponding author. Centre de Recherche de l'Hôpital du Sacré-Coeur, Hôpital du Sacré-Coeur de Montréal, 5400 Boulevard, Gouin O., Montreal, Quebec, Canada H4J 1C5. Tel.: +1-514-338-2222 ext. 3696; fax: +1-514-338-2694.

E-mail address: n-lemarec@crhsc.umontreal.ca (N. Le Marec).

[1] These two authors contributed equally to this work.

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manipulations, even if they have a promising potential (Babcok et al., 1997; Wilson and Roof, 1997), will not be readily available for patients before some time.

The aim of the present study was to further examine the role of 5-HT neurotransmission on motor coordination in an animal model of cerebellar atrophy, i.e., the *Lurcher* mutant. This mutant can be considered as an adequate experimental model of autosomal dominant spinocerebellar ataxia. Indeed, this mutant is characterized by degeneration of Purkinje neurons and granule cells of the cerebellum, as well as neuronal loss in deep cerebellar nuclei and inferior olive (Caddy and Biscoe, 1979; Heckroth, 1992, 1994; Heckroth and Eisenman, 1991), leading to overt alterations in equilibrium and balance, with manifest cerebellar ataxia (Lalonde, 1996; Lalonde et al., 1993; Le Marec et al., 1997). For this purpose, the behavior of *Lurcher* mutant mice undergoing serotoninergic therapy was evaluated with several differential tests. Animals were tested successively in four different motor tasks, as previously reported (Thifault et al., 1996). The order effect was not to be tested here, since we intended to screen the serotoninergic treatment effect, and document which tasks were sensitive to this treatment in order to further analyze this in detail. Also, and as it is well known, wild type mice of the same background strain as *Lurcher* mutant mice present higher motor performances (Le Marec and Lalonde, 1997; Thifault et al., 1996), and therefore, only a small number of wild type mice were trained. The treatments were given during the entire behavioral session, i.e., for 20 consecutive days using either the amino acid L-tryptophan, the precursor of both 5-HTP and 5-HT, or with the 5-HT_{1A} agonist buspirone. Four different motor function tests were used in the present study: (1) the coat hanger, (2) the inclined screen, (3) the vertical grid and (4) the rotorod (Lalonde, 1996; Lalonde et al., 1993; Le Marec and Lalonde, 1997; Le Marec et al., 1997). In addition, brain concentrations of 5-HT and its main metabolite, 5-hydroxyindole-acetic acid (5-HIAA), were measured by high-performance liquid chromatography in frontal cortex, neostriatum, thalamus, brainstem, cerebellum and spinal cord.

2. Materials and methods

2.1. Animals

Female *Lurcher* mutants (*Lc*/+, $n=20$) and wild type mice (+/+, $n=14$) of the B6CBACa-$A^{w\text{-}J}$/A strain were purchased from the Jackson Laboratory (Bar Harbor, ME) and housed in a temperature- and humidity-controlled room. On arrival at the laboratory, the mice were handled daily for a few days in order to permit their adaptation both to the experimenters and to the environment before the actual testing sessions. At the time of testing, the mice were between 2.5 and 3.5 months of age. All procedures were in strict accordance with the Canadian Council of Animal Care "Guide to the Care and Use of Experimental Animals" (2nd edn) and the protocol approved by *Comité Institutionnel pour la Protection des Animaux de l'Hôtel-Dieu de Montréal*.

2.2. Drug administration and dosage

Lurcher mutants received daily intraperitoneal injections of either L-tryptophan methyl ester (50 mg/kg; Sigma, St. Louis, MO), buspirone (1 mg/kg; Hoffmann-La Roche, Nutley, NJ) or an equivalent volume (2 ml/kg) of 0.9% saline during the 20 consecutive days of the behavioral sessions; the compounds were always administered at the same time every morning. The agonist buspirone and the precursor L-tryptophan were chosen because in some cases, these compounds have been shown to be beneficial in human cerebellar ataxia, in spite of controversial effects on motor coordination in some of the clinical trials (Currier et al., 1995; Lou et al., 1995; Trouillas et al., 1995, 1997, 1998; Wessel et al., 1995). The dosages were established based on their known peak behavioral and neurochemical effects (Carli et al., 1989; Fernandez-Guasti and Picazo, 1997; Fernstrom and Wurtman, 1971, 1972; Geller and Hartmann, 1982; Gardette and Crepel, 1993; Le Marec et al., 1998, 1999; Lookigland et al., 1986; Pecknold, 1994; Reader et al., 1999). In the present investigation, the amino acid L-tryptophan was preferred to the immediate precursor 5-HTP, since *Lurcher* mice do not seem to have a deficit in the activity of the rate-limiting enzyme tryptophan hydroxylase, and can indeed synthesize 5-HT when challenged with a load of L-tryptophan (Reader et al., 1999, 2000). Whenever wild type mice were used, they were subjected to the same drug injection protocol.

2.3. Behavioral testing

The coat hanger test consisted of a horizontal steel wire (diameter: 2 mm, length: 40 cm) placed at a height of 80 cm from a cushioned table and flanked by two diagonal side bars (length: 19 cm, orientation: 35° from the horizontal). The inclined screen consisted of a wooden frame and a wire-mesh grid (38×38 cm, 5 cm^2) that could be inclined at any angle. The bottom part of the screen was placed at a height of 15 cm from the table. The vertical grid (height: 25 cm, width: 20 cm, 1 cm^2) was placed at a height of 80 cm from the mat-covered floor. The accelerating rotorod (Model 7650; Stoelting, Wood Dale, IL) consisted of a horizontal rod (3 cm in diameter) turning around its longitudinal axis at a height of 15 cm from the table. This rod was made of a knurled material; it was divided into five sections of 5.5 cm by means of a plastic barrier, thus permitting adequate traction and the capability of testing five mice simultaneously.

The *Lurcher* mice were first pre-trained on the coat hanger test without receiving any injection on four trials per day (intertrial interval of 15 min) for seven consecutive

days, in order to stabilize their performances. At the end of this pre-training period, *Lurcher* mutants were distributed into one of the three treatment groups, and received daily intraperitoneal injections of L-tryptophan ($n=7$), buspirone ($n=7$) or saline ($n=6$). All subsequent behavioral performances were evaluated by an observer blind to the nature of the treatments for two trials per day, with an intertrial interval of 15 min. Throughout all these studies, the mice were trained for 60 min after L-tryptophan administration and 15 min after buspirone or saline injections. The latencies before injecting serotoninergic compounds were chosen, taking into account the time needed for the drug (by intraperitoneal injection) to have their maximal effect within the organism, i.e., 15 min for buspirone (Algeri et al., 1988; Gammans et al., 1986; Jann, 1988) and 60 min for L-tryptophan (Fernstrom and Wurtman, 1971, 1972).

On every trial, the mice were placed upside down in the middle part of the horizontal string and allowed to stay on it for 60 s. The mice were not released until their paws firmly gripped the bar. In addition to measuring latencies before falling, seven types of movement time (MT) were documented. These measurements were: the latencies before reaching (snout criterion) the first segment of 10 cm (MT-1) or the end (MT-2) of the horizontal string, the latencies before reaching (paw criterion) one of the two diagonal bars with two (MT-3), three (MT-4) or four paws (MT-5), and the latencies before reaching (snout criterion) either the half-way point (MT-6) or the top (MT-7) of either diagonal bar. When any mouse succeeded in reaching the top of the diagonal bar, it was immediately retrieved, and given the maximal score of 60 s for latencies before falling. The data were compiled in three blocks of trials; the first trial block comprised the last day of pre-training (baseline), while the second and third blocks comprised the first 5 days and the last 5 days of testing, as previously implemented (Lalonde et al., 1993; Le Marec et al., 1997; Thifault et al., 1996).

After 8 days of injections, immediately after the coat hanger test, the mice were evaluated on two additional tests: the inclined screen and the vertical grid. The mice were placed in the middle part of the inclined screen with the snout facing downward at one of two slopes (45° or 60°). The time taken before turning upward and before reaching the top of the screen, i.e., snout criterion, was measured for four trials of 60 s (two for each inclination). In alternating trials with the previous test, the mice were placed in the bottom part of the vertical grid facing upward and latencies before reaching the top (snout criterion) were measured during four trials, with an intertrial interval of 15 min.

From days 11 to 20 of injections, the mice were evaluated on the rotorod. The mice were placed on the rotating beam while it was in motion, on the opposite orientation from where the mice stood facing, so that forward locomotion was necessary in order to avoid a fall. The speed of rotation was gradually increased at each 30-s interval by 4 revolutions/min (rpm), and the range of rotating speed was from 4 to 40 rpm; the cut-off point was established at 5 min per trial. There were four trials per day during 10 consecutive days of testing, with an intertrial interval of 15–20 min. On most trials, the *Lurcher* mice clung to the rod without moving and were therefore passively rotated. Thus, in addition to latencies before falling, the time spent walking was compiled. Passive rotation was tabulated only on those trials when this strategy was used up to the end of any trial. None of the animals deliberately jumped from the apparatus at any time, thereby providing valid measurements of motor coordination.

Another group of 26 mice (14 wild type and 12 *Lurcher*) was submitted, at the 20th day of treatment with either saline or L-tryptophan, to a spontaneous locomotion test. The animals were positioned in a familiar environment with two novel objects, and were videotaped during 10 min of free locomotion. The data analyzed were the time during which the animals explored objects and the number of movements; this task allowed us to determine whether serotoninergic treatment implies an overall side effect, such as an increase in motor activity.

2.4. Measurements of 5-HT and 5-HIAA

After the termination of the behavioral studies, drug administration was continued for an additional period of 20 days. At the same post-injection interval, the mice were killed by swift decapitation, their brains and spinal cord rapidly removed, frozen in *N*-methylbutane at -40°C and then kept at -80°C. The brains were dissected on a cold plate under a binocular microscope following stereotaxic coordinates (Franklin and Paxinos, 1997) and classical anatomical landmarks (Ase et al., 1999; Reader et al., 1999, 2000; Strazielle et al., 1996). The frontal cortex included the motor and prefrontal cortices from the poles of the hemispheres to the anterior commissure. The neostriatum (caudate–putamen) was dissected rostral to the anterior commissure, and the entire thalamus was removed as a separate sample. The cerebellum included cerebellar cortices from the vermis and hemispheres as well as the deep cerebellar nuclei. The brainstem sample consisted of the mesencephalon, pons and medulla oblongata, while the spinal cord, removed as complete as possible from the medullary canal, was analyzed in its entirety.

The dissected samples were weighed (10–100 mg wet weight), homogenized in 40–50 vol of monochloroacetic acid 0.1 N with 2.15 mM Na_2EDTA with a Tissumizer (Tekmar, Cincinnati, OH) and centrifuged at $39{,}000 \times g$ for 45 min at 4°C. The pellets were dissolved overnight for protein determinations (Lowry et al., 1951), while the supernatants were filtered through 0.45-μm pores (GS; Millipore, Bedford, MA) and stored at -80°C until analysis by high-performance liquid chromatography with electrochemical detection (Ase et al., 1999; Reader et al., 1999, 2000). The mobile phase was composed of 0.1 M monochloroacetic acid, adjusted to pH 3.3 with 1 N NaOH, containing 800 mg/l of Na_2EDTA, 230 mg of sodium octyl

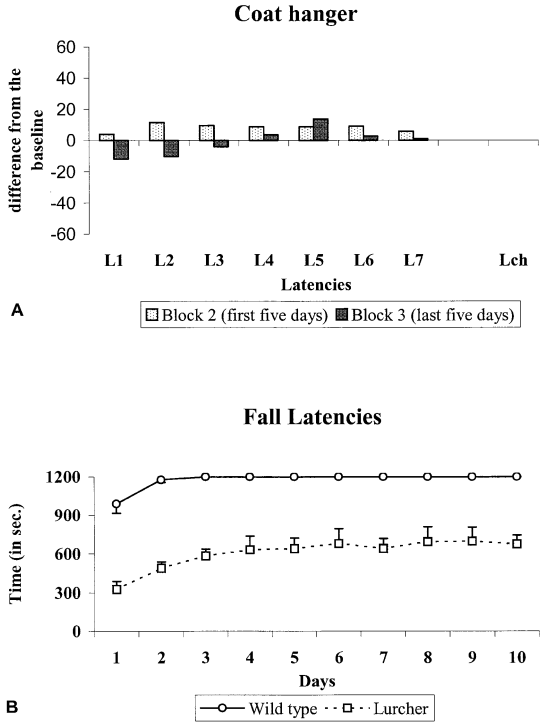

Fig. 1. (A, B) Wild type performances in (A) coat hanger test and (B) rotorod task. (A) Difference in time needed by wild type to reach each latencies (L1 to L7 and fall latency) in blocks 2 (□) and 3 (■) compared to baseline (block 1). (B) Mean (±S.E.M.) fall latency in rotorad task in wild type (○) and in *Lurcher* (□) mutant group.

sulfate (Sigma) and 10% methanol. The flow was kept at 0.6 ml/min and the samples in 100-μl aliquots were injected into a 3-μm particle column (100.0 × 4.6 mm; Adsorbosphere Catecholamine; Alltech, Deerfield, IL). The substances were oxidized with a glassy carbon electrode at a potential of 680 mV relative to a Ag/AgCl reference electrode, and the electrochemical detector (EE&G M-400; Princeton Applied Research, NJ) set at a gain of 10 nA full scale. The peaks generated were recorded and their areas integrated with a Hewlett Packard 3392A instrument. Since there were no pre-column purification or recovery steps, only external standards were used. They consisted of noradrenaline HCl, 3,4-dihydroxyphenylacetic acid, 5-hydroxy-L-tryptophan, 5-hydroxytryptophol, 5-HIAA, dopamine HCl, homovanillic acid, L-tryptophan, 5-HT HCl and 3-methoxytyramine; however, only the 5-HT and 5-HIAA levels are reported here. For every chromatographic session, the external standards containing 0.5–1.0 ng of each monoamine were injected, i.e., every four to six samples, to quantify peak areas and to determine retention times; these values had coefficients of variation never exceeding 5%.

2.5. Statistical analyses

Analyses of variance (two-factor ANOVA) were used in all cases, with repeated measure on the second factor for coat hanger and rotorod tests. The least significant difference (LSD) (Barlow, 1983; Frank and Althoen, 1994) test was used for the coat hanger test. Paired *t* tests were used to compare performances of mutant mice on block trial measurements in the coat hanger task, while unpaired *t* tests were applied to compare the performances between groups on the coat hanger task.

3. Results

3.1. Motor coordination

3.1.1. Wild type mice

The wild type mice used in this study did not show during the length of the experiments any drug-related problems such as weight loss, changes in spontaneous motor activity and increases in either aggressiveness or sedation. Because the performances of wild type mice are already well known (Le Marec et al., 1997), they will only be very briefly reported here (see Fig. 1A and B and Table 1).

On the coat hanger task, wild type mice were able to reach the middle or the end of the inclined bar (latencies 6 or 7, respectively) at the end of the pre-training session. The performances of wild type mice on block 2 (first 5 days of training session) or in block 3 (last 5 days of training session) were not different from block 1 (baseline level) for the eight latencies measured, showing stability of performances (Fig. 1A). On the grid test, the animals exhibited a relatively important time to reach the top of the apparatus (Table 1), as previously reported (Le Marec et al., 1997). On the rotorod task, wild type mice reached maximal levels of performance (1200 s and 40 rpm) on the third day of testing (Fig. 1B) and never used a *grasping strategy*; indeed, they only used a walking strategy.

3.1.2. Lurcher mutant mice

3.1.2.1. Coat hanger test. Among the seven MT measurements, the only one showing a significant group difference was MT-2, as defined by the time taken to reach the end of the horizontal bar (see Fig. 2A–C for the first three MT values) as the Group × Trial Block interaction was significant ($F_{2,4} = 3.26$, $P < .05$). The MT-2 value of the L-trypto-

Table 1
Mean (±S.E.M.) time taken (in seconds) before turning or before reaching the top of the inclined screen or the time taken before reaching the top of the vertical grid in wild type mice

Test	Saline
Inclined screen	
45° turn (s)	31 ± 10
60° turn (s)	27 ± 09
45° top (s)	83 ± 13
60° top (s)	44 ± 10
Vertical grid top (s)	107 ± 20

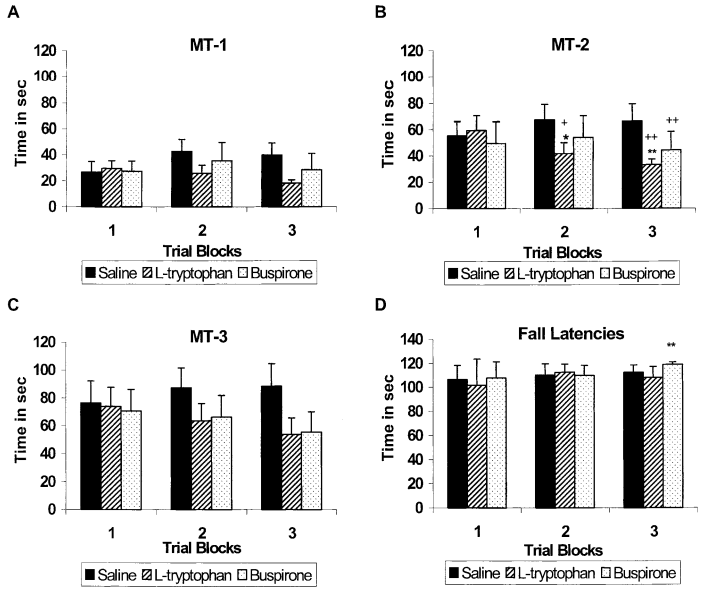

Fig. 2. Mean (±S.E.M.) movement time (MT) for *Lurcher*-treated group (A–C) in the coat hanger test, (■) saline (▨), L-tryptophan or (◧) buspirone. (A) MT-1 (reach the middle of the horizontal bar). (B) MT-2 (reach the end of the horizontal bar). (C) MT-3 (put the two front paws on the diagonal bar). Graph D: Latencies before falling, mean time in seconds (±S.E.M.). Trial block 1 = baseline; trial block 2 = first 5 days of testing and trial block 3 = last 5 days of testing. * $P<.05$, ** $P<.01$, compared to baseline; $^{+}$ $P<.05$, $^{++}$ $P<.01$, compared to saline.

phan group was lower than that of the saline-treated group on trial blocks 2 and 3 ($P<.05$ and $P<.01$, respectively), while the buspirone group differed from saline only on trial block 3 ($P<.01$). The L-tryptophan-treated animals also had lower MT-2 values in comparison to baseline, i.e., trial block 1 ($P<.05$ and $P<.01$, respectively, in trial blocks 2 and 3). In contrast, the performances of the buspirone-treated mice did not differ from baseline values (Fig. 2B).

On fall latency measurements (Fig. 2D), there is an effect on trial blocks ($F_{2,2}=4.14$, $P<.05$). A paired *t* test showed that there was a difference between baseline and trial block 3 ($P<.05$). An unpaired *t* test revealed that while latencies before falling for the saline- and the L-tryptophan-treated groups remained stable across time (Fig. 2D) ($P>.05$), the buspirone-treated group had significantly higher fall latencies in trial block 3, when compared to baseline values ($P<.05$). However, no significant difference was found by comparison to the saline group ($P>.05$).

3.1.2.2. Grid tests. By contrast to the results found in the previous test, no significant intergroup differences ($P>.05$) were observed in terms of the latencies before turning, as well as before reaching the top of the inclined screen or before reaching the top of the vertical grid (Table 2).

3.1.2.3. Rotorod test. On the rotorod test, the day effect was significant ($F_{2,9}=23.76$, $P<.001$) for latencies before falling, but not the group effect ($F_{2,9}=0.19$, $P>0.05$) or the Group × Day interaction ($F_{2,18}=1.06$, $P>.05$). As shown

Table 2
Mean (±S.E.M.) time taken (in seconds) before turning or before reaching the top of the inclined screen or the time taken before reaching the top of the vertical grid in *Lurcher* mutants injected with L-tryptophan, buspirone or saline

Test	Saline	L-Tryptophan	Buspirone
Inclined screen			
45° turn (s)	19±10	27±33	20±14
60° turn (s)	22±11	29±17	17±07
45° top (s)	48±20	54±33	75±42
60° top (s)	64±31	55±34	71±39
Vertical grid top (s)	190±57	162±60	187±59

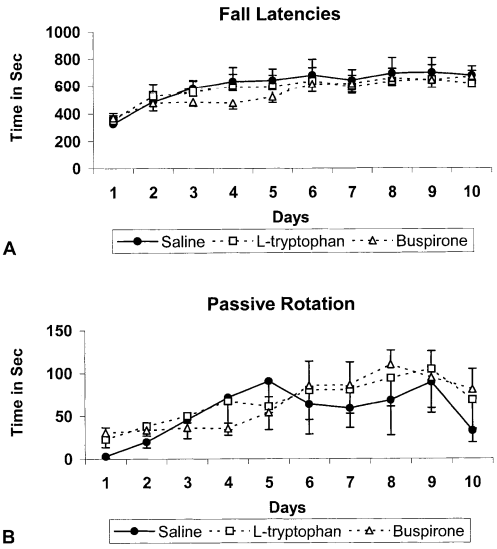

Fig. 3. Mean (±S.E.M.) latencies before falling (A) and time spent in passive rotation (B) of *Lurcher* mutant mice injected with (●) saline, (□) L-tryptophan or (△) buspirone in the rotorod test.

Table 3
Mean (±S.E.M.) for number of object visits and for moving time (in seconds) in wild type and *Lurcher* mice in free exploration of a familiar environment in the presence of two novel objects during 10 min

Group	Wild type		*Lurcher*	
Treatment	Saline	L-Tryptophan	Saline	L-Tryptophan
Exploration				
Number	41±08	46±03	45±06	43±09
Time	44±10	43±05	50±09	48±13

in Fig. 3A, all three groups remained on the rotorod for longer periods of time, as a function of repeated days of testing. The time spent in passive rotation (Fig. 3B) was also augmented with repeated trials ($F_{2,9}=5.89$, $P<.001$) in the absence of group ($F_{2,9}=0.09$, $P>.05$) or interaction ($F_{2,18}=0.72$, $P>.05$) effects.

3.1.2.4. Spontaneous exploration. There were no significant differences ($P>.05$) in exploration time between animals treated with or without serotoninergic compounds. Similarly, the number of movements made during the entire 10 min of the test was not significantly different ($P>0.05$) between animals injected with either the active compound or with saline solution (Table 3).

Table 4
Tissue contents of 5-HT and 5-HIAA in brain regions of *Lurcher* mutants injected with L-tryptophan, buspirone or saline

Region	Saline (ng/mg protein)	L-Tryptophan (ng/mg protein)	Buspirone (ng/mg protein)
Frontal cortex			
5-HT	4.61±0.45	6.34±0.89	5.66±0.69
5-HIAA	3.38±0.50	7.40±2.20[a]	3.71±0.43
Neostriatum			
5-HT	5.73±0.67	7.07±1.03[b]	5.42±0.63
5-HIAA	4.46±0.79	6.88±1.18[a]	4.79±0.50
Thalamus			
5-HT	8.26±1.04	10.58±1.37	7.24±0.60
5-HIAA	12.85±2.16	23.31±1.74[a]	11.73±1.66
Brainstem			
5-HT	10.22±1.41	15.20±1.92[b]	10.81±1.40
5-HIAA	14.02±1.43	20.91±2.09[b]	14.71±1.55
Cerebellum			
5-HT	2.53±0.28	4.28±0.56[a]	4.28±0.73[b]
5-HIAA	2.47±0.16	5.39±0.40[a]	3.21±0.31
Spinal cord			
5-HT	5.76±0.82	5.37±0.55	4.68±0.57
5-HIAA	6.99±0.53	11.63±0.76[a]	10.18±0.85[b]

The values are the means±S.E.M. ($n=6-7$) in nanograms per milligram of protein (ng/mg protein).
[a] $P<.01$ vs. saline-treated animals.
[b] $P<.05$ vs. saline-treated animals.

3.1.2.5. Tissue indoleamine measurements. The administration of L-tryptophan augmented brain 5-HT and 5-HIAA concentrations in the *Lurcher* mutants (Table 4). The levels of endogenous 5-HT were increased in neostriatum, brainstem and cerebellum. The tissue levels of the metabolite 5-HIAA were increased in all brain regions examined, including the frontal cortex and spinal cord, where 5-HT contents were unchanged. In contrast, the effects of buspirone on indoleamine concentrations were limited to the cerebellum and spinal cord; by comparison to saline-treated *Lurcher* mutants, buspirone increased 5-HT contents in the cerebellum and 5-HIAA levels in the spinal cord.

4. Discussion

This study was carried out to test effects of putative serotoninergic replacement therapies (Currier et al., 1995; Lou et al., 1995; Trouillas et al., 1995, 1997, 1998) on an animal model of ataxia due to cerebellar degeneration; indeed, in this study, both motor coordination as well as neurochemical modifications were taken into account. The results show that L-tryptophan and buspirone did not improve motor coordination in *Lurcher* mutant mice, as previously reported in some of the clinical trials with ataxic patients (Currier, 1995; Currier et al., 1995; Wessel et al., 1995). The chronic administration of L-tryptophan increased 5-HIAA levels in most regions, namely, in frontal cortex, neostriatum, brainstem, cerebellum and spinal cord, and tissue 5-HT contents only in neostriatum, brainstem and cerebellum. In contrast, the treatment with the partial 5-HT_{1A} agonist buspirone increased the levels of 5-HT in cerebellum and those of 5-HIAA in spinal cord.

Among the eight latencies measured, the treatment with L-tryptophan only decreased the time spent before reaching the end of the horizontal bar (MT-2) of the coat hanger by 44%, in comparison to baseline levels. There are several reasons why this increase in performance after chronic L-tryptophan treatment could not be due to a global increase of motor activity. In the first place, this increase was not observed with the wild type mice that had also received a chronic L-tryptophan treatment (Fig. 4). Secondly, the

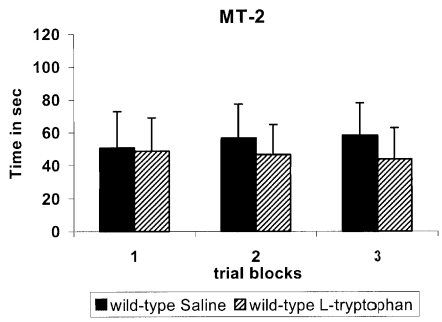

Fig. 4. Mean (±S.E.M.) of MT-2 for wild type mice injected with (■) saline or (▨) L-tryptophan in the coat hanger test.

Lurcher mutant mice have been tested (Joseph et al., 2000) in a familiar environment in the presence of two novel objects. The L-tryptophan-treated mice, *Lurcher* or wild type, did not exhibit more exploratory time when compared to the saline-treated, as one would have expected if they had become hyperactive (Table 3). Thus, the L-tryptophan treatment did not lead to a global elevation of motor activity, and the differences documented with the *coat hanger* test can be interpreted as a slight and restricted improvement in motor coordination. In spite of the decrease in MT-2 values, animals that had received the treatment with L-tryptophan did not climb faster onto the side bars, as discerned by latencies 3–5, which were not significantly modified after treatment. Moreover, the first latency (MT-1) was not increased, thus indicating that the drugs used here did not improve movement initiation, in contrast to amantadine (Lalonde et al., 1993); indicating very limited effects of L-tryptophan on motor coordination.

The buspirone-treated *Lurcher* had lower MT-2 values than the saline-treated *Lurcher* mutants; however, these results did not differ from baseline levels ($P>.05$). The reason for these results is a slight decrease in performance on the part of the saline-treated group for this measure, significant ($P<.05$) when comparing block 1 to baseline, but not significant ($P>.05$) when comparing block 2 to baseline. Although the cerebellum has been hypothesized to play a role in motivational processing (Caston et al., 1998; D'Agata et al., 1993), the performance of the saline-treated group was relatively stable over time on all measures, except MT-2, in a task where no positive reinforcement is provided aside from removing the mice from the apparatus. It remains to be determined whether serotoninergic therapies stabilize motor performances in tasks causing low motivational levels (Lou et al., 1995; Massana, 1998; Steckler and Sahgal, 1995).

The chronic treatment with buspirone led to increased (+11%) latencies before falling from the coat hanger, but without compromising any MT measure. As illustrated in Fig. 2D, the increased latencies before falling induced by buspirone nearly reached the maximal value of 120 s/day. These improved latencies before falling, documented in the buspirone-treated group, were not caused by an increase in the time spent in immobility, as shown by MT values equal to baseline. Conversely, treatments with L-tryptophan or buspirone did not improve motor functions in the other tests performed here, namely, the inclined screen, the vertical grid and the rotorod. In contrast to the coat hanger test, the animals could not be pre-trained in those tests, as this would have entailed the use of a prohibitively high number of rare and expensive mutant mice. The absence of drug-induced improvement may be explained by the specific functions that are measured by each test. The inclined screen and vertical grid require climbing, and this behavior was not affected in the coat hanger test by either drug. In contrast to the other three tests, the rotorod test requires continuous shifts of equilibrium in response to a mobile apparatus, i.e., the animal now needs to construct a new motor strategy, which will permit him to adapt his behavior to a new environment. The present results show that serotoninergic therapies have a very moderate and circumscribed effect; indeed, more important amelioration could eventually be discerned with longer treatment schedules, since stabilization of symptoms may more readily be improved than symptom regression.

The present results are not congruent with some of the clinical trials indicating improvements of motor functions after long-term treatment with 5-HTP, the immediate precursor of 5-HT, or following buspirone administration (Lou et al., 1995; Trouillas et al., 1995, 1997, 1998). In fact, moderate test-selective improvements in motor functions have been reported after long-term administration of 5-HTP (Trouillas et al., 1995, 1998) or buspirone (Lou et al., 1995; Trouillas et al., 1997) in patients suffering from heredodegenerative ataxias. However, our results are more in line with other studies that found no improvement in motor function (Currier, 1995; Currier et al., 1995; Wessel et al., 1995). Trouillas et al. (1995) reported that kinetic scores, i.e., in finger–nose and heel–tibia tests, were improved after 5-HTP, whereas static scores, such as stance and gait, were not. The present series of tests in mice is indeed comparable to those used for kinetic measurements in human patients, and we did not find that there was a clear treatment improvement in this animal model. The beneficial influence of 5-HT-modulating agents may not be limited to measures of limb ataxia, as suggested in a previous study by the improvements of cerebellar tremors due to several kinds of pathology, including multiple sclerosis, documented after a treatment with the 5-HT_3 receptor antagonist ondansetron (Rice et al., 1997). It is already well established that cerebellar pathology could be caused by multiple neurochemical disorders; therefore, future drug therapies may have to be based on several chemical compounds (Botez et al., 1998). In fact, it has been shown that a multiple drug cocktail partially based on serotoninergic drugs seemingly protects ataxic patients from the typical nocturnal obstructive apnea and can improve their dysarthria as well (Botez et al., 1997).

Cerebellar 5-HT afferent fibers originate from the medullary reticular formation and the pontine dorsal raphe nucleus (Batini, 1993; Bishop et al., 1993; King et al., 1993). In an effort to determine the role of the 5-HT system in cerebellar-related motor disorders, the effects of L-tryptophan, the precursor of 5-HT, and buspirone, a selective 5-HT_{1A} receptor agonist, were evaluated in *Lurcher* mice. By comparison to controls, *Lurcher* and other cerebellar mutants have regionally selective increases of 5-HT uptake site labelling (Le Marec et al., 1998, 1999; Strazielle et al., 1996) and 5-HT concentrations (Reader et al., 1999, 2000; Strazielle et al., 1996), probably as a secondary adaptation following cerebellar and inferior olive degeneration (Caddy and Biscoe, 1979; Heckroth, 1992, 1994; Heckroth and Eisenman, 1991). Indeed, the already high 5-HT and 5-HIAA

concentrations in *Lurcher* mutants, compared to the levels found in wild type mice, were further increased after a 40-day L-tryptophan treatment (Reader et al., 1999) or a 15-day combined treatment with L-tryptophan, amantadine and thiamine (Le Marec et al., 1999). In the present study, chronic L-tryptophan administration increased concentrations of these indoleamines in most of the brain areas examined, indicating that in *Lurcher* mice, the conversion of L-tryptophan into 5-HT and its oxidation after synaptic release into 5-HIAA were not impaired. In contrast, buspirone had lesser effects on 5-HT and 5-HIAA levels. Some of the behavioral effects of buspirone can be caused by a decrease in the release of 5-HT, mediated through 5-HT_{1A} autoreceptors (Sharp et al., 1989; Soderpalm et al., 1993). In the present study, the drug led to increased levels of 5-HT in cerebellum and of 5-HIAA in spinal cord. These results indicate that the behavioral effects of buspirone cannot be attributed to a generalized accumulation of 5-HT in nerve terminals throughout the entire brain. It is plausible that after L-tryptophan treatment, there is an increased 5-HT transmission in the cerebellum and/or brainstem, as 5-HT and 5-HIAA concentrations were augmented in both regions. In addition to this neurotransmitter effect, 5-HT may reduce abnormal neurochemical processes caused by cellular degeneration in the cerebellum and inferior olive, perhaps mediated by the excitatory amino acid glutamate; indeed, applications of 5-HT in cerebellum have been shown to modulate glutamate-induced excitations (Gardette and Crepel, 1993; Reader et al., 1999; Strahlendorf et al., 1984). On the other hand, the motor effects of buspirone are likely to be mediated outside the cerebellum, as 5-HT_{1A} receptors are only transiently expressed during developmental stages in this brain region; however, they are highly expressed in the adult inferior olive and other motor regions (Miquel et al., 1994; Vergé et al., 1993). Another possibility is that some of the motor effects of buspirone are mediated by other neuromodulators, particularly the catecholamines and/or inhibitory amino acid neurotransmitters (Algeri et al., 1988; Scuvee-Moreau et al., 1987).

Finally, we adhere to the statement of Currier (1995) that "... the levorotatory form of 5-hydroxy-tryptophan may have an effect that is minimal, selective, and difficult to detect." In the present study, the amino acid L-tryptophan was used to augment 5-HT synthesis, and this is a more reliable biochemical approach (Reader et al., 1999, 2000) than the use of L-5-hydroxy-tryptophan (L-5-HTP), since the latter compound may increase serotoninergic neurotransmission in a rather non-specific way. Indeed, conversion of L-5-HTP into 5-HT is by a simple decarboxylation step, and this can be carried out even by non-serotoninergic neurons as well as by non-neuronal elements such as glial and/or endothelial cells. In contrast, in our studies, the administration of L-tryptophan to enhance 5-HT levels requires the conversion by the rate-limiting enzyme tryptophan hydroxylase, which is present in serotoninergic neurons and nerve terminals. As indoleamine levels were increased, it shows that L-tryptophan as a precursor was certainly capable from a neurochemical perspective to increase neurotransmitter contents, but failed to modify most of the behavioral parameters measured here. In other words, 5-HT therapies alone may not be an effective treatment for amelioration of symptoms of ataxia in cerebellar mutant mice. Although 5-HT therapies could eventually maintain the level of certain performances, they may not increase them beyond a certain threshold; therefore, the possible beneficial effects still warrant further examination. Future studies, including extended duration of drug administration, combinations with other compounds that exhibit trophic and/or neuroprotection properties or an earlier onset of therapy, particularly during the critical period of Purkinje cell degeneration on the second to the fourth postnatal weeks (Caddy and Biscoe, 1979), may be required to fathom some of these possibilities.

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