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Magali Hernandez, Guillaume Harlé, Robert Lalonde, Catherine Strazielle. Effects of corticosterone injections in mid-to-late mouse postnatal development on adult motor activity and coordination. *Neuroscience Research*, 2021, 164, pp.22-32. 10.1016/j.neures.2020.03.012 . hal-03219976

HAL Id: hal-03219976

<https://hal.univ-lorraine.fr/hal-03219976>

Submitted on 6 May 2021

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Neuroscience Research

Effects of corticosterone injections in mid-to-late mouse postnatal development on adult motor activity and coordination

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Manuscript : 49 pages, 6 figures and 4 tables

Declaration of interest: none

ABSTRACT

Glucocorticoids are involved in the developing brain but, in excessive amounts, may depress its growth and cause psychomotor development disorders. To test the long-term vulnerability of motor structures such as the cerebellum to supraphysiological corticosterone (CORT), the hormone was subcutaneously delivered at a dose of 20 mg/kg from postnatal day (P) 8 to P29 in C57BL/6 male mice evaluated for sensorimotor functions at P15, P22, P29, and 3 months. Relative to placebo, CORT increased motor activity in the open-field at P29 and 3 months as well as facilitating rotorod acquisition and visuomotor control necessary for swimming towards a visible goal without affecting spatial learning in the Morris water maze. CORT caused lobule-specific effects on cerebellar morphology by decreasing granule cell layer thickness in simplex lobule but increasing molecular and granule cell layer thickness in crus 2. The functional impact of these changes is indicated by significant correlations found between cerebellar size and activity levels or proficiency on the rotorod test of motor coordination.

Keywords: Corticosterone - Brain development - Cerebellum - Motor coordination - Spatial learning

1. Introduction

Glucocorticoids and stress affect brain development (Oitzl et al., 2010; Heim and Binder, 2012; Bali and Jaggi, 2015). On rat postnatal day (P) 7, dexamethasone administration depleted brain glucocorticoid receptors (Benesova and Pavlik, 1985) and cortisol on P14–P18 lowered corticosterone binding in frontal cortex, hippocampus, and hypothalamus (Ordyan et al., 2001). But opposite results have been obtained on different administration schedules and different postnatal days, as when corticosterone (CORT)-nursed rat offspring had higher (not lower as in the two previous studies) hippocampal glucocorticoid receptors at P30 (Casolini et al., 1997) and adult (Catalani et al., 2000) periods. Various neurochemical effects have been described even with a single glucocorticoid dose (Contestabile, 2000; Katyare et al., 2003; Li et al., 2014).

Glucocorticoid receptors are expressed on cerebellar fetal neurons and maintained in the mature structure (McEwen, 1987; Ozawa, 2005). CORT administered to rat dams on 17, 18, and 19 days of gestation accelerated the decrement of the external granular layer in the offspring at P12 (Velazquez and Romano, 1987), affecting as a consequence the dendritic arbor and spine density of Purkinje cells (Ruggerio-Vargas et al., 2007). A CORT injection on P7 induced cerebellar neural progenitor cell death at the external granule cell layer (Noguchi et al., 2008) in a manner mimicked by inhibiting the activity of hydroxysteroid-dehydrogenase-type 2 (HSD2), a glucocorticoid-metabolizing enzyme (Noguchi et al., 2011), on a non-sonic hedgehog pathway (Noguchi 2014;

Noguchi et al., 2015). But opposite actions on cell survival occurred when CORT injected on P1-P12 increased cholinergic and gamma-aminobutyric acid (GABA) neuron numbers in the rat diagonal band of Broca (Roskoden et al., 2005).

Many experimenters have focused on the stress-hyporesponsive period during the first ten postnatal days when blood corticosterone levels are minimal (Sapolsky and Meaney, 1986). We sought instead to examine the effects of CORT administration during mid-to-late mouse postnatal development (Brydges, 2016), corresponding to granule cell layer formation, Purkinje cell dendritic arborization, and synaptic organization with both climbing and parallel fibers from P8 to P29 (Altman, 1972), on adult cerebellar-related morphology and behavior, comprising exploratory activity in tests of spontaneous alternation (Dember and Fowler, 1958; Lalonde, 2002), open-field (Walsh and Cummins 1976), elevated plus-maze (Pellow et al., 1985; Cole and Rodgers 1993), and emergence (Paré et al., 2001), motor coordination in stationary beam, coat-hanger, and rotorod tests (Caston et al., 1995; Le Marec et al., 1997; Lalonde and Strazielle, 2007, 2015), as well as spatial learning in the Morris water maze (Morris et al., 1982; DiMattia and Kesner, 1988; Joyal et al., 1996; Noblett and Swain, 2003; Lalonde and Strazielle, 2007).

The effects of CORT on cerebellar morphology were determined on simplex, crus 1, crus 2, and paramedian lobules, the hemispheric part of the dorsal posterior lobe. The adult cerebellar motor syndrome occurs after lesions of anterior lobules I to V and of

simplex and paramedian posterior lobules (respectively, VI and VIIb), disrupting two-way pathways with neocortical and spinal regions, whereas the cerebellar cognitive and mood syndrome occurs after lesions of posterior lobules VI and VII, including crus 1 and crus 2, disrupting two-way pathways with neocortex (Timmann et al., 2008; Stoodley and Schmammann, 2010; Buckner, 2013).

2. Materials and methods

2.1 Animals

Four-week-old C57BL/6J mice were purchased from Charles River (L'Arbresle, France) and coupled to obtain pups. Mice were reared inside plastic transparent cages (32 cm x 21 cm x 17 cm for length, width and height, respectively) with woodchip bedding under a 12/12 hr light-dark cycle, a temperature of 23-25°C, a relative humidity of 27-30 %, and with food and water available at all times.

Six litters were used in each group and the mouse pups (n=3-4 males in each litter) were injected with CORT (Sigma-Aldrich, France) at a supra-physiological concentration of 20 mg/kg (CORT-treated mice; n=11), based on a medium dose range where behavioral effects were obtained (Harlé et al., 2017) or else injected with dimethylsulfoxide (DMSO) used as the vehicle (control mice; n=10) from P8 to P29, day of birth being recorded as P1. In each litter, both types of treatment were distributed equally between the males to minimize a litter effect. Female mouse pups were injected with physiological serum but excluded from the study. The mice were identified by felt

pen markings on their tail. All drugs were delivered subcutaneously once a day in a volume of 5 μ L per gram body weight of the animal between 8 and 10 AM for 21 consecutive days. At P15 and P22, the mice were evaluated blindly in the SHIRPA (SmithKline/Harwell/Imperial college/Royal hospital/Phenotype Assessment) primary neurological screen (Rogers et al., 1999), which comprises a battery of neurobehavioral tests on several reflexes, sensorimotor functions, anxiety, and motor activity that reflect the integrity and maturation of the central nervous system. At P29, the end of the injections, male mice were separated and reared inside plastic transparent cages. The animals were kept in groups (animals of same litter) but with different treatments (3-4 animals from the same litter per cage treated with CORT or placebo). At 3 months of age, the mice were assessed on tests evaluating spontaneous motor activity, anxiety, motor coordination, and spatial learning during 16 consecutive days. The body weight of each mouse was recorded once a day during the injection procedure and once a week from P29 to 3 months. This research protocol adhered to guidelines of the European Council Directive (2010/63/UE) and animal care regulations at the University of Lorraine (CELMEA-2012-0008).

2.2 Behavioral studies

The overall experimental design is illustrated in Fig. 1. [Insert Figure 1].

Behavioral tests were conducted during the afternoon (between 13:00 to 17:00) by an experienced observer blind to group designation. The order of behavioral tests was

conducted to minimize order effects by starting with the least stressful to the most stressful test. We also minimized a possible inter-test interaction by starting each new test on a different day while submitting both groups to the same procedure, glucocorticoid administration being the only variable differing between the two groups.

2.2.1 Evaluation during the postnatal developmental period (first month)

The mice were evaluated in the SHIRPA primary screen at P15 and P22 and in the actimeter at P29.

The *SHIRPA primary screen* protocol was reported in detail by Lalonde et al. (2005). The mice were first observed under a viewing jar during 5 min for motor parameters and number of fecal boli, then transferred in an arena to record transfer arousal, locomotor activity, body and tail positions, eye opening, piloerection, auditory startle, and touch escape. Lifted by the tail, the mice were then assessed for postural reflexes. After landing on the horizontal grid, the mice were evaluated for grasping the grid, grip strength when dragged by the tail across the grid, body tone, pinna and corneal reflexes, as well as toe-pinch withdrawal of a hindlimb after squeezing with a handheld forceps. The forepaws were then placed on a horizontal bar and the ability of the mice to hang suspended was evaluated. Autonomic function such as skin color, heart rate, hindlimb tone, abdominal tone, lacrimation, salivation, and provoked biting in response to a plastic probe gently put in their mouth were observed. The drop righting reflex was then evaluated according to landing position followed by contact righting when placed inside

a small plastic tube. In the final SHIRPA test, the mice were placed on a horizontal grid rotated towards a vertical position and assessed for negative geotaxis (turns and climbs, turns and freezes, moves without turning, no movement, falls off the grid). These observations were completed by noting whether any sign of fear (none, freezes during transfer arousal), irritability (none, struggling during restraint), aggression (none or either provoked or non-provoked biting), and vocalization (none or provoked while handling, as heard by the experimenters) had occurred, together with measurements of body weight and length from tip of nose to base of tail. Any other neurologic sign such as abnormal reflexes, notably limb-clasping or a bat-like posture, and seizure activity, was also noted.

The *photocell actimeter* (Leticia model LE8811, Bioseb, France) provided an evaluation of exploratory activity. It contained a floor made of black perspex 45 cm × 45 cm in size and 36-cm high transparent perspex walls. The device distinguished between fast (>10 cm/s) and slow (<10 cm/s) ambulatory, stereotyped, and rearing movements. Stereotyped movements included grooming and rearing movements included jumping. The mice were placed in the center of the apparatus and the number of movements was recorded in a 5-min session at P29.

2.2.2 Evaluation at 3 months of age

The testing schedule for adult mice, executed during the afternoon, included the following tests: spontaneous alternation in a T-maze (days 1-10), actimeter (days 1-3),

elevated plus-maze (day 4), emergence (day 5), stationary beam (day 6), coat-hanger (day 7), rotorod (day 8-10), and Morris water maze (days 11-16). No mouse was pre-exposed to any apparatus except for the actimeter at P29. We reported this battery in several articles and described the tests in detail (e.g. Lalonde et al., 2008; Jacquelin et al., 2013)

The *T-maze* made of plywood consisted of two arms (length 30 cm, width 10 cm) perpendicular to a central stem (length: 80 cm, width: 10 cm). Each part of the maze contained walls 20 cm in height. On the initial trial, the mice were placed in the stem of the T-maze with the right arm blocked by a plastic barrier (forced choice). After entering the available arm (4-paw criterion), the mice were kept in it for 60 s by closing the barrier behind them. The mice were then retrieved and, after removing the barrier, were immediately placed back in the stem for a free-choice trial. On the following 9 days, the same 2-trial procedure was repeated except that the blocked arm on the first trial was changed from right on odd days to left on even days. The number of alternations and the latencies before responding during the choice trial were measured with a cut-off period of 60 s per trial, after which time the mice were gently prodded from behind until responding.

The *elevated plus-maze* (Leticia model LE 840, Bioseb, France) consisted of four cross-shaped arms and a 10 cm × 10 cm central region. The enclosed arms and the open arms faced each other on opposite sides. Entries, as defined by a 4-paw criterion, and

time spent in enclosed and open arms were measured together with open/total arm entry and duration ratios. The mice were placed in the center, considered as enclosed time until an open arm was entered. The mice were evaluated in a single 5-min session.

In the *emergence test*, the mice were placed inside a novel small toy object (orange-colored plastic shoe, length: 13 cm, width: 6 cm, height: 7.5 cm) perforated with three holes (diameter: 3 cm) and situated in the middle of a 41 cm × 27 cm enclosure made of white plastic and surrounded by 18-cm high walls. Latencies before emerging with two or four paws in contact with the floor were determined in two 5-min trials with an intertrial interval of 60 min. Irrespective of whether emergence occurred or not, the mice were allowed to explore the large enclosure for 10 s. In the three tests of exploratory activity, the apparatus was wiped clean with a damp cloth and dried before evaluating the next mouse.

In the *stationary beam test*, the mice were placed in the center of a round wooden beam 2.5 cm in diameter, covered with adhesive tape, divided into 11 segments of 10 cm, and situated at a height of 70 cm from a mat-covered floor, to cushion any fall. The number of segment crossed (4-paw criterion) and the latencies before falling were measured in a single 4-trial session with a 1 min cut-off period and a 30-min intertrial interval.

In the *coat-hanger test*, the mice were placed upside down in the middle of a horizontal wire (diameter: 2 mm; length: 40 cm) situated at a height of 60 cm from a

mat-covered floor. The mice were released only after firmly gripping the bar with all four paws. Seven types of movement time (MT) were compiled: latencies before reaching (snout criterion) the first 10-cm segment (MT-1) or the extremity (MT-2) of the horizontal bar, latencies before reaching one of the two diagonal bars with two, three, or four paws (MT-3 to MT-5), as well as latencies before reaching (snout criterion) the midway point (MT-6) or the top (MT-7) of either diagonal bar. Latencies before falling and the number of falls were also recorded. Whenever a mouse reached the top of the diagonal bar, it was immediately retrieved and a score of 60 s given for latencies before falling. When a mouse fell, a score of 60 s was given for all MTs not attained. The mice were observed during a single 4-trial session with a 1 min cut-off period and a 20-min intertrial interval.

In the *rotorod* (Leticia Rota-Rod/RS, Bioseb, France) test, the mice were placed on top of a revolving beam moving at an initial speed of 4 rpm. The rotorod gradually accelerated from 4 to 40 rpm over a 2-min trial. Latencies before falling were measured in 8-trial sessions per day with a 15-min intertrial interval. Whenever the mice rotated passively for two complete turns, the mouse was removed and the trial counted as if it fell.

The Morris water maze consisted of a round basin (diameter: 86 cm, height of each wall: 30 cm) made of opaque white plastic and filled with water (22°C). Yellow plastic beads covered the water surface to camouflage the escape platform (diameter: 8 cm).

The platform was made of plastic and pierced with holes for the purpose of providing a firm grip. The acquisition of the spatial task consisted of placing the mice next to and facing the wall successively in north (N), east (E), south (S), and west (W) positions, with the escape platform hidden 1 cm beneath water level in the middle of the NW quadrant. When reaching the platform, the mice stayed on it for 5 s. Because mice sometimes bump against the platform and continue swimming during the early trials, the criterion adopted for reaching it throughout training was to remain on it for 5 s. The experimenter was hidden from the view of the animal, but able to follow swimming trajectories on a video-monitor, on which the pool was separated into 4 equally spaced quadrants. The number of quadrant entries, defined as an entry into any of the 4 quadrants with a 4-paw criterion, and escape latencies were measured for 4 trials per session for 5 days, with an intertrial interval of 15 min. After their swim, the mice were kept dry in a plastic holding cage filled with paper towels. Whenever the mice failed to reach the escape platform within the maximally allowed time of 60 s, it was manually placed on it for 5 s. The day after the acquisition phase, a probe trial was conducted by removing the platform and placing the mouse next to and facing the N side. The time spent in the previously correct quadrant (4-paw criterion) was measured during a single trial of 60 s. Ninety minutes later, the visible platform version of the Morris maze was performed, when the escape platform was raised to a height of 1 cm above water level and shifted to the SE quadrant, with a pole (7 cm in height) inserted on top of it to

facilitate viewing by animals swimming with their head up. As in the place learning task, quadrant entries and escape latencies were measured for 4 trials per session with a cut-off period of 60 s per trial and an intertrial interval of 15 min, except that the test was conducted on a single day.

2.3 Cerebellar morphology

2.3.1 Tissue preparation

At the end of the water maze test, the animals were rapidly anesthetized in a chamber saturated with halothane. Blood samples were collected by cardiac puncture within less than 3 min to avoid changing CORT levels; brains were rapidly removed after decapitation and snap-frozen in cooled 2-methylbutane. The blood was centrifuged at 3500 x g for 20 min at room temperature. Sera and brains were stored at – 80°C until use.

2.3.2 Histological procedure

For each brain, freshly cut frozen sections 20 µm in thickness were stained with a 0.5% cresyl violet (Sigma-Aldrich, France) solution for 30 min following the protocol proposed by Bolam (1992). Images of half the left side of the cerebellum were digitalized with a camera on a light microscope (Olympus, France). The cerebellar lobules were identified by means of the mouse brain stereotaxic atlas of Franklin and Paxinos (1997). Layer thicknesses were measured by means of Image J software (version 1.37, from the National Institutes of Health) after calibration with a

micrometric slide converting pixels into micrometers. Planimetry of the cerebellar cortex on the sections was verified by observing that Purkinje cells formed a continuous line parallel to the lobule fissure (Yamasaki et al., 2001; Zhang et al., 2006). In each lobule, measures were performed in its central segment with 6 measures per section from 2 sections per mouse. The measures integrated granular and molecular layers, the latter including the Purkinje cell layer consisting of perikarya. The simplex lobule was measured on two sections, 60 to 100 μm separated from each other (between 5.8 and 6.2 mm posterior to the bregma point). The paramedian lobule was similarly measured on two sections at a level between 6.7 and 7.0 mm posterior to bregma. For crus 1 and crus 2 lobules, largely extended, two sections between 6.2 and 6.5 mm posterior to bregma were chosen. As comparable sections among animals were used, morphometric data were obtained from 7 to 9 mice in each animal group depending on the lobule.

2.4 Serum CORT assay

CORT serum levels were evaluated by an enzyme-linked-immunosorbent assay (ELISA) kit (Arbor Assays, Euromedex, France) according to instructions provided by the manufacturer. All samples were analyzed together in duplicate. The CORT concentration, calculated from the standard curve, was expressed in ng/mL. The intra-assay and inter-assay coefficient variations were respectively below 11% and 6%.

2.5 Statistical analyses

The results obtained on the SHIRPA in CORT- or control-treated mice were compared with the chi-square test. Unpaired-t tests were used to compare the groups in the actimeter at P29 as well as adult behaviors. In spontaneous alternation testing, control and CORT were each compared to a theoretical control group performing at 50% chance by the Mann-Whitney U test. A two-way ANOVA was used for three tests containing repeated measures (actimeter, rotorod, Morris water-maze at 3 months of age). For the morphometric study, unpaired-t-tests were used for intergroup comparisons and the data presented in the form of means \pm standard errors (SEM). Linear correlations were tested in accordance with the restrictive standards proposed by Rousselet and Pernet (2012) between the altered behavioral performances and cerebellar morphometric values of those lobules that differed from normal values. For all statistics, the level of significance was set at $P < 0.05$ and analyzed with StatviewTM 4.1 software.

3. Results

3.1 Body weight

For the entire period of drug injection (P8 to P29), body weight was similar in the two groups of mice ($F(1,19)=0.1, P > 0.05$), with only the day effect being significant ($F(1,20)=22.7, P < 0.0001$) as a result of normal mouse growth. Body weight was also unchanged on the final day of testing in adults ($26.2 \text{ g} \pm 0.4$ and $26.1 \text{ g} \pm 0.4$ for CORT- and placebo-treated mice respectively).

3.2 SHIRPA

As presented in Table 1, CORT-treated mice were distinguishable from controls on only one aspect of the SHIRPA battery: locomotor activity inside the perspex jar, where they appeared more active at P22 ($\chi^2(2)=7.97$; $P < 0.05$). On the contrary, the two groups were equivalent ($P > 0.05$) in squares traversed in the arena at P15 (3.5 ± 0.7 vs 2.9 ± 0.6 respectively for CORT and controls) and P22 (5.4 ± 0.8 vs 3.5 ± 1.1 respectively for CORT and controls). [Insert Table 1]

3.3 Exploratory activity

In contrast to the negative result in the SHIRPA arena conducted for only 1 min, a hormone effect was observed on 3 of 6 actimeter measures over a 5-min period at P29 (Fig. 2), where CORT-treated mice were more active than controls in regard to slow ambulatory movements ($t(19)=2.4$, $P < 0.05$), slow stereotypies ($t(19) = 2.13$, $P < 0.05$), and fast rears ($t(19)=2.34$, $P < 0.05$). [Insert Figure 2] As seen in Fig. 3, CORT-treated mice were also more active than placebo-treated mice at 3 months of age in regard to slow ($F(1,19)=6.37$, $P < 0.05$) and fast ($F(1,19)=6.13$, $P < 0.05$) ambulation, slow ($F(1,19)=6.03$, $P < 0.05$) and fast ($F(1,19)=6.61$, $P < 0.05$) rears, and fast stereotypies ($F(1,19)=5.03$, $P < 0.05$), slow stereotypies being at borderline significance ($F(1,19)=4.34$, $P = 0.05$). [Insert Figure 3],

During the entire 10-day spontaneous alternation period, CORT-treated mice and controls alternated above chance (respectively $60\% \pm 5$, $p < 0.05$ and $74\% \pm 4$, $p <$

0.0001, Mann-Whitney). Although CORT-treated mice (111.6 ± 18.65 s) had almost half the choice latencies of control-treated mice (204.4 ± 52.4 s), the results fell short of significance ($t(19)=1.73$, $P > 0.05$), possibly due to high variations. In the elevated plus-maze (Table 2), CORT-treated mice had slightly more open arm entries than controls at borderline significance ($t(19)=2.05$, $P = 0.054$), but these entries relative to the total number of arm entries showed no intergroup difference ($P > 0.05$). Latencies before emerging from the small compartment with two or four paws were equivalent between the two groups of mice (Table 2). Only a trial effect was observed, emergence latencies being higher on the second trial than the first ($F(1,19)=15.9$, $P < 0.001$). [Insert Table 2]

3.4 Motor coordination

Motor coordination was assessed on stationary beam, coat-hanger, and rotorod tests. On the stationary beam (Table 3), none of the mice fell off the bar and distance travelled was similar for CORT-treated mice and controls ($P > 0.05$). Likewise, CORT-treated mice did not differ from controls ($P > 0.05$) on any measure of the coat-hanger (Table 3). [Insert Table 3] However, CORT-treated mice remained on the rotorod (Fig. 4) longer than control-treated mice ($F(1,19)=4.83$, $P < 0.05$). There was a borderline interaction ($F(11,209)=1.69$, $P = 0.078$) but no trial effect ($F(11,209)=1.52$, $P > 0.05$). In view of the borderline interaction, we examined each day separately. A trial effect

appeared on day 1 ($F(3,57)=7.7, P < 0.001$) but tapered off on the two subsequent days in the control group. [Insert Figure 4].

3.5 Spatial learning and visuomotor control

Only the day factor was significant for quadrants traversed ($F(4,76)=2.79, P < 0.01$) and escape latencies ($F(4,76)=10.18, P < 0.001$) during acquisition of the hidden platform version of the Morris water maze (Fig. 5). In the probe trial, CORT-treated (19.73 ± 2.46 sec) and controls (23.7 ± 2.9 sec) did not differ ($t(19)=1.05, P > 0.05$). But when compared with a theoretical value of 15 s, control mice spent more time in the target quadrant than chance ($P < 0.05$, Mann-Whitney) while CORT-treated mice did not ($P > 0.05$). In contrast, CORT-treated mice had a lower number of quadrant entries (- 25 %, $p < 0.05$) and faster escape latencies (- 41 %, $P < 0.05$) than controls in the visible platform subtask. [Insert Figure 5]

3.6 Serum CORT levels

CORT-treated mice had equivalent serum corticosterone levels relative to placebo-treated mice ($42.8 \text{ ng/mL} \pm 15.2$ and $40.7 \text{ ng/mL} \pm 9.5$, respectively $P > 0.05$).

3.7 Cerebellar morphometry

CORT-treated mice displayed smaller volumes in the simplex lobule (Fig. 6) and larger volumes in crus 2. In particular, simplex granule cell layer thickness decreased, while both molecular and granule cell layer thickness increased in crus 2 (Table 4). No morphological changes were uncovered in motor cortex or striatum (data not shown).

The morphological alterations of the cerebellum were correlated with actimeter measures. Highly significant correlations were observed between granule cell thickness of the simplex lobule and five parameters altered in the actimeter (r values between -0.74 and -0.83, $P < 0.001$) when the two groups of mice were considered together, attesting to the link between structure and motor activity. More especially in CORT-treated mice, the decrease in granule cell thickness of the simplex lobule was inversely correlated with fast ambulation movements ($r = -0.77$, $P < 0.05$); correlation factors were somewhat high for slow ambulation movements ($r = -0.74$, $P = 0.052$), fast rears ($r = -0.74$, $p = 0.052$), and slow rears ($r = -0.73$, $P = 0.06$) without reaching significance, perhaps because of the small sample size ($n = 7$). The increased volume in the molecular layer of crus 2 was also positively correlated with slow rears ($r = +0.86$, $P < 0.01$), both results indicating that mice with higher morphological changes were more active. Besides, the increasing volume in the molecular layer of crus 2 was correlated with higher fall latencies of the rotorod when considering the two groups of mice together ($r = +0.59$, $p < 0.05$), but more particularly in CORT-treated mice ($r = +0.82$, $P < 0.05$).
[Insert Table 4 and Figure 6]

4. Discussion

CORT-infused mice from P8 to P29 acquired the rotorod task more swiftly as adults than placebo-treated mice. Possible mediation of this effect by the cerebellum is indicated by the positive correlation found between latencies before falling off the rod

and increased molecular layer thickness of crus 2, possibly caused by glucocorticoid-mediated alterations in growth factors (Mocchetti et al., 1996). In contrast, rotorod acquisition slowed down in adult mice injected with CORT on P2-P14 (Howard and Granoff, 1968), adult rats injected with dexamethasone on P7 (Benesova and Pavlik, 1989), or mice injected and tested with CORT as adults (Harlé et al., 2017). CORT given on P2-P14 also slowed down the cerebellar-mediated conditioned eye-blink reflex in adult rats (Wilber et al., 2011), but when given on P15-P17 facilitated at P28 trace eye-blink conditioning, a hippocampal-mediated task (Wentworth-Eidsaune et al., 2016), though the latter result depended on type of hormone delivery (Claflin et al., 2005, 2014). On the contrary, dexamethasone injected on P7, P9, and P11 had no effect in mice performing the rotorod at P28 or P29 (Maloney et al., 2011). Rotorod acquisition was likewise undisturbed at P27-P29 in rats injected with dexamethasone on P7 (Ferguson et al., 2001) or adult rats injected with dexamethasone on P4 (Vicedomini et al., 1986). In summary, rotorod acquisition appears especially vulnerable after early onset and prolonged administration of glucocorticoids, as in the Howard and Granoff (1968) study when cerebellar volume diminished in size (Howard, 1968), illustrating a defect on Purkinje cell maturation, a major one during the first postnatal week for proximal dendrite maturation (Lee et al., 2007).

Positive associations have been obtained between cerebellar size or weight and other types of behavioral performances. In particular, heavier cerebellar weight was correlated

with better active avoidance learning in normal rats (Villescas et al., 1979) and larger cerebellar size was correlated with better sensorimotor performance in normal human subjects (Dimitrova et al., 2008) or patients with spinocerebellar atrophy (Hernandez-Castillo et al., 2016, 2017; Richter et al., 2005; Selvadurai et al., 2016). In an opposite manner, crus 1 volume was negatively associated with balance times in a mixed population of young and old human subjects (Bernard and Seidler, 2013). Another factor to consider is that sensorimotor training itself may lead to changes in cerebellar morphology (Ben-Soussan et al., 2015; Kleim et al., 1998, 2007).

In contrast to the rotorod, we found no effect on stationary beam and coat-hanger tests or on any SHIRPA test of postural control. Nevertheless, glucocorticoids administered during the first postnatal week are liable to cause deficits in postural control. Dexamethasone injected on P7, P9, and P11 diminished wheel-running skills in mice tested at P75 or P88 and reduced their latencies before falling from an inverted screen at P25, though not at P26 (Maloney et al., 2011). In addition, latencies before turning up on an inclined surface increased at P8 in rats injected with dexamethasone on P7 (Ferguson et al., 2001) and rats injected with dexamethasone on P3-P6 had lower scores at P7 for forelimb placing and postural extension reflexes and at P14 for postural and vibrissa reflexes (Flagel et al., 2002). Pellet-reaching was less precise after CORT in rats injected and tested as adults (Metz et al., 2005) or after combined administration of dexamethasone and aldosterone (Jadavji et al., 2011).

The body weight of our CORT-treated mice was equivalent to that of controls. Likewise, body weight was unchanged in rats at P11-P90 (Casolini et al., 1997; Catalani et al., 2002) and adult mouse offspring (De Filippis et al., 2013) of CORT-administered lactating dams. However, body weight may decrease when glucocorticoids are given prior to the second postnatal week. Rat pups injected with dexamethasone on P3-P6 weighed less than controls at P4-P8, P14, and P20 (Flagel et al., 2002) and those injected on either P4 (DeKosky et al., 1982) or P7 (Benesova and Pavlik, 1989; Ferguson and Holson, 1999; Ferguson et al., 2001) weighed less as adults. Other neonatal treatments affect only neonatal measures, as when rat pups injected with dexamethasone on P3 weighed less at P7-P28 but not P112 (Ferguson and Holson, 1999) and mouse pups injected with dexamethasone on P7, P9, and P11 weighed less at P9-P28 but not P43 (Maloney et al., 2011). CORT given to mouse dams in drinking water on gestational days 14-21 reduced offspring weight at P25-37 but not P112-P170 (Ceci et al., 2014).

Motor activity increased in our CORT-injected mice exploring the viewing jar at P22 and the open-field at P29 and 3 months. Likewise, open-field horizontal and vertical activities augmented in adult mouse offspring of dams receiving CORT in drinking water from the beginning of the third week of pregnancy until weaning (Pechnick et al., 2006), as did wheel-running activity at P14 and P16-P26 in CORT-injected mice on P2-P14 (Howard and Granoff, 1968). Moreover, a single dexamethasone dose on P7

increased rat open-field activity and rears at P18-P21 (Ferguson and Holson, 1999) and 8 months (Benesova and Pavlik, 1989). Yet the same dexamethasone injection on P7 diminished rat open-field activity at P48-P51 without affecting such behavior at P105-P107 (Ferguson et al., 2001). Moreover, open-field ambulation and rears declined in adult rats after dexamethasone injections on P1, P3, and P5 (Felszeghy et al., 1993), as did open-field activity at 2 and 4 months of age in CORT-injected mice on P2-P14 (Howard and Granoff, 1968). However, CORT given to mouse dams in drinking water on gestational days 14-21 reduced home cage but not open-field activity (Ceci et al., 2014) and dexamethasone injected on P7, P9, and P11 had no effect on open-field activity in mice at P21 (Maloney et al., 2011), nor did open-field or home cage activity change in adult mouse offspring of dams given CORT in drinking water on P1-P7 (De Filippis et al., 2013) or open-field activity in rats at P21 after dexamethasone injections on P3-P6 (Flagel et al., 2002). Thus, motor activity effects differ depending on time of administration and evaluation. Our findings of hyperactivity are in line with those of Pechnick et al. (2006), Howard and Granoff (1968), Ferguson and Holson (1999), and Benesova and Pavlik (1989), but either no effect or hypoactivity is found when glucocorticoids are administered prior to the second postnatal week.

Our finding of elevated ambulatory mobility was correlated with a thinner granule cell layer of the simplex lobule. But higher slow-paced vertical mobility was correlated with a thicker molecular layer of crus 2, illustrating a bidirectional structuro-functional

link. In support of a cause-and effect relation is the finding of hyperactivity in several mouse mutants with early-onset cerebellar atrophy and ataxia (Lalonde and Strazielle, 2007) as well as in rats with cerebellar cortical depletion caused by repeated X-ray exposures on P8-P15 or P12-P15 (Pellegrino and Altman, 1979). Known regulators of dendritic spine development and plasticity (Liston and Gan, 2011), glucocorticoids may play a direct role in the architectonic organization of the cerebellar cortex or an indirect one via changes in calcium currents, extracellular glutamate, and NMDA receptors (Jacobs et al., 2006; Li et al., 2014; Mori and Mishina, 2003).

Unlike the open-field, no hormone effect was discerned on our other exploration tests such as spontaneous alternation, though this assay derives from brain development occurring during the course of the second postnatal week (Egger et al., 1973). Likewise, adult spontaneous alternation in rats was intact when CORT was administered in drinking water on P1-P10 (Macri et al., 2018). In a similar manner, emergence latencies were unchanged in our study and at P57 in rats injected with dexamethasone on P7 (Ferguson et al., 2001) or at P28 when injected on P3-P6 (Flagel et al., 2002). Moreover, no change occurred on the elevated plus-maze in our study or on the elevated zero-maze in adult mouse offspring of dams given CORT in drinking water on P1-P7 (De Filippis et al., 2013). In contrast, open arm duration of the elevated plus-maze augmented in adult rat offspring of dams supplemented with CORT in drinking water from day 1 postpartum to weaning (Catalani et al., 2000, 2002). But when CORT was

given to mouse dams in drinking water only on gestational days 14-21, open sector duration of the elevated zero-maze diminished in the offspring at P80 (Ceci et al., 2014). Thus, a change in anxiety levels in elevated plus- or zero-mazes has so far been detected only with glucocorticoid administration started prior to the second postnatal week. In different tests, rat offspring of dams given CORT in drinking water on P2–P14 or pups given the hormone via osmotic micropumps on P7-P14 increased adult conditioned freezing and extinction (Callaghan and Richardson 2014).

In a similar manner to rotorod facilitation, CORT administration facilitated visuomotor control in the visible platform subtask in the Morris water maze without affecting acquisition of the hidden platform subtask. This facilitation occurred in the form of lower escape latencies and quadrant entries, indicating that the mice reached the platform sooner by swimming a more direct course. Likewise, CORT infused in drinking water to rat dams on P5-P9 or P13-P17 improved the visible platform subtask of the pups at P21 (McCormick et al., 2001). Also as in our study, CORT administered in drinking water to lactating rat dams had no effect on the hidden platform subtask of 16-day old offspring (Casolini et al., 1997) and even shortened latencies before reaching the hidden platform in 1, 2, and 3-month-old offspring (Catalani et al., 1993, 2002), though no effect occurred on the visible platform subtask conducted at 3 months (Catalani et al., 1993). But when glucocorticoids were administered during the first postnatal week, water maze performances worsened (DeKosky et al., 1982; Ferguson et

al., 2001; Howard and Granoff, 1968; Vicedomini et al., 1986), such results underlining again that Purkinje cell maturation is particularly vulnerable during the first postnatal week while it is crucial for the following development of the cerebellar cortex. We only found a slight deficit in the probe trial. Despite the lack of an intergroup difference, CORT-injected mice had chance levels of performance in contrast to placebo-injected mice performing above chance, an indication of a spatial memory deficit worth exploring in future studies. Probe trial performance diminished in juvenile mice treated with CORT combined with restraint stress, but not CORT alone (Ngoupaye et al., 2018).

The present findings clearly demonstrate the long-term impact of CORT administration during the postnatal period. Several studies in animals and humans recently highlighted the role of the cerebellum on psychomotor or psychiatric disorders (Frye and Llaneza, 2010; Peng et al., 2011; Stoodley et al., 2017). An ongoing neurochemical study should permit linking the behavior phenotype to brain modifications.

Acknowledgements: This research received intra-mural funding from the University of Lorraine.

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Table 1 Number of control- (n=10) and CORT-treated mice (n=11) displaying specific behaviors of the SHIRPA primary screen measured in the viewing jar and the open-field at P15 and P22.

TESTS		P15		P22	
		Control	CORT	Control	CORT
		%	%	%	%
Jar (5 min)					
Body position	Sitting and standing	60	46	90	82
	Rearing	40	54	10	18
Activity	Slow mvt	20	37	50	0*
	Moderate mvt	80	54	50	82*
	Vigorous darting mvt	0	9	0	18*
Tremor	None	90	91	90	73
	Mild	10	9	10	27
Open-field (30 s)					
Arousal transfer	Prolonged freeze, slight	20	0	10	9
	mvt	0	0	20	0
	Extended freeze,	10	0	20	17

	moderate mvt	60	73	50	55
Startle	Brief freeze, active mvt	10	27	0	19
	Momentary freeze, swift mvt	30	27	30	0
	No freeze, immediate	0	0	30	45
	mvt	70	73	40	55
Touch escape	None	0	0	50	37
	Preyer reflex	0	8	0	18
	Jump less than 1 cm	0	0	0	9
	No response				
	Mild				
	Moderate				
	Vigorous				
Body	Weight (g)	6.7	6.5	7.6	7.7
measurements	Length (cm)	5.2	5.1	5.6	5.7

* $P < 0.05$ vs. controls (χ^2 test); mvt = movement

Table 2. Mean (\pm SEM) number of arm entries and duration by control- and CORT-treated mice at 3 months of age in the elevated plus-maze and latencies before emerging from a small toy object with two or four paws for 2 trials

	Control	CORT
Elevated plus-maze		
Open arm entries	2.2 \pm 0.6	4.1 \pm 0.7
Enclosed arm entries	4.6 \pm 0.6	5.8 \pm 0.8
Open/total arm entries	0.36 \pm 0.08	0.45 \pm 0.06
Open arm duration (s)	68.6 \pm 28.0	97.8 \pm 23.5
Enclosed arm duration (s)	221.4 \pm 27.7	202.2 \pm 23.5
Open /total arm duration (s)	0.23 \pm 0.09	0.33 \pm 0.08
Emergence		
Trial 1-2 paws (s)	92.7 \pm 31.6	62.1 \pm 18.8
Trial 1-4 paws (s)	137.5 \pm 41.7	74.7 \pm 20.1
Trial 2-2 paws (s)	172.6 \pm 40.6	137.3 \pm 30.1
Trial 2-4 paws (s)	174.0 \pm 40.3	158.8 \pm 33.6

Table 3. Motor coordination (means \pm SEM) of control- and CORT-treated mice at 3 months of age in stationary beam and coat-hanger tests

	Control	CORT
Stationary beam		
Segments	84.9 \pm 6.7	75.8 \pm 8.9
Fall latencies (s)	60 \pm 0	60 \pm 0
Fall number	0 \pm 0	0 \pm 0
Coat-hanger		
MT-1 (s)	55.5 \pm 9.3	43.9 \pm 11.3
MT-2 (s)	94.2 \pm 11.7	82.1 \pm 13.6
MT-3 2 paws (s)	158.8 \pm 13.1	124.8 \pm 12.3
MT-4 3 paws (s)	204.6 \pm 8.5	191.2 \pm 12.9
MT-5 4 paws (s)	220.0 \pm 4.6	207.3 \pm 9.4
MT-6 Midway (s)	234.7 \pm 2.7	227.6 \pm 6.2
MT-7 Top (s)	236.1 \pm 3.6	234.6 \pm 2.9
Fall latencies (s)	212.2 \pm 13.9	210.3 \pm 11.0
Fall number	0.7 \pm 0.3	0.9 \pm 0.2

Table 4. Thickness variations for granular and molecular layers of dorsal posterior lobules of the cerebellar hemispheric cortex between control- and CORT-treated mice at 3 months of age. Purkinje cell layer is included in the molecular layer measure

CEREBELLUM				
	Simplex	Crus 1	Crus 2	Paramedian
Molecular	- 8%	- 10%	+ 13% *	- 6%
Granular	- 21% **	- 10%	+ 12% **	- 4%

* $P < 0.05$; ** $P < 0.01$ – unpaired *t*-test

Figure legends

Fig.1 Protocol design for the CORT injections during the postnatal period and behavioral study with the different tests. (Day of birth corresponding to P1).

Fig. 2 Exploratory activity in the actimeter (means \pm SEM) for 5 min at P29 by control- and CORT-treated mice.

FM = fast ambulation movements; FR = fast rears; FS : fast stereotypies; SM = slow ambulation movements; SR = slow rears; SS = slow stereotypies (* $p < 0.05$ – unpaired t-test).

Fig. 3 Exploratory activity in the actimeter (means \pm SEM) for 5 min in adults (3 months) by control- and CORT-treated mice.

FM = fast ambulation movements; FR = fast rears; FS = fast stereotypies; SM = slow ambulation movements; SR = slow rears; SS = slow stereotypies (* $p < 0.05$ – unpaired t-test).

Fig. 4 Latencies before falling (means \pm SEM) from the rotorod by control- and CORT-treated mice at 3 months of age (8 trials/day with 2-trial combined latencies for each score).

Fig. 5 Total quadrant entries and escape latencies (s) over 4 trials (means \pm SEM) during acquisition of the hidden platform version of the Morris water maze and during the visible platform version by control- and CORT-treated mice at 3 months of age (* $p < 0.05$ – unpaired t-test).

Fig. 6 Nissl-stained sections of cerebellar cortical simplex lobule of control- (a) and CORT-treated (b) mice at 3 months of age (scale bar = 160 μm). We observe a thinner granular layer (white asterisk) in regard to the primary fissure in the CORT-treated mouse. The thickness is also lower in the molecular layer but without reaching the statistical significance.

Gr = granular layer; Mol = molecular layer; Pc = Purkinje cell layer; prf = primary fissure; Sim = Simplex lobule.