

Familiarization effects on the behavioral disinhibition of the cerebellar Lurcher mutant mice: use of the innovative Dual Maze

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- 1 Familiarization effects on the behavioral disinhibition of the cerebellar Lurcher mutant
- 2 mice: use of the innovative Dual Maze.
- 3
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1 ABSTRACT

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3 Anxiety-related behaviors in mice are often assessed over short periods starting 4 immediately after introducing the animals in a dedicated apparatus. In these usual 5 conditions (5 to 10 minutes periods), the cerebellar Lurcher mutants showed 6 disinhibited behaviors characterized by abnormally high exploration of the aversive 7 areas in the elevated plus-maze test. We nevertheless observed that this disinhibition 8 sharply weakened after 10 minutes. We therefore decided to further investigate the 9 influence of the disinhibition on the intrinsic and anxiety-related exploratory behaviors 10 in Lurcher mice, with a special focus on familiarization effects. To this end, we used 11 an innovative apparatus, the Dual Maze, permitting to tune the familiarization level of 12 animals to the experimental context before they are faced with more (open 13 configuration of the device) or less (closed configuration of the device) aversive 14 areas. Chlordiazepoxide administration in BALB/c mice in a preliminary experiment confirmed both the face and the predictive validity of our device as anxiety test and 15 16 its ability to measure exploratory motivation. The results obtained with the Lurcher 17 mice in the open configuration revealed that 20 minutes of familiarization to the 18 experimental context abolished the behavioral abnormalities they exhibited when not 19 familiarized with it. In addition, their exploratory motivation, as measured in the 20 closed configuration, was comparable to that of their non-mutant littermates, 21 whatever the level of familiarization applied. Exemplifying the interest of this 22 innovative device, the results we obtained in the Lurcher mutants permitted to 23 differentiate between the roles played by the cerebellum in exploratory motivation 24 and stress-related behaviors.

25

1 1. **INTRODUCTION**

2 The experimental procedures used in behavioral neuroscience always 3 challenge a wide range of brain systems and functions including attention, motivation 4 and emotions in addition to those they are primarily to be examined. These factors 5 contribute to inconsistency and irreproducibility of results. Therefore, it is of 6 importance to consider the general way the animals cope with the experimental 7 environment as a whole before interpreting the specific behavioral aspect under 8 investigation (as suggested, for instance, in [1]). Stress is at the forefront of the 9 factors impeding the tests specificity [2]. Unfortunately, in spite of refined methods 10 aiming at reducing suffering in laboratory animals, virtually all the experimental 11 manipulations remain more or less stressful. The resulting stress-induced behaviors 12 and hormone rise impact animal's performances in behavioral tests, regardless of the 13 nature of the variable of interest: cognitive, emotional and even motor [3-7]. Such 14 biases are particularly confounding when investigating emotional reaction with preset 15 controlled anxiogenic factors (i.e. more or less open space, level of illumination or 16 height) in standardized conditions. It obviously becomes of critical importance when 17 testing animals having abnormal stress reactivity.

18 Novelty is a crucial component driving the rodents' behaviors in the classical 19 exploration-based anxiety tests [8, 9]. Nevertheless, experimental procedure 20 (manipulation) as well as novelty are also stressors [10], the effects of which depend 21 on both the intrinsic features of the novel environment and the individual's sensitivity. 22 The latter is largely determined by genetic factors and numerous mutant mice display 23 specifically impaired reactivity to novelty [11-13], notably those modeling 24 neuropsychiatric diseases such as autism spectrum disorders [14, 15]. In addition, 25 reaction to novelty is a dynamic process influenced by habituation and familiarization 26 mechanisms that can considerably change the coping strategies of animals over 27 time. For instance, behaviors adopted by mice forcibly exposed to the elevated plus-28 maze first resulted from escape tendency, with long time spent in the aversive open 29 arms. The approach-avoidance conflict, more in line with anxiety theory and 30 characterized by a clear preference for the closed arm, progressively appeared only 31 in a second phase [16].

32 The cerebellum plays a role in attention to novel stimuli [17]. As a 33 consequence, cerebellar mutant mice could improperly manage novelty in the course

1 of tests evoking exploratory behaviors. Nevertheless, it would be hazardous to 2 attribute a unique cause to behavioral abnormalities observed in cerebellar mice in a 3 given test because of the various and deeply intertwined functions carried out by the 4 cerebellum [18, 19]. Although the precise mechanisms involved are still elusive, all 5 these functions, which range from motor coordination to cognitive processing and 6 from control of emotions to vegetative regulations, are sustained by complex, 7 bidirectional projections between the cerebellum and diverse brain structures [20-22]. 8 So, the behavioral abnormalities of cerebellar mutants in any experimental situation 9 very likely result from dysfunctional interaction between several of these cerebello-10 cerebral circuits. Considering such dysfunctions, the non-specific interfering effect of 11 stress could have a particular resonance in cerebellar mutants and even aggravate 12 their multiple deficits.

Lurcher mutant mice [23] are ones of the most commonly used cerebellar mutants. Almost all of their Purkinje cells are extinct by the age of 3 months due to a mutation in the delta2 glutamate receptor-encoding gene [24, 25]. The loss of Purkinje cells is accompanied by massive secondary degeneration of cerebellar cortex interneurons and inferior olive [24, 26] and by mild reduction of the cerebellar nuclei [27]. These histological defects result in marked cerebellar ataxia and cognitive impairments [17, 19, 28-32].

20 It was also extensively demonstrated that Lurcher mice exhibited profound 21 behavioral and physiological emotional-related disturbances [32-37]. For instance, 22 they did not avoid the aversive open arms of the elevated plus-maze test as much as 23 their littermate controls did [34]. Although such a behavior is classically interpreted as 24 attenuated anxiety, the paradoxical dramatic corticosterone rise observed in these 25 mutants in response to any manipulation (including the exposure to the elevated 26 plus-maze) led us to hypothesize that their functional cerebellar decortication actually 27 resulted in pathological behavioral disinhibition [34, 35, 38]. Further experiments 28 conducted in our laboratories revealed that the significant differences between the 29 behaviors of Lurcher and control mice noticed in the first 10 minutes of a 15-minutes 30 session in the elevated plus-maze test were no longer observed in the last 5 minutes 31 (see supplementary material). This clearly indicates that the conclusions of studies 32 comparing the Lurcher mutants and control mice behaviors strongly depend on the 33 duration of exposure to experimental conditions and the timing of data collection.

Moreover, it reinforces the idea that the Lurcher behavioral profile is shaped by the dynamic of novelty-induced stress provoked by forcible exposure to an unknown context. In line with these observations, we hypothesized that adding a resting period in the experimental environment before starting the test itself could lead to the normalization of the anxiety-related behaviors in Lurcher mice by neutralizing the effects of handling- and novelty-induced stress.

7 The first aim of this study was to test this hypothesis by comparing the effects 8 of prior familiarization to the general experimental context on the behavioral reaction 9 of heterozygous Lurcher cerebellar mutants and their healthy littermates facing an 10 aversive and suddenly unveiled area they were kept to freely explore. We also 11 sought to investigate their behaviors in the same conditions but facing a much less 12 aversive environment in order to evaluate hypothetical genotype-dependent 13 differences in pure exploratory motivation, which could affect the anxiety variables. 14 Taking into consideration such a possible bias seemed to us particularly important 15 since Lurcher mice were previously described to suffer from exploratory impairments 16 in the hole board test [39]. Several well-documented tests based on exploratory 17 activity, such as the classical open field or elevated plus-maze or the more recent 18 zero maze, are available to investigate anxiety-related behaviors in mice. Nevertheless, none of these apparatuses allows to properly carry out prolonged 19 20 familiarization of mice to experimental conditions or to separately evaluate the effects 21 of confrontation to aversive and less aversive places on their behaviors. We thus 22 designed an innovative apparatus coming in highly or lowly aversive versions: the 23 Dual-Maze. This new device permits 1/ to familiarize animals, for a controlled 24 duration, to experimental context before being tested and 2/ to investigate behavioral reaction of animals confronted to more or less aversive environment. Before 25 26 submitting Lurcher mice and control littermates to this new apparatus, we first tested 27 its face validity (the mice would avoid open space) and predictive validity (well known 28 anxiolytic agent would reduce this avoidance) by measuring the chlordiazepoxide 29 effects on behaviors of BALB/c mice exposed to it.

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31 2. Experimental procedure

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2.1. Experimental design

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1 The study was split into two distinct experiments: the first one consisted in 2 validating the Dual maze as a new apparatus for anxiety and exploratory behavior 3 assessment in mice. In this first experiment, we measured the effects of 4 chlordiazepoxide (CDP), anxiolytic benzodiazepine-type an substance. in 5 pathologically anxious BALB/c mice [40]. The second experiment evaluated the 6 impact of familiarization on anxiety- and spontaneous exploration-related behaviors in 7 the Lurcher mutant mice.

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2.1.1. Apparatus: The Dual Maze test (Figure 1)

10 Two black-painted IffaCredo® individual cages 20 x 7 x 8 (length x width x 11 height in cm) were assembled to construct the apparatus. It consisted of a starting 12 closed arm facing a testing arm. The latter was either open (open configuration) or 13 closed (closed configuration: in this case, the testing arm was identical to the starting 14 arm). A guillotine door separated the starting and testing arms. Each of the arms was 15 virtually divided into two segments of the same surface. The segments adjacent to 16 the limit between the two arms of the device were called "proximal" segments, the 17 other ones "distal segments". All the closed arms (e.g. the starting arm in both 18 configurations and the testing arm in the closed configuration) were covered with 19 plastic plates with ventilation holes. These plates were aimed at preventing the 20 escape of mice out of the arms. The whole apparatus was screwed to a pole and was 21 elevated to a height of 50 cm above the floor level.

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2.1.1.1. <u>Closed configuration (named CC)</u>

In this configuration, the Dual maze is a parallelepiped box opened on its upper face. This box is made of two contiguous individual Iffa Credo cages. Their joined small sides were cut to allow the mice to stroll from one to another. Two transverse partitions 1 cm long, located on both sides of the device, mark the limit between these two cages.

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2.1.1.2. <u>Open configuration (named OC)</u>

In this configuration, the testing arm is opened and has the same surface as in
 the closed configuration. We added 3 mm high edges to this open arm to prevent the

fall of animals, especially that of ataxic Lurcher mice. In open configuration, the mice
are therefore confronted, from the familiar starting arm where they were deposited a
few seconds or minutes before, with an aversive open environment.

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2.1.2. Animals

6 Adult male BALB/c mice, aged 3 months +/- 1 week, were used in the 7 experiment investigating the sensitivity of the Dual Maze to the anxiolytic effects of 8 CDP. We chose mice of this strain because they were described as a model of 9 pathological anxiety [40]. This first experiment was aimed at verifying the face validity 10 of the test (that is the occurrence of anxiety-related behaviors in it) and its predictive 11 validity (as indicated by the sensitivity of the variables recorded to the injection of an 12 anxiolytic agent). In the second experiment, adult male and female (approximately 13 1:1 ratio) heterozygous Lurcher mutant (+/Lc) and littermate wild type (+/+) mice from 14 B6CBA strain, aged 3 months +/- 10 days, were submitted either to the open or to the closed configuration of the Dual Maze, after or not a 20 min familiarization period. 15 16 The detail of the number of mice per group (n) is given in figure 2.

17 All mice were obtained and bred in the lab under standard temperature- and 18 humidity-controlled conditions, with water and commercial pellet food available *ad* 19 *libitum* and 12:12 hours light:dark cycle. They were tested during the light period.

All the experimental procedures were performed in compliance with EU guidelines about protection of animals used for scientific purposes and with the permission of the Ethical Commission. All efforts were made to minimize the number of animals used and their suffering.

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2.1.3. **Drugs**

CDP hydrochloride was obtained from Sigma–Aldrich. It was dissolved in 0.9% physiological saline, which, alone, served as vehicle control. CDP hydrochloride (Sigma–Aldrich) dissolved in 0.9% saline was injected intraperitoneally in a dose of 5 mg/kg 20 min prior scoring the mouse behaviors. Control mice were treated by injection of adequate volume (10 ml/kg) of saline (see Procedure – Experiment 1 for details). 1

2

2.2. Principle of the Dual Maze test

3 The test is based on the resolution of approach/avoid conflict which balance 4 the innate motivation of rodents to explore their environment with their aversion for 5 hazardous places. In line with what was repeatedly demonstrated in other devices [5, 6 41-43] we expected that our open testing arm was much more aversive for mice than 7 closed one. In accordance with the classical meaning of this kind of variables in the 8 literature, we therefore considered the number of entries and the time spent in the 9 open testing arm as indices of anxiety. We also measured the number of protected 10 stretched attend postures (pSAP: the body of the mouse is stretched from the starting 11 arm toward the testing arm then retracted to the original position) and the number of 12 protected head dips (pHD: downward visual screening over the edge of the open 13 testing arm achieved from the testing arm) as additional markers indicating an 14 elevated level of anxiety when they are high [44-46]. While rare pSAPs could be 15 observed in the closed configuration of our device, we proposed the latter, 16 considering its physical features not much aversive, was more suitable for isolating 17 the motivational component of the mice exploratory activity. In both configurations, 18 the total number of segments crossed was interpreted as an index of general activity.

19 Because both tests are based on the same principle, the variables measured 20 in the Dual Maze test were similar to those usually measured in the plus-maze test. 21 Nevertheless, the former allows to overcome some problems of interpretation 22 encountered in the latter, especially those related to the center of the device [8, 47]. 23 Incidentally, the zero maze was partly designed to answer this issue [41]. But the 24 Dual Maze is even simpler than this alternative to the plus-maze and, as an important 25 consequence for our purpose, it strongly limits the influence of Lurcher mouse motor 26 disorders on their exploratory behaviors. So, the conformation of the device ensures 27 that an entry into an aversive zone can hardly be accidental as it can happen in mice 28 with motor disabilities in more complex devices. Indeed, ataxic mice have problems 29 with straight trajectory maintenance and can enter initial part of an open arm of the 30 elevated plus maze accidentally due to lateropulsions when just transiting from one 31 closed arm to the opposite one. They also show typical backwards movements 32 particularly confusing in the zero-maze, in which the closed arm is surrounded by two 33 open arms.

1 The size of the starting arm, identical to that of an individual transport cage, 2 seemed to us ideal to carry out the familiarization of the animals. Lastly, the strict 3 segregation of the highly and lowly aversive testing arms in two separated 4 configurations facilitates the discrimination between pure exploratory motivation and 5 anxiety-related behaviors.

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2.3. Procedure

8 Common Protocol

9 First, all animals were habituated for 30 minutes to the experimental room. At 10 the beginning of the experiment, each mouse was individually placed in the distal 11 segment of the starting arm. Whatever the level of prior familiarization (0 or 20 min.), 12 the following parameters were recorded for 5 min after removing the guillotine door: 13 the number of entries (four paws criterion) and the time spent in the different 14 segments of the apparatus and the number of pSAPs and pHD performed from the 15 starting arm. The number of entries and the time spent in the testing arm were 16 calculated from the data obtained for each segment. For more clarity, all the temporal 17 variables are expressed as relative values that is as a percentage of the total 18 experiment duration. Experimental sessions were recorded by a miniaturized camera placed 1.5 m above the device and connected to a video recorder located in a room 19 20 next to that where the mice were tested. The experimenter (always the same) could 21 follow the experiment proceedings from this adjacent room by watching the screen 22 connected to the video recorder. All the behaviors of the mice were quantified later 23 from the camera footage using the ethological analysis software Etholog [48].

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2.3.1. Experiment 1 - The effects of CDP on BALB/c behaviors

The mice were allocated to two different cohorts. The first one was submitted to the closed configuration of the DualMaze and the second one to its open configuration. Each mouse was intraperitoneally injected with saline or CDP (5mg/kg) and was immediately placed in the starting arm of the device. Twenty minutes later, the guillotine door was removed, and the mouse could freely access to the testing arm. The dose of CDP chosen was determined using data from the literature [43, 44]. Moreover, in preliminary experiments, we observed that CDP was highly sedative at a dose of 7.5 mg / kg (data not shown). Animals were randomly tested in a simple
blind procedure so that the experimenter did not know the compound administered to
the mice.

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2.3.2. Experiment 2 - Examination of Lurcher mice behaviors

All the animals were tested randomly for the genotype and the configuration.
Due to the obvious ataxic gate of the Lurcher mice, the experimenter could not be
unaware of the genotype tested.

9

2.3.2.1. Without familiarization

10 In this procedure, animals were only habituated for 30 minutes to the 11 experimental room. Then, they were individually placed in the closed starting arm of 12 the device, either in its closed or open configuration, and the guillotine door was 13 immediately removed.

14

2.3.2.2. With familiarization

15 This procedure was similar to the previous one except that the mice were 16 familiarized to the starting arm of the device for 20 minutes before the removing of 17 the guillotine door.

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2.4. Statistical analysis

19 The normality of residuals was assessed using normal probability plots. 20 Variances homogeneity was verified by comparing the externally studentized 21 residuals of groups to each other. For experiment 1, these assumptions were met 22 and the variables of interest were analyzed with a two-way ANOVA (treatment x 23 configuration) followed, where appropriate, by post-hoc t-test corrected for multiple 24 comparison with the Benjamini-Hochberg method. As most of the data of experiment 25 2 did not meet the parametric assumptions, we submitted them to a non-parametric 26 analysis. So, we first performed a Kruskal-Wallis test in order to detect any significant 27 heterogeneity in our results for each variable. When appropriate, multiple 28 comparisons were achieved with Fisher-Pitman permutation tests corrected with the 29 Benjamini-Hochberg method. The avoidance or preference for the testing arm was 30 assessed by comparing the relative time spent in it to the chance level with one-31 sample t- (experiment 1) or Mann-Whitney (experiment 2) test. In all cases, the sex

factor was blocked (after checking it did not interact with any other factor). P<0.05
was used as significance threshold in all analyses.

3

4 3. RESULTS

5 6

3.1. Experiment 1 - Effects of CDP injection on BALB/c mice behavior (table 1, figure 3)

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8 The statistical analysis presented in table 1 revealed that the mice behaviors 9 were dependent on the configuration they were submitted to. So, the configuration 10 factor exerted a significant effect upon the total number of segments crossed (fig.3A), 11 the number of entries in the testing arm (fig. 3B) and in its distal segment (fig. 3 C) 12 and the number of rears (fig. 3F). All these parameters were clearly decreased in the 13 open configuration when compared with what observed in the closed one, 14 demonstrating that both configurations had a very different impact on the exploratory 15 activity of animals. Interestingly, significant configuration x treatment interactions 16 were detected concerning the relative time spent in the testing arm (fig. 3D) and in its 17 distal segment (fig. 3E) (this segment being supposed to be the most aversive part of 18 the device in its open configuration).

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20 Post hoc comparisons revealed that NaCl-injected mice spent more time in the 21 testing arm (fig. 3D) (p<0.001) and in its distal segment (p<0.01) (fig. 3E) when CC rather OC was used, confirming that the closed testing arm is less aversive than the 22 23 open one. In accordance with this analysis, one-sample t-test showed the mice 24 tested in OC had a preference for the starting arm (t= -3.0112, p<0.05, compared to 25 chance level). In CC, the preference for the testing arm (fig. 3D) probably reflected 26 the strong motivation of mice to explore new areas after prolonged familiarization to 27 the starting arm (t= 5.1468, p<0.05, compare to chance level). In accordance with its 28 anxiolytic effects, CDP significantly increased the time spent in the distal segment of 29 the testing arm (fig. 3E) in OC (p<0.01) while it did not significantly change it in CC 30 (p>0.05). By the way, the responsivity of this parameter to pharmacological anxiolysis 31 was such that CDP suppressed the significant difference previously noticed between 32 both configurations in NaCl-mice (p>0.05). Although the time spent in the open 1 testing arm as a whole (fig. 3D) did not sufficiently respond to the anxiolytic drug to 2 lead to a significant difference between NaCl and CDP-mice in OC (p>0.05), the 3 dramatic reduction of the number of pSAPs (fig.3G) by the treatment in this 4 configuration (p<0.05) and, at a lesser extent, that of the number of pHDs (p>0.05) 5 (fig. 3H), were another evidence of the potency of the anxiolysis induced by the 6 substance. It can be noted that pSAPs were only exceptionally observed in CC (for 7 this reason, they were not included in the statistical analysis), confirming the 8 extremely low aversiveness of this configuration.

9 As a whole, our results demonstrated that, at least after familiarization to the 10 starting arm: 1/ our device in OC was appropriate to precisely detect pharmacological 11 anxiolysis 2/ our device in CC, due to the virtually complete absence of any aversive 12 aspect (as indicated by the anxiety variables *per se* and the lack of CDP effect on 13 them), was appropriate to specifically investigate the intrinsic exploratory motivation 14 of mice.

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3.2. Experiment 2 -examination of Lurcher mice (tables 2-5, figure 4)

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18 In accordance with the experiment 1 results, the B6CBA strain mice behaviors 19 strongly differed in CC and OC of the Dual Maze. In addition, our data patently 20 indicated that this configuration effect was influenced by both the genotype and the 21 level of familiarization. The data are shown on figure 4 and tables 2-5.

22

For more clarity, we decided to first present the results obtained in OC and CC without any prior familiarization. The data collected after 20 minutes of familiarization in the starting arm are described in the second paragraph.

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27

3.2.1. Without familiarization

The analysis of the time spent in the testing arm clearly showed that mice of both genotypes avoided this arm in OC (U=0; p<0.01, compared to chance level for both genotypes) but not in CC where no significant preference for any arm was observed (control mice: U=35; p>0.05 / Lurcher mice: U=20; p>0.05, compared to

1 chance level) (fig. 4D). Accordingly, the time spent in the open testing arm (fig. 4D) 2 was significantly shorter than that spent in the closed testing arm in control 3 (p<0.0001) and Lurcher mice (p<0.05). Nevertheless, the avoidance behavior 4 induced by the testing arm in OC was more marked in the former than in the latter 5 (number of entries in the open testing arm: p<0.005, fig. 4B / time spent in the open 6 testing arm: p<0.05, fig. 4D). In contrast, no significant difference between the 7 animals of both genotypes was noticed concerning the time spent in the closed 8 testing arm (p>0.05) (fig. 4D), although the motor deficits of the mutants reduced the 9 number of entries they made in this arm (p<0.05 when compared with the control 10 mice, fig. 4B) as well as the total number of segments they crossed (p<0.05 when 11 compared with the control mice, fig. 4A). The results reported above, consistent with 12 the behavioral disinhibition previously reported in Lurcher mice, were confirmed by 13 the parameters regarding the distal segment of the open testing arm: the mutants 14 entered more often (p<0.01) (fig. 4C) and spent more time (p<0.001) (fig. 4E) in this 15 segment than their littermate controls. The disrupted aversion of the cerebellar mutants for open spaces was particularly well illustrated by the fact they entered as 16 17 often in the testing arm (p>0.05) (fig. 4B) and spent as much time in its distal 18 segment (p>0.05) in OC as in CC (fig. 4E), contrary to wild type mice which entered 19 less this arm (p<0.0001) (fig. 4B) and spent less time in its distal segment in OC 20 (p<0.0005) (fig. 4E). In a more general way, the aversive configuration of our device 21 provoked a significant inhibition of locomotor activity only in wild type animals (control 22 mice: p<0.001 / Lurcher mice: p>0.05) strongly reflecting the impaired behavioral 23 reaction of the Lurcher mice to aversive environments (fig. 4A). Such an abnormal 24 reaction was also suggested by the higher proportion, albeit not significant (p>0.05), 25 of mice performing some pSAPs (fig. 4G) in OC in the control group (8/12) than in the 26 Lurcher group (4/12). By contrast, the higher number of pHDs (fig. 4H) in the control 27 mice than in the mutant mice is only slight and very probably due to statistical noise 28 (p>0.05). As expected, the number of rears (fig. 4F) were always significantly lower in 29 Lurcher mutants compared to control mice (p<0.05 in OC and p<0.001 in CC) and decreased in both genotypes in open configuration (obviously due to the absence of 30 31 wall to support the rears; p<0.001 for control mice and p<0.05 for Lurcher mice). 32 Thus, in their all, the data we collected in the Dual Maze without prior familiarization 33 are consistent with those presented above regarding the BALB/c mice and those 34 previously obtained in the elevated plus-maze test with the Lurcher mutant mice.

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3.2.2. With familiarization

3 The familiarization considerably influenced the mice behaviors in the Dual Maze. 4 While they still significantly avoided the open testing arm (U=0; p<0.01, compared to 5 chance level for both genotypes), they displayed a clear preference for the closed 6 testing arm where they spent about 75% of the total experiment time whatever their 7 genotype (control mice: U=91; p<0.01 / Lurcher mice: U=44; p<0.05, compared to 8 chance level) (fig. 4D). As a consequence, the control as well as the Lurcher mice 9 spent about 8 times less time in the testing arm in OC than in CC (p<0.0001 for both 10 genotypes) (fig. 4D). But the most relevant of our results was that the cerebellar mice 11 were statistically indistinguishable from their littermate controls in terms of number of 12 entries (fig. 4B) and time spent in the open testing arm (fig. 4D) and in its distal 13 segment (fig. 4E) (p>0.05 for all the comparisons). These observations, contrasting 14 with those made in unfamiliarized animals, suggested a kind of normalization of the 15 anxiety-related behaviors in Lurcher mice by familiarization. Such an hypothesis was 16 reinforced by the direct comparison of the data obtained with or without 17 familiarization: while familiarized and unfamiliarized control mice did not behave 18 differently in front of the open testing arm and its distal segment (p>0.05 for all 19 comparisons), the 20-minutes period of familiarization was sufficient to lead to a 20 drastic decrease in the number of entries and time spent in both areas (fig. 4B, 21 4C,4D, 4E) in Lurcher mice (number of entries in the testing arm / its distal segment: 22 p=0.001 / p<0.0001; time spent in the testing arm / its distal segment: p<0.01 / 23 p<0.001). A hypothetical impairment of exploratory motivation could hardly account 24 for this modification since the already evoked preference for the testing arm in CC 25 had the same magnitude in mice of both genotypes (p>0.05 for the time spent in the 26 closed testing arm between Lurcher and control mice). Furthermore, familiarization 27 significantly decreased the number of segments crossed (p<0.001) (fig. 4A) and 28 rears (p<0.001) (fig. 4F) in Lurcher mice only in OC (p>0.05 for all the comparisons in 29 CC), suggesting an anxiety-induced inhibition of general activity. Although the mean 30 number of pSAPs (fig. 4G) was not significantly changed by prior familiarization in 31 Lurcher mutants (p>0.05), it is important to note that the proportion of the latter 32 displaying this behavior in OC was much higher after familiarization than in the case 33 of forced exposure without any prior familiarization (9/10 of the familiarized Lurcher

1 mice vs 4/12 of the unfamiliarized Lurcher mice). This tendency was also detectable 2 in control mice, but at a lesser extent (10/10 of the familiarized control mice vs 8/12 of 3 the unfamiliarized control mice). So, pSAPs, as well as the other variables 4 considered, indicated that 20-minutes familiarization prior testing mice in the aversive 5 version of the Dual Maze led to the convergence of the behavioral expression of 6 anxiety in Lurcher and control mice. The only exception to this picture is the number 7 of pHDs (fig. 4H) which is very higher in the mutants than in their control littermates 8 after familiarization (p<0.05) while no significant difference was noticed in the 9 unfamiliarized animals (p>0.05). The explanation for this exception is not obvious. 10 But we could hypothesize that the cerebellar deficiencies in Lurcher mice make the 11 information processing during their assessment of the height harder. This impairment 12 could be due to motor deficits (instability in head dipping and visual anchoring 13 resulting in interrupted information intake) as well as to specific cognitive difficulties. 14 Whatever the origin of the deficit, the mutants could have to repeat their scanning 15 behaviors to estimate the height of the open arm more often than control mice to compensate it. Even so, the number of pHDs performed confirmed that the 16 17 familiarization to the experimental conditions radically changed the way the Lurcher 18 mice investigated the aversive environments (familiarized vs unfamiliarized mice: 19 Lurcher mice: p<0.05 / control mice: p>0.05).

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22 4. DISCUSSION

In this work, we have 1) designed and tested an innovative apparatus to investigate anxiety-related behavior, intrinsic exploratory behavior and the effect of familiarization on them in mice, and 2) shown the impact of prior familiarization with experimental apparatus on the behavioral disturbances expressed by cerebellar Lurcher mutant mice.

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4.1. Validation of apparatus and protocol

The types of behavior we took into consideration in the Dual Maze were essentially the same as those measured in the elevated plus-maze test and are thought to result from an approach/avoidance conflict [42]. Beyond this similarity, the absence of a center in our device nevertheless eliminates an ambiguous area, often
discarded from the results analysis in the studies using the plus-maze [41, 45, 49]. It
permits to avoid any loss of information about the emotional status of animals or any
distortion in the appraisal of their real anxiety level.

5 The drop of horizontal and vertical exploratory activities observed in the open 6 configuration (figure 3) in both NaCl- and CDP-injected BALB/c mice obviously 7 resulted from their avoidance of the open testing arm (in which, moreover, they could 8 not lean on any wall). This avoidance was accompanied by numerous pSAPs from 9 the starting towards the testing arm, limiting the time the animals could allocate to 10 their locomotor movements. In closed configuration, pSAPs, which reflect the 11 approach-avoidance conflict evoked by an aversive space [50], were exceptional. As 12 expected, our results showed the open testing arm of our device was much more 13 aversive for mice than the closed one.

14 The profound modifications of mouse behaviors caused by CDP in the open 15 configuration of the apparatus further demonstrated the latter was particularly 16 valuable to measure anxiety level and detect anxiolysis. Thus, the drug specifically 17 elevated the time spent in the open testing arm (as denoted by the significant configuration x treatment interaction). The time spent in the distal segment of this arm 18 19 seemed an especially reliable anxiety-related variable since CDP significantly 20 increased it. Although the lack of significant configuration x treatment interaction did 21 not permit us to statistically test it using post-hoc comparisons, it should be noted the 22 anxiolysis was also reflected by the dramatic increase in the number of entries in the 23 open testing arm as a whole and in its distal segment (+85% and +261%) 24 respectively). The almost complete elimination of the pSAPs provoked by CDP 25 administration confirmed the open version of the Dual Maze was highly sensitive in 26 determining the level of anxiety in mice.

In the closed configuration, whatever the treatment administered, animals exhibited a clear preference for the testing arm as indicated by the time they spent (two thirds of the total time of the experiment) and the number of rears they achieved into it (70% of the total number of rears). Such a preference for novelty has already been observed in mice (especially in Swiss strain mice) which could freely explore an environment after having been familiarized with a starting area [51]. In our experimental conditions, 20 minutes of familiarization to a starting box was enough

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1 for animals to mark a preference for an unfamiliar space. The procedure and the 2 device used in this study did not allow us to notice any significant influence of CDP 3 on the spontaneous exploratory activity of the mice suggesting that sedative effect of 4 CDP can be excluded in this study. To detect anxiolytic effects of a pharmacological 5 compound, it is needed that mice exhibit a sufficient baseline level of anxiety [8, 46]. 6 So, the lack of behavioral modifications in BALB/c mice injected with CDP in the 7 closed configuration, confirmed that the latter induced only negligible anxiety in 8 animals.

9 Taken as a whole, the results we obtained in the BALB/c mice strongly 10 suggested that the Dual maze test was efficient at assessing the anxiety level of mice 11 in a clear-cut way (open configuration) and at specifically measuring their intrinsic 12 motivation to explore environment (closed configuration). A comprehensive 13 pharmacological study designed to test its bidirectional sensitivity (with anxiogenic 14 and anxiolytic drugs) in several strains of mice with more animals per group is 15 certainly needed to add value to it as an anxiety test. Nevertheless, our data are 16 sufficient to reasonably interpret the behaviors of Lurcher mice in the Dual maze in 17 terms of disinhibition. It is worthy to note that all the variables of anxiety we reported 18 here are influenced by CDP in the expected direction. In addition, the effect size, in 19 most cases, is particularly strong even when differences were not found significant 20 due to our limited sample sizes (that is the case for the number of entries in the 21 whole testing arm and in its distal part and for SAPs. The effect for pHD is a little bit 22 less strong). It is why we decided to make use of all the parameters measured in the 23 part of our work dedicated to the Lurcher mice.

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4.2. Examination of the Lurcher mutant mice

26 The Lurcher mice display a very particular behavioral profile in experimental 27 devices, which can lead to bias and misinterpretation of classical test results. This 28 profile was proposed to be described by the concept of behavioral disinhibition. It 29 was defined as the paradoxical combination of inappropriate, risky behaviors with an 30 abnormal physiological reactivity to stressors [19, 34-37]. For instance, Lurcher mice 31 were consistently found to visit much more the aversive open arms of the elevated 32 plus-maze than their littermate controls and to realize fewer behaviors evocative of 33 anxiety-related inhibition like pSAPs, although being more physiologically stressed 1 [34, 38]. Such a disinhibition probably affects behaviors of the mutants in a wide 2 range of tests. So, their lack of immobility responses in the forced swimming test 3 could be a consequence of the impairment of the inhibition system rather than an 4 evidence of resistance to resignation [19, 52]. In the Morris water maze test, the 5 abnormally high levels of stress hormones reported in Lurcher mice [37] could denote 6 the disinhibition to which they are usually associated to. This disinhibition would 7 probably interfere with the selection of the most efficient strategy to complete the task and could contribute to the poor performances of the mutants. 8

9 Whether it be the Lurcher or control mice, they all behave very differently in 10 the CC and OC of the Dual Maze when tested without any prior familiarization. 11 Nevertheless, the precise effect of the change of configuration depended on the 12 genotype of animals. In the CC, the Lurcher mice were distinguishable from their 13 littermate controls only by their lowest locomotor and rearing activity, likely due to 14 their motor deficits. On the other hand, the animals of both genotypes spent 15 equivalent time in the starting and closed testing arm, showing the device as a whole 16 appeared to them as a new, homogeneous space. The physical characteristic of the 17 testing arm in the OC deeply changed the exploratory strategy in control mice. The 18 sharp reduction of their locomotor activity coincides with a strong expression of 19 pSAPs, indicating the aversive valence of the open testing arm. Consistently, they 20 entered much less the latter and its distal segment than the control mice tested in the 21 CC. The same kind of changes were noticeable in the Lurcher mutants but at a much 22 lesser extent. Their preference for the starting arm in the OC clearly showed they 23 perceived the open arm as aversive. Nevertheless, compared to the control mice, 24 they exhibited reinforcement of the approach tendency (and / or attenuation of the 25 avoidance tendency) relative to the aversive area of the device as previously 26 observed in the plus-maze apparatus. This reinforcement (and / or this attenuation) 27 together with the fact that mutants very rarely used stretched postures, illustrate their 28 behavioral disinhibition in tests involving a forced confrontation to a novel 29 environment. Thus, in their all, the data we collected with the Lurcher mutant mice in 30 the Dual Maze without familiarization were in line with those previously obtained in 31 the elevated plus-maze test.

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4.3. Effects of familiarization on the behavioral disinhibition of the Lurcher mice

In this study, we hypothesized that familiarization to the starting arm of the apparatus prior to testing phase could lower the novelty-induced stress and reduce the associated behavioral modifications in the animals. Although paradoxical behaviors caused by forcible exposure to novelty were observed in early periods of testing even in mice not suffering from any pathological disinhibition [16], it was expected the familiarization effects would be particularly strong in Lurcher mice.

9 20 minutes of familiarization in the starting arm were sufficient to strongly 10 modify exploratory activity of mice of both genotypes into the Dual Maze in CC. 11 Contrary to what observed without any prior familiarization, mutants and non-mutants 12 showed a marked preference for the testing arm: they spent more than 2/3 of the 13 total experiment time into it and reared much more in this area of the apparatus than 14 in the starting arm. If such a preference was expected considering the data of the 15 literature about the free exploration paradigm [51, 53], it is of particular interest 16 regarding the Lurcher mutants. It has been previously shown that their exploratory 17 activity was impaired in the hole board test [39, 54]. According to Caston and his 18 collaborators [39], this deficit would primarily result from the alteration of the 19 motivation to explore. Contrasting with these previous results, our data indicated that 20 exploratory behavior of the Lurcher mutant mice was similar to that of their littermate 21 controls. Such discrepancy could be explained by the simpler structure of the Dual 22 Maze when compared to the hole-board apparatus. Consequently, the exploration of 23 the latter could be more demanding and more sensitive to the visuospatial 24 disturbances of the mutants [55, 56]. Be that as it may, our results strongly suggested 25 there was actually not any significant impairment of the intrinsic exploratory 26 motivation in the Lurcher mice.

As it was the case without any prior familiarization, open testing arm evoked passive avoidance behavior in all the familiarized mice, whatever their genotype. Nevertheless, the effect of familiarization in this configuration was very different in control and Lurcher mice. While this effect was moderate in the former, it led to a large reinforcement of the approach-avoidance conflict in the latter, as indicated by the (although non-significant) increase in the number of pSAPs they achieved. In addition, exploration of the open arm showed the resolution of the conflict resulted in

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similar avoidance in both control and Lurcher mice. Actually, our results strongly suggested that familiarization to the starting arm of the Dual Maze restored the inhibition process in the mutants. The main consequence of familiarization is probably the decline of the novelty-induced stress. Thus, it is reasonable to postulate that the behavioral disinhibition only observed in non-familiarized Lurcher mice is triggered by their intense stress in the experimental situation (as in our device without familiarization or the elevated plus-maze in its classical version).

8 This means, *in fine*, that the cerebellum as a whole, and the cerebellar cortex 9 in particular, could participate in the modulation of coping strategies adopted by 10 animals confronted to a stressful experience. The multiple reciprocal connections the 11 cerebellum has with some limbic structures involved in the behavioral inhibition [57, 12 58], such as the septo-hippocampal system [59, 60], together with those it has with 13 the central noradrenergic [61-63] and serotoninergic circuits [63-66] represent 14 candidates of choice as neurobiological substrates for such a function.

15 Using the Dual maze, an innovative device with face and predictive validity, we 16 could compare the impact of prior familiarization to the experimental context on both 17 anxiety-related behaviors and pure exploratory motivation in Lurcher mutant mice and littermate controls. In its all, our experiment showed the behavioral disinhibition 18 19 of the Lurcher mice in a novel environment they were forcibly exposed to was 20 abolished by prior familiarization to the same environment. Such results support the 21 hypothesis that the cerebellum is involved in novelty-induced stress response and 22 contributes to the regulation of associated coping strategies. Beyond the 23 interpretation we proposed about the cause of the Lurcher disinhibition, our work 24 more generally underlines the fact that performance of laboratory mice, particularly 25 mice with abnormal stress reactivity, is a product of complex factors that must be 26 included in the interpretation of measured data in a given task.

27

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- 3

4 6. REFERENCES

- 5 [1] D.H. Simmons, H.K. Titley, C. Hansel, P. Mason, Behavioral Tests for Mouse
- 6 Models of Autism: An Argument for the Inclusion of Cerebellum-Controlled Motor7 Behaviors, Neuroscience (2020).
- 8 [2] K. Gouveia, J.L. Hurst, Optimising reliability of mouse performance in behavioural 9 testing: the major role of non-aversive handling, Sci Rep 7 (2017) 44999.
- 10 [3] G.S. Anderson, P.M. Di Nota, G.A.S. Metz, J.P. Andersen, The Impact of Acute
- Stress Physiology on Skilled Motor Performance: Implications for Policing, FrontPsychol 10 (2019) 2501.
- 13 [4] A.J. Dunn, A.H. Swiergiel, Effects of acute and chronic stressors and CRF in rat 14 and mouse tests for depression, Ann N Y Acad Sci 1148 (2008) 118-26.
- 15 [5] S. Hogg, A review of the validity and variability of the elevated plus-maze as an
- 16 animal model of anxiety, Pharmacol Biochem Behav 54(1) (1996) 21-30.
- 17 [6] S.M. Korte, Corticosteroids in relation to fear, anxiety and psychopathology,
- 18 Neurosci Biobehav Rev 25(2) (2001) 117-42.
- 19 [7] G. Luksys, W. Gerstner, C. Sandi, Stress, genotype and norepinephrine in the
- prediction of mouse behavior using reinforcement learning, Nat Neurosci 12(9)
 (2009) 1180-6.
- 22 [8] K.R. Bailey, J. Crawley, Anxiety-Related Behaviors in Mice, in: J.J. Buccafusco
- 23 (Ed.), Methods of Behavior Analysis in Neuroscience, CRC Pess/Taylor & Francis,
- 24 Boca Raton, 2009.
- [9] J.N. Crawley, Exploratory behavior models of anxiety in mice, Neurosci Biobehav
 Rev 9(1) (1985) 37-44.
- [10] R. Misslin, M. Cigrang, Does neophobia necessarily imply fear or anxiety?,
- 28 Behav Processes 12(1) (1986) 45-50.
- 29 [11] R. Madani, S. Kozlov, A. Ákhmedov, P. Cinelli, J. Kinter, H.P. Lipp, P.
- 30 Sonderegger, D.P. Wolfer, Impaired explorative behavior and neophobia in
- 31 genetically modified mice lacking or overexpressing the extracellular serine protease 32 inhibitor neuroserpin, Mol Cell Neurosci 23(3) (2003) 473-94.
- 33 [12] J.R. Parkitna, M. Sikora, S. Golda, K. Golembiowska, B. Bystrowska, D.
- 34 Engblom, A. Bilbao, R. Przewlocki, Novelty-seeking behaviors and the escalation of
- 35 alcohol drinking after abstinence in mice are controlled by metabotropic glutamate
- receptor 5 on neurons expressing dopamine d1 receptors, Biol Psychiatry 73(3)
 (2013) 263-70.
- 38 [13] V.M. Pogorelov, R.M. Rodriguiz, M.L. Insco, M.G. Caron, W.C. Wetsel, Novelty
- 39 seeking and stereotypic activation of behavior in mice with disruption of the Dat1 40 gene, Neuropsychopharmacology 30(10) (2005) 1818-31.
- 41 [14] T. Sarowar, S. Grabrucker, T.M. Boeckers, A.M. Grabrucker, Object Phobia and
- Altered RhoA Signaling in Amygdala of Mice Lacking RICH2, Front Mol Neurosci 10
 (2017) 180.
- 44 [15] T. Sarowar, S. Grabrucker, K. Fohr, K. Mangus, M. Eckert, J. Bockmann, T.M.
- 45 Boeckers, A.M. Grabrucker, Enlarged dendritic spines and pronounced neophobia in 46 mice lacking the PSD protein RICH2, Mol Brain 9 (2016) 28.
- 47 [16] A. Arabo, C. Potier, G. Ollivier, T. Lorivel, V. Roy, Temporal analysis of free

- 1 exploration of an elevated plus-maze in mice, J Exp Psychol Anim Learn Cogn 40(4)
- 2 (2014) 457-66.
- 3 [17] P.E. Dickson, J. Cairns, D. Goldowitz, G. Mittleman, Cerebellar contribution to
- 4 higher and lower order rule learning and cognitive flexibility in mice, Neuroscience
 5 345 (2017) 99-109.
- 6 [18] J.D. Schmahmann, The cerebellum and cognition, Neurosci Lett 688 (2019) 62-7 75.
- 8 [19] J. Tuma, Y. Kolinko, F. Vozeh, J. Cendelin, Mutation-related differences in
- 9 exploratory, spatial, and depressive-like behavior in pcd and Lurcher cerebellar
 10 mutant mice, Front Behav Neurosci 9 (2015) 116.
- 11 [20] M. Adamaszek, F. D'Agata, R. Ferrucci, C. Habas, S. Keulen, K.C. Kirkby, M.
- 12 Leggio, P. Marien, M. Molinari, E. Moulton, L. Orsi, F. Van Overwalle, C. Papadelis, A.
- 13 Priori, B. Sacchetti, D.J. Schutter, C. Styliadis, J. Verhoeven, Consensus Paper:
- 14 Cerebellum and Emotion, Cerebellum 16(2) (2017) 552-576.
- 15 [21] O. Baumann, R.J. Borra, J.M. Bower, K.E. Cullen, C. Habas, R.B. Ivry, M.
- 16 Leggio, J.B. Mattingley, M. Molinari, E.A. Moulton, M.G. Paulin, M.A. Pavlova, J.D.
- 17 Schmahmann, A.A. Sokolov, Consensus paper: the role of the cerebellum in 18 perceptual processes, Cerebellum 14(2) (2015) 197-220.
- 19 [22] L.F. Koziol, D. Budding, N. Andreasen, S. D'Arrigo, S. Bulgheroni, H. Imamizu,
- 20 M. Ito, M. Manto, C. Marvel, K. Parker, G. Pezzulo, N. Ramnani, D. Riva, J.
- Schmahmann, L. Vandervert, T. Yamazaki, Consensus paper: the cerebellum's role in
 movement and cognition, Cerebellum 13(1) (2014) 151-77.
- [23] R.J.S. Phillips, "Lurcher", a new gene in linkage group XI of the house mouse.,
 Journal of Genetics 57 (1960) 35-42.
- 25 [24] K.W. Caddy, T.J. Biscoe, Structural and quantitative studies on the normal C3H
- and Lurcher mutant mouse, Philos Trans R Soc Lond B Biol Sci 287(1020) (1979)
 167-201.
- 28 [25] J. Zuo, P.L. De Jager, K.A. Takahashi, W. Jiang, D.J. Linden, N. Heintz,
- Neurodegeneration in Lurcher mice caused by mutation in delta2 glutamate receptor gene, Nature 388(6644) (1997) 769-73.
- 31 [26] S.H. Zanjani, F. Selimi, M.W. Vogel, A.M. Haeberle, J. Boeuf, J. Mariani, Y.J.
- 32 Bailly, Survival of interneurons and parallel fiber synapses in a cerebellar cortex
- deprived of Purkinje cells: studies in the double mutant mouse Grid2Lc/+;Bax(-/-), J
 Comp Neurol 497(4) (2006) 622-35.
- 35 [27] F. Sultan, T. Konig, M. Mock, P. Thier, Quantitative organization of
- neurotransmitters in the deep cerebellar nuclei of the Lurcher mutant, J Comp Neurol
 452(4) (2002) 311-23.
- 38 [28] J. Cendelin, J. Tuma, I. Korelusova, F. Vozeh, The effect of genetic background
- 39 on behavioral manifestation of Grid2(Lc) mutation, Behav Brain Res 271 (2014) 218-40 27.
- 41 [29] P. Hilber, J. Caston, Motor skills and motor learning in Lurcher mutant mice
- 42 during aging, Neuroscience 102(3) (2001) 615-23.
- 43 [30] P. Hilber, F. Jouen, N. Delhaye-Bouchaud, J. Mariani, J. Caston, Differential roles
- 44 of cerebellar cortex and deep cerebellar nuclei in learning and retention of a spatial
- 45 task: studies in intact and cerebellectomized lurcher mutant mice, Behav Genet 28(4)
 46 (1998) 299-308.
- 47 [31] J.C. Lopez-Ramos, Z. Houdek, J. Cendelin, F. Vozeh, J.M. Delgado-Garcia,
- 48 Timing correlations between cerebellar interpositus neuronal firing and classically
- 49 conditioned eyelid responses in wild-type and Lurcher mice, Sci Rep 8(1) (2018)
- 50 10697.

- 1 [32] E. Porras-Garcia, J. Cendelin, E. Dominguez-del-Toro, F. Vozeh, J.M. Delgado-
- 2 Garcia, Purkinje cell loss affects differentially the execution, acquisition and prepulse
- 3 inhibition of skeletal and facial motor responses in Lurcher mice, Eur J Neurosci 4 21(4) (2005) 979-88.
- 5 [33] F. Frederic, T. Chautard, R. Brochard, C. Chianale, E. Wollman, C. Oliver, N.
- 6 Delhaye-Bouchaud, J. Mariani, Enhanced endocrine response to novel environment
- 7 stress and endotoxin in Lurcher mutant mice, Neuroendocrinology 66(5) (1997) 341-8 7.
- 9 [34] P. Hilber, T. Lorivel, C. Delarue, J. Caston, Stress and anxious-related behaviors 10 in Lurcher mutant mice, Brain Res 1003(1-2) (2004) 108-12.
- 11 [35] T. Lorivel, M. Gras, P. Hilber, Effects of corticosterone synthesis inhibitor
- 12 metyrapone on anxiety-related behaviors in Lurcher mutant mice, Physiol Behav 13 101(2) (2010) 309-14.
- 14 [36] T. Lorivel, V. Roy, P. Hilber, Fear-related behaviors in Lurcher mutant mice 15 exposed to a predator, Genes Brain Behav 13(8) (2014) 794-801.
- 16 [37] J. Tuma, Y. Kolinko, D. Jelinkova, P. Hilber, J. Cendelin, Impaired spatial
- performance in cerebellar-deficient Lurcher mice is not associated with their 17
- 18 abnormal stress response, Neurobiol Learn Mem 140 (2017) 62-70.
- 19 [38] T. Lorivel, P. Hilber, Effects of chlordiazepoxide on the emotional reactivity and
- 20 motor capacities in the cerebellar Lurcher mutant mice, Behav Brain Res 173(1) 21 (2006) 122-8.
- 22 [39] J. Caston, C. Chianale, N. Delhaye-Bouchaud, J. Mariani, Role of the cerebellum 23 in exploration behavior, Brain Res 808(2) (1998) 232-7.
- 24 [40] C. Belzung, G. Griebel, Measuring normal and pathological anxiety-like
- behaviour in mice: a review, Behav Brain Res 125(1-2) (2001) 141-9. 25
- 26 [41] A.A. Braun, M.R. Skelton, C.V. Vorhees, M.T. Williams, Comparison of the
- 27 elevated plus and elevated zero mazes in treated and untreated male Sprague-
- 28 Dawley rats: effects of anxiolytic and anxiogenic agents, Pharmacol Biochem Behav 29 97(3) (2011) 406-15.
- 30 [42] R.G. Lister, Ethologically-based animal models of anxiety disorders, Pharmacol 31 Ther 46(3) (1990) 321-40.
- [43] S. Pellow, P. Chopin, S.E. File, M. Briley, Validation of open:closed arm entries in 32
- 33 an elevated plus-maze as a measure of anxiety in the rat, J Neurosci Methods 14(3) 34 (1985) 149-67.
- 35 [44] A. Holmes, R.J. Rodgers, Influence of spatial and temporal manipulations on the
- anxiolytic efficacy of chlordiazepoxide in mice previously exposed to the elevated 36 37 plus-maze, Neurosci Biobehav Rev 23(7) (1999) 971-80.
- [45] R.J. Rodgers, A. Dalvi, Anxiety, defence and the elevated plus-maze, Neurosci 38 39 Biobehav Rev 21(6) (1997) 801-10.
- 40 [46] R.J. Rodgers, B. Davies, R. Shore, Absence of anxiolytic response to
- 41 chlordiazepoxide in two common background strains exposed to the elevated plus-
- 42 maze: importance and implications of behavioural baseline, Genes Brain Behav 1(4) 43 (2002) 242-51.
- 44 [47] P.M. Wall, C. Messier, Ethological confirmatory factor analysis of anxiety-like
- 45 behaviour in the murine elevated plus-maze, Behav Brain Res 114(1-2) (2000) 199-46 212.
- 47 [48] E.B. Ottoni, EthoLog 2.2: a tool for the transcription and timing of behavior
- 48 observation sessions, Behav Res Methods Instrum Comput 32(3) (2000) 446-9.
- [49] M. Komada, K. Takao, T. Miyakawa, Elevated plus maze for mice, J Vis Exp (22) 49
- 50 (2008).

- 1 [50] A.M. Van der Poel, A note pn "stretched attention", a behavioural element
- 2 indicative of an approach-avoidance conflict in rats., Anim Behav 27 (1979) 446-450.
- 3 [51] A. Agmo, C. Belzung, Interactions between dopamine and GABA in the control of
- ambulatory activity and neophobia in the mouse, Pharmacol Biochem Behav 59(1)
 (1998) 239-47.
- [52] R. Lalonde, Immobility responses in Lurcher mutant mice, Behav Genet 28(4)
 (1998) 309-14.
- 8 [53] C. Ducottet, C. Belzung, Correlations between behaviours in the elevated plus-
- 9 maze and sensitivity to unpredictable subchronic mild stress: evidence from inbred
 10 strains of mice, Behav Brain Res 156(1) (2005) 153-62.
- 11 [54] C. Monnier, R. Lalonde, Elevated (+)-maze and hole-board exploration in lurcher 12 mutant mice, Brain Res 702(1-2) (1995) 169-72.
- 13 [55] I. Korelusova, J. Cendelin, F. Vozez, Motor and visuospatial abilities in a model
- of olivocerebellar and retinal degeneration--Lurcher mutant mice of C3H strain,
 Prague Med Rep 108(1) (2007) 37-48.
- [56] R. Lalonde, Y. Lamarre, A.M. Smith, Does the mutant mouse lurcher have
 deficits in spatially oriented behaviours?, Brain Res 455(1) (1988) 24-30.
- 18 [57] J.A. Gray, N. McNaughton, The Neuropsychology of Anxiety: An Enquiry into the
- 19 Functions of the Septo-Hippocampal System, 2 nd edition ed., Oxford University
- 20 Press, New York, 2000.
- 21 [58] N. McNaughton, P.J. Corr, A two-dimensional neuropsychology of defense:
- 22 fear/anxiety and defensive distance, Neurosci Biobehav Rev 28(3) (2004) 285-305.
- 23 [59] P. Bohne, M.K. Schwarz, S. Herlitze, M.D. Mark, A New Projection From the
- Deep Cerebellar Nuclei to the Hippocampus via the Ventrolateral and Laterodorsal
 Thalamus in Mice, Front Neural Circuits 13 (2019) 51.
- 26 [60] R.G. Heath, C.W. Dempesy, C.J. Fontana, A.T. Fitzjarrell, Feedback loop
- between cerebellum and septal-hippocampal sites: its role in emotion and epilepsy,
 Biol Psychiatry 15(4) (1980) 541-56.
- 29 [61] T.J. Gould, C.E. Adams, P.C. Bickford, Beta-adrenergic modulation of GABAergic
- inhibition in the deep cerebellar nuclei of F344 rats, Neuropharmacology 36(1) (1997)
 75-81.
- 32 [62] T. Hokfelt, K. Fuxe, Cerebellar monoamine nerve terminals, a new type of
- 33 afferent fibers to the cortex cerebelli, Exp Brain Res 9(1) (1969) 63-72.
- 34 [63] N. Schweighofer, K. Doya, S. Kuroda, Cerebellar aminergic neuromodulation:
- towards a functional understanding, Brain Res Brain Res Rev 44(2-3) (2004) 103-16.
- 36 [64] P.A. Cumming-Hood, H.K. Strahlendorf, J.C. Strahlendorf, Effects of serotonin
- and the 5-HT2/1C receptor agonist DOI on neurons of the cerebellar
- 38 dentate/interpositus nuclei: possible involvement of a GABAergic interneuron, Eur J
- 39 Pharmacol 236(3) (1993) 457-65.
- 40 [65] S. Dieudonne, A. Dumoulin, Serotonin-driven long-range inhibitory connections
- 41 in the cerebellar cortex, J Neurosci 20(5) (2000) 1837-48.
- 42 [66] C.W. Kerr, G.A. Bishop, Topographical organization in the origin of serotoninergic
- 43 projections to different regions of the cat cerebellar cortex, J Comp Neurol 304(3)
 44 (1991) 502-15.
- 45

1 Figure legends

2 Fig. 1

Pictures of the Dual Maze: Picture A: open configuration / Picture B: closed configuration. The floor of the arms was covered with sawdust. Each arm was symbolically subdivided into distal and proximal segments of same dimensions measuring 10 cm each, delimited here with dotted line. The closed arms were covered with transparent plastic plates with ventilation holes. The open arm was surrounded by 3 mm high plastic rods.

9

10 **Fig. 2**

Scheme of the experiment 1 and experiment 2: experimental groups and number of mice per group (n) for BALB/c mice treated with chlordiazepoxide (CDP) or vehicle tested in open of closed configuration, and for B6CBA Lurcher mutant (+/Lc) and wild type (+/+) mice tested in open or closed configuration either with 20 min familiarization (F) or with no familiarization (No F).

16

17 Fig. 3

18 Total number of crossed segments (A), number of testing arm entries (B), number of 19 entries into the distal segment of the testing arm (C), relative time in the testing arm 20 (D), relative time in the distal segment of the testing arm (E), number of rearings (F), 21 number of protected SAPs (stretched attend postures) in the open configuration (G), 22 number of protected head dips in the open configuration (H) in chlordiazepoxide- and 23 saline-treated BALB/c mice. Data are shown as means; error bars represent standard 24 error of the mean. Horizontal bars connect groups between which statistically 25 significant differences were found. For P values, see text.

26

27 **Fig. 4**

Total number of crossed segments (A), number of testing arm entries (B), number of entries into the distal segment of the testing arm (C), relative time in the testing arm (D), relative time in the distal segment of the testing arm (E), number of rearings (F), number of protected SAPs (stretched attend postures) in the open configuration (G),
 number of protected head dips in the open configuration (H) in Lurcher mutant and
 wild type B6CBA mice. Data are shown as means; error bars represent standard
 error of the mean. Horizontal bars connect groups between which statistically
 significant differences were found. For P values, see tables 2-5

Testing Arm



Picture A

Picture B

Experiment 1







Daramatar -	Configura	ation effect	Treatmen	nt effect	Configuration by treatment interaction		
Taranicter	$F_{(1,35)}$	Р	$F_{(1,35)}$	Р	$F_{(1,35)}$	Р	
Number of crossed segments	23.5	< 0.001	2.61	NS	0.45	NS	
Number of entries into the testing arm	26.54	< 0.001	2.18	NS	1.38	NS	
Number of entries into the distal part of the testing arm	42.75	< 0.001	3.83	NS	1	NS	
Relative time in testing arm	16.65	< 0.001	0.12	NS	5.09	< 0.05	
Relative time in the distal part of the testing arm	6.78	< 0.05	3.95	NS	4.12	< 0.05	
Number of rearings	55.03	< 0.001	0.16	NS	0.67	NS	
Number of pSAPs	-	-	Z = -2.83	< 0.001	-	-	
Number of pHD	-	-	Z = -1,53	NS	-	-	

Tab.1: Statistical significance of the effects of configuration (open/closed), treatment (chlordiazepoxide/saline) and their interaction on parameters measured in BALB/c mice in the Dual Maze. NS = statistically non-significant, - = not applicable (measured only in open configuration).

Relative time in testing arm ($\chi^2 = 69.822$; p < 0.0001)									
	WT CC N	WT CC F	WT OC N	WT OC F	Lc CC N	Lc CC F	Lc OC N	Lc OC F	
Lc OC F				NS		Z = 3.8228 P < 0.0001	Z = 2.8805 $P \le 0.01$		Lc OC F
Lc OC N			Z = -2.463 P < 0.05		Z = 2.5616 P < 0.05			Z = 3.2984 P < 0.0005	Lc OC N
Le CC F		NS			Z = -2.5767 P < 0.01			Z = 3.5569 $P \le 0.0005$	Lc CC F
Lc CC N	NS					NS	NS		Lc CC N
WT OC F		Z = 4.4305 P < 0.0001	NS					NS	WT OC F
WT OC N	Z = 3.9414 P < 0.0001			NS			Z = -3.1121 P < 0.001		WT OC N
WT CC F	Z = -3.8985 P < 0.0001			Z = 4.2856 P < 0.0001		NS			WT CC F
WT CC N		Z = -4.0715 P < 0.0001	Z = 3.6065 P < 0.0005		NS				WT CC N
	WT CC N	WT CC F	WT OC N	WT OC F	Lc CC N	Lc CC F	Lc OC N	Lc OC F	
		Relative	e time in the dis	tal segment of th	ne testing arm (χ	² = 64.117; p <	0.0001)		

Table 2

Statistical significance of differences in relative time spent in the testing arm (grey fields) and in relative time spent in the distal part of the testing arm (white fields) between individual experimental groups of mice (post hoc tests – planned comparison). χ^2 and p-value of the Kruskal-Wallis analysis is shown next to the parameter title.

WT = wild type mice, Lc = Lurcher mice, CC = closed configuration, OC = open configuration, F = with familiarization, N = no familiarization, NS = statistically non-significant

	Number of entries into the testing arm ($\chi^2 = 61.336$; p < 0.0001)								
	WT CC N	WT CC F	WT OC N	WT OC F	Lc CC N	Lc CC F	Lc OC N	Lc OC F	
Lc OC F				NS		Z=3.7408 P < 0.0005	Z=3.1209 P < 0.005		Lc OC F
Lc OC N			Z = -2.932 $P \le 0.005$		NS			Z = 3.5696 $P \le 0.001$	Lc OC N
Lc CC F		Z = 3.0222 P < 0.0005			NS			Z = 3.8343 P < 0.0005	Lc CC F
Lc CC N	Z = 2.4306 $P \le 0.05$					NS	Z = 2.3688 $P \le 0.05$		Lc CC N
WT OC F		Z = 4.1089 $P \le 0.0001$	NS					NS	WT OC F
WT OC N	Z = 3.8794 P < 0.0001			NS			Z = -3.1762 P < 0.005		WT OC N
WT CC F	NS			Z = 4.2090 $P \le 0.0001$		NS			WT CC F
WT CC N		NS	Z = 3.9164 P < 0.0001		Z = 2.2594 P < 0.05				WT CC N
	WT CC N	WT CC F	WT OC N	WT OC F	Lc CC N	Le CC F	Lc OC N	Lc OC F	

Number of entries into the distal segment of the testing arm ($\chi^2 = 68.235$; p < 0.0001)

Table 3

Statistical significance of differences in number of entries into the testing arm (grey fields) and in number of entries into the distal part of the testing arm (white fields) between individual experimental groups of mice (post hoc tests – planned comparison). χ^2 and p-value of the Kruskal-Wallis analysis is shown next to the parameter title.

WT = wild type mice, Lc = Lurcher mice, CC = closed configuration, OC = open configuration, F = with familiarization, N = no familiarization, NS = statistically non-significant

Number of crossed segments ($\chi^2 = 55.79$; p < 0.0001)										
	WT CC N	WT CC F	WT OC N	WT OC F	Lc CC N	Le CC F	Lc OC N	Lc OC F		
Lc OC F				NS		Z = 3.5420 P < 0.0005	Z = 3.266 P < 0.005		Lc OC F	
Lc OC N			NS		NS			Z = 3.0463 $P \le 0.001$	Lc OC N	
Lc CC F		Z = 2.2923 $P \le 0.05$			NS			Z = 3.3929 P < 0.0001	Lc CC F	
Lc CC N	Z = 2.4826 $P \le 0.05$					NS	Z = 2.1920 $P \le 0.05$		Lc CC N	
WT OC F		Z = 4.0010 $P \le 0.0001$	NS			-		Z = 3.6521 P < 0.0001	WT OC F	
WT OC N	Z = 3.6249 P < 0.0005			NS			Z = 2.3444 $P \le 0.05$		WT OC N	
WT CC F	Z = 2.2772 $P \le 0.05$			Z = 4.1492 $P \le 0.0001$		Z = 3.4174 P < 0.0005			WT CC F	
WT CC N		NS	Z = 3.817 P < 0.0001		Z = 3.2867 P < 0.0005				WT CC N	
	WT CC N	WT CC F	WT OC N	WT OC F	Lc CC N	Le CC F	Lc OC N	Lc OC F		
	Number of rearings ($\chi^2 = 64.481$; p < 0.0001)									

Table 4

Statistical significance of differences in number of crossed segments (grey fields) and in number of rearings (white fields) between individual experimental groups of mice (post hoc tests – planned comparison). χ^2 and p-value of the Kruskal-Wallis analysis is shown next to the parameter title.

WT = wild type mice, Lc = Lurcher mice, CC = closed configuration, OC = open configuration, F = with familiarization, N = no familiarization, NS = statistically non-significant

Number of protected SAPs ($\chi^2 = 39.862$; p < 0.0001)									
	WT OC N	WT OC F	Lc OC N	Le OC F					
Lc OC F		NS	NS		Lc OC F				
Lc OC N	NS			Z = -2.8272 P < 0.05	Lc OC N				
WT OC F	NS			Z = -2.3059 P < 0.05	WT OC F				
WT OC N		NS	NS		WT OC N				
	WT OC N	WT OC F	Lc OC N	Le OC F					
Number of protected head dips ($\chi^2 = 16.83$; p < 0.0001)									

Table 5

Statistical significance of differences in number of protected SAPs (stretched attend postures; grey fields) and in number of protected head dips (white fields) between individual experimental groups of mice (post hoc tests – planned comparison). χ^2 and p-value of the Kruskal-Wallis analysis is shown next to the parameter title.

WT = wild type mice, Lc = Lurcher mice, OC = open configuration, F = with familiarization, N = no familiarization, NS = statistically non-significant