Measure of anxiety and exploration behaviours in cerebellar mice using a simple and unique apparatus: influence of habituation on behavioural disinhibition.

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ABSTRACT

Stress is one of the prominent factors driving exploration showed by rodents in behavioural tests. Such an influence is exacerbated in mice like the Lurcher cerebellar mutants which are hyper reactive to experimental handling. In order to precisely delineate this influence of stress on explorative behaviours, we designed an innovative apparatus (the I maze) existing under two configurations (aversive and less aversive) and permitting to habituate animals more or less to experimental conditions before being tested. Exemplifying the interest of this device, the results we obtained in the Lurcher mutant mice permitted to differentiate between the roles played by the cerebellum in motivation for exploration proper and stress-related behaviours.

INTRODUCTION

The cerebellar Lurcher mutant mice (+/Lc) exhibit severe and obvious motor deficits and also cognitive and emotional-related behaviours disturbances [1]. Thus, when tested in the elevated plus maze, these mutants did not avoid the open spaces (the time they spent in the open arms is similar to that measured in the closed arms). As revealed by corticosterone measures, they are also more stressed than their littermate controls when placed in a novel environment (i.e. in a novel cage or tested in the elevated plus maze). For this reason, we proposed that the total degeneration of the cerebellar cortex caused by the Lurcher mutation led to pathological behavioural disinhibition [2]. On the other hand, some authors showed the mutants were less motivated to explore their environment in the hole board test and concluded from this that the cerebellar cortex plays a role in exploration motivation [3]. In the present study we evaluated the impact of experimental manipulation (environmental changes) on anxiety- and spontaneous exploration-related behaviours in the Lurcher mice. For this purpose we used a unique apparatus with two potential configurations which permits in particular to maintain mice in a starting arm for a habituation period.

MATERIAL AND METHODS

Apparatus: the I maze (see Figure 1)

All the components of the apparatus were made from black-painted IffaCredo® individual cages 20 x 7 x 8 (length x width x height in cm). It consisted of a starting closed alley facing either an open alley (open configuration) or another similar closed alley (closed configuration). The starting and testing alleys were separated by a guillotine door. The whole apparatus was elevated to a height of 50 cm above floor level.

Animals

Adult male Lurcher mutant and control mice from the same strain (B6CBA) were tested in the open and the closed configuration, with or without a habituation period (H or NH; see Table 1). We also verify the validity of the test

(open versus closed configuration) with mice from other strains (BALB/C and C57BL/6) as well as its sensitivity to a pharmacological agent (the benzodiazepine chlordiazepoxide, CDP, 5 mg/kg).

Genotype	Treatment	Closed configuration	Open configuration
C57BL/6	NaCl, NH	N=10	N=9
BALB/C	NaCl, NH	N=10	N=9
BALB/C	Chlordiazepoxide: 5mg/kg, NH	N=10	N=10
Lurcher B6CBA	NH NH	N=10 N=11	N=12 N=12
Lurcher B6CBA	H H	N=9 N=13	N=11 N=10

Table 1: groups and protocols

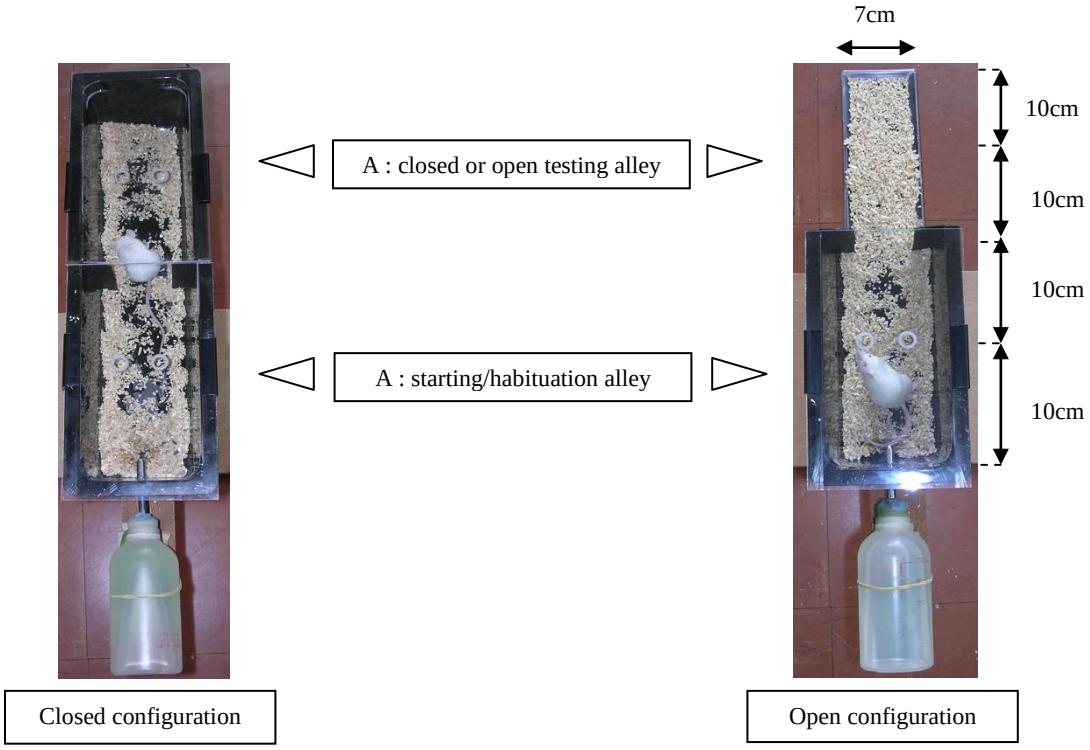

Figure 1: the I maze under its two configurations

Procedures

All the mice tested were randomly subjected to the I maze in either its open or closed configuration. In the classical procedure (no habituation), at the beginning of the test each mouse was placed in the starting closed alley and its behaviours were then measured during 5 minutes with the free EthoLog® Software. Additional Lurcher mice and littermate controls were submitted to the habituation procedure in which each mouse was placed during 20 minutes

in the starting closed alley with water available (as it can be seen in the figure 1) before the opening of the separation door and the assessment of behaviours for 5 minutes. For each condition, we measured the locomotor activity, the time spent and the number of entries into the different areas, the number of SAPs and rears. The floor of the apparatus (starting and testing alleys) was covered with sawdust.

Ethical statement

All the experiments reported here were conducted in accordance with the ethical recommendations of the Directive 2010/63/EU of the European Parliament and of the Council for the protection of animals used for scientific purposes.

Statistical Analysis

Data were analysed with Statistica® software. Two ways ANOVAs with genotype (or drug) and configuration as factors were performed, followed by LSD tests for post hoc comparisons when allowed.

RESULTS AND DISCUSSION

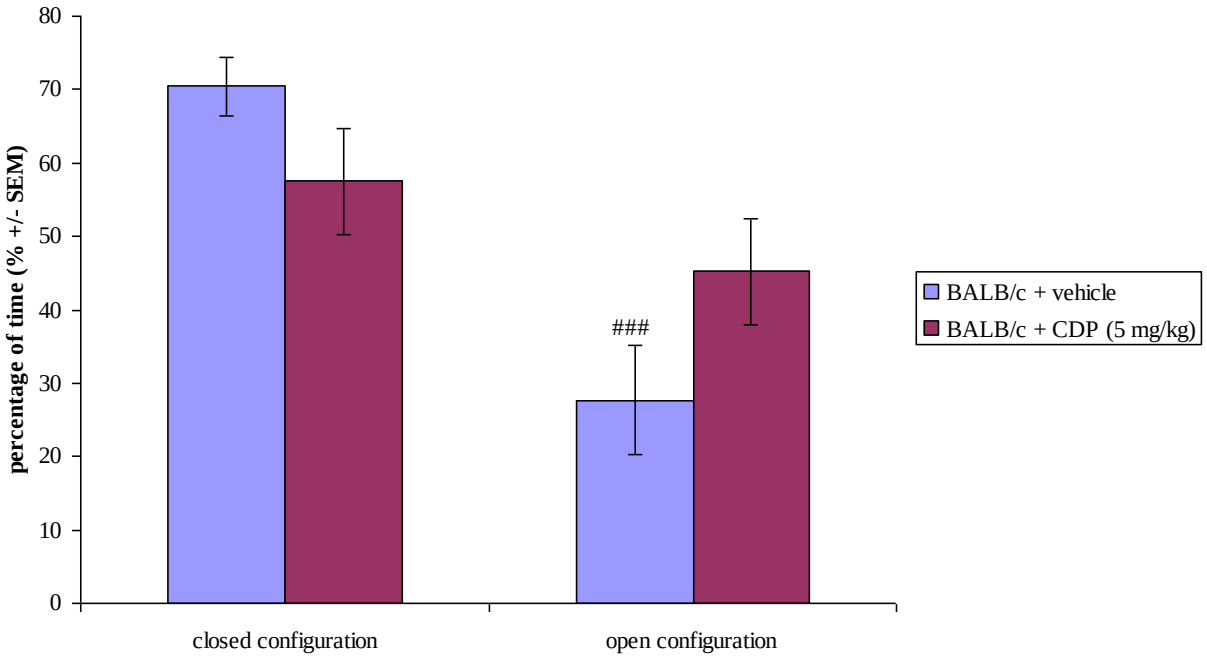

Figure 2: percentage of time spent into the testing arm under the closed and open configuration by BALB/c mice injected or not with chlordiazepoxide (### = closed vs open configuration in BALB/c + vehicle, $p<0.001$)

Consistently with the data obtained in the elevated plus maze, we showed that all the mice we tested (B6CBA, BALB/C, C57BL/6 mice) in the open configuration of the I maze spent less time in the open testing alley than in the closed starting alley (see Figure 2). Such avoidance behaviour was reduced in mice injected with benzodiazepine. On the contrary, mice whether administred with the anxiolytic or not did not show any place preference in the closed configuration of the apparatus.

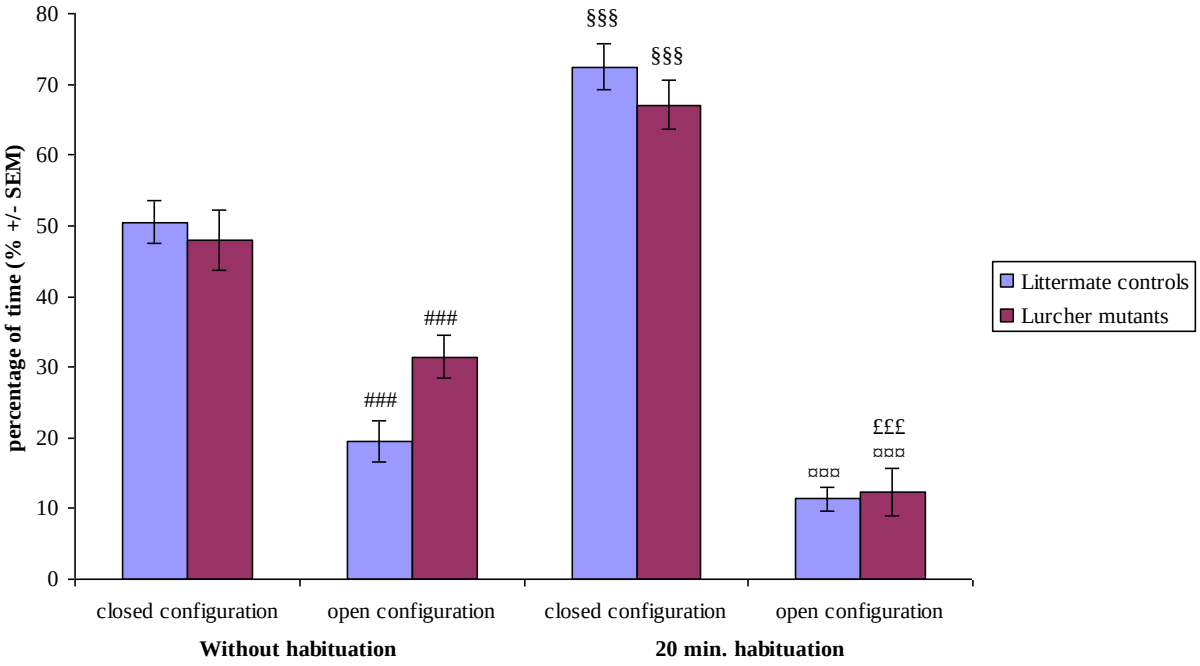

Figure 3: percentage of time spent in the testing arm by Lurcher mutants and littermate controls without prior habituation or after a 20 min. habituation period (### = open vs closed configuration without habituation, $p<0.001$; §§§ = closed configuration without habituation vs closed configuration after 20 min. of habituation, $p<0.001$; ¤¤¤ = open vs closed configuration after 20 min. of habituation, $p<0.001$; £££ = open configuration without habituation vs open configuration after 20 min. of habituation, $p<0.001$) Lurcher mutants versus controls in open configuration without habituation, $p<0.01$.

As previously noticed in the elevated plus maze and in accordance with the disinhibition hypothesis, we observed that compared to their littermate controls , the cerebellar Lurcher mice did not avoid significantly the testing alley when placed in the open configuration of the I maze (see Figure 3). As expected, we showed that 20 minutes habituation to experimental design were sufficient to abolish the stress-related behavioural disinhibition of the mutants. In contrast with precedent results we did not observe reduced exploration motivation in the Lurcher mice when tested in the closed configuration of our device.

CONCLUSION AND PERSPECTIVES:

Our results clearly suggested that cerebellar damages potentially altered behavioural expression of anxiety in stressfull conditions but changed neither anxiety-related behaviours in neutral conditions nor spontaneous exploration motivation in mice.

The novel I maze prototype is now developed (in collaboration with Intellibio® and Novall® see Figure 4) and new protocols are in process to investigate the influence of habituation and drug treatments in both spontaneous and anxiety-related exploration behaviours in mice. The results are compared to those obtained with the elevated + maze. With this novel apparatus behaviours are videotracked during 5 minutes (ANYMAZE®, Stoelting).

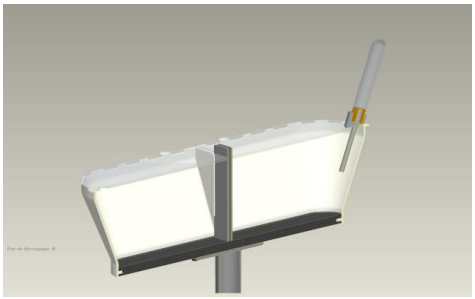

Figure 4: longitudinal section of the I maze (closed configuration)

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