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Effects of corticosterone synthesis inhibitor metyrapone on anxiety-related behaviors in Lurcher mutant mice

Thomas Lorivel [a,*], Marjorie Gras [b], Pascal Hilber [a]

[a] Laboratoire de Psychologie et Neurosciences de la Cognition et de l'Affectivité, EA4306, Université de Rouen, 76134 Mont-Saint-Aignan, France
[b] Laboratoire «Différenciation et Communication Neuronale et Neuroendocrine», INSERM U482/EA4310, IFRMP23, 76134 Mont-Saint-Aignan, France

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ABSTRACT

Beyond their motor impairments, the cerebellar Lurcher mutant mice show an alteration of the anxiety-related behaviors we called "behavioral disinhibition". This is characterized by a low avoidance towards the open arms of the elevated plus-maze device paradoxically combined with a dramatic blood corticosterone level rise induced by the exposure to the experimental conditions. The present study was aimed at determining if the disinhibition of the mutants could be caused by their stress-induced high corticosterone rate. For this purpose, we compared the behaviors of Lurcher and control mice in the elevated plus-maze test after injection of either 2-methyl-1.2-di-3-pyridil-1-propanone (metyrapone; 75 mg/kg), a corticosterone synthesis inhibitor, or vehicle alone (Tween 80, 5%). Our results showed that metyrapone, although efficiently reducing their blood corticosterone rate, provoked only modest modifications of the anxiety-related behaviors in mice of both genotypes. As a result, the behavioral distance between the Lurcher and control mice slightly decreased, without being totally abolished. Thus, it seems that the behavioral disinhibition of the mutants is caused only in part by their stress-provoked high corticosterone level. As a complementary hypothesis, we propose that the behavioral disturbances observed in the Lurcher mice also might arise from dysfunctions of the neural pathways connecting the cerebellum with some limbic structures known to be highly involved in the regulation of emotions.

1. Introduction

Animal studies contributed to show that the behavioral expressions of emotions did not depend on a unique brain region but rather resulted from the activity of networks of spread and interconnected structures. In addition, it was proposed that those networks were rostro-caudally organized in hierarchical systems [1]. Several structures included in the latter like the amygdala and the hippocampus, have been known for a long time to be involved in emotions-related processes. More recently, data obtained in human strongly suggested that the cerebellum might be also involved in the regulation of emotions [2–6]. To date, only few studies using animal models were conducted aiming to elucidate this issue [7,8]. Among them, our works on the Lurcher mutants permitted to show that the cerebellar impairment exhibited by these mice had harmful repercussions on their anxiety-related behaviors [8,9]. The Lurcher mutation affects the δ2 glutamatergic channel receptor gene [10]. It results in constitutively open channels leading to the early, complete and specific loss of the Purkinje cells of the cerebellum [11,12] followed by a significant retrograde degeneration of the olivo-cerebellar pathway [13]. In the elevated plus-maze test, one of the most used tests to assess anxiety in rodents [14], the Lurcher entered more frequently into [8,9] and spent more time in [8,9,15] the aversive areas of the device than the non-mutant mice. Although this kind of data is usually interpreted as a low level of anxiety [14], some indices of high stress reported in mutants seem to contradict such conclusion. Indeed, it was demonstrated that, compared to control mice (+/+), the Lurcher mice (+/Lc) exhibited a hyperresponsiveness of the Hypothalamo-Pituitary-Adrenal axis (HPA axis), as expressed by a dramatic raise of ACTH and corticosterone levels, to a low stressful event (a mere change of homecage) [16]. Since the blood level of corticosterone (cortisol in humans) is largely accepted as a reliable plasmatic index of stress, such results indicated that the mutants were probably more stressed by regular handling including change of homecage and experimental conditions than the non-mutants. Considering the powerful anxiogenic effect of stress [17], it seemed a bit "hasty" to state that Lurcher mice were just less anxious than control mice. So, in order to reconcile the exaggerated stress response exhibited by the mutants with our behavioral data in the elevated plus-maze, we previously proposed that the Lurcher were not really less anxious than the control mice but were actually disinhibited in comparison with them when confronted to aversive stimuli [8]. In our opinion, this proposition, referring to the neurobiological mechanism underlying behavioral inhibition as described by Gray and McNaughton [18], translated the behaviors of

* Corresponding author. Tel.: +33 2 35 14 67 22; fax: +33 2 35 14 63 49.
E-mail address: thomas.lorivel@gmail.com (T. Lorivel).

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mutants in a more precise and restrictive way than the idea of a simple "low anxiety", incompatible, in addition, with high stress.

Corticosterone is not just an index of stress in animals; it is thought to be crucial in coping with the energetic consumption and the homeostatic imbalance linked to the emotions-related behaviors [19]. Furthermore, it would play a crucial role in the consolidation [20,21] and the extinction [22] of conditioned fear as well as in the ontogeny and spontaneous expression of freezing (immobility exhibited in response to a threat) [23–25]. The whole of these effects would be mediated by the mineralocorticoid and glucocorticoid receptors localized in brain structures involved in emotions (i.e. the hippocampus, the septum or the amygdala) [26]. In addition to the genomic delayed action of corticosterone resulting from the activation of these receptors, some evidence suggests that the compound might also promote some anxiety or fear-related behaviors very quickly [26–28]. Giving these potential rapid effects of corticosterone on behaviors, we proposed that the "disinhibition" of the Lurcher mice could be paradoxically due to the hypersecretion of the hormone following the exposure to the novelty of the experimental conditions [8]. If it was confirmed, this hypothesis would mean that the implication of their cerebellar damages for their emotional alterations would be, at best, only indirect.

Up to now, no data permitted to test the possible causal influence of the physiological special feature of the mutants on their behavioral alteration. We therefore decided to complete our previous studies by investigating the influence of the corticosterone level raise on the anxiety-related behaviors in Lurcher and control mice. For this purpose, we compared the effects of 2-methyl-1.2-di-3-pyridil-1-propanone (metyrapone), an inhibitor of the corticosterone synthesis, on the behaviors of Lurcher and control mice in the elevated plus-maze test.

2. Material and methods

All the experiments were conducted in agreement with the ethical recommendations of the European Community Council directive 86/609/EEC.

2.1. Animals

The mice used in this study were heterozygous Lurcher (+/Lc; $n=18$) and control (+/+; $n=21$) males of the same strain (B6CBA), born in our laboratory (3 months old +/− 10 days at the beginning of the experiments). Mutants and non-mutants came from the same litters, +/+ females being crossed with +/Lc males. Weaned by the age of 21 days, they were housed 5 per cage and were bred under standard conditions: 12 h light (00 h00–12 h00)/12 h dark (12 h00–00 h00), 20–22 °C, water and food *ad libitum*.

2.2. Drug and injections

Metyrapone, purchased from SIGMA-ALDRICH, was dissolved in Tween 80 (5% in saline, SIGMA-ALDRICH), which alone served as vehicle control. For each genotype, one group of mice received an i.p. injection of metyrapone (+/Lc-mety; $n=10$/+/+-mety; $n=10$) at the dose of 75 mg/kg and a second group an i.p. injection of Tween (+/Lc-Tween; $n=8$/+/+-Tween; $n=11$). After being injected, each animal returned to its homecage for 90 min before being submitted to the elevated plus-maze test. This waiting time as well as the dose of metyrapone administered was determined in order to obtain an effective corticosterone synthesis inhibition during the behavioral testing [29,30].

2.3. The elevated plus-maze test

The elevated plus-maze device was made of painted wood and consisted in two open (28 cm × 5 cm) and two enclosed arms (28 cm × 5 cm) extended from a central platform (5 cm × 5 cm), the arms of the same kind facing each other. The whole apparatus, screwed to a pole, was elevated to a height of 50 cm above floor level and was indirectly lit (20 lux). An edge 1 mm in height was added to the perimeter of the open arms in order to avoid falls of the ataxic mice. At the beginning of the test, each mouse was individually placed in the central area of the device, facing an open arm, and the following parameters were recorded for 10 min: the number of open, enclosed and total arm entries and the time spent in the different parts of the device (center, open and enclosed arms). As in most studies [8,9,15], the 4-paw criterion was used to count the number of entries in each arm (mice had to have their four paws in the considered arm). The percentage of open arm entries was calculated as follows: (open arm entries/total arm entries) × 100. We also considered the number of stretched attempt postures (SAPs). During SAPs, mice stretch forward their body in order to scan their environment then retract it to the original position. This behavior is considered as an ethological index of anxiety, sometimes divided in protected SAPs achieved from the enclosed arms or the center of the device and unprotected SAPs achieved from the open arms [31]. Because the number of unprotected SAPs was very rare in our experiments (and did not have specific impact on the statistical results), we chose not to differentiate them from the protected SAPs. So, only the total number of SAPs achieved by the mice is mentioned in the Results section. The 10 min duration chosen for the plus-maze test in the present study was aimed to allow comparisons with our previous work about alterations of anxiety in Lurcher mice [8]. We however wish to point out that data obtained after 5 min (a more traditional duration for this test) were in compliance with the main results reported hereinafter. All experimental sessions were conducted during active phase of animals (between 2:00 pm and 6:00 pm) and were videotaped by a camera placed 50 cm above the center of the maze. Videotapes were later scored blind by using an ethological software: Etholog [32].

2.4. Corticosterone level assay

The plasma corticosterone level of each animal was measured after it was submitted to the elevated plus-maze test. The mice were decapitated immediately after the completion of the test and their blood was tipped into hemolysis tubes containing 30 µl of 5% EDTA.

The blood samples were centrifuged (4 °C, 5000 rpm, 20 min) and plasma was taken and stored at −20 °C until determination of the corticosterone rate. After its extraction in absolute ethanol, the corticosterone concentration was measured by radioimmunoassay as previously described by Leboulenger et al. [33].

2.5. Statistical analysis

Results were analysed by a two-way ANOVA (genotype × compound) and subjected to post-hoc comparisons with Fisher's Least Significance Difference test. $P<0.05$ was chosen as threshold of significativity.

3. Results

3.1. Corticosterone levels (see Fig. 1)

The genotype of the mice ($F(1,35)=28.61$, $p<0.001$) and the compound injected ($F(1,35)=14.86$, $p<0.001$) exerted significant influences on the corticosterone levels measured (Fig. 1). These levels were more elevated in the mutants than in the non-mutants, whether mice were administered with Tween ($p<0.001$) or metyrapone ($p<0.01$). Coherently with its reported effects, metyrapone injection lowered the rate of corticosterone in both genotypes (+/+: $p<0.05$; +/Lc: $p<0.01$). This lowering was similar in +/+ and +/Lc so that no significant interaction was detected between genotype and compound