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Report

Assessment of Two Unknown Compounds in Depression-like and Anxiety Behaviour in Wild-Type SWISS mice

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Abstract

Major depressive disorder (MDD) and generalised anxiety disorder (GAD) are among the most common mental disorders today, which affect approximately 5% of the population worldwide, making them a leading cause of disability and mortality. Their prevalence increased by 25% after pandemic stressors by creating a conducive environment for poor mental health conditions [1,2]. MDD and GAD are characterised by anhedonia, loss of pleasure, depressed mood, loss of interest, and fatigue over a prolonged period of at least two weeks [3,4]. Although our understanding and treatment of MDD and GAD has improved over the last decade, several people are or have become drug resistant, leading to relapse and recurrence of the diseases. Thus, new treatment options and a better understanding of the pathology are needed, and research in this area remains highly relevant. In this study, we examine the therapeutic potential of two unknown compounds in a murine model using three established behavioural paradigms of depression in mice: Open Field test, Elevated Plus Maze, and Tail Suspension test. These tests have shown to be robust in assessing depression-like and anxiety-like behaviour in mice and will allow to infer the effect of the two treatments [5,6,7,8].

Introduction

Depression is the second most common psychiatric disorder in the world, which is characterised by the persistence of low mood, anhedonia, and cognitive dysfunction that interferes with the patient's daily life [9]. Despite progress in understanding clinical features and treatment, a significant proportion of patients do not respond to medication [10]. For this reason, animal models, especially rodents, have continued to be used and refined to study the underlying mechanisms of depression and to select potential therapeutic compounds. Although no model can fully replicate human depression, tests such as Elevated Plus Maze (EPM), Open Field Test (OFT), and Tail Suspension Test (TST) are widely used to assess the depression-like behaviour and prove their usefulness in testing neuropharmacological effects of drugs [11].

In this paper, we report our laboratory experiments on depression- and anxiety-like behaviour in rodents. The substances were administered in a blind design—neither the identities nor the expected effects of A and B were known at the time of testing. By using established behavioural tests with EPM, OFT, and TST, we sought to observe the responses of the mice to each treatment and thus to deduce the potential effects of these substances. Our aim was to identify the behavioural patterns associated with each solution and to speculate on the potential identity or pharmacological action of the unknown compounds. We will first describe our experimental setup and materials, followed by observed results and discussion of the likely identity and effects of the two solutions. A brief consideration of limitations will also be given, followed by a conclusion.

Materials

In this experiment, we used SWISS mice aged approximately one month. A total of 96 mice were given to 16 groups of students from the University of Toulouse, with six mice per group. In each group, three mice were assigned to OFT, three to EPM, and all six were tested in TST. Mice were injected intraperitoneally with either NaCl (control), compound A, or B, whose identity was unknown to the experimenters. The amount of solution injected was adjusted based on each mouse's body weight at a concentration of 0.1 mL per 10 g. To ensure correct identification of each mouse and its corresponding injected solution, tails were marked with specific symbols (see Fig. 1). Three mice were excluded from the data set due to their abnormal performance, since they can potentially bias the results of the test. TST data from four mice were corrected due to errors in data collection, as confirmed by other experimental groups. All procedures were carried out under the supervision of experienced

researchers to guarantee animal welfare and respect for ethical guidelines for handling laboratory animals.

	Weight	Qte	Ident	
Mice 1	29	0,29		Substance A
Mice 2	30,2	0,30		Substance A
Mice 3	27	0,27		Substance B
Mice 4	28,1	0,28		Substance B
Mice 5	29,9	0,30		NaCl
Mice 6	30,7	0,30		NaCl

Fig. 1. Example of mouse identification marking and corresponding injection volumes used by the authors' group

Experiment protocol

The experiment followed a fixed timeline for the injection and behavioural testing. Both solutions were administered at least 30 minutes prior to behavioural assessment, except for NaCl groups, which could be injected at the beginning of the experiment. Here we proposed the timeline that was used by our group. At t_0, mice 5 and 6 received NaCl. Thirty minutes later (t_{30}), the remaining mice were injected with their respective test solutions, either A or B.

Behavioural tests were scheduled as follows:

- Between t_{30} and t_{60}: mouse 5 was tested in EPM, while mouse 6 was in OFT
- Between t_{60} and t_{90}: mouse 1 was tested in the EPM, and mouse 2 in the OFT
- From t_{90} onward: Mouse 3 was tested in the EPM, and Mouse 4 in the OFT

Once individual tests were completed, all six mice were tested for TST.

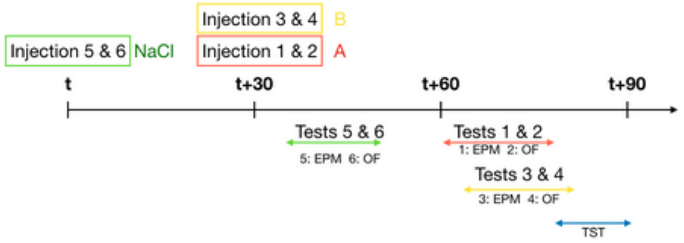

Fig. 2. Procedural timeline used by authors' group. Animals received either substances or saline injection at timepoint t (minutes), followed by behaviour testing (EPM, OF, and TST). Different groups may conduct differently but follow the strict protocol of injection 30 minutes before testing.

Behavioural evaluations

In this study, we used three behavioural assessments: OFT, EPM, and TST (Fig. 3). The OF test was used to assess general locomotor activity and anxiety-like behaviour. Mice were placed in a circular arena with four quarters and a centre. We measured the time spent in the centre, corresponding to the anxiogenic zone, the time spent in the periphery, corresponding to the safe zones, and the number of quadrants the mice crossed. Mice that remained close to the periphery were considered to exhibit higher anxiety levels, whereas increased time in the centre was seen as a sign of low anxiety.

The second test is EPM, which consists of two open arms and two closed arms raised above the floor. Each mouse was placed in the middle of the cross. We monitored the time and number of entries into both open and closed arms. If the mouse spends more time in the open arms, it is considered less anxious, as these arms are seen as riskier and generally avoided by anxious mice.

The final test is TST, which measures depressive-like behaviour, specifically behavioural resignation. Mice were suspended by their tails for six minutes. We recorded the total duration of immobility and the latency to the first immobility. A longer duration of immobility is an indicator of a lack of escape behaviour and is similar to a depressive state.

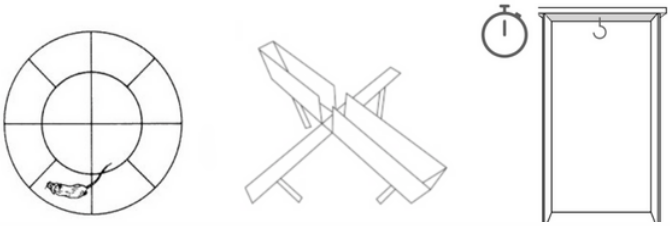

Fig. 3. Visualisation of paradigms (left - right): OFT, EPM, TST

Results

We set the significance level at $\alpha = .05$. The Shapiro-Wilk test indicated that the NaCl group deviated significantly from normality in all three behavioural tests ($p<.05$). In addition, the assumption of homogeneity of variances was violated in the TST. Because of these violations of parametric assumptions, we decided to use non-parametric statistical analysis.

Elevated Plus Maze test (EPM)

The descriptive analysis shows that the mice in solution A entered more frequently and spent more time in the open arms compared to control and solution B groups (Fig. 4). Nevertheless, the Kruskal-Wallis H test shows no statistically significant difference between groups, with $\chi^2 = 5.84$, $p = .054$. Although the result did not reach

statistical significance, it approached the conventional threshold.

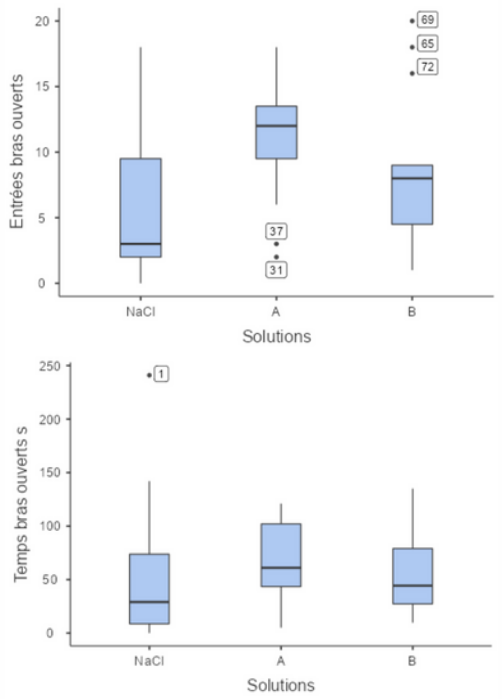

Fig. 4. Results of EPM test with the number of entries in open arms in accordance to each group (n = 43), with indication of outliers.

Open Field Test (OFT)

In OF test, the time spent in the centre is crucial for defining the depressive-like behaviour. Overall, in our data, the box plot shows no clear difference in the time spent in the centre nor the periphery across three groups (Fig. 5). The number of quadrant crossings, which represents locomotor activity, shows little variation between groups (Fig. 6). A further Kruskal-Wallis test confirmed that there was no statistically significant difference in the time spent in the centre between the groups, χ^2=0.502, p=.778, as well as the frequency of crossing quadrants, χ^2=3.00, p=.223.

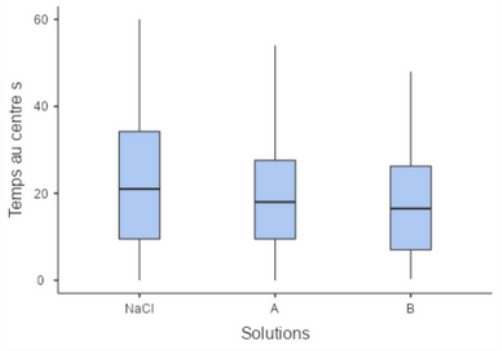

Fig. 5. OFT results for each injected group (n = 47) with time spent in the centre and the periphery of the arena over the 5-minute test duration.

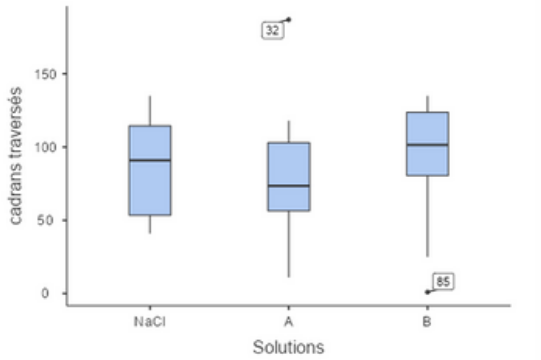

Fig. 6. OFT results for each injected group (n = 47) indicating the number of quadrant crossings during the full test duration. Data is shown with outliers.

Tail suspension test (TST)

More pronounced differences were observed between groups of treatment. Mice treated with solution A showed a shorter latency to first immobility and a higher total immobility time compared to the NaCl group, while the opposite trend was observed for solution B. Statistical analyses support these observations; a Kruskal-Wallis test revealed significant group differences in both latency to first immobility ($\chi^2(2) = 17.8$, p<.001, ε^2=0.196) and total immobility time ($\chi^2(2) = 30.3$, p<.001, $\varepsilon^2 = 0.333$). Post-hoc analyses showed that Solution A led to a shorter latency and longer immobility time compared to NaCl and Solution B groups (p<.05). Meanwhile, solution B showed the opposite patterns (p<.05).

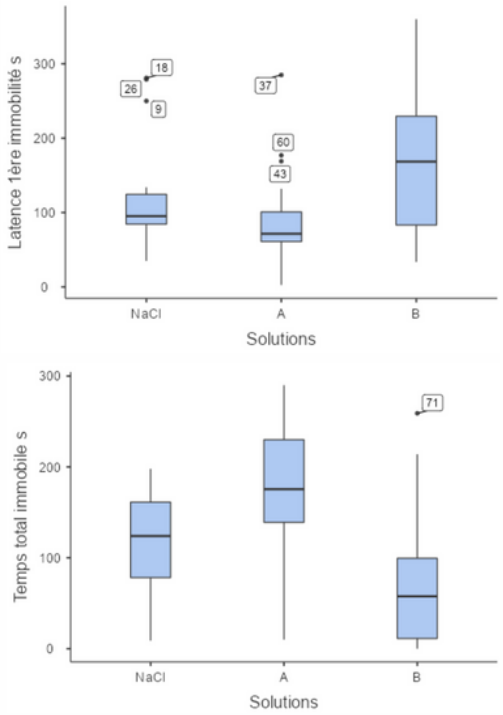

Fig. 7. Results of TST (n = 92). The first immobilisation was defined when mice stopped moving for at least three seconds. Data is shown with outliers.

Discussion

To estimate the potential impact of our two novel substances, we used a cohort of 93 control wild-type SWISS mice aged one month administered with intraperitoneal injection of either a control physiological saline solution or with one of the two drug solutions at a concentration of 0.1 mL/10 g of body weight. Mice were tested using three widely employed behavioural paradigms commonly used for assessing depressive-like and anxiety-like behaviours in rodents. The EPM and OFT allowed us to gauge the locomotion and anxiety behaviour by measuring both time passed in the secure areas (closed arms for the EPM and close to the edge in the periphery for the OFT) and the amount of crossing of mice from one zone to another. Meanwhile, TST was used to assess behavioural resignation, hence the depressive state of mice, by quantifying the delay for the first immobilisation and the total time passed unmoving during the full course of the test.

We found that in the TST, mice injected with solution A passed significantly more time immobile ($p<0.01$) and shorter latency to first immobility ($p=0.034$) compared to the control group NaCl. Contrarily, mice administered with solution B were significantly half as immobile as control mice ($p=0.007$) and had a greater latency for their first immobilisation ($p=0.026$) compared to control mice. These findings suggest that compound A has an anxiolytic effect on mice, while compound B would be stimulating and having antidepressant effects on mice.

Our results align with previous studies using various antidepressants such as tricyclic antidepressants imipramine and desipramine, as well as selective serotonin reuptake inhibitor paroxetine or citalopram and dopamine reuptake inhibitor bupropion, all of which have been shown to significantly reduce the time spent immobile in the SWISS strain in TST [12,13].

Nevertheless, these two results are not significant in the two other paradigms. In EPM, while mice injected with solution A tend to visit open arms more often compared to control mice, it fails to reach significance, and neither of the three other parameters showed a difference with control mice.

Mice injected with solution B also showed no significant contrast in their variables compared to control mice. Additionally, OFT proved to have the least significant results of all the behavioural paradigms used, with highly similar results for all conditions. This suggests an absence of any measureable anxiolytic or exploratory effects in this paradigm. Thus, we cannot draw any conclusions from these experiences about the outcome of the inoculation of the two drugs in depressive-like and anxiety behaviours.

Although all experiments aimed to maximise the peak threshold for the drug's effect, poor reactions from the separate groups of mice may have resulted from the concentrations of compounds A and B being too low to trigger behavioural changes and from potential faulty intraperitoneal injections performed by inexperienced personnel despite being supervised by professional researchers. Another limitation of our study might originate from the time of experiments, as all behavioural tests were performed from 10 a.m. to 4 p.m., yet it is established that mice's preferred activity period is set during the night and ends early in the day, as shown by several experiments using free wheel running quantification during the light/dark cycle [14].

Even though compounds A and B might have shown potential for treating depressive-like behaviour, more research is needed to rationalise their effects, whether positive or negative. On that account, we propose to re-examine the consequences of administering these drugs in other cohorts of mice with different concentration gradients in order to best inspect their effective dose as well as to investigate the behavioural response of mice in another valid behavioural paradigm for depressive-like behaviour in mice, such as the Porsolt forced swimming test [15,16], and in a distinct group of mice with and without depressive-like states, such as injection in control wild-type mice and chronically stress-induced mice to compare the impact of the compound in each case as antidepressant drugs are meant and prescribed for people with MDD and GAD.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships and without any conflict of interest with other researchers.

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