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A proposal for refining the forced swim test in Swiss mice

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ABSTRACT

The forced swim test (FST) is a preclinical test to the screening of antidepressants based on rats or mice behaviours, which is also sensitive to stimulants of motor activity. This work standardised and validated a method to register the active and passive behaviours of Swiss mice during the FST in order to strength the specificity of the test. Adult male Swiss mice were subjected to the FST for 6 min without any treatment or after intraperitoneal injection of saline (0.1 ml/10 g), antidepressants (imipramine, desipramine, or fluoxetine, 30 mg/kg) or stimulants (caffeine, 30 mg/kg or apomorphine, 10 mg/kg). The latency, frequency and duration of behaviours (immobility, swimming, and climbing) were scored and summarised in bins of 6, 4, 2 or 1 min. Parameters were first analysed using Principal Components Analysis generating components putatively related to antidepressant (first and second) or to stimulant effects (third). Antidepressants and stimulants affected similarly the parameters grouped into all components. Effects of stimulants on climbing were better distinguished of antidepressants when analysed during the last 4 min of the FST. Surprisingly, the effects of antidepressants on immobility were better distinguished from saline when parameters were scored in the first 2 min. The method proposed here is able to distinguish antidepressants from stimulants of motor activity using Swiss mice in the FST. This refinement should reduce the number of mice used in preclinical evaluation of antidepressants.

1. Introduction

The forced swim test (FST) in rats or mice is pre-clinically employed to evaluate drugs being screened for putative antidepressant activity (Castagné et al., 2009; Porsolt et al., 1977, 1978). The FST is quick test to run, very reliable across laboratories, sensitive, and relatively selective for antidepressant drugs (Cryan et al., 2005; Petit-Demouliere et al., 2005). The original protocol of the FST (Porsolt et al., 1977, 1978) consisted of placing the animal into a receptacle filled with water once (mice) or twice (rats) while recording the amount of time spent in a posture of immobility. Mice or rats, after 2 min of vigorous struggle, adopted a typical posture of immobility (floating in the water making only the slight movements necessary to keep the head above the water), alternated with swimming or paddling movements (Porsolt et al., 1977, 1978). This stress-induced failure in escape performance was named "behavioural despair" and has been consistently prevented by the treatment of rats or mice with different types of antidepressants (Porsolt et al., 1977, 1978).

Modifications of the catalogued behaviours, scoring methods and protocols have improved the predictive validity of the FST in rats (Borsini et al., 1989; Cryan et al., 2005; Dal-Zotto et al., 2000; Detke et al., 1997) and could suggest an approach to develop a more robust FST in mice. A main modification of the FST in rats was the analysis of active behaviours in addition to immobility (Cryan et al., 2005; Detke et al., 1997; Lucki, 1997; Vieira et al., 2008). Indeed, it was reported that antidepressants in general reduced rats' immobility posture, whereas norepinephrine selective reuptake inhibitors of (NSRIs) increased climbing and selective serotonin reuptake inhibitors (SSRIs) increased swimming (e.g. Detke et al., 1997). These changes of the FST in rats enabled discrimination between different types of antidepressants (Cryan et al., 2005; Detke et al., 1997; Lucki, 1997). Moreover, in rats a tricyclic antidepressant increased the duration of climbing, whereas a stimulant of motor activity (caffeine) increased the frequency of climbing (Lino-de-Oliveira et al., 2005; Vieira et al., 2008). These changes of the FST in rats enabled exclusion of the confounding effects of motor stimulants in FST itself (Kitada et al., 1981; Lino-de-Oliveira et al., 2005; Vieira et al., 2008). In

Abbreviations: ANOVA, Analysis of Variance; CD-1, Outbred Strain of mice from Charles River Laboratory; CEUA, Ethics Committee on Animal Use; C57Bl6/J, Inbred Strain of mice from The Jackson Laboratory; EV, Eingenvectors; FST, Forced Swimming Test; MANOVA, Multivariate Analysis of Variance; NMRI, Outbred strain of mice from Charles River Laboratory; NSRIs, Norepinephrine Selective Reuptake Inhibitors; PCA, Principal Component Analysis; S.E.M., Standard Error of the Mean; SSRIs, Selective Serotonin Reuptake Inhibitors.

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mice, active and passive behaviours during the FST is currently utilised to study several potential antidepressant compounds and to develop hypotheses about their mechanisms of action (Nguyen et al., 2013; Perona et al., 2008; Sanmukhani et al., 2011; Szewczyk et al., 2009). The specificity for the effects of antidepressants in the FST in mice has been verified only by an additional open field test to discard motor confounds (David et al., 2003; Lucki, 1997; Petit-Demouliere et al., 2005).

Improvement of the FST in mice might facilitate the study of relevant new molecular targets of potential antidepressants because genetically engineered strains (transgenic or knockouts, e.g. Easton et al., 2003) are more often available in this animal species than they are in rats. A comprehensive review of the FST in mice (Petit-Demouliere et al., 2005) mentioned a number of procedural aspects that should be taken into account to accurately employ the test. Experimental alterations in cylinder diameter, water depth, water temperature, the interval between treatment and test, the treatment schedule and scoring methods are all sources of data variability and inter-laboratory variation (Petit-Demouliere et al., 2005). Housing conditions (enrichment or isolation (Guo et al., 2004; Voikar et al., 2005; Xu et al., 2009), circadian rhythm (Easton et al., 2003), dietary factors (restriction or ad libitum) or previous experience in the cylinder (e.g. Alcaro et al., 2002) modify the behaviour of the mice. In addition, animal-specific characteristics such as gender (Guo et al., 2004), age (David et al., 2001) and strain (Alcaro et al., 2002; David et al., 2003; Lucki et al., 2001) affect the amount of time spent immobile and therefore affect the evaluation of antidepressant effects. In contrast to the unpredictable responses across inbred strains, treatment with a range of different antidepressants reduces the immobility of Swiss or Swiss-derived mice (see, e.g., CD-1 and NMRI) during the FST (David et al., 2003; Lucki et al., 2001; Petit-Demouliere et al., 2005), indicating that this strain is valuable for the preclinical selection of monoaminergic antidepressants (Bourin et al., 2005). Indeed, applying the FST in Swiss mice was suggested as the first step in investigating new potent antidepressant drugs before searching for downstream mechanisms of action (Bourin et al., 2005).

Hence, the main aim of the present work was to characterise the temporal and factorial profile of active and passive behaviours of male Swiss mice in the FST to identify variables useful in discriminating between different antidepressants and to distinguish them from stimulants of motor activity. If the scorings of active and passive behaviours during the FST explained most of the variability in the response of Swiss mice to drug treatment, it would be possible to use them to discriminate between drugs. Therefore, the effects of antidepressants (imipramine, desipramine, and fluoxetine) and stimulants of motor activity (caffeine and apomorphine) on several passive and active behaviours of Swiss mice during the FST were compared. In addition, behavioural latencies were recorded because, in NMRI and C57BL6/J mice, latency to immobility seems to be a useful parameter for differentiating antidepressants from stimulants of motor activity (Castagné et al., 2009).

2. Methods

2.1. Animals

Male Swiss mice, 90 days old, were kept in groups of 10 per cage (33 cm × 40 cm × 18 cm) under controlled lighting (12 h light/dark cycle with lights on at 07:00 am) and temperature (22 ± 2 C) conditions with free access to food and water, except during the behavioural test. The animals were provided by UFSC animal facilities. All experiments were carried out in accordance with the principles of ethics and animal welfare recommended by Brazilian Law (# 11.794 — 10/08/2008) and the procedures approved by the local Ethics Committee on Animal Use (CEUA/UFSC # 23080.024594/2010-87). All efforts were made to minimise suffering and to reduce the number of animals used in the experiments.

2.2. Experimental design: descriptive behaviour analysis during the FST (Experiment 1) and the effects of antidepressants on Swiss mice during FST (Experiment 2)

In Experiment 1, the FST (Duarte et al., 2006, 2007; Gavioli et al., 2003a, 2003b; Hellion-Ibarrola et al., 2008; Herrera-Ruiz et al., 2006) consisted of individually placing the mouse into a cylindrical tank (height 18.5 cm, diameter 12.5 cm) containing clean water at 25 C (13.5 cm deep). After the test (6 min), the mice were taken out of the water and allowed to dry under a lamp (40 W, 15 min) before being returned to their home cages. The experimental room was illuminated by indirect red light (15 W). The FST took place between 1:00 and 6:00 p.m. All test sessions were videotaped using an infrared video camera (GeoVision Inc. GV-800 system, Taipei, Taiwan) located 20 cm above the tank, to enable subsequent evaluation of the latency, frequency of episodes and amount of time spent in swimming, climbing and immobility. Latency was defined as the amount of time that elapsed between placing the mouse in the tank and the first instance of each behavioural occurrence. Frequencies represent the number of incidences of each type of behaviour, while duration reflects the total time spent in all bouts of that behaviour within a given period. Immobility was defined as a lack of motion of the whole body, when mice ceased struggling and remained floating motionless in the water, making only those movements necessary to keep the head above water. Swimming was recorded when large and horizontal movements of the forepaws were performed, leading to displacement of the body around the cylinder. Climbing was recorded when vigorous vertical movements of the forepaws, directed against the wall of the tank, were displayed, leading to displacement the body around the cylinder. These parameters were recorded and summarised in either one block of 6 min (i.e., the total time of the test), or in one block of 4 min (i.e. the last 4 of the test as previously proposed, e.g. (Petit-Demouliere et al., 2005) or in the remaining block of 2 min (the first 2 min of the test). An additional minute-by-minute description provided a temporal distribution of each parameter and was analysed using a MANOVA ($p < 0.05$).

In Experiment 2, mice were tested in the FST (as described before) 30 min after intraperitoneal (i.p.) treatment with saline (NaCl 0.9%, 0.1 ml/10 g of body weight), antidepressant drugs (imipramine, desipramine, and fluoxetine, 30 mg/kg) or caffeine (30 mg/kg) ($n = 10$ mice/group). These doses were chosen according to the literature (Bernardi et al., 1989; Steru et al., 1987; O'Neill et al., 1996; Rodrigues et al., 2002). The behavioural parameters were recorded by experimenters blind to treatment and are summarised as follows: in one block of 6 min, in one block of the last 4 min, in one block of the first 2 min, or minute-by-minute.

2.3. Statistical analysis

2.3.1. Principal components analysis

Parameters summarised in one block of 6 min of Experiment 1 were submitted to Principal Components Analysis (PCA) followed by orthogonal Varimax rotation (Statistical Package®, 1995) was performed as published by (Espejo, 1997; Lino-de-Oliveira et al., 2005; Mezadri et al., 2011). A component was defined by an Eigenvalue greater than one. Eigenvectors (EVs) of ±1 indicate a perfect correlation of the variable with the component. EV values ranging from ±0.40 to ±0.60 indicate a moderate correlation, and values lower than ±0.40 indicate a poor correlation. On the basis of the EV values (EV > ±0.4), variables were divided into groups of putatively similar parameters. Positive EVs indicate that a behavioural parameter correlates with the corresponding component. A negative EV value indicates that the behavioural variable is inversely correlated with the component. Multivariate analysis requires the number of animals to be at least three times the number of variables. Therefore, PCA was applied only on the data of Experiment 1. Shapiro–Wilk's W and Levene's tests were used to check the normality and the homogeneity of variance, respectively.

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