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Automation of the free-exploratory paradigm

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ABSTRACT

The free-exploratory paradigm (FEP) is currently the only proposed animal model of trait anxiety, making it highly valuable to behavioural neuroscience. However, FEP has not yet been automated, so its results depend on human scoring, which can be quite imprecise. The aim of this study was, therefore, to validate an automated version of FEP, using a commercially available video-tracking system (ANY-maze© – Stoelting Co., USA). To achieve this, two experiments were performed. The first one evaluated the reliability of the video-based automation of FEP, and the second, assessed whether the zeolites, used as a bedding material in the first experiment to facilitate video-tracking, influenced the animals' behaviour in FEP. In experiment I, 15 drug-naive, adult, male rats were tested in FEP, while their behaviour was simultaneously evaluated by ANY-maze© and two human observers. Subsequently, the intraclass correlation coefficient (ICC) was calculated for the automated and manual results of the parameters "percentage of time in the novel side" (%TNS) and "total units visited" (TUV). The analysis resulted in high, significant values of ICC (%TNS: 0.9962 and TUV: 0.9453). In experiment II, 18 drug-naive, adult, male rats were allocated to two different groups: (1) tested in FEP with zeolites; and (2) tested in FEP with sawdust. The data obtained were analysed using the Student's *t*-test, which revealed no significant differences between the groups for the parameters %TNS and TUV. In conclusion, the data presented here show that automation of FEP, using a video-based tracking system, is not only possible, but also highly reliable.

1. Introduction

The free exploratory paradigm (FEP) has been proposed as a model of trait anxiety. In this situation, animals are given the opportunity to move around freely within an environment containing both familiar and novel parts. This approach allows the evaluation of neophobic responses. As the animals have a choice between novelty and familiarity, it is expected that individuals with low trait anxiety will exhibit a preference for novelty, whereas high trait anxiety subjects will prefer familiarity (Griebel et al., 1993). This free choice paradigm was first described by Hughes (1965, 1968), who observed that Wistar rats actually preferred the novel environment, spending more time in it. Subsequently, Griebel et al. (1993), compared two strains of mice, BALB/c and C57BL/6, known respectively as "emotional" and "non-emotional", and observed that BALB/c mice presented a marked preference for the familiar environment, while C57BL/6 mice exhibited a preference for novelty, indicating that FEP could differentiate traits of anxiety. Furthermore, it has been shown that there is no change in state anxiety during this test situation. Misslin and Cigrang (1986) and Misslin et al. (1982) observed that Swiss mice did not present physiological signs of fear unless they were forced into the novel environment, while Belzung and Le Pape (1994), using a principal component analysis, demonstrated that variables measured in FEP were not described by the same factors as variables measured in models where the animals were confronted with anxiety provoking situations by being forced into a novel environment, i.e., models of state anxiety. In addition, FEP has proved to be stable over time (Teixeira-Silva et al., 2009), a sine qua non condition for any model proposing to measure trait anxiety, which, by definition, does not vary from moment to moment (Spielberger et al., 1970).

Taken together, these data strongly suggest that FEP is an animal model of trait anxiety and, to the best of our knowledge, it is the only model proposed as such; from which, one can gauge its importance to behavioural neuroscience. However, FEP is still dependent on human scoring, which is time-consuming and labour intensive and which can yield imprecise results, due to fatigue and inter-rater inconsistencies. An automation system could abolish these problems, as computer algorithms always work in the same way, without fatigue or bias and can score many apparatuses at the same time. Also, automation improves the evaluation of locomotor

Abbreviations: %TNS, percentage of time in the novel side; CTRL, control group; FEP, free-exploratory paradigm; ICC, intraclass correlation coefficient; TUV, total units visited; ZEO, zeolite group.

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