Measurement of Akinesia in Rats: Design and Validation of a Side Effect Paradigm in Freely Moving Animals

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ABSTRACT
In this paper we describe a method to assess akinesia, a side effect of antipsychotic drug treatment, in freely moving rats. Animals were placed on a circular platform and the latency to initiate exploration of the novel environment was recorded using image processing software. This was done by video tracking of the rat to determine time of first entry into a defined area, 15 cm away from the starting position. Vehicle treated rats immediately initiated exploration, while rats treated with antipsychotic drugs showed a dose-dependent increase in latency to start exploration. It is concluded that drug-induced akinesia can be accurately assessed in rats using the present setup.

Author Keywords
Akinesia, antipsychotics, rat, behaviour, video tracking.

INTRODUCTION
Apart from their therapeutic benefit, CNS-medications are also known to potentially induce extra pyrimidal side-effects such as catalepsy, akinesia etc ... [1]. Akinesia is the inability of a subject to smoothly initiate a voluntary movement. Drug-induced akinesia can be assessed in rodents in several ways, but is generally based on qualitative observations of animal behaviour. The purpose of the present study was to develop and validate a non-invasive set-up to obtain quantitative measures of akinesia induced by antipsychotics in freely moving unrestrained rats.

METHODS

Animals
Male Sprague–Dawley rats (Harlan, The Netherlands), weighing 230–260 g at the time of the experiment, were used. Rats were housed in individual ventilated cages (25cm×33cm×18 cm; Tecniplast) in groups of four. The animals were allowed to acclimate prior to the experiment for at least 7 days after receipt from the supplier. All animals were maintained under controlled environmental conditions throughout the study: 22±2 °C ambient temperature, relative humidity at 60%, with a 12/12 h light/dark cycle (6PM lights off, 6AM lights on). Food and water were available ad libitum.

Experimental Setup
The setup was designed and constructed with key principles such as ease of use, multi functionality, low cost and robustness. A stable circular black platform of 120 cm diameter was positioned 75 cm above ground level, in a room with low light intensity (1 lux). The centre of the platform was marked with a 2 cm^2 red spot. A video camera was mounted 165 cm above the platform.

Image Analysis
Video images were captured using Ethovison 3.0 (EV [2]) on a Windows PC. By means of the EV-software, the image of the platform area was divided into 4 circular areas with identical width (Figure 1). The position of a rat on the platform was detected in real time using a manually set grey-threshold in EV: the centre of gravity of the region of interest (=rat) was taken as the rat's actual position in each video frame. At the start of a recording session, a rat was gently placed in the middle of the platform on the central mark in area 1. When an object (=the rat) was detected by EV in this area 1, the recording automatically started after 0.5 sec to allow the experimenter to with draw his arm. Recording was programmed to stop when the rat was first detected in the outer area of the platform (area 4), or after 30 seconds.

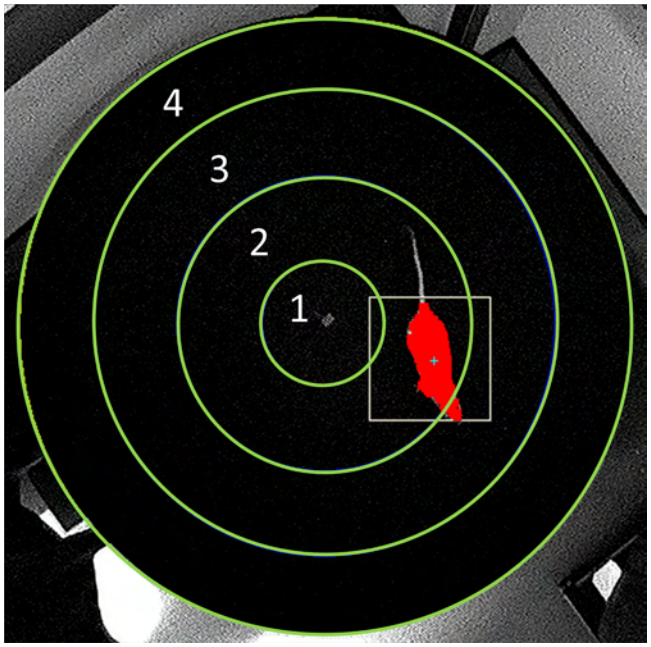

Figure 1. Top view of platform area showing the 4 circular sub-areas identified by the green lines, and the threshold image of a rat (red area). A white cross, that marks the centre of gravity in the threshold image, is taken as the actual position of the rat on the field.

Study Design

Each individual rat was evaluated in this setup just prior to administration of a dose of the test compound or its vehicle (t=0h), and after 0.5, 1, 2, 3 and 4 h. Tracking data from EV were stored on disk and analysed off-line: the primary parameter of interest was latency until first occurrence of the rat in area 2, which was considered as an indication that the rat had uninitiated a movement away from its starting position. In addition, also latency to first entry into area 3 and 4 respectively, total distance travelled, and total recording time were calculated.

RESULTS

Vehicle treated rats almost immediately started to explore the novel environment, and were detected in area 2 after a latency time of 0.7±0.1 to 1.1±0.4 sec (mean±SEM, n=25) during the first hour. A small increase in latency was observed at later time points (up to 9.0±2.6 sec at 4h). Haloperidol (0.01-0.63 mg/kg, n=5) and risperidone (0.08-2.5 mg/kg, n=5) showed a dose-dependent increase in latency time up to 30 sec during the 4h observation period, while MP10, a novel PDE10-inhibitor (0.16-10 mg/kg), showed a maximum of only 16 sec at 2.5 mg/kg (n=5).

CONCLUSIONS

The size of the starting area (area 1) was chosen such that the centre of gravity of the rat as identified by EV was detected in area 2 when the rat had made two or three steps away from the centre of the platform. This occurs almost immediately in control rats, as they will voluntary explore a novel environment [3]. This initiating behaviour was further facilitated by the low light intensity environment. This allowed an accurate assessment of the inability of a rat to initiate a movement as is the case in drug-induced akinesia. The present results show that drug-induced akinesia can be accurately assessed in rats using the present setup, and that novel therapies with a more favourable side effect can be identified. Whether akinesia elicited under these conditions involves motor deficits or impairment of motivation remains to be elucidated.

Ethical Statement

The study was performed in strict accordance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) and was approved by the Institutional Ethical Committee.

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