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Research article

Pharmacological validation of individual animal locomotion, temperature and behavioural analysis in group-housed rats using a novel automated home cage analysis system: A comparison with the modified Irwin test

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ABSTRACT

Background: The ActualHCA™ system continuously monitors the activity, temperature and behavior of group-housed rats without invasive surgery. The system was validated to detect the contrasting effects of sedative and stimulant test agents (chlorpromazine, clonidine and amphetamine), and compared with the modified Irwin test (mIT) with rectal temperature measurements.

Methods: Six male Han Wistar rats per group were used to assess each test agent and vehicle controls in separate ActualHCA™ recordings and mIT. The mIT was undertaken at 15, 30 mins, 1, 2, 4 and 24 h post-dose. ActualHCA™ recorded continuously for 24 h post-dose under 3 experimental conditions: dosed during light phase, dark phase, and light phase with a scheduled cage change at the time of peak effects determined by mIT.

Results: ActualHCA™ detected an increase stimulated activity from the cage change at 1-2 h post-dose which was obliterated by chlorpromazine and clonidine. Amphetamine increased activity up to 4 h post-dose in all conditions. Temperature from ActualHCA™ was affected by all test agents in all conditions. The mIT showed effects on all 3 test agents up to 4 h post-dose, with maximal effects at 1-2 h post-dose. The maximal effects on temperature from ActualHCA™ differed from mIT. Delayed effects on activity were detected by ActualHCA™, but not on mIT.

Conclusions: Continuous monitoring has the advantage of capturing effects over time that may be missed with manual tests using pre-determined time points. This automated behavioural system does not replace the need for conventional methods but could be implemented simultaneously to improve our understanding of behavioural pharmacology.

1. Introduction

Neurobehavioral assessments of drugs in rodents provide insights into pharmacological effects on the central nervous system (CNS) and are often conducted using manual measurements at pre-selected time points. Automated methods of monitoring locomotor activity and behavior in laboratory rats have been emerging that can continuously assess the responses to test agents (Alexandrov, Brunner, Hanania, & Leahy, 2015; Dunne, O'halloran, & Kelly, 2007; Van de Weerd et al., 2001). Automated systems have advantages over conventional manual observations that are often susceptible to observer bias, are of shorter duration and performed during the light phase only (Alexandrov et al.,

Abbreviations: ActualHCA™, Actual Home Cage Analyzer; AUC, Area under the curve; CNS, Central nervous system; mIT, Modified Irwin test; RFID, Radiofrequency identification; % MPS, Percentage of maximum possible score

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Table 1
A study design table, illustrating the different experimental objectives, tested by ActualHCA™ and the conventional modified Irwin test.

Test agent	Animal cohort	ActualHCA™ system	Animal cohort	Modified Irwin test
Chlorpromazine	1	Dosed and assessed on 3 different experimental conditions: "Light phase", "dark phase" & "cage change"	4	Dosed and assessed conventionally
Clonidine	2	Dosed and assessed on 3 different experimental conditions: "Light phase", "dark phase" & "cage change"	5	Dosed and assessed conventionally
Amphetamine	3	Dosed and assessed on 3 different experimental conditions: "Light phase", "dark phase" & "cage change"	6	Dosed and assessed conventionally

A table illustrating the study design and the cohort of animals used for each experiment and each test agent. The objective was to compare the capability of ActualHCA™ vs. the conventional modified Irwin test, in detecting functional effects of test agents with known pharmacological effects on the CNS in group-housed rats.

2015; Dunne et al., 2007; Van de Weerd et al., 2001). However, both these and more conventional locomotor activity systems require the use of single-housing (Redfern et al., 2017). In contrast, modern laboratory practices have moved away from single-housing rats as it affects their behavior and welfare (Balcombe, 2006). Moreover, automated methods such as continuous temperature monitoring require invasive surgical implantation of radiotelemetry transmitters (Ansah, Wade, & Shockley, 1996; Bishop et al., 2001; Deveney, Kjellström, Forsberg, & Jackson, 1998; Harkin, O'Donnell, & Kelly, 2002; Ossenkopp, Rabi, Eckel, & Hargreaves, 1994). A recent approach that mitigates these concerns is the ActualHCA™ system (Actual Analytics, UK), which was developed as part of the "Rodent Big Brother project" funded by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), UK, to continuously monitor the activity and temperature of individual rats simultaneously when group-housed in their home cage environment on a cage rack, without the need for invasive surgery (Redfern et al., 2017).

The present study was conducted to validate the ActualHCA™ system by assessing its ability to detect changes in activity, temperature and behaviors in response to stimulant and sedative test agents, and to compare the results to the conventional manual approach: the modified Irwin test. The Irwin test is a comprehensive, systematic qualitative observational assessment that was introduced to evaluate the neurobehavioral effects of drugs on mice (Irwin, 1968). It has since been modified for use in rats and is currently used in safety pharmacology studies recommended by the International Conference on Harmonisation (ICH) S7A guideline for assessing the effects of new chemical entities, to help protect clinical trials participants and patients from potential adverse effects ("International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) (2000),"; Redfern & Wakefield, 2006). It is also used as a general observational test on rodents for assessing the neurobehavioral effects in disease models (Blokland, Hinz, & Schmidt, 1995; Hunter et al., 2000; Roux, Sablé, & Porsolt, 2004).

Pharmacological validation of the system was conducted using well-characterised sedatives (chlorpromazine and clonidine), and a stimulant (amphetamine). Chlorpromazine was originally introduced as a neuroleptic agent in humans, and causes reduced activity, ataxia and lowered body temperature in rats (Mattsson, Spencer, & Albee, 1996; Moscardo, Maurin, Dorigatti, Champeroux, & Richard, 2007). Clonidine is a centrally-acting α_2-adrenoreceptor agonist, which results in reduced activity and lowered body temperature in rodents (Drew, Gower, & Marriott, 1977; Drew, Gower, & Marriott, 1979; Ewart et al., 2013; Moscardo et al., 2007; Van der Laan & De Groot, 1988). Amphetamine is a CNS stimulant first synthesised in 1927 for the treatment of narcolepsy and mild depression (Heal, Smith, Gosden, & Nutt, 2013). Since then it has been useful for the treatment of attention deficit hyperactivity disorder (Heal et al., 2013). Amphetamine works predominantly by increasing and sustaining the level of extracellular dopamine (Calipari & Ferris, 2013), and causes increase in locomotion, rearing and temperature in rats (Fog, 1970; Mattsson et al., 1996; Moscardo et al., 2007).

The objective of this study was to explore how the capability of this new technology compares to that of the conventional modified Irwin test as a means in detecting functional effects of test agents with known pharmacological effects on the CNS (Table 1). We also propose how the ActualHCA™ system could be integrated into the modified Irwin test to augment it.

2. Materials and methods

2.1. Drugs

Chlorpromazine hydrochloride was purchased from Sigma Aldrich, UK and was formulated with sterile water. The formulated chlorpromazine was stored in the dark at 4 °C. Clonidine hydrochloride was purchased from Sigma Aldrich, UK and was formulated with sterile water. The formulated clonidine was stored in the dark at room temperature. D-amphetamine sulfate salt was purchased from Tocris, UK and was formulated with sterile water. The formulated amphetamine was stored in the dark at room temperature.

2.2. Ethical statement

The sample size of n = 6 per treatment group was selected to have sufficient power to detect the effects reported on the modified Irwin test (Ewart et al., 2013). The Irwin test is designed to identify rare event symptoms as such we infrequently observe these within the control data and therefore seeing multiple symptoms within the treatment is a significant effect. This design also gives a good sensitivity on subcutaneous temperature measured using ActualHCA™, which was achievable with n = 6 because of the low variability of the data. Two tailed Student's *t*-test for temperature gives a power = 0.9 to detect a 1 °C change with a variability of 0.48 °C (Lenth, 2009). The activity measure using ActualHCA™ has lower sensitivity when time points are considered in isolation due to high variation, this means the screen can detect median to large-sized activity effects that are sustained over multiple time points. A total of 72 rats were used for the work described in this paper. All procedures were conducted in accordance with the United Kingdom Animal (Scientific Procedures) Act 1986 and associated guidelines, approved by institutional ethical review committees (Alderley Park Animal Welfare and Ethical Review Board; Babraham Institute Animal Welfare and Ethical Review Board) and conducted under the authority of the Project Licences (40/3368 and 70/8307, respectively). All animal facilities have been approved by the United Kingdom Home Office Licensing Authority and meet all current regulations and standards of the United Kingdom. This manuscript has been prepared to meet the ARRIVE reporting guidelines (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010).

2.3. Animals

Twelve male Han Wistar (Crl:W1 (Han)) rats were used for the assessment of each drug and assessment methodology: with 6 animals in