Journal of Pharmacological and Toxicological Methods 70 (2014) 241–245

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Repeated measurements of motor activity in rats in long-term toxicity studies

V. Golozoubova *, T.K. Brodersen [1], S. Klastrup, M. Oksama, J. Løgsted, A. Makin

CiToxLAB Scantox A/S, Hestehavevej 36A, Ejby, DK-4623 Lille Skensved, Denmark

ARTICLE INFO

Available online 19 July 2014

Keywords:
Open field test
Behavioural test battery
Safety pharmacology endpoints
Regulatory toxicology study

ABSTRACT

Introduction: In the light of 3R (replace, reduce, refine) principles in animal experimentation and increased focus on delayed effects of treatment on central nervous system, the incorporation of behavioural tests into standard toxicology studies as a complement or substitution of a stand-alone safety pharmacology study appears very attractive, but poses some challenges. In the present study, we evaluated the results of an open field test (standard part of the behavioural test batte- ries) incorporated into the 3-month regulatory toxicology study. **Methods:** The study was performed in two rat strains most commonly used in toxicology studies (Wistar and Sprague Dawley (SprD)). Open field test was performed according to the standard protocol for stand-alone behavioural test (modified Irwin test) before the start of treat- ment (Day-7, "naïve" animals), on Day 2, inWeek 6 and inWeek 13 of treatment with saline. **Results and Discussion:** There was no overall difference between strains, and onlyminor differenceswere detected at the individual time points. With regard to time effect, the average values for most of the parameters were comparable throughout the study but individual variability in the performance in the arena was increased at repeated measurements compared to the start. In conclusion, performance in the open field arena did not differ principally betweenWistar and SprD rats of both genders. However, individual variability in the behaviour in the open field arena increased with time. This has clear implications for deciding the appropriate group size for this type of study and has to be taken into account in the design of a toxicology study with integrated safety pharmacology endpoints.

1. Introduction

Functional observation battery (FOB) and the modified Irwin test are two behavioural test batteries commonly utilised to assess the effects of test substances on the central nervous system in accordance with regulatory guidelines for pharmaceuticals (Ewart et al., 2013; S7A, Safety Pharmacology Studies for Human Pharmaceuticals) and chemicals (OECD Guidelines for the Testing of Chemicals, test 408). The importance of including a functional observation battery test as part of the safety pharmacology 'core battery' also for the compounds which do not have central nervous system as a target was emphasised earlier (Redfern et al., 2005).

In the light of the biomedical industry's focus on the 3R approach to animal experimentation and increased attention to the delayed effects on safety pharmacology parameters, an integrated approach, incorporating neurological evaluation into the standard repeat-dose toxicology study, appears attractive. However, this approach may present some logistical and interpretational challenges.

The behavioural test battery typically includes three types of observations: cage-side observations, hand-held observations and open field observations. While the settings of cage-side observations and hand-held observations can be standardized throughout the study, open field observations may be more of a challenge, as they include an additional factor, namely, the novelty factor. In the open field test, which, per definition, "… consists of the measurement of behaviors elicited by placing the subject in a novel open space from which escape is prevented by a surrounding wall" (Walsh & Cummins, 1976), the validity of the results of repeated measurements of motor activity performed in the course of a long-term toxicity study may be questioned on the basis of limited "novelty" of the test arena when repeated measurements are performed in the same animals. Therefore, our efforts were concentrated on this part of the test battery.

Generally, the repeated open field test is regarded as an acceptable option as long as due care is taken to assure that both control and treatment group(s) undergo exactly the same procedures performed in the same manner. However, this is correct only under the assumption that variation in the data would be the same after repeated measurements

* Corresponding author. Tel.: +45 56861500.
E-mail address: vg@dk.citoxlab.com (V. Golozoubova).
[1] Present address: Novo Nordisk A/S, Denmark.

http://dx.doi.org/10.1016/j.vascn.2014.06.007
1056-8719/

as it is at a single recording. This, however is not necessarily the case in long-term studies, as the absolute values of the parameters may be different (e.g. due to the age of the animals (Himmel, 2008)). The mean values and the standard deviations of the parameters at the later phases of the study have a direct influence on the study design (group size in particular) for the integrated study, and therefore have to be evaluated. Further, the majority of the single-dose stand-alone behavioural tests are performed in male rats. The incorporation of the test in toxicology studies would typically mean that the test has to be performed both in male and in female animals, and therefore knowledge of the possible gender difference is important. In the present study, we compared the results of repeated open field test performed in both genders of Wistar and Sprague Dawley (SprD) rats integrated into a 3 month-regulatory toxicology study design, and, based on the data obtained, attempted to evaluate whether group size known to be sufficient at acute measurements would give reliable data at repeated dosing with a multiple open field measurement paradigm.

2. Materials and methods

2.1. Animals and housing

The study was performed in 40 animals: 10 male and 10 female SPF Wistar rats of the strain HanTac:WH (GALAS), and 10 male and 10 female SPF Sprague Dawley rats of the Ntac:SD strain, all delivered from Taconic Europe A/S, Ejby, Denmark.

The rats were approximately 5 weeks old upon arrival to the facility. At arrival, the animals were health checked and a pupil reflex-test was performed to confirm that they were not blind. The rats were kept in polycarbonate cages (floor area: 1500 cm^2 — height 21 cm) with two animals/cage. For environmental enrichment, each cage was supplied with bedding (softwood sawdust), wood wool (Aspen Wood Wool (Tapvei Estonia OÜ, Estonia)), autoclaved wood bricks (Tapvei Estonia OÜ, Estonia) and a rat house (Tecniplast Gazzada S.a.r.l., 21020 Buguggiate-Va, Italy). As stereotypic behaviour was observed in three animals towards the end of the study, additional environmental enrichment was offered to these animals (and their cage mates) from Day 71 and until the termination of the study.

The study took place in an animal room provided with filtered air at a temperature of 21 °C ± 3 °C and relative humidity of 55% ± 15%. The room was illuminated to give a 12:12 light:darkness cycle (light on from 06:00 h to 18:00 h). Animals had an ad libitum access to complete pelleted rodent diet (Altromin, Gesellschaft für Tierernährung mbH, D-32791 Lage, Germany) and water. Animals were weighed once weekly. The type of study was approved by the Animal Experiments Inspectorate, Ministry of Justice, Denmark.

2.2. Treatment

In order to reconstruct the experimental settings of the typical toxicology study, animals were treated daily for the whole period of the study. The daily dose of sterile physiological saline (Fresenius Kabi, Denmark) was given subcutaneously (5 ml/kg body weight).

2.3. Open field test

All animals were examined in an open field test (5 min recording in ActiMot Motility Measuring System (TSE, Germany), arena size 96 × 96 cm) before the start of treatment (Day-7), after the second dosing (Day 2), in Week 6 (Day 37) and in Week 13 (Day 86) of daily treatment with saline. The animals were tested at each testing occasion by the same operators, at least 1 h after dosing, at approximately the same time, in the same sequence. At the start of the session, the animal was placed in the centre of arena.

On each occasion, the test was performed in parallel in two arenas first in all males, then in all females (same sequence of individual animals on all occasions), at 10.00–12.30 (±15 min).

The level of illumination was kept similar to that in the home cage. Parameters evaluated were the same as in a stand-alone behavioural test — either automatically recorded by ActiMot system (time moving, total distance, numbers of rearings (vertical activity), time in centre/periphery, total corner visits, total activity (moves/counts)), or visually evaluated (abnormal behaviour (e.g. aggression, stereotypic behaviour, cataleptic behaviour) and number of faecal pellets at the end of each session).

2.4. Statistical analysis

Overall strain differences (separate for males and females) were assessed by an analysis of covariance (ANCOVA) with pre-dose measurement as a covariate (where applicable). In addition, Student's t-test (unpaired) was used for the direct comparison of strains on the individual days. Time effect (using Day-7 values as a reference) was evaluated by paired t-test. For all tests, the level of significance was defined as $p < 0.05$. The statistical analyses were made with SAS® procedures (version 8.2) described in "SAS/STAT® User's Guide, SAS OnlineDoc®, 1999, SAS Institute Inc., Cary, North Carolina 27513, USA".

3. Results

3.1. General

As expected, there was a substantial weight gain in the course of the study (gain of approx. 240 g for males and approx. 100 g for females). Body weights were significantly different between the strains from the study start and until Day 29 for males, and throughout the study for the females (SprD heavier than Wistar).

Open field measurements were performed successfully in all animals. No abnormal behaviour was observed during the test in any of the animals at any of the time points. No overall difference between the strains was identified either for males or for females.

3.2. First exposure to the arena

Male Wistar rats appeared to have a more pronounced reaction to the novelty of the environment at the first exposure to the test arena compared to SprD males: their total activity count and number of rearings were higher, they spent more time moving, spent more time in the centre of arena (Table 1), and had more faecal pellets. For the females, the difference between strains was minimal, the only parameter which differed statistically significantly between strains was total distance travelled (longer in Wistar females).

3.3. Repeated exposure to the arena

In both strains time moving, time in the centre and total activity tended to be higher at the first measurement (before, Day-7) compared to the following measurements (Day 2, Week 6 and Week 13), the difference being more pronounced in males than in females (Table 1).

The pattern of reaction to the exposure to arena was very similar on all occasions, the peak of activity was, as expected, observed within the first minute in the arena, and was gradually reduced thereafter. Due to an increased individual variation, this trend was more clear after the measurements in the beginning of the study (before and Day 2) compared to Week 6 and Week 13. At all measurement occasions, individual variation was least pronounced during the first minute of exposure to the arena and was increased during the following period (2–5 min) (data not shown).