Introduction

Validity of the results of repeated measurements of motor activity performed in the course of a long-term toxicity study is often questioned on the basis of limited "novelty" of the test arena when the measurements are repeated in the same animals. Interpretation of the results of motor activity evaluation (open field test) is further complicated by time-dependent effects in the control group, which may differ between strains and gender. In the present study, we compared the results of repeated open field test performed in both genders of Wistar and Sprague Dawley (SprD) rats in the course of a 3 months period.

Materials and methods

The study was performed in 60 animals: 10 male and 10 female Wistar rats (HanTac:WH (GALASS)) and 10 male and 10 female SprD rats (Ntc:SD). The rats were kept in polycarbonate cages (floor area: 1500 cm² - Height 21 cm) with two in each cage. Each cage was supplied with bedding (softwood sawdust), wood wool, wood bricks and rat house. All animals were examined in open field test (5 min recording in ActiMot Motility Measuring System (TSE, arena size 96x96 cm)) before the start of treatment, on Day 2, in Week 6 and in Week 13 of daily treatment with saline.

Results

General. There was a substantial weight gain in the course of the study (approx. 240 g for males and approx. 100 g for females). Three Wistar rats (1 male and 2 females) showed stereotypic behaviour in the home cage at the end of the second month of the study.

First exposure to the arena. Male Wistar rats appeared to be more stressed by the first exposure to the test arena than the Sprague Dawley males (see Figure 1): their total activity count and number of rearings were higher, they spent more time moving in general and being in the centre of arena, and they 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).

Repeated exposure to the arena. There was no systematic difference between the first measurement (Before) and the following measurements (Day 2, Week 6 and Week 13) in most of the parameters. In Week 13, male Wistar rats showed slightly higher reaction to the arena than Sprague Dawley males as they spent significantly more time in the centre of the cage and had higher incidence of defecation.

Individual variation. While activity level in naïve animals was rather homogenous within the groups, individual variation between animals of the same group was more pronounced at repeated measurements (Day 2, Week 6 and Week 13). The reason for increased variation could not easily be categorised by separation into broad categories such as "active" and "lazy" animals; animals showing low level of activity at one of the measurements showed normal or even relatively high activity at the other time points (Figure 2).

The difference between strains at repeated measurements was minor (see Figure 1) and not statistically significant for most of the parameters. In Week 13, male Wistar rats showed slightly higher reaction to the arena than Sprague Dawley males as they spent significantly more time in the centre of the cage and had higher incidence of defecation.

Sterotypic behaviour. The three animals showing stereotypic behaviour in the home cage did not show such behaviour in the open field arena in Week 13, probably due to the novelty of the environment. Males compared to the other animals, the male showing stereotypy had lower than average number of rearings, and the females did not distinguish themselves from the average in any of the parameters.

Conclusion

- The pattern of behaviour and activity observed in the repeated measurements of motor activity in open field test in rats in a long-term toxicity study is very similar to the observations after a single measurement.
- Although pre-study measurement may be advantageous, care should be taken not to place this measurement too close to the study start (at least a week or, preferably, more) to avoid reduced reaction to the arena at the start of the study.
- Difference between the two strains and genders in behaviour in the open field was expressed only in males at the pre-dose measurement and, to a much smaller degree, in Week 13 (reaction to the arena more pronounced in Wistar males). The behaviour of male SprD rats indicated less stress at the initial exposure to the arena. There was slightly less individual variability in SprD rats compared to Wistar rats.
- Due to time-dependent increases in the individual variation in reaction to the open field, a relatively large group of animals has to be tested in the arena in order to obtain reliable data in long term studies.

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