Characterization of Behavior and Voluntary Alcohol Intake in Wistar and Lister Hooded Rats

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Introduction

Preclinical researchers are faced with the challenge of selecting the optimal model organism based on the research question addressed. Few comparisons between different rat strains are available [1]. Sometimes anecdotal reports and laboratory traditions guide the choice. Therefore, studies comparing different rat strains may refine preclinical research.

The multivariate concentric square field™ (MCSF) test is based on forced exploration, but the animal can freely choose between different environments aiming at provoking behaviors associated with activity, risk taking and shelter seeking [2, 3]. In the final interpretation, the descriptive parameters are brought together into the functional categories general activity, exploratory activity, risk assessment, risk taking and shelter seeking [2].

When comparing Wistar rats from different vendors, pronounced differences in voluntary alcohol intake have been demonstrated [4, 5]. However, with the aim of using Lister hooded rats in future experiments, little information about voluntary alcohol intake in this strain was available.

This study aimed to evaluate behavioral profiles and voluntary alcohol intake in Wistar rats after an interruption of breeding at the vendor, and to compare Wistar rats with Lister hooded rats.

Material and methods

Animals

Male rats of two different strains, Lister hooded (HsdOla:LH) and Wistar (RccHan:WI) rats (Envigo, Horst, the Netherlands) were delivered at 6 weeks of age (n=21 of each strain). The animals were housed 3 per cage in transparent type IV cages with raised lids, wood-chip bedding and two pieces of paper as enrichment. The cages were kept in an animal room on reversed light/dark cycle (lights off at 6:00 am) with a masking background noise. Once the alcohol intake studies began the rats were single housed in high type III cages with wood-chip bedding and two pieces of paper until the end of the study. The experimental protocol and use of animals in this study was approved by the Uppsala Animal Ethical Committee, and was consistent with the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS1998:56) and the European Union Directive on the Protection of Animals Used for Scientific Purposes (2010/63/EU).

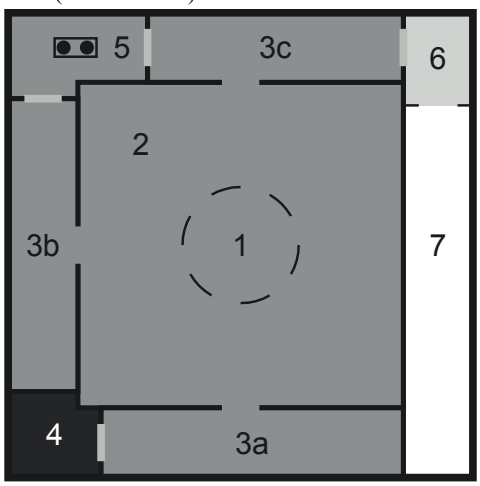

Figure 1. Schematic layout of the MCSF arena. The shading of the zones represents illumination levels in the arena. The arena is divided into zones: 1, the central circle; 2, the central field; 3a-c, corridors; 4, the dark corner room; 5, the hurdle with hole board; 6, the slope; and 7, the bridge [6].

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The multivariate concentric square field™

Before the alcohol intake studies began the animals underwent testing in the multivariate concentric square field™ test (MCSF) (Figure 1). The MCSF is used for behavioral profiling of rodents [2, 3].The animal is placed in an arena and able to explore and freely choose between different environments during 20 minutes. The MCSF contains a dark corner room (DCR) which represents a safe area were the animal can seek shelter. It also has two risky areas, a brightly illuminated bridge and a central circle. Lastly the animal can explore an elevated hole board in the hurdle. The different areas are connected with corridors which also provide areas for the animal to explore. At the start of the trial the animal is placed in the central field facing the wall without any openings.

The room that the MCSF is placed in has the same background noise as the animal room and the experimenter leaves the room during the trial, to make sure not to influence the animal's behavior. The trials are recorded using a camera mounted in the ceiling above. Noldus Ethovision® XT 12.0 (Noldus Information Technology, Wageningen, The Netherlands) is used to track the animal's movements during the trial. Behaviors that can't be tracked automatically is manually scored by the experimenter. These behaviors are rearing, grooming, stretch attend posture (SAP), boli and urine left in the arena after the trial.

Voluntary alcohol intake

A modified intermittent two-bottle free-choice paradigm was used, with three consecutive 24-hour sessions per week for six weeks [7]. Each rat had access to two bottles, one with tap water and the other with a 20% (v/v) alcohol solution. Bottle positions were changed for each session to avoid position preference. After each 24-hour session the bottles were weighed to measure intake of water and alcohol. Alcohol preference was calculated as alcohol intake (g) divided by total fluid intake (g). Drinking sessions took place on Tuesdays, Wednesdays and Thursdays. Changing of cages was done on Fridays to make sure not to disturb the animals during alcohol intake sessions.

Statistical analyses

One Lister hooded animal was excluded due to health reasons. Statistical analyses were carried out using Statistica 13.2 (Dell Inc., Tulsa, OK, USA). Differences were considered statistically significant at $p<0.05$. Most of the data did not show normal distribution according to the Shapiro-Wilk's W test, hence nonparametric tests were used. Between group differences were analyzed with Mann-Whitney U-test. Intragroup differences over time was analyzed with Friedman ANOVA followed by Wilcoxon Matched Pairs Tests where appropriate. Behavioral profiles in the MCSF test were presented using the trend analysis, which is based on grouping behaviors within the same context and then ranking the individuals against each other. The rank values are then summed into a sum rank for the functional categories general activity, exploratory activity, risk assessment, risk taking and shelter seeking [2].

Results

MCSF

The result from the trend analysis is shown in Figure 2. The only functional behavior category that differed between the strains was risk taking, where Lister hooded rats exhibited higher level of risk taking behavior.

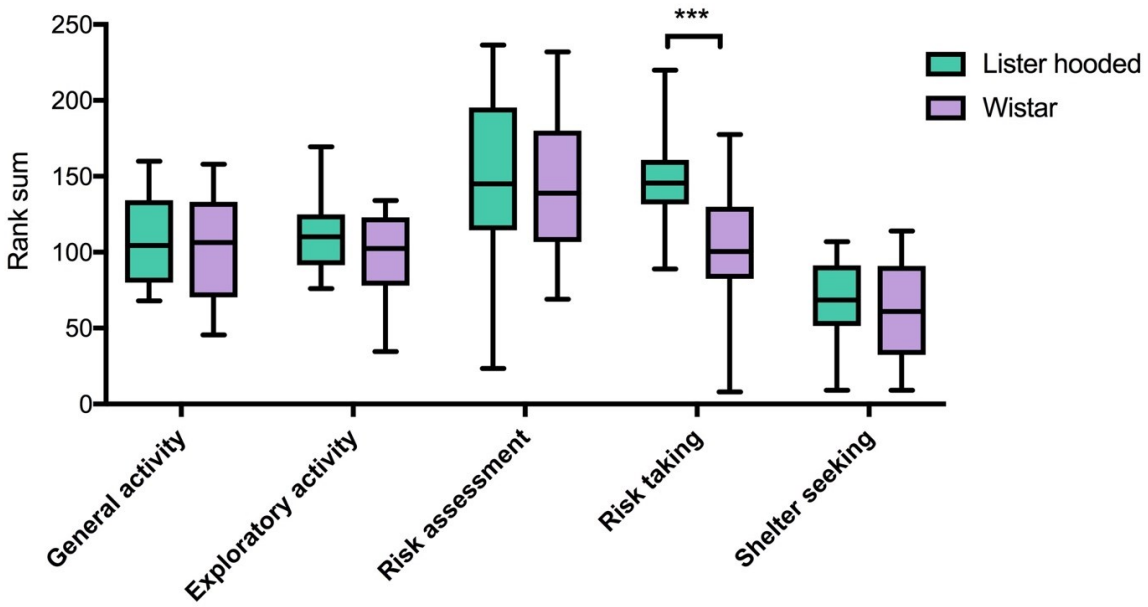

Figure 2. Rankings of the functional behavioral categories in the MCSF trend analysis. Data are shown as median with upper and lower quartiles, min and max. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ comparing Lister hooded and Wistar rats (Mann-Whitney U-test).

Voluntary alcohol intake

Alcohol intake (g/kg) and preference (%) for the two strains are shown in Figure 3. All 18 sessions are represented in the figure. No significant differences in voluntary alcohol intake was found during the six weeks of access. Lister hooded rats displayed a significantly higher preference during six of the sessions. The variance within the group was larger for Lister hooded rats, most obvious in alcohol preference on the first drinking sessions of each week.

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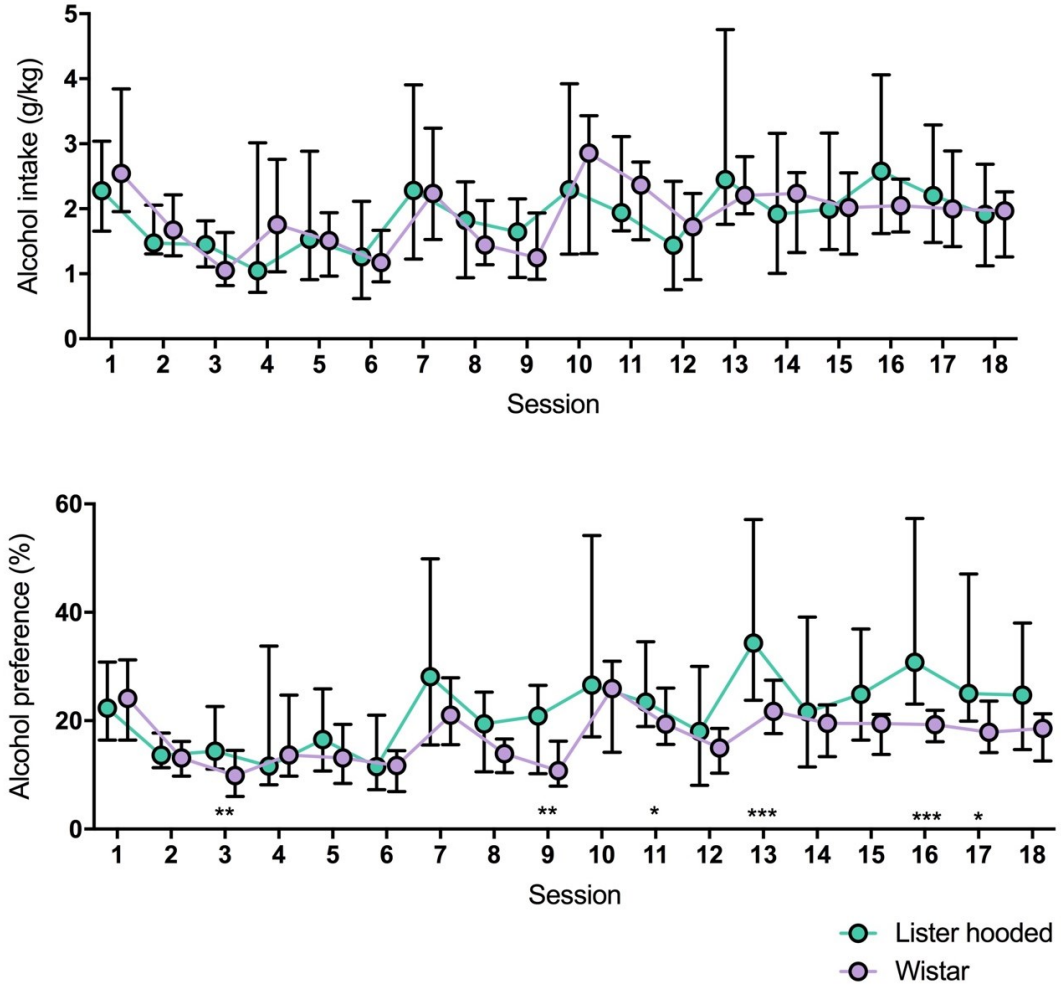

Figure 3. Alcohol intake (g/kg) and preference (%) in Lister hooded and Wistar rats during the 18 sessions of voluntary alcohol intake during a period of 6 weeks. Data are shown as median with interquartile range. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ comparing Lister hooded and Wistar rats (Mann-Whitney U-test).

Data representing the average intake and preference on the first, second and third day of access is shown in Figure 4. No significant difference between the strains was found when considering the alcohol intake. Lister hooded had significantly higher preference than Wistar rats on the second and third alcohol session of the week. When comparing the difference between alcohol sessions within the respective strains, significant differences were found between all alcohol sessions for both strains with significantly higher intake and preference on the first day of access.

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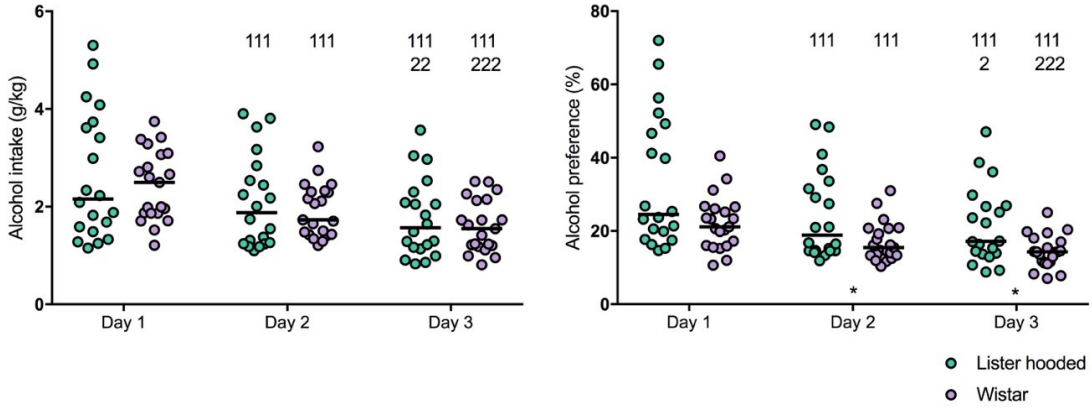

Figure 4. Average alcohol intake (g/kg) and preference (%) in Lister hooded and Wistar rats during the three weekly drinking sessions. Data are shown as scatter dot plots with the line at median. *p<0.05, **p<0.01, ***p<0.001 comparing Lister hooded and Wistar rats (Mann-Whitney U-test), ^{1}p<0.05, ^{11}p<0.01, ^{111}p<0,001 compared to the intake on the first day of access within the respective strain (Wilcoxon's matched pairs test), ^{2}p<0.05, ^{22}p<0.01, ^{222}p<0.001compared to the intake on the second day of access within the respective strain (Wilcoxon's matched pairs test).

Discussion

The results from this study revealed smaller differences between Wistar and Lister hooded rats, than previously have been demonstrated when comparing Wistar rats from different vendors [4, 5, 8]. The behavioral difference that could be seen in the trend analysis was that the Lister hooded rats displayed a higher level of risk taking behavior relative to Wistar rats. The parameters that make up risk taking behavior are related to the illuminated bridge and the central circle. The behavior of Lister hooded and Wistar rats in the MCSF has been evaluated previously [3]. The study found that Wistar rats spent more time in the DCR and took longer to enter the central circle. The trend analysis was not used at that time but these behaviors are related to shelter seeking and low risk taking behavior, which thus are in agreement with the findings in this study. In a previous comparison, Wistar rats had high thigmotaxis in the open field test [9]. On the other hand, with large enough sample size individual differences have been demonstrated, and both high and low risk taking individuals, respectively, among outbred Wistar rats [10]. The Lister hooded rats' behavior are not as well characterized as for example Wistar rats. They are commonly used in operant techniques that require high attentional function [11]. A more risk taking behavior is also beneficial when faced with such a task. The operant chamber is an unfamiliar environment and to learn the requirements of the task they need to be active and nose poke the illuminated holes to eventually succeed and get the reward.

When it comes to voluntary alcohol intake no difference between Wistar and Lister hooded rats were found during the 18 sessions. However, Lister hooded rats had a higher alcohol preference towards the end of the study. Moreover, pronounced individual differences within the Lister hooded rats were noted. The alcohol intake of Wistar rats in this study as well as in a parallel, unpublished study, indicates a lower median intake as well as a lower variance within the group compared to previous studies [7, 12]. Based on these results the Lister hooded rats would be more suitable for phenotypic division into high- and low-drinking subgroups, which previously has been useful in studies using the Wistar strain [7, 12]. The reason for this change in drinking behavior seen in the Wistar rats is not known, but it could be due to an interruption in breeding caused by an outbreak of parvo virus at the vendor, or a change in breeding material. However, despite several previous studies utilizing the large individual differences in voluntary alcohol intake among the Wistar rats [4, 7, 12], a batch-dependent effect cannot be excluded.

In conclusion, comparisons between laboratory animal strains, as here when comparing behavioral profiles and voluntary alcohol intake are important and may facilitate the choice of animal strain used in preclinical research.

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