Biological Models Research and Technology
ISSN 9675-9225X (Online)

BMRT

RESEARCH ARTICLE

One-capillary lane-maze test in flies: exploratory studies

Fabiola Boz Eckert[1], Dhiozer de Brittos Valdati[2], José Marino Neto[3], Daniela Cristina de Toni[4], Cilene Lino de Oliveira[5*]

[1] Doutorado em Farmacologia, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil.
[2] Graduação em Engenharia Química, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil.
[3] Instituto de Engenharia Biomédica, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil.
[4] Departamento de Biologia Celular, Embriologia e Genética, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil.
[5] Departamento de Ciências Fisiológicas, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil.

How to cite: Eckert FB, Valdati DB, Marino Neto J, Toni DC, Lino-de-Oliveira C. One-capillary lane-maze test in flies: exploratory studies. Bio M Res Tech. 2023;3: e00142022. https://doi.org/10.4322/2675-9225.00142022

Abstract

Drosophila melanogaster is a candidate species to replace rodents in some neurobiological studies, encouraging attempts to develop behavioural tests for these flies. This study aimed to develop a sucrose preference test in flies that simultaneously assesses the ethological aspects of motor and fluid intake activities in several experimental units. Therefore, a lane-maze with 14 lanes was printed three-dimensionally to accommodate up to 14 individual flies in a single trial. Each lane had a capillary filled with a 5% sucrose solution attached to one of the extremities, simulating a one-bottle preference test in rodents. Similarly, the test was named the one-capillary lane-maze. Male and female flies (adults, 5–6 days of age) were subjected to 0 (control), 2, 8, or 20 h of food deprivation (FD, n= 9–11/group) before testing. The one-capillary lane-maze test consisted of 10 min of habituation and 30 min of trial, which were video-recorded for further evaluation. The duration of locomotion, immobility, and grooming in the lane, capillary, or corner (zone in extreme opposition to the capillary) was scored using EthoWatcher software. A 'preference index' for the capillary zone was calculated as a proxy for sucrose preference. Independent of the lane zone, locomotion was the most prevalent behaviour in flies, followed by immobility and grooming. All flies were proportionally longer in the corners than in the middle of the lane, indicating that sex or FD had a minor influence on fly behaviour in the lane-maze test. The preference index revealed that flies of both sexes avoided the capillary zone, even when food-deprived. These data suggest that, contrary to the primary hypothesis, the capillary was aversive to the flies. In summary, although the apparatus was suitable for high-throughput assessment of flies' behaviours, more studies are required to develop a sucrose preference test in the lane-maze.

Keywords: behaviour, motor activity, preference, replacement, sexual differences.

INTRODUCTION

The scientific community has criticised models and behavioural tests in rodents owing to concerns regarding their clinical validity, application, and animal welfare, impelling the research field to find alternatives to using of vertebrates[1]. Thus, the principle of 3Rs (refine, reduce, and replace) may help scientists in conducting better animal research[2]. Refinement may minimise animal suffering and improve welfare during experimentation. Reduction may guide methods that minimise the number of animals used per experiment. Replacement can lead to methods with full or partial substitution

***Corresponding author:** cilene.lino@ufsc.br
Competing interests: No conflicts of interest declared concerning the publication of this article.
Received: November 30, 2022. **Accepted:** April 21, 2023.

of animals for alternative methods. Total replacement avoids using non-human animals in research by substituting them with alternatives, such as human volunteers, tissues and cells, mathematical and computer models, and established cell lines. Partial replacement avoids the use of vertebrates in research by substituting them with invertebrates, such as nematodes, social amoebae, and flies. Although invertebrates react to stimuli, they have no brain circuitry to experience the cognitive and emotional aspects of suffering[3].

In this context, *Drosophila melanogaster* (*Drosophila)* has been considered a candidate organism to replace laboratory rodents or other vertebrates in neuroscientific studies[4,5,6,7]. *Drosophila* has a well-known anatomy, physiology, genome, proteome, and natural behaviour[4,8]. As vertebrates, *Drosophila* has monoaminergic receptors and neural circuits controlling feeding, sleep, aggression, preferences, learning, memory, and responses to stress[6,9,10,11,12]. In addition, different strains of *Drosophila* with various phenotypes may be maintained and reproduced quickly in the laboratory, allowing for planning well-powered experiments as required in neurobiological research. Nevertheless, standardization of models and behavioural tests for psychopharmacology or neurobiology of stress in *Drosophila* are incipient as compared to rodents[7,13].

This study aimed to develop a preference test for flies. Preference tests, such as the sucrose preference test, have been employed to assess the behavioural consequences of stress or pharmacological treatments in a variety of laboratory animals, including *Drosophila*[14,15,16]. In *Drosophila,* the preference for sucrose may be assessed by measuring fluid intake in paradigms such as fly Proboscis and Activity Detector[17], capillary feeding (CAFE)[18,19], proboscis extension[20], dyed abdomen[20], Fly Liquid-Food Interaction Counter (FLIC)[21] or 'Activity Recording Capillary Feeder' (ARC)[22]. Except for ARC[22], most of the above methods have been developed to investigate a single experimental unit at a time (an individual fly or a group of flies). ARC[22] employs a positional tracking system, allowing for the simultaneous assessment of sleeping and fluid intake in 60 individual flies in the same trial. The aim in the present study was to obtain a paradigm to assess simultaneously the ethological aspects of motor and fluid intake activities in several independent flies. Therefore, inspired by ARC[22], a horizontal apparatus with parallel, separate lanes (lane-maze) was printed in plastic to accommodate up to 14 individual flies in a single behavioural trial[23]. In contrast to a vertical apparatus such as ARC[22], the horizontal lane-maze offers the possibility of attaching a capillary in both extremities of each lane. Thus, the lane-maze apparatus may offer a large variety of behavioural testing compared to other apparatuses. In this context, capillaries may be filled with different palatable fluids, which may be used to simulate one-bottle or two-bottle preference tests, as performed in rodents[24,25,26]. Here, sucrose solution 5% was selected as an attractor in the present study because of previous literature indicating sucrose preference in flies[14,15,18].

In the present study, a single capillary filled with 5% sucrose solution was attached to each lane, simulating a single-bottle preference test in rodents[24]. Hence, the test was named a one-capillary lane-maze test. The sucrose preference test outcome in rodents often involves calculating a preference index, which depends on the volume of fluid intake in 24 h[24,25,26]. Ideally, the capillary allows for the quantification of the palatable fluid intake of flies[18,19]. However, the volume of fluid intake of an individual fly for 24 h is too low to be measured accurately. Herein, instead of sucrose intake, the behavioural scores in the capillary zone filled with sucrose were used to calculate a 'preference index' as a proxy for sucrose preference in the lane-maze. In addition, the preference index based on behavioural scores would also permit a behavioural test shorter than 24 h, which is habitual in neuroscience[24,27,28,29].

The primary hypothesis in this study was that flies stay longer near the capillary than the other regions of the lane-maze. A corollary hypothesis is that food deprivation induces hyperactivity and increases flies' preference for sucrose[16]. Another secondary hypothesis is that flies' preference for sucrose in the lane-maze is sexually dimorphic, and flies' preference for the capillary zone may increase over the behavioural test. To test these hypotheses, adult virgin male and female flies were deprived of food for 0 (control), 2, 8, or 20 h before the one-capillary lane-maze test. At present, a 30-min version of the one-capillary lane-maze test was planned. In addition, behavioural scores were pooled into 5-min blocks to investigate the temporal pattern of the test. Finally, the behavioural scores were used to calculate the preference index for each independent fly in the experimental groups.

MATERIALS AND METHODS

Flies

Drosophila melanogaster (Canton-S) was obtained from Stock Center Tucson (Arizona, USA). Flies of both sexes were kept in house glasses (300 mL glass bottles) containing standard food (supplementary methods) sealed with a foam stopper until eclosion. House glasses were in a vivarium with controlled temperature and photoperiod (20 ± 1 °C, 12 h light-dark cycle, lights on at 6 am, 60-80% of relative humidity) in the Biomedical engineering laboratory at the Federal University of Santa Catarina.

Experimental design

See the experimental timeline in the Figure 1B. On the day of eclosion, flies were collected, anaesthetised on ice (-4ºC) for ±1 min, sexed, and transferred to house tubes separated by sex (7.5 cm x 1.1 cm). Virgin male or female flies were maintained in groups of 8-10 under identical medium conditions (1 g/tube) for five days before allocation to the experimental groups (control, C or food deprivation, FD). Flies (adults, 5-6 days of age) were transferred from the house tubes to the experimental tubes with food (C) or without food containing a filter paper saturated with water (FD). Experimental tubes were kept under vivarium conditions until the behavioural tests. For preliminary studies, only flies of C groups were analysed. For experiments, flies of C or FD groups were kept in the experimental tubes for two (experiment 1), eight (experiment 2), or twenty hours (experiment 3) before behavioural testing. Each independent experiment was carried out within several days to complete the final sample size (4 groups per experiment, n= 10 flies per group, n= 40 flies per experiment). Each experimental day consisted of testing up from ten to twenty flies, males and ten females of C or FD groups, simultaneously in the lane-maze. Afterwards, flies were euthanized by immersing the tubes in ice (-4 ºC, for ±5 min).

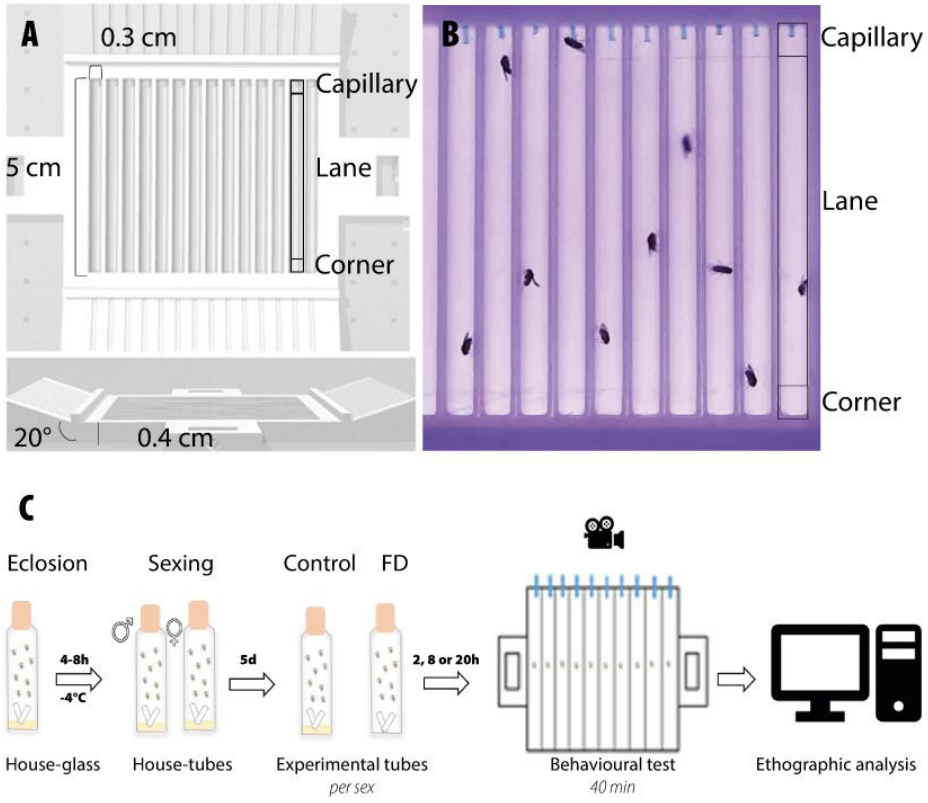

Figure 1. A- Lane-maze in a top (upper figure) and a lateral view (lower figure); B-Experimental timeline. Abbreviations: FD= food deprivation.

Lane-maze apparatus

Lane-maze apparatus is printed in Acrylonitrile butadiene styrene filaments (Figure 1A, supplementary methods)[23]. Apparatus consisted of a squared arena (external dimensions: 6.6 x 9.2 cm, internal dimensions: 5 x 5.5 cm) with 14 internal subdivisions (lanes) of identical dimensions (length: 5 cm, width: 0.3 cm) covered in a transparent acrylic plate. At both extremities of each lane, a hole (diameter: 0.05 cm) allowed inserting a glass capillary at an angle of 20 degrees provided by the inclined edges of the external arena. Capillaries allow access to palatable fluids during behavioural tests. During the tests, the apparatus was placed in an experimental environment, which consisted of a plastic support with illumination (a string of LED lamps, 490 lux) and a camera (a USB Digital Microscope Camera, Lenovo ® positioned 42 cm above the apparatus allowing for simultaneous recording of 10 lanes). The lane-maze was maintained in a fixed position relative to the camera from test to test on a platform (30 cm length, 4.5 height) made from white translucent acrylic with a pair of plastic sockets to fit the lane-maze. An opaque screen covered the experimental environment to avoid illumination variations from the experimental room.

One-capillary lane-maze test procedures

Procedures for testing in the lane-maze were as follows: 1- the lane-maze was placed on an ice plate for ± 5 min; 2-flies were anaesthetised on ice (-4ºC) for ± 1 min and transferred carefully, using tweezers, to the respective lane of the cold lane-maze; 3-each lane had a glass capillary (diameter: 0.04 cm) filled with blue sucrose solution (5%, dissolved in filtered water plus 0.1 mL of blue dye, food-grade colouring, Arcolor®, São Paulo-SP, Brazil) inserted in one of the extremities; 4-the lane-maze was covered with the acrylic plate before flies recovered from the anaesthesia and were transferred to the test platform; 5-video camera recording started at the insertion of the lane-maze into the test environment; 6-after the testing, flies were euthanised on ice (-4ºC, ± 5 min); 7-after euthanasia, flies were examined to check for the presence of blue dye in their abdomen; 8- acquisition of behavioural outcomes occurred after finishing the experiments by examining video-recordings. Blue dye was added to the sucrose solution to facilitate the capillary visualisation in the video recordings and recognition of sucrose intake. Behavioural trials lasted a maximum of 40 min and occurred between 12 am and 4 pm in a room at 23±3 ºC. In the pilot study, behavioural outcomes were scored during the video recording (40 minutes) to identify the maximum time flies required to recover from anaesthesia. In experiments 1, 2, and 3 behavioural outcomes scoring began after the first movement a fly made, indicating that anaesthesia was over, until the end of the trial comprising 30 min of data collection.

Selection of behavioural outcomes, data acquisition, and calculations

Behavioural scores were acquired using Ethowatcher open-source software package[30], available upon request. Behavioural data acquisition requires a description of each item to be scored. Preliminary analyses of the control groups helped select the more appropriate outcomes of the test. Due to inconsistent measures, the presence and absence of the blue dye in the abdomen of flies were discarded as an indicator of fluid intake in the present study (more details in the Supplementary Material). Shortly, behavioural outcomes of the one-capillary lane-maze were selected according to the quality and reliability of their assessment, which varied from almost perfect (Cohen's *kappa* upper 80%), substantial (Cohen's *kappa* upper 75%) to weak (Cohen's *kappa* below 50%). The latency (s), frequency (number of bouts), and duration (s) of locomotion, immobility, and grooming were scored in the different sectors of the lane (50 mm). The latencies for locomotion or grooming were used to determine the end of the anaesthesia effect, consequently, the onset of behavioural scoring. Frequencies were used to calculate Cohen's *kappa* indices. Duration (s) was the primary outcome of the test used to compare treatments and to the preference index (P-index) calculations. Sectors of the lane with different lengths (mm) were arbitrarily defined as follows: capillary (5 mm), opposite corner (5 mm), and lane (40 mm). To fit scores in the same timescale, the durations (s) in each sector (capillary, corner, lane) were normalised by the length of the sector (capillary=5 mm; corner=5 mm; lane=40 mm) to obtain the proportional occupancy of each sector (normalised durations, s/mm). Raw (s) or normalised (s/mm) durations for each sector (capillary, corner, lane) were presented in

blocks of 5 min. The P-index was estimated as a proxy for the sucrose preference index. P-index was conceived to separate sucrose preference (capillary) from the place preference (corner). Therefore, the duration in the lane is not part of the P index calculation. The P-index was calculated by doing the following steps: 1-calculation of the duration (D) in each zone (capillary= D capillary, corner= D corner) by summing up the duration of all behaviours scored in either zone (locomotion, immobility, grooming); 2- calculation of the proportional occupancy (P) in each zone (capillary= P capillary, corner= P corner) by division of the D in the zone by the total duration (P capillary= D capillary/(D capillary + D corner); P corner= D corner/(D capillary + D corner); 3- P index was the difference between P capillary and P corner (P index= P capillary – P corner). The P-index ranges from + 1 (total preference for the capillary zone, i.e., preference for sucrose) to – 1 (total aversion to the capillary zone, i.e., aversion to sucrose). A p-index equal to zero indicates the absence of preference. See Supplementary Material for raw data, normalised data of individual flies or means, standard errors, standard deviations, and detailed calculations.

Statistical analysis

Raw duration, normalised duration, or preference indices lacked normal distribution (significant Kolmogorov-Smirnov test) and homogeneous variances (significant Levene and Brown-Forsythe tests). Kruskal–Wallis, a non-parametric one-way analysis of variance, was used to compare the four groups (F-C, F-FD, M-C, M-FD; female=F; male=M; control=C, food deprived= FD) within independent experiments (Experiment 1, 2 or 3). These analyses were performed with Statistica 8.0, and GraphPad Prism 8 (San Diego, CA, USA). The P-index of every experimental group (F-C, F-FD, M-C, M-FD; female=F; male=M; control=C, food deprived= FD) was compared to zero (i.e., absence of preference) with Sign test and Wilcoxon matched-pairs signed-ranks test[31] (Excel software, Microsoft Office, version 16.37). Sex and treatment were masked for an unbiased statistical analysis of data. Data are expressed as the mean(s) ± standard error (SE). Comparisons with a p-value<.05 were considered statistically significant.

RESULTS

Effects of food deprivation on behavioural outcomes of the one-capillary lane-maze test

Except for the items "immobility in the corner zone" and "grooming in the capillary zone", the other items included in the behavioural catalogue were measured with substantial reliability (locomotion in the lane or capillary or corner zones; immobility in the lane or capillary zones; grooming in the lane or corner zones). The mean duration, standard deviation, or standard error for each experimental group may be found in Table S4 (raw data) and Table S5 (normalised data). Raw or normalised locomotion, grooming, and immobility durations were similar in the lane, capillary, and corner zones in all experiments' 30-min one-capillary lane-maze test (Figure 2, Figures S6, S7, S8). Food deprivation for 2 h, 8 h, or 20 h had no visible effect on locomotion, immobility, or grooming of flies of both sexes in the lane-maze (Figure 2, Figures S6, S7, S8).

Indeed, in experiment 1 (2h FD, Figure 2, Figure S6), no significant differences were seen among the groups (F-C, F-FD, M-C, M-FD) for the raw duration of locomotion ($H(3) = 2.41$, $p = 0.49$), immobility ($H(3) = 4.37$, $p = 0.22$) or grooming ($H(3) = 0.38$, $p = 0.94$) in the capillary zone. In the lane, no significant differences were seen among the groups for the raw duration of locomotion ($H(3) = 2.86$, $p = 0.41$), immobility ($H(3) = 5.53$, $p = 0.13$), grooming ($H(3) = 5.08$, $p = 0.16$). In the corner, no significant differences were seen among the groups for the raw duration of locomotion ($H(3) = 1.05$, $p = 0.79$), immobility ($H(3) = 2.66$, $p = 0.44$) and grooming ($H(3) = 2.55$, $p = 0.46$). Normalised durations were not conclusively different among the groups for any category in the capillary zone (locomotion: $H(3) = 2.41$, $p = 0.49$, immobility: $H(3) = 4.37$, $p = 0.22$; grooming: $H(3) = 0.38$, $p = 0.94$) or in the lane ($H(3) = 2.86$, $p = 0.41$; immobility: $H(3) = 5.53$, $p = 0.13$; grooming: $H(3) = 5.08$, $p = 0.16$) or in the corner (locomotion: $H(3) = 1.05$, $p = 0.79$; immobility: $H(3) = 2.66$, $p = 0.44$; grooming: $H(3) = 2.55$, $p = 0.46$).

In experiment 2 (8h FD, Figure 2, Figure S7), no significant differences were seen among the groups (F-C, F-FD, M-C, M-FD) for the raw duration of locomotion ($H(3) = 0.61$ $p = 0.89$), immobility ($H(3) = 1.20$ $p = 0.75$) and grooming ($H(3) = 1.83$ $p = 0.6$) in the capillary zone. In the lane, no significant

differences were seen among the groups for the raw duration of locomotion (H (3) =3.34 p =0.34), immobility (H (3) =2.63 p =0.45), and grooming (H (3) =2.43 p =0.48). In the corner, no significant differences were seen among the groups for the raw duration of locomotion (H (3) =1.06 p =0.78), immobility (H (3) =3.21 p=0.36), and grooming (H (30) =5.90 p =0.11). Normalised durations were not conclusively different among most of the groups for any category in the capillary zone (locomotion: H (3) =0.61, p =0.89, immobility: H (3) =1.20, p =0.75; grooming: H (3) =1.83, p =0.60) or in the lane (locomotion: H (3) =3.34, p =0.34; immobility: H (3) =2.63, p =0.45, grooming: H (3) =2.43, p =0.48) or in the corner (locomotion: H (3) =1.06, p =0.78; immobility: H (3) =3.21, p =0.36; grooming: H (3) =5.90, p =0.11).

In experiment 3 (20h FD, Figure 2, Figure S8), no significant differences were seen among the groups (F-C, F-FD, M-C, M-FD) for raw durations of locomotion (H (3) =0.71, p =0.87), immobility (H (3) =5.40, p =0.14) and grooming (H (3) =2.68, p =0.44) in the capillary zone. In the lane, no significant differences were seen among the groups for raw durations of locomotion (H (3) =2.79 p =0.42), immobility (H (3) =0.2, p =0.98), and grooming (H (3) =1.06, p =0.78). In the corner, no significant

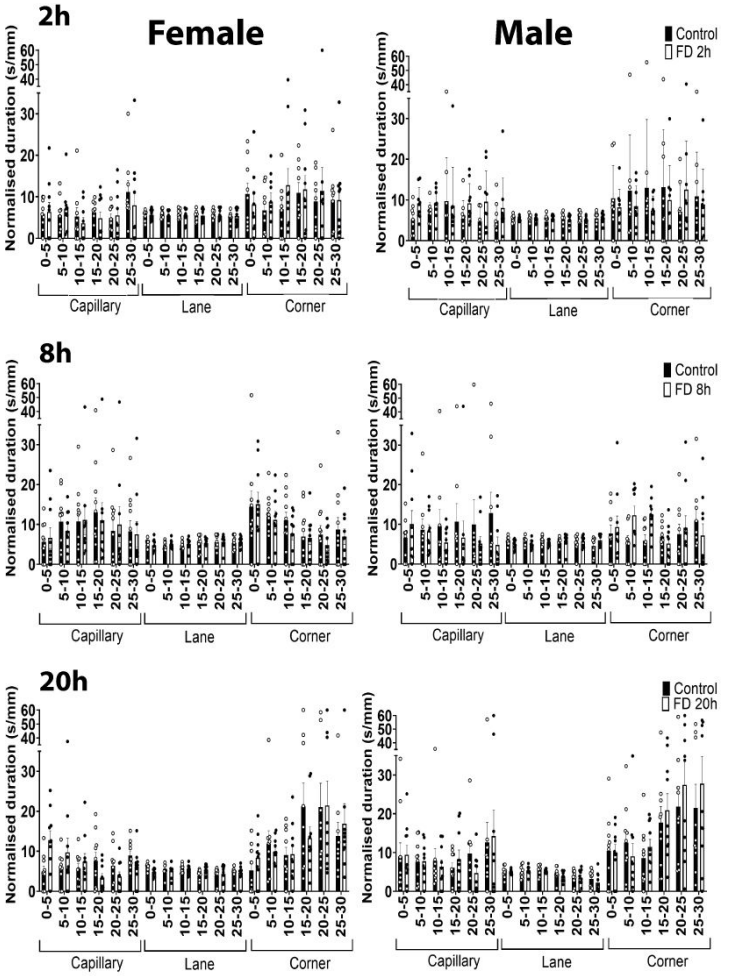

Figure 2. Normalised duration (seconds *per* millimetre, s/mm) in each lane sector (capillary, lane, corner) summarized in blocks of 5 min over 30 min of the one-capillary lane-maze test. Female (left panels) or male (right panels) flies were food-deprived (FD) for 0 h (control in experiments 1-3), 2 h (experiment 1, upper panels), 8 h (experiment 2, middle panels), or 20 h (experiment 3, lower panels) before behavioural testing. Sample sizes *per* group- (Experiment 1): Control, females, n=9; males, n=9; FD, females, n=10; males, n=9. (Experiment 2): Control, females, n=11; males, n=9; FD, females, n=10; males, n=10. (Experiment 3): Control, females, n=10; males, n=10; FD, females, n=10; males, n=10. Data are expressed as mean ±SEM. White circles = individual data of the control group; black circles= individual data of the FD group. Kruskal–Wallis statistical test indicated no conclusive difference between groups (F-C, F-FD, M-C, M-FD).

differences were seen among the groups for locomotion (H (3) =1.09, p =0.78), immobility (H (3) =3.43, p =0.33), and grooming (H (3) =3.42, p =0.33). Normalised durations were not conclusively different among the groups for any category in the capillary (locomotion H (3) =0.71, p =0.87; immobility: H (3) =5.40, p =0.14; grooming: H (3) =2.68, p =0.44) or in the lane (locomotion: H (3) =2.79, p =0.42; immobility: H (3) =0.2, p =0.98; grooming: H (3) =1.06, p =0.78) or in the corner (locomotion: H (3) =1.09, p =0.78, immobility: H (3) =3.43, p =0.33, grooming: H (3) =3.42, p =0.33).

Effects of food deprivation on the P index of the one-capillary lane-maze test

The value of the P-index varied from group to group (Figure 3, Figure S9, Table S6). Most of the P-indices were negative except for a slight, no conclusive, positive P-index in the control group of male flies in experiment 2 (M, C 8h, Figure 3). The P-index was significantly (Wilcoxon test, p< 0.05) different from zero (P index=-0.33±0.12) in the group of female flies food-deprived for 2 hours (F, FD 2h Figure 3). Between-group comparisons (F-C, F-FD, M-C, M-FD) indicated no conclusive differences in any experiment (Kruskal–Wallis: 2 h FD: H (3) =3.4, *p*=0.3; 8 h: H (3) =2.7, *p* =0.4; 20 h FD: H (3) =0.3 *p* =0.9; Figure 3, Figure S9). For all statistical results, see supplementary results.

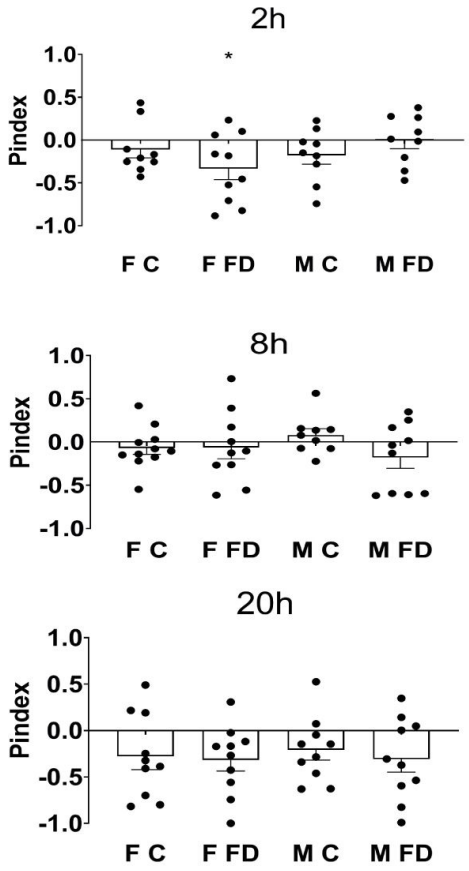

Figure 3. P-index of female (F) or male (M) flies of the control (C) or food-deprived (FD) groups in experiment 1 (2 h FD; left panel), experiment 2 (8 h FD; middle panel) and experiment 3 (20 h FD; right panel). For sample sizes *per* group, see the legend of Figure 2. Data are expressed as mean ±SEM. Circles= individual data. (*) significantly different from zero according to the Wilcoxon test, p< 0.05.

DISCUSSION

The lane-maze allowed for the simultaneous scoring of the durations of locomotion, immobility, and grooming of 10 flies in a trial. The behaviour of each fly was observed in different zones of the apparatus. However, contrary to the primary hypothesis, flies avoided exploration of the zone of

the capillaries filled with sucrose during the one-capillary lane-maze test. Independent of sex, flies stayed, on average, longer in the corner opposite the capillary, generating negative preference indices. Negative preference indices indicate aversion to the capillary zone. Moreover, food deprivation failed to induce a preference for capillaries filled with sucrose in the one-capillary lane-maze test. These results indicate that the lane-maze apparatus was helpful in simultaneously evaluating several behavioural outcomes in multiple flies. Nevertheless, the one-capillary lane-maze test failed to identify sucrose preference in flies of either sex. Instead, the one-capillary lane-maze test revealed that the flies avoided exploration of the capillary zone of the lane. The unanticipated results in the one-capillary lane-maze test can be related to the experimental design, procedures, biology of the flies, or a combination of these factors, as discussed below.

Concerning the experimental design, for example, many previous studies tested flies collectively in the same test environment (CAFE[14,15,18,19] or dyed abdomen[20]). In the present study, individual flies were used as the experimental units, as in the experiments using ARC[22]. Beyond the consequences of the statistical analysis (see litter effect[32]), flies may behave differently when tested in groups or individually in mnemonic tests[33]. However, Ja et al.[18] found a similar preference for sucrose in flies tested individually or in groups. Another relevant aspect related to the experimental design is the assessment of outcomes. In the present study, experimenters and analysts were unaware of the identity of the experimental groups, a characteristic that was unclear in some previous studies[14,15,16,17,18]. In addition, most previous studies included only males in the experimental groups[14,15,18] or a mixture of male and female flies[16]. Here, based on the hypothesis of sexual dimorphism in the sucrose preference test, male and female flies were assigned to independent groups. Moreover, the experimental flies were virgin, that is, grown in bottles separated by sex, which could influence behavioural outcomes. In future studies, comparing virgin and non-virgin flies of both sexes in the lane-maze test would be an interesting inquiry.

Beyond sex, other aspects of fly biology, such as strain, age, appetite for sugar, aversion to novelty, and motor and sensory skills, could affect behavioural outcomes. Sugar solution is a natural attractor for different strains of *Drosophila* (e.g. Harwich, Canton-S)[14,15,18]. The sucrose solution was used as a reward or a physiologically relevant reinforcement in paradigms of appetitive olfactory conditioning in *Drosophila* with different genetic backgrounds (e.g. Canton-S, amnesic mutants, several mutants of dorsal paired medial neurones, *others*)[34,35,36]. Moreover, flies have demonstrated a preference for different concentrations of sucrose in various tests such as CAFE (5% or 0.1 M)[18], proboscis extension and dyed abdomen (100 and 4 mM)[20], or FLIC (1%, 10%)[21]. The addition of blue dye to the sucrose solution seem not affect the attractive properties of sugar to the flies once it was previously used to estimate sucrose intake in *Drosophila*[20,37]. Therefore, the aversion to the capillary zone observed in the one-capillary lane-maze test in flies was unlikely due to the presence of sucrose solution, blue dye, or their mixture. In the lane-maze preference test, future studies should use other types of attractive substances, such as vinegar, fructose, or ethyl acetate, which were deemed attractive to *Drosophila* larvae or adults[38,39,40]. Novelty suppressed feeding could also explain the aversion to the capillary zone. However, whether flies such as rodents[41] display 'novelty-suppressed feeding' remains unclear. In future experiments, a longer period of acclimation before testing could reduce putative novelty-suppressed feeding in flies. Moreover, new studies in flies with different ages or lacking olfactory receptor neurons[42] would contribute to the understanding of preference mechanisms in adult *Drosophila* when tested in the lane-maze.

Procedural factors may explain these results. The orientation of the testing apparatus, for example, may play a role in the exploratory behaviour of flies. *Drosophila* displays a negative geotaxis, an innate response of climbing to the top of a vertical cylinder after being tapped to its bottom[43]. A test such as ARC[22] performed in a vertical cylinder may facilitate flies exploration because of negative geotaxis. In contrast, the one-capillary-lane-maze test was conducted in a horizontal apparatus, which does not allow negative geotaxis to occur. A role for negative geotaxis in the preference test would require further study. Other procedural factors potentially relevant to explain the present results include the configuration of the preference test, duration of the trial, and calculation of the sucrose preference index. In rodents, the one-bottle or two-bottle configuration test may be used to assess sucrose preference[25,26]. Nevertheless, the two-bottle configuration test was more sensitive in detecting taste

aversion than the one-bottle configuration[44]. In future studies, a two-capillary lane-maze test may be more sensitive than the one-capillary lane-maze test in detecting sucrose preference in flies.

In rodents, sucrose preference indices are often calculated using the proportion of sucrose intake in the total fluid consumed in 24 h[24,25]. In *Drosophila*, studies using CAFE[14,15,18,19] measured fluid intake within 24 h when a group of flies was the experimental unit. Because the experimental unit in the one-capillary lane-maze test was an individual fly, the fluid intake was too low (less than 1 µL) to be accurately measured from the capillary during the test. Hence, future experiments should consider the extended periods of the lane-maze test. Owing to the above-mentioned limitations originating from an individual fly as an experimental unit, the preference index in the one-capillary lane-maze test was a proxy for sucrose preference. The preference index was based on the hypothesis that flies would stay longer near the capillary than near the other regions of the lane-maze. Consequently, the sucrose preference index, as calculated in this study, may be biased by the motor skills of the flies, as it is dependent on the exploration of the environment. For example, small, imperceptible lesions in the paws due to manipulations or sequelae of anaesthesia may disable the flies from exploring the lane. However, the scoring of locomotion, immobility, and grooming during the one-capillary-lane-maze test suggested that the experimental flies had optimal motor abilities. Future studies should also exclude the influence of anaesthesia on behaviour by transferring flies from the experimental tubes to the lane-maze using a mouth aspirator[45]. Future studies measuring sucrose intake or licking behaviour to calculate the preference index would provide a more reliable estimation of sucrose preference in flies. A practical solution to detect the interaction of an individual fly with sucrose would be to use a sensor for the proboscis extension at the tip of the capillary[17].

According to previous literature, food deprivation may induce hyperactivity, centrophilic behaviour, and sucrose preference in flies[16]. Remarkably, results by Vogt et al.[38] suggested that the nutritional state affects the preference for odours in *Drosophila* larvae. For example, geranyl acetate and menthol, which are innately aversive to fed larvae, are attractive to food-deprived larvae[38]. Studies by Ko et al.[40] showed that starved female flies exhibited enhanced sensitivity to attractive odours and reduced sensitivity to aversive odours. In the present study, no pronounced effect of food deprivation was observed in the one-bottle lane-maze test in either female or male flies. Indeed, food deprivation failed to promote hyperactivity compared to control flies, perhaps because locomotion is already a high-incident behaviour in the lane-maze. Moreover, the duration of food deprivation in flies was not a determinant of preference for the capillary zone. Independent of the food deprivation time, 2, 8, or 20 h, flies of either sex stayed, on average, longer in the corner without capillaries. Short exploration of the capillary zone generated negative preference indices, which indicated aversion to the capillary zone by most of the flies. In the present conditions, food deprivation or sex of the flies may have had a moderate to low effect size on sucrose preference in the lane-maze test. Present experiments were powered to detect large effect sizes. Future studies should use power analysis to calculate sample sizes to detect the small effects of sex, food deprivation, or other interventions on the preference index.

In conclusion, in the present study, flies avoided the zone near the sucrose capillaries in the lane-maze test, indicating that sex and food deprivation had smaller effects on the behaviours of flies tested in the one-capillary-lane-maze test than anticipated in the experimental plan of this study. This exploratory research forms the basis of more conclusive research helping to set the research design, sampling methodology, and method for data collection in future studies.

Identification of dissertation-originated material

This material is part of an academic dissertation developed at the Post Graduate Program of Pharmacology of the Federal University of Santa Catarina, Florianópolis, Brazil.

ACKNOWLEDGEMENTS

The authors thank Fabiani F Triches for the support in the maintenance of the vivarium, Tamires M Marchetto for the blinding of the video recordings of behavioural testing, and João A Marcolan for the assistance with the Ethowatcher software. Juliana A. Bolzan for the assistance with the figures. We would like to thank Editage (www.editage.com) for English language editing.

Financial support: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001, Conselho Nacional de Desenvolvimento Científico e Tecnológico– Brasil (CNPq), Alexander von Humboldt Foudation (Germany).

REFERENCES

1. Robinson NB, Krieger K, Khan F, et al. The current state of animal models in research: a review. Int J Surg. 2019;72:9-13. http://dx.doi.org/10.1016/j.ijsu.2019.10.015. PMid:31627013.
2. National Centre for the Replacement Refinement and Reduction of Animals in Research [Internet]. London: NC3Rs; c2023. [cited 2023 Apr 21]. https://www.nc3rs.org.uk/the-3rs
3. Ridder D, Adhia D, Vanneste S. The anatomy of pain and suffering in the brain and its clinical implications. Neurosci Biobehav Rev. 2021;130:125-46. http://dx.doi.org/10.1016/j.neubiorev.2021.08.013. PMid:34411559.
4. Bellen HJ, Tong C, Tsuda H. 100 years of *Drosophila* research and its impact on vertebrate neuroscience: a history lesson for the future. Nat Rev Neurosci. 2010;11(7):514-22. http://dx.doi.org/10.1038/nrn2839. PMid:20383202.
5. Jennings BH. *Drosophila*–a versatile model in biology & medicine. Mater Today. 2011;14(5):190-5. http://dx.doi.org/10.1016/S1369-7021(11)70113-4.
6. Ries AS, Hermanns T, Poeck B, Strauss R. Serotonin modulates a depression-like state in *Drosophila* responsive to lithium treatment. Nat Commun. 2017;8(1):15738. http://dx.doi.org/10.1038/ncomms15738. PMid:28585544.
7. Moulin TC, Covill LE, Itskov PM, Williams MJ, Schiöth HB. Rodent and fly models in behavioral neuroscience: an evaluation of methodological advances, comparative research, and future perspectives. Neurosci Biobehav Rev. 2021;120:1-12. http://dx.doi.org/10.1016/j.neubiorev.2020.11.014. PMid:33242563.
8. Harris KP, Littleton JT. Transmission, development, and plasticity of synapses. Genetics. 2015;201(2):345-75. http://dx.doi.org/10.1534/genetics.115.176529. PMid:26447126.
9. Hermanns T, Graf-Boxhorn S, Poeck B, Strauss R. Octopamine mediates sugar relief from a chronic-stress-induced depression-like state in *Drosophila*. Curr Biol. 2022;32(18):4048-56.e3. http://dx.doi.org/10.1016/j.cub.2022.07.016. PMid:35914533.
10. Hidalgo S, Molina-Mateo D, Escobedo P, et al. Characterization of a novel *Drosophila* SERT mutant: insights on the contribution of the serotonin neural system to behaviors. ACS Chem Neurosci. 2017;8(10):2168-79. http://dx.doi.org/10.1021/acschemneuro.7b00089. PMid:28665105.
11. Kasture AS, Hummel T, Sucic S, Freissmuth M. Big lessons from tiny flies: *Drosophila melanogaster* as a model to explore dysfunction of dopaminergic and serotonergic neurotransmitter systems. Int J Mol Sci. 2018;19(6):1788. http://dx.doi.org/10.3390/ijms19061788. PMid:29914172.
12. Mohammad F, Aryal S, Ho J, et al. Ancient anxiety pathways influence *Drosophila* defense behaviors. Curr Biol. 2016;26(7):981-6. http://dx.doi.org/10.1016/j.cub.2016.02.031. PMid:27020741.
13. Vollmayr B, Gass P. Learned helplessness: unique features and translational value of a cognitive depression model. Cell Tissue Res. 2013;354(1):171-8. http://dx.doi.org/10.1007/s00441-013-1654-2. PMid:23760889.
14. Araujo SM, Poetini MR, Bortolotto VC, et al. Chronic unpredictable mild stress-induced depressive-like behavior and dysregulation of brain levels of biogenic amines in *Drosophila melanogaster.* Behav Brain Res. 2018;351:104-13. http://dx.doi.org/10.1016/j.bbr.2018.05.016. PMid:29803654.
15. Araujo SM, Bortolotto VC, Poetini MR, et al. γ-Oryzanol produces an antidepressant-like effect in a chronic unpredictable mild stress model of depression in *Drosophila melanogaster.* Stress. 2021;24(3):282-93. http://dx.doi.org/10.1080/10253890.2020.1790519. PMid:32723199.
16. Ramos-Hryb AB, Ramirez MF, Lino-de-Oliveira C, Pagani MR. Stress-mediated hyperactivity and anhedonia resistant to diazepam and fluoxetine in *Drosophila.* Stress. 2021;24(1):96-106. http://dx.doi.org/10.1080/10253890.2020.1759547. PMid:32319840.
17. Itskov PM, Moreira JM, Vinnik E, et al. Automated monitoring and quantitative analysis of feeding behaviour in *Drosophila.* Nat Commun. 2014;5(1):4560. http://dx.doi.org/10.1038/ncomms5560. PMid:25087594.
18. Ja WW, Carvalho GB, Mak EM, et al. Prandiology of *Drosophila* and the CAFE assay. Proc Natl Acad Sci USA. 2007;104(20):8253-6. http://dx.doi.org/10.1073/pnas.0702726104. PMid:17494737.
19. Diegelmann S, Jansen A, Jois S, et al. The CApillary FEeder assay measures food intake in *Drosophila melanogaster.* J Vis Exp. 2017;121(121):55024. PMid:28362419.
20. Gordesky-Gold B, Rivers N, Ahmed OM, Breslin PA. *Drosophila melanogaster* prefers compounds perceived sweet

by humans. Chem Senses. 2008;33(3):301-9. http://dx.doi.org/10.1093/chemse/bjm088. PMid:18234713.

21. Ro J, Harvanek ZM, Pletcher SD. FLIC: high-throughput, continuous analysis of feeding behaviors in *Drosophila*. PLoS One. 2014;9(6):e101107. http://dx.doi.org/10.1371/journal.pone.0101107. PMid:24978054.

22. Murphy KR, Park JH, Huber R, Ja WW. Simultaneous measurement of sleep and feeding in individual *Drosophila*. Nat Protoc. 2017;12(11):2355-59. http://dx.doi.org/10.1038/nprot.2017.096. PMid:29022943.

23. Eckert FB, Valdati DB, Toni DC, et al. Lane-maze for behavioral tests in flies. Drosoph Inf Serv. 2020;103:77-80.

24. Towell A, Muscat R, Willner P. Effects of pimozide on sucrose consumption and preference. Psychopharmacology. 1987;92(2):262-4. http://dx.doi.org/10.1007/BF00177926. PMid:3110851.

25. Rygula R, Abumaria N, Flügge G, Fuchs E, Rüther E, Havemann-Reinecke U. Anhedonia and motivational deficits in rats: impact of chronic social stress. Behav Brain Res. 2005;162(1):127-34. http://dx.doi.org/10.1016/j.bbr.2005.03.009. PMid:15922073.

26. Frie JA, Khokhar JY. An open source automated two-bottle choice test apparatus for rats. HardwareX. 2019;5:e00061. http://dx.doi.org/10.1016/j.ohx.2019.e00061. PMid:31245655.

27. Domingues K, Lima FB, Linder AE, et al. Sexually dimorphic responses of rats to fluoxetine in the forced swimming test are unrelated to the function of the serotonin transporter in the brain. Synapse. 2020;74(1):e22130. PMid:31449695.

28. Remus JL, Stewart LT, Camp RM, Novak CM, Johnson JD. interaction of metabolic stress with chronic mild stress in altering brain cytokines and sucrose preference. Behav Neurosci. 2015;129(3):321-30. http://dx.doi.org/10.1037/bne0000056. PMid:25914924.

29. Wang Z, Gu J, Wang X, et al. Antidepressant-like activity of resveratrol treatment in the forced swim test and tail suspension test in mice: the HPA axis, BDNF expression and phosphorylation of ERK. Pharmacol Biochem Behav. 2013;112:104-10. http://dx.doi.org/10.1016/j.pbb.2013.10.007. PMid:24125781.

30. Marcolan JA. Ferramenta de código aberto para análise de comportamento e aquisição de vídeo em "tempo real" usando técnicas de visão computacional e processamento paralelo [dissertation]. Florianópolis: Universidade Federal de Santa Catarina; 2017.

31. Lehner PN. Handbook of ethological methods. 2nd ed. Cambridge: Cambridge University Press; 1998.

32. Golub MS, Sobin CA. Statistical modeling with litter as a random effect in mixed models to manage "intralitter likeness". Neurotoxicol Teratol. 2020;77:106841. http://dx.doi.org/10.1016/j.ntt.2019.106841. PMid:31863841.

33. Chabaud MA, Isabel G, Kaiser L, Preat T. Social facilitation of long-lasting memory retrieval in *Drosophila*. Curr Biol. 2009;19(19):1654-9. http://dx.doi.org/10.1016/j.cub.2009.08.017. PMid:19781943.

34. Tempel BL, Bonini N, Dawson DR, Quinn WG. Reward learning in normal and mutant *Drosophila*. Proc Natl Acad Sci USA. 1983;80(5):1482-6. http://dx.doi.org/10.1073/pnas.80.5.1482. PMid:6572401.

35. Krashes MJ, Waddell S. Rapid consolidation to a radish and protein synthesis-dependent long-term memory after single-session appetitive olfactory conditioning in *Drosophila*. J Neurosci. 2008;28(12):3103-13. http://dx.doi.org/10.1523/JNEUROSCI.5333-07.2008. PMid:18354013.

36. Schwaerzel M, Monastirioti M, Scholz H, Friggi-Grelin F, Birman S, Heisenberg M. Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in *Drosophila*. J Neurosci. 2003;23(33):10495-502. http://dx.doi.org/10.1523/JNEUROSCI.23-33-10495.2003. PMid:14627633.

37. Deshpande SA, Carvalho GB, Amador A, et al. Quantifying *Drosophila* food intake: comparative analysis of current methodology. Nat Methods. 2014;11(5):535-40. http://dx.doi.org/10.1038/nmeth.2899. PMid:24681694.

38. Vogt K, Zimmerman DM, Schlichting M, et al. Internal state configures olfactory behavior and early sensory processing in *Drosophila* larvae. Sci Adv. 2021;7(1):eabd6900. http://dx.doi.org/10.1126/sciadv.abd6900. PMid:33523854.

39. Fishilevich E, Domingos AI, Asahina K, Naef F, Vosshall LB, Louis M. Chemotaxis behavior mediated by single larval olfactory neurons in *Drosophila*. Curr Biol. 2005;15(23):2086-96. http://dx.doi.org/10.1016/j.cub.2005.11.016. PMid:16332533.

40. Ko KI, Root CM, Lindsay SA, et al. Starvation promotes concerted modulation of appetitive olfactory behavior via parallel neuromodulatory circuits. eLife. 2015;4:e08298. http://dx.doi.org/10.7554/eLife.08298. PMid:26208339.

41. Ramaker MJ, Dulawa SC. Identifying fast-onset antidepressants using rodent models. Mol Psychiatry. 2017;22(5):656-65. http://dx.doi.org/10.1038/mp.2017.36. PMid:28322276.

42. Elmore T, Ignell R, Carlson JR, Smith DP. Targeted mutation of a *Drosophila* odor receptor defines receptor requirement in a novel class of sensillum. J Neurosci. 2003;23(30):9906-12. http://dx.doi.org/10.1523/JNEUROSCI.23-30-09906.2003. PMid:14586020.

43. Gargano JW, Martin I, Bhandari P, Grotewiel MS. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in *Drosophila*. Exp Gerontol. 2005;40(5):386-95. http://dx.doi.org/10.1016/j.exger.2005.02.005. PMid:15919590.

44. Batsell WR, Best MR. One bottle too many? Method of testing determines the detection of overshadowing and retention of taste aversions. Anim Learn Behav. 1993;21(2):154-8. http://dx.doi.org/10.3758/BF03213395.

45. Barradale F, Sinha K, Lebestky T. Quantification of *Drosophila* grooming behavior. J Vis Exp. 2017;125:e55231. PMid:28745619.

Supplementary Material

Supplementary material accompanies this paper.

Figure S1. Agreement calculations.
Figure S2. First version of the catalogue.
Figure S3. Second (final) version of the catalogue.
Figure S4. Calculation of the normalized duration.
Figure S5. Calculation of the preference index (P-index).
Figure S6. Raw duration of behaviours in the lane-maze test.
Figure S7. Raw duration of behaviours in the lane-maze test (experiment 1).
Figure S8. Normalized duration of behaviours in the lane-maze test (experiment 1).
Figure S9. Raw duration of behaviours in the lane-maze test (experiment 2).
Figure S10. Normalized duration of behaviours in the lane-maze test (experiment 2).
Figure S11. Raw duration of behaviours in the lane-maze test (experiment 3).
Figure S12. Normalized duration of behaviours in the lane-maze test (experiment 3).
Figure S13. Preference index segmented in pools of 5 minutes (experiments 1, 2, 3).
Figure S14. Latency to recovery from anaesthesia.
Figure S15. Behavioural outcomes of the lane maze test without the capillary.
Table S1. First catalogue: intra-observer agreement and agreement by behaviour.
Table S2. Second (final) version of the behavioural catalogue.
Table S3. Raw duration of behaviours in the lane-maze test.
Table S4. Raw durations: means, standard deviations, sample sizes (experiments 1, 2, 3).
Table S5. Normalized durations: means, standard deviations, sample sizes (experiments 1, 2, 3).
Table S6. Preference index (P index) and sample sizes (experiments 1, 2, 3).

This material is available as part of the online article from https://doi.org/10.4322/2675-9225.00142022