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Severity assessment of experimental procedures by means of conditioned place preference/aversion - improving the procedure

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Summary

It has been proposed to use conditioned place aversion and place preference tests to assess the severity of different experimental procedures and behavioural tests. However, this method was not used in this context before. After first experiments (see DOI: 10.17590/asr.0000112) we will now conduct further experiments to improve the procedure. For this, two experimental procedures (unconditioned stimuli) are paired with two kinds of flooring (neutral stimuli becoming conditioned stimuli). After conditioning, the flooring materials will be tested in conditioned place preference tests (or conditioned place aversion, respectively) in order to derive information about the valence of the experimental procedures.

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1. General Information

Keywords

Preference test, conditioned place preference, conditioned place aversion, severity assessment, mice, flooring, material, choice test

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Not provided

Additional remarks

proceeding of the experiments described in DOI: 10.17590/asr.0000112

2. Study design

Introduction

“Severity assessment in animal experimentation is a complex biomedical and ethical issue and largely biased by uncertainty. The interpretation of physiological and behavioural measures in relation to animal welfare is difficult and often reflecting an educated gut feeling rather than scientifically sound conclusions. It is thus utmost important to include the perspective of the animals themselves into severity assessment. Choice and preference tests are a straightforward approach in asking the appraisal of different goods” (Habedank et al. 2018; DOI: 10.2376/0005-9366-18007). To assess the severity of different experimental procedures and behavioural tests, a conditioned place preference test is a promising test procedure. However, this method has not been used in this context before, thus, a first study shall be conducted to test the applicability. Conditioned place preference (or aversion, respectively) is a form of classical (Pavlovian) conditioning in which a previously neutral stimulus becomes associated with a motivationally significant stimulus. As a consequence of this learning procedure, the conditioned stimulus is able to evoke a similar response to the one that was caused by the unconditioned stimulus. In the subsequent preference test, the animal is offered a choice between a spatial location near the conditioned stimulus and a location with a different stimulus which had not be paired with the same unconditioned stimulus. If the subject chooses the location with the conditioned stimulus, the unconditioned stimulus is interpreted to be positively associated. If it chooses the location with the unpaired stimulus, the unconditioned stimulus seems to be negatively associated. Hence, with the help of a conditioned place preference test, the valence of the unconditioned stimulus can be investigated.

After the first experiments (see DOI: 10.17590/asr.0000112) we will now conduct further experiments to improve the procedure. To do so, two experimental procedures (restraint by hand vs. weighing, unconditioned stimuli) shall be paired with two kinds of flooring material (neutral stimuli becoming conditioned stimuli). After the conditioning phase the valence of the conditioned testing procedures will be tested by means of analysing the preference for the flooring materials that have been associated with the respective procedures. Preference testing will be performed in a two-choice test comparing the two paired stimuli and thus allowing a ranking between the tested experimental procedures.

Type of research

Exploratory

Hypothesis of your study

Using conditioned place preference it is possible to test mice for their preference for different experimental procedures.

Study design

Twelve C57BL/6J mice live together in a home cage. One test procedure lasts 6 days, with day 1 for habituation and assessment of a baseline preference between two neutral stimuli, day 2-5 for conditioning of stimulus A and B, with two conditioning sessions per day. (Which stimulus is A and which is B differs for half of the group). On day 6 the place preference test will take place. On every experimental day, only one conditioning session will be performed.

As unconditioned stimuli, two different experimental methods, namely restraint by hand or weighing, will be tested. As conditioned stimuli, two different flooring materials will be used, e.g. two different kinds of grid. Previous preference for the floorings will assessed in the baseline test a few days before the start of the conditioning sessions. Only, if there is no significant preference for either of the floorings before conditioning, they will be used. Otherwise, baseline testing will be repeated with a different set of floorings.

Method of blinding

The researcher has no contact with the mice during the final preference test (data is recorded automatically) and thus, cannot influence the choice of the mice.

Method of randomization

The pairing of bedding stimulus and experimental procedure will be randomized for all mice (e.g., half of the mice will associate flooring A with procedure A and half of the mice flooring A with procedure B). Also, the mice will be randomly assigned to two groups, of which one will start with procedure A and one with procedure B. In addition, the order in which the mice are taken out of the home cage for the sessions will be randomized, as well as the order of mice for the preference test. Also, the cage (left or right cage) into which the mice is placed for the habituation session or the preference test is randomized.

3. Methods

3. 1. Home Cage

Description of the method

One group of mice (12 mice) is kept in a home cage system consisting of two type IV macrolon cages (Tecniplast) connected by a Perspex tube. They live in this system since they were around 2 months old (for other research purposes). As a change of this home cage setting could possibly influence their behavior we decided to not apply any changes. The second group of mice (12 mice) will be kept in one type IV macrolon cage (Tecniplast).

For both groups applies: Food (LASvendi, LAS QCDiet, Rod 16, autoclavable) and tab water are available *ad libitum* in both cages, as well as bedding material (JRS Lignocel FS14, spruce/ fir, 2,5-4 mm), a red house (TheMouseHouse, Tecniplast), two wooden bars to chew on, two papers, two cotton rolls and 6 strands of additional nesting material.

For the habituation and condition sessions, as well as for the preference test the mice are taken out of the home cage into a transport cage, to be moved to the experimental room. Here they stay for 30 min before the start of the sessions to habituate to the environment. After the sessions the mice are transported back to the housing room and returned to their home cage. If not noted otherwise, mice are always handled by tunnel handling.

Narcotic/analgesic treatment

Not provided

Drugs/substances

Not provided

Antibodies

Not provided

Cell lines, viruses, DNA or RNA constructs and bacteria

Not provided

3. 2. Transponder Implantation

Description of the method

For other research purposes, all twelve mice have a subcutaneous transponder. At the age of around 5 weeks the transponders (Planet ID, FDX-B transponder according to ISO 11784/85) were implanted under the skin in the neck, a procedure performed under anesthesia (see below). A second transponder transplantation (group 1: 56 days old, group 2: not yet known) had to be performed because two mice lost their transponder after the first implantation.

Narcotic/analgesic treatment

Two hours before (group 1) or the evening before (group 2) the transponder implantation, all mice obtained an analgesic (Meloxicam). For the implantation itself, mice were anesthetized with isoflurane. Afterwards, mice were placed in a separate cage with bedding and paper until they were fully awake again. Then, they were returned to their home cages.

Drugs/substances

Not provided

Antibodies

Not provided

Cell lines, viruses, DNA or RNA constructs and bacteria

Not provided

3. 3. Conditioning Procedure

Description of the method

Habituation session (day 1)

The habituation session takes place in the morning, in a transportation cage the mouse group is moved to the testing room. The mice get 30 min of habituation time to the new room. One type III cage is divided by a small barrier (2 cm high) into two areas of the same size containing the different floorings (material A and B). Mice are placed inside the system (whether left or right cage is randomized) and activity is recorded for 10 min. The results of stay time on each flooring serve as a baseline preference. If one of the mice does not change cages during the habituation session (and therefore, did not experience both conditions), the habituation phase is to be repeated with this mouse.

Activity is recorded using a webcam (Logitech C930e, Switzerland) and the open source recording program iSpy 64 (version 7.0.3.0). Stay time on each flooring is only measured when all four paws of the mice are in one of the halves.

Conditioning sessions (day 2-5)

Mice are randomly assigned to a conditioning group. In conditioning group 1, flooring A is paired with procedure A and flooring B with procedure B, while in conditioning group 2, flooring A is paired with procedure B and the other way round. There are two conditioning sessions per day, the second one taking place directly after the first one. To prevent a time effect (early morning versus late morning), the procedure tested last one day will be the first to be tested the next day (e.g. day 2: procedure A, procedure B, day 3: procedure B, procedure A, day 4: procedure A, procedure B, …)

The conditioning sessions take place in the morning, the mouse group is moved in a transportation cage to the testing room. The mice get 30 min of habituation time to the new room. For each session, a mouse is taken out of the home cage and placed into one half the type III cage also used during habituation session for 3 min. During this time, access to the other side is blocked by an opaque plate so the mouse has only direct contact with one of the conditioned stimuli (floorings). Afterwards, the mouse performs the experimental procedure (unconditioned stimulus, restraint by hand or weighing). Afterwards the mouse is taken directly back to the transportation cage.

Preference test (day 6)

The preference test will be performed the same way as the habituation session (only order and start side of the mice are again randomized).

Narcotic/analgesic treatment

Not provided

Drugs/substances

Not provided

Antibodies

Not provided

Cell lines, viruses, DNA or RNA constructs and bacteria

Not provided

3. 4. Experimental Procedures (Unconditioned Stimuli)

Description of the method

Restraint by hand

The mouse is taken out of the conditioning cage by tube handling and placed on top of an upside down type III cage (this was chosen to have smooth flooring similar to the glass jar of the weighing procedure). While holding the tail with one hand, the animals gets restraint by taking the loose skin of the scruff between thumb and index finger of the other hand and lifting the mouse of the lid (as described, e.g., by Hurst & West 2010). The mouse is held for 20 s, before it gets released straight into the conditioning cage again. The surface will be cleaned with 70 % ethanol in order to eliminate any olfactory cues for subsequently tested mice.

Weighing

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The mouse is taken out of the conditioning cage by tube handling and placed into a glass jar on top of a scale. A lid is placed on the jar to prevent the mouse from climbing out. After weight has been noted, the lid is lifted and the mouse is taken back to the conditioning cage by tube handling. The glass jar will be cleaned with 70 % ethanol in order to eliminate any olfactory cues for subsequently tested mice.

Narcotic/analgesic treatment

Not provided

Drugs/substances

Not provided

Antibodies

Not provided

Cell lines, viruses, DNA or RNA constructs and bacteria

Not provided

3. 5. Flooring Material (Conditioned Stimuli)

Description of the method

As flooring material (conditioned stimuli), two different metal sheets (obtained from a local do it yourself store) are placed on the floor of the conditioning cage, one with holes and one with slits. Thus, the flooing material comprises a visual and a haptic cue. In addition, the side of the conditioning cage in which the stimulus is presented can work as a spatial cue.

Narcotic/analgesic treatment

Not provided

Drugs/substances

Not provided

Antibodies

Not provided

Cell lines, viruses, DNA or RNA constructs and bacteria

Not provided

4. Statistics

4. 1. Conditioned Preference Test

Assigned method(s)

Conditioning Procedure

Main endpoints

For the conditioned preference test the main endpoint is a percentage of stay duration in either of the halves with different flooring of the conditioning cage (in contact with the conditioned stimulus). If it differs significantly from 0.5 (chance level), there is a preference.

Secondary endpoints

Not provided

Sample size calculation

In the protocol for conditioned place preference, Cunningham et al. 2006 stated that based on experience, 12 to 16 mice per treatment group would provide enough statistical power to detect treatment effects. Here, there is only one group whose behavior should be compared to chance level, so one group of twelve mice should be sufficient. In addition, we plan to repeat the test with group 2 to achieve a sample size of 24 mice.

Primary statistical analysis

During the habituation session, a baseline preference is measured, consisting of the total duration spent in each of the two halves of the conditioning cage. The first minute in the conditioning cage is counted as habituation time, the remaining 9 of the 10 minutes will be analysed. For every mouse, the stay time per cage side can be calculated and this dataset will then be used for statistical tests. To test for normal distribution, a Shapiro Wilk-Test will be used. If p > 0.05 normal distribution is assumed and a one sample t-test will be performed. If p <= 0.05 a sign test will be performed. Significance level is set to 0.05. The same will be repeated for the analysis of the preference test. In addition, a comparison of the results before and after the conditioning (habituation session vs. preference test) can be achieved by conducting a paired t-test.

Exclusion criteria

Not provided

5. Animals

5. 1. Mice (Mus musculus)

Animal strain/breed

C57BL/6J by Charles River, Sulzfeld

Genetically modified

No

Sex

Female

Further characteristics of the animals (e.g. age, body weight, size)

Group 1:

Female C57BL/6J mice were purchased from Charles River Sulzfeld in December 2017. The mice arrived at the institute at the age of three weeks. At the age of 33 and 56 days the transponders

(Planet ID, FDX-B transponder according to ISO 11784/85) were implanted under the skin in the neck, a procedure performed under anesthesia. For this study, twelve mice at the age of 18 months were used. Beforehand, the mice took part in the development of an automated home cage based choice test.

Group 2:

Twelve female C57BL/6J mice are purchased from Charles River Sulzfeld for June 2019. The mice will arrive at the institute at the age of four weeks. At the age of 5 weeks the transponders (Planet ID, FDX-B transponder according to ISO 11784/85) will be implanted under the skin in the neck, a procedure performed under anesthesia. For this study, twelve mice at the age of presumably 8 weeks will be used. Beforehand, the mice took part in the development of an automated home cage based choice test.

Housing conditions

One group of mice (12 mice) is kept in a home cage system consisting of two type IV macrolon cages (Tecniplast) connected by a Perspex tube. They live in this system since they were around 2 months old (for other research purposes). The second group of mice (12 mice) will be kept in one type IV macrolon cage (Tecniplast).

For both groups applies: Food (LASvendi, LAS QCDiet, Rod 16, autoclavable) and tab water are available *ad libitum* in both cages, as well as bedding material (JRS Lignocel FS14, spruce/ fir, 2,5-4 mm), a red house (TheMouseHouse, Tecniplast), two wooden bars to chew on, two papers, two cotton rolls and 6 strands of additional nesting material.

Room temperature is maintained at 22°C +/- 3°C, the humidity at 55% +/- 15%. Animals are kept at 12/12 dark/light cycle with the light phase starting at 8 am. Between 7:30 and 8:00 a sunrise is simulated. Once per week, the home cage is cleaned and all mice are scored and weighed. If not noted otherwise, mice are always handled by tunnel handling.

Refinement

To reduce anxiety, mice are handled by tunnel handling.

In the morning, before the top light at 8 a.m. goes on, a simulated sunrise started at 7:30 a.m. increasing smoothly the light intensity.

To encourage digging behaviour, cages were provided with bedding material of 4 cm in height minimum.

In between experiments (e.g. three weeks in May), a running wheel (igloo house with running disc on top, Zoonlab, Germany) was provided in each cage.

6. Updates

2019-07-10

During the implementation of the first experiment, some observations were made which caused us to alter the second experiment in three points:

1) 1) Mice of the first group urinated and defecated a lot inside the conditioning cage which could be a sign of stress. Therefore, the second group of mice will be habituated to the conditioning cage

before performing the baseline test. The habituation will take place the days before the baseline test and is going to involve 1 min of time in the conditioning cage with metal plates on the floor (these will be the same for both sides and differing from the plates used in the actual conditioning experiment). In-between mice, the metal plates will be cleaned with 70 % ethanol.

2) 2) The procedures tested are not going to be weighing (as supposedly neutral procedure) and fixation (as supposedly aversive procedure) but weighing and a food reward (a supposedly attractive procedure). Thus, the mice will be less stressed during the experiment, while we search for the optimized method to associate the procedure with the stimulus during presumably repeated experiments. In addition, during a food reward procedure the focus of the mice might not be as strongly on the flooring material used during the procedure as it is during fixation (because the mice are pressed against the floor to get the scruff). Therefore, it hopefully will be more successful in producing results.

Description of the food reward procedure:

Millet is used as food reward. Mice are habituated to the millet before the experiment by offering it for three days in the morning, three times 2 g in different places in the cage. During the conditioning, the mouse is taken out of the conditioning cage by tunnel handling and placed in a type III cage filled with bedding material (from the kind used during normal husbandry). On one end the cage contains a small amount of millet (0,1 g) as food reward. If the mouse does not start feeding until 1 min has passed, the mouse is taken out of the cage by tunnel handling and returned to the transport cage. If it starts feeding before that, the mouse is taken out of the cage immediately after it stops feeding. Feeding is defined as sitting beside the millet for some time and eating the grains audibly. In-between mice, the cage is cleaned with 70 % ethanol and bedding material is changed.

3) Because the metal plates turned very cold after cleaning (due to the evaporation of ethanol), the start temperature of the metal plates when the mouse is placed in the conditioning cage may differ between mice (depending on how long it took to get the mouse out of the cage). To reduce this difference, for all mice start time will be minimum 90 s after cleaning the metal plate.