Lars Lewejohann, 2019-04-16
DOI 10.17590/asr.0000112

Severity assessment of experimental procedures by means of conditioned place preference/aversion

Prof. Dr. Lars Lewejohann
German Federal Institute for Risk Assessment, Diedersdorfer Weg 1, 12277 Berlin, Germany

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, and thus, a first study shall be conducted to test the applicability. Here, four experimental procedures (unconditioned stimuli) are paired with four kinds of bedding material (neutral stimuli becoming conditioned stimuli). After conditioning, the bedding 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.

Registration details	
Status of the study	Accessible
Date of registration	2019-04-16
Date of publication	2024-02-19
DOI	10.17590/asr.0000112
Planned start of the study	2019-02-18
Planned end of the study	2019-05-15
License	

1. General Information

Keywords

Preference test, conditioned place preference, conditioned place aversion, severity assessment, mice, bedding

Funding sources

DFG research group 2591

International code of classification

Not provided

Additional remarks

This study is a proof of concept study and may have to be repeated after all possible error sources have been eliminated.

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 sbject 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. For this study, four experimental procedures (unconditioned stimuli) shall be paired with four kinds of bedding 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 bedding materials that have been associated with the respective procedures. Preference testing will be performed in a two-choice test comparing two of the 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

Thirteen C57BL/6J mice live together in a home cage. One test procedure lasts two weeks, with day 1 for habituation and assessment of a baseline preference between two neutral stimuli, day 2, 4, 8 and 10 for conditioning of stimulus A and day 3, 5, 9 and 11 for conditioning of stimulus B (which stimulus is A and which is B differs for half of the group). On day 12 the place preference test will take place. Onvery experimental day, only one conditioning session will be performed.

As unconditioned stimuli, different kinds of experimental methods, e.g., restraint, weighing procedure, Open Field or access to a running wheel will be tested. As conditioned stimuli, different kinds of bedding materials will be used. Previous preference for the bedding materials will be seen in the

baseline test at the beginning so that a potential preference can be taken into account (e.g., by pairing this with the stimulus which is assumed to be more aversive).

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 bedding A with procedure A and half of the mice bedding 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

The mice are kept in a home cage system consisting of two type IV macrolon cages (Tecniplast) connected by a Perspex tube. They live in such a 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. 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 (The MouseHouse, 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.

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 thirteen mice were implanted with a subcutaneous transponder. At the age of 33 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 (see below). A second transponder transplantation (56 days old) had to be performed in two mice due to loss of their transponder after the first implantation.

Narcotic/analgesic treatment

Two hours before 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. Two type II cages (Tecniplast) are connected via a short Perspex tube. Each of the two cages contains a different kind of bedding material (A or B). Mice are placed inside the system (whether left or right cage is randomized) and activity is recorded for 10 min. The results here serve as a baseline preference. If one of the mice does not change the 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 by an automated tracking system (containing two light barriers, an RFID reader which are connected to an Arduino computer) installed around the tube to detect the cage changes. This was, however, not tested before, and for this reason we use the video recordings as a back-up: Using a webcam (Logitech C930e, Switzerland) and the open source recording program iSpy 64 (version 7.0.3.0) each session is recorded for later analysis of the stay time in each cage.

Conditioning sessions (day 2-5, 8-11)

Mice are randomly assigned to a conditioning group. In conditioning group 1, bedding A is paired with procedure A and bedding B with procedure B, while in conditioning group 2, bedding A is paired

with procedure B and the other way round. One procedure is conditioned on days 2, 4, 8 and 10, whereas the other is conditioned on days 3, 5, 9 and 11.

The conditioning sessions take 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. For each session, a mouse is taken out of the home cage and placed into a type II cage (Tecniplast) containing the neutral stimulus (bedding) for 5 min. Afterwards, the mouse performs the experimental procedure (unconditioned stimulus) and is then placed again into the cage containing the same bedding (neutral stimulus becoming the conditioned stimulus). After another 5 min the mouse is taken back to its home cage.

Preference test (day 12)

The preference test 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. Similar to the habituation, two type II cages (Tecniplast) are connected via a short Perspex tube to form the test apparatus. Each of the two cages contains a different kind of bedding material (A or B). Mice are placed inside the system (whether left or right cage is randomized) and the activity and the duration spent in each compartment is recorded for 10 min. Recording is performed by an automated tracking system (containing two light barriers, an RFID reader which are connected to an Arduino computer) installed around the tube to detect the cage changes. In addition, each session is recorded using a webcam (Logitech C930e, Switzerland) and the open source recording program iSpy 64 (version 7.0.3.0) for later analysis of the stay time in each cage.

Experimental procedures (unconditioned stimulus)

In the first conditioned place preference test, restraint by hand will be compared with a weighing procedure. Depending on observations during this first test, in the second test either restraint by an restrainer, the Open Field or access to running wheel will be compared.

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 used as 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 a grid cage lid. 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 lid will be cleaned with 70 % ethanol in order to eliminate any olfactory cues for subsequently tested mice.

Weighing

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.

Restraint by a Restrainer

The mouse is taken out of the conditioning cage by tube handling and placed into a restrainer (Perspex tube in which movements are restricted) for 3 min. It is then taken out of the restrainer and placed back into the conditioning cage. The restrainer will be cleaned with 70 % ethanol in order to eliminate any olfactory cues for subsequently tested mice.

Open Field

The open field apparatus (rectangular arena 75 x 75 cm, with 57 cm high walls) is cleaned with 70 % ethanol and dried before testing. The mouse is taken out of the conditioning cage and placed into a start barrel in one corner for 1 min, than the barrel is lifted and the mouse is free to explore the maze for 5 min, during which its behavior is video recorded. Afterwards the mouse is returned to the conditioning cage by tube handling.

Access to running wheel

All mice had beforehand access to a running wheel because of previous experiments obtained. Thus, they do not have to learn how to use a running wheel. For the conditioning, a single mouse will be taken out of the conditioning box by tube handling and placed into a cage (containing also the to-be-conditioned bedding) with a running wheel (igloo house with running disc on top, Zoonlab, Germany). For 5 min the mouse has free access to the running wheel. Afterwards it is taken out by tube handling and returned to the conditioning box.

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. Conditioned Stimuli

Description of the method

There are four types of bedding material which will be used as conditioned stimuli: BC4 (cellulose, 4 x 4x 1,5 mm, AsBe-wood), pure (cellulose, JRS), comfort white (cellulose, JRS) and MK3500 (maize, 2,5-3,5 mm, JRS).

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 Place 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 cages of the conditioning box (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 13 mice should be sufficient.

Primary statistical analysis

During the habituation session, a baseline preference is measured, consisting of the total duration spent in each of the two compartments of the conditioning box. For every mouse, this duration as a percentage of the total time in the box can be calculated and this data set 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. During the preference test, the conditioned place preference is measured, consisting of the total duration spent in each of the two compartments of the conditioning box. For every mouse, this duration as a percentage of the total time in the box can be calculated and this data set 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. This result can then also be compared to the baseline results.

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)

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, thirteen mice at the age of fourteen months were used. Beforehand, the mice took part in the development of an automated home cage based choice test.

Housing conditions

All thirteen mice are kept in a group in a home cage system of two type IV macrolon cages (Tecniplast) connected with a Perspex tube. They live in such a system since they were around 2 months old (for other research purposes). 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 (The MouseHouse, 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 7 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.

Refinement

Not provided

6. Updates

2019-06-05

For the second experiment, two baseline tests were performed until two types of gravel were found for which no significant preference was measured. Between the second baseline test and the conditioning procedure, for organisatory reasons two weeks passed. In this time, one of the thirteen mice had to be euthanised due to health problems independent from the experiments, leaving only twelve mice for the condtioning phase.

2019-02-14

Changes regarding the analysis of the first experiment:

For the first experiment, we decided to analyse not the whole 10 min of the preference test (or the baseline test, respectively), but to use only the last 9 in, to provide 1 min of habitation time. This was done because in the course of the testing, we noticed that some mice had to be awakened before the test.

In addition, a supplementary exlusion criteria was added: If a mouse did not change compartments at all during the final preference test, the data of this mouse could also not be analysed thoroughly. Due to the design of the test setup (the separation of the compartments by a tube), it could not be ruled out that this mouse had not noticed the option of the other compartment.

Changes planned for the second experiment:

The first conditioned place preference test revealed a bedding preference of the mice independent from the experimental procedure (weighing or fixation). Due to that we decided not to move on directly to the next procedures but repeat this one under slightly altered conditions:
1. Instead of bedding material as conditioned stimulus, we will use different stones/gravel as flooring. Thus, direct interaction like digging (and a potential preference influenced by it) should get reduced, while still using multiple cues (visual, tactile, possibly also olfactory) for conditioning.
2. Some studies report that the presentation pre or post the conditioning may have oppsing effects. Thus, the two presentations of flooring before and after the procedure may have neutralized each other. Therefore, for the second experiment, we will only present the flooring before the procedure (pre-conditioning).
3. The duration of the presentation of the conditioned stimulus (flooring) will be shortened to 3 instead of 5 minutes. This allows us to perform two conditioning sessions on one day, reducing the whole procedure to 6 instead of 10 days. To prevent a time effect, for each mouse the beginning procedure will be alternated, meaning on day 1, procedure A will be conditioned first and afterwards B and on day 2, procedure B will be conditioned first and A second.
4. The baseline test (habituation) will take place few days before the start of the conditioning sessions. In this manner, we will gain the time to analyse the data first and, if again a flooring preference is already apparent in the baseline test, will not start conditioning but choose another stimulus pair (and also test it for baseline preference first).
All other parts of the experiment remained the same as for the first trial.

Lars Lewejohann, 2019-04-16
DOI 10.17590/asr.0000112