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Protocol

Learned helplessness: Validity and reliability of depressive-like states in mice

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Abstract

The learned helplessness paradigm is a depression model in which animals are exposed to unpredictable and uncontrollable stress, e.g. electroshocks, and subsequently develop coping deficits for aversive but escapable situations (J.B. Overmier, M.E. Seligman, Effects of inescapable shock upon subsequent escape and avoidance responding, J. Comp. Physiol. Psychol. 63 (1967) 28–33 [15]). It represents a model with good similarity to the symptoms of depression, construct, and predictive validity in rats. Despite an increased need to investigate emotional, in particular depression-like behaviors in transgenic mice, so far only a few studies have been published using the learned helplessness paradigm. One reason may be the fact that—in contrast to rats (B. Vollmayr, F.A. Henn, Learned helplessness in the rat: improvements in validity and reliability, Brain Res. Brain Res. Protoc. 8 (2001) 1–7)—there is no generally accepted learned helplessness protocol available for mice. This prompted us to develop a reliable helplessness procedure in C57BL/6N mice, to exclude possible artifacts, and to establish a protocol, which yields a consistent fraction of helpless mice following the shock exposure. Furthermore, we validated this protocol pharmacologically using the tricyclic antidepressant imipramine. Here, we present a mouse model with good face and predictive validity that can be used for transgenic, behavioral, and pharmacological studies.

Theme: Neural basis of behavior
Topic: Motivation and emotion

Keywords: Learned helplessness; Depressive-like behavior; Antidepressive treatment; Mice

1. Type of research

(1) The protocol described here allows murine studies of molecular, neurochemical, stress-physiological, and behavioral consequences of the learned helplessness paradigm, a behavioral model of depression.
(2) A detailed characterization of this model, defining a time course of coping deficits (helplessness) and employing clearly structured definitions of helpless behavior, also evaluated by pharmacological treatment, supplies good validity and reliability of this model.
(3) As a result, this protocol can be used for transgenic and pharmacological approaches to study depression in mice.

2. Time required

The course of the learned helplessness paradigm starts with an inescapable shock procedure on two consecutive days, each of these shock sessions lasting approximately 52 min. Learned helplessness is assessed 24 h after the second shock procedure and lasts up to 24 min, depending on the animals' performance in the shuttle box. More then 80% of helpless animals remain helpless for at least 1 week (Fig. 1).

3. Materials

3.1. Special equipment

The shock procedure was applied in a transparent plexiglas shock chamber ($18 \times 18 \times 30$ cm^3), equipped

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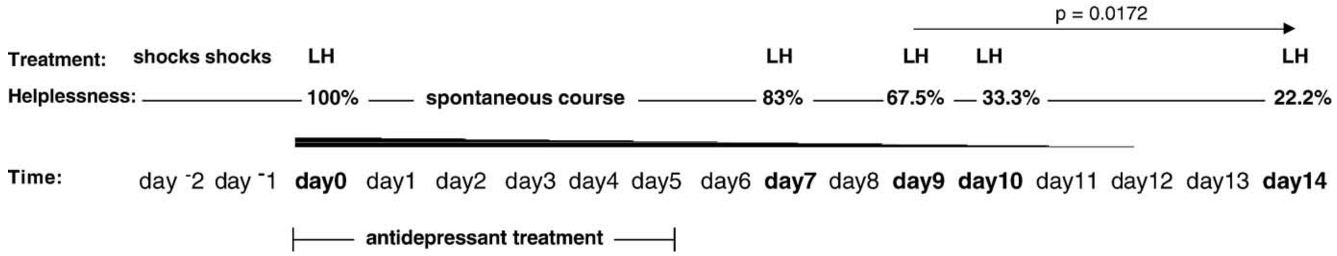

Fig. 1. Time course of the learned helplessness model. 360 footshocks with an intensity of 0.150 mA at two consecutive days (day-2, day-1) result in about 30% of all shocked animals in significant coping deficits 24 h later (day 0), i.e. increased failures and escape latencies in the shuttle box ($P < 0.05$). From 100% helpless mice at day 0, 83% of the mice are still helpless at day 7, with rapid improvement thereafter. The time-period between day 9 and 13 represents a critically labile condition in which a significant improvement becomes detectable as indicated by a sign-test ($P = 0.0172$).

with a stainless steel grid floor (diameter of each grid: 0.5 cm, spacing: 0.6 cm) (Coulborn precision regulated animal shocker, Coulborn Instruments, Düsseldorf, Germany).

The two-way avoidance test was conducted in a two-compartment shuttle box (Coulborn Instruments, Düsseldorf, Germany), equipped with infrared-light beams at the bottom of each of the two compartments to monitor spontaneous shuttling as well as behavioral responses to a light (conditioned) or an aversive footshock (unconditioned) stimulus, respectively. The shuttle box consisted of equal-sized compartments ($18 \times 18 \times 30$ cm^3) that were separated by a small gate (6 cm wide and 7 cm high). Both compartments of the shuttle box contained a grid floor (diameter of each grid: 0.5 cm, distance: 0.6 cm), through which the current was applied, and a signaling light at the top of each compartment. Protocol charts for both the shock procedure and shuttle box testing, respectively, were designed using Graphic State software (Version 1.014-00, Coulborn Instruments, Düsseldorf, Germany).

3.2. Hotplate

To exclude altered pain sensitivity as a confounding factor, all mice were tested on the hotplate (ATLab, Vendargues, France).

3.3. Pharmacological treatment

The model was pharmacologically validated with the tricyclic antidepressant imipramine in two different doses (10 and 30 mg/kg body weight). We assessed the capacity of imipramine to revert helpless behavior in the shuttle box. NaCl served as vehicle control.

4. Detailed procedure

4.1. Animals

10-week-old male C57BL/6N mice were purchased from Charles River, Sulzfeld, Germany, and acclimatized to single housing in macrolon cages (type II) at constant conditions with a 12 h dark–light cycle and an average room temperature of 22 °C for 2 weeks prior to the experiments, with food and water ad libitum. All experiments were approved by German animal welfare authorities (Regierungspräsidium Karlsruhe).

4.2. Inescapable shock procedure

(1) Mice were exposed to inescapable shocks during their active (dark) phase. Animals had to be transported in their home cages to the experimental room, and were then placed into the shock chamber.
(2) The shock procedure comprised 360 scrambled footshocks (0.150 mA) on two consecutive days. The footshocks were unpredictable with varying duration (1–3 s) and interval-episodes (1–15 s), amounting to a total session duration of approximately 52 min. During the shock exposure, lights were turned off.
(3) Control animals underwent the same handling and contextual procedures without receiving the footshocks. By thorough cleaning with 70% ethanol, we took care that control animals, which did not receive electroshocks, were exposed to the shock chambers without being distressed by the smell of shocked mice. Daily cleaning with soap should prevent fixation of potential alarm substances.

4.3. Assessment of learned helplessness

24 h after the second the shock procedure, learned helplessness was assessed in the dark phase of the animals by testing shuttle box performance (Graphic State Notation, Coulborn Instruments, Düsseldorf, Germany). Each trial started with a light stimulus of 5 s, announcing a subsequent footshock of maximum 10 s duration (intensity: 0.150 mA). The intertrial interval was 30 s. The following behavioral reactions were defined: *avoidance* as adequate reaction to the light stimulus by changing to the other compartment immediately, *escape* as shuttling to the other compartment as reaction to the electric shock, and *failure* when no attempt to escape was made. Furthermore, the parameter *escape latency* was recorded as the time needed to shuttle into the other compartment after onset of the footshock. For determination of the general activity, the shuttles before the first footshock

(*initial activity*), as well as the activity in-between the trials (*intertrial interval activity* or *ITI*) were recorded. Total time of testing for helplessness lasted about 20–24 min, the exact time period depending on the animal's ability to learn the paradigm and to respond properly. Before each trial, the apparatus was thoroughly cleaned with 70% ethanol.

To underscore the assessment of "true" learned helplessness effects, which relies on the uncontrollability of the stress, an additional cohort of animals was tested for immunization [11–13,18,27]. These animals were exposed to a pre-session, identical to the learned helplessness test in the shuttle box, in which they experienced a controllable shock condition. Moreover, initial activity during the pre-exposure was monitored.

4.4. Pain sensitivity

To exclude potential artifacts by altered pain sensitivities, which could influence the effect of the electroshocks, a subgroup of mice was tested on the hotplate prior to the learned helpless procedure at a temperature of 52 °C. The latency to first reaction (jumping or licking the hind paws) was monitored.

4.5. Defining helplessness

Following the evaluation of the behavioral parameters, the shocked animals were classified as "helpless" or "resistant", depending on their performance in the shuttle box test. Failures and escape latencies were taken as indicators for helplessness, and a k-means ($k = 2$) clustering algorithm was applied to a data pool including 212 mice subjected to the described protocol (see Section 5.3). The number of failures and the escape latencies were used as performance scores of the individual animals, because these are the most commonly reported indices of helplessness [2,3,17,19,22,24,25]. These behavioral indices were normalized (i.e. transformed to Z scores) to prevent differences in the range of each variable, which could produce a bias, and then inadvertently be used to implement a clustering process. This classification was further refined by means of a two-step discriminant-canonical analysis, which also provided classification equations for identification of helpless/non-helpless mice following this protocol [7,8,23].

4.6. Pharmacological validation

94 additional C57BL/6N mice were trained and tested in our protocol. Prior to any pharmacological treatment, these mice were classified as "helpless" or "non-helpless" using the classification equations previously obtained (see Sections 4.5 and 5.1) which takes into account the number of failures and the latency to escape.

The duration of helplessness for approximately 10 days dictated a short-timed, antidepressive treatment interval of 5–6 days. Thus, the animals underwent 5 days of vehicle (NaCl), 10 or 30 mg/kg b.w. imipramine regimen. On day 6, animals were retested in the escape task. The classification equations were used again to classify each subject, but now the values of the retest session were used in for the calculation. The changes in this categorical classification (i.e. mice moving from "helpless" to "non-helpless" group) after imipramine treatment were considered as an index of sensitivity of the provided operational definition of helplessness. This analysis was complemented by the assessment of variations in the squared mahalanobis distance (for detailed explanation, see Section 5.3) to the centroid of the non-helpless group before/after the pharmacological treatment to have a continuous rather than categorial index of imipramine effects.

5. Results

5.1. Definition of helplessness

Cluster analysis (Fig. 2) is a group of exploratory data analysis tools, which aim at sorting different objects into groups in a way that the degree of association between two objects is maximal if they belong to the same group and minimal otherwise. Thus, cluster analysis can be used to discover structures in data without providing an explanation or interpretation. Among the different clustering algorithms available, we used the k-means procedure, because it allows the a priori specification of the number of clusters suspected to be in the sample. In general, the k-means method will produce exactly k different groups of greatest possible distinction. In our case, this number was restricted to two clusters, aimed at separating helpless and non-helpless mice. In our sample, 65 subjects (approximately 30% of the sample) were categorized as our "helpless" cluster, and 149 subjects were in the "non-helpless" cluster, respectively. The Euclidean distance between both clusters was 2.007933 (squared Euclidean distance = 4.031235) and two independent ANOVAs revealed that the obtained clusters significantly differed in the number of failures [$F_{(1,212)} = 668.716$, $P < 0.000001$] and latencies to escape [$F_{(1,212)} = 643.243$, $P < 0.000001$ (for descriptive statistics see Table 1A).

To further confirm that these empirically defined clusters were mainly reflecting differences in the variables of interest (failures and escape latencies) and to obtain a classification equation for new cases, we performed a two-step discriminant-canonical analysis (Fig. 2). Discriminant function analysis is used to determine which variables discriminate between two or more naturally occurring groups, and when applied to two groups its computation and interpretation are almost identical to a multiple linear regression. We used our clusters as naturally occurring groups and different dependent variables of our escape procedure (failures, escape latency, intertrial interval activity, and initial activity) as possible discriminant factors. The number of escapes and avoidances was not included in this procedure, because

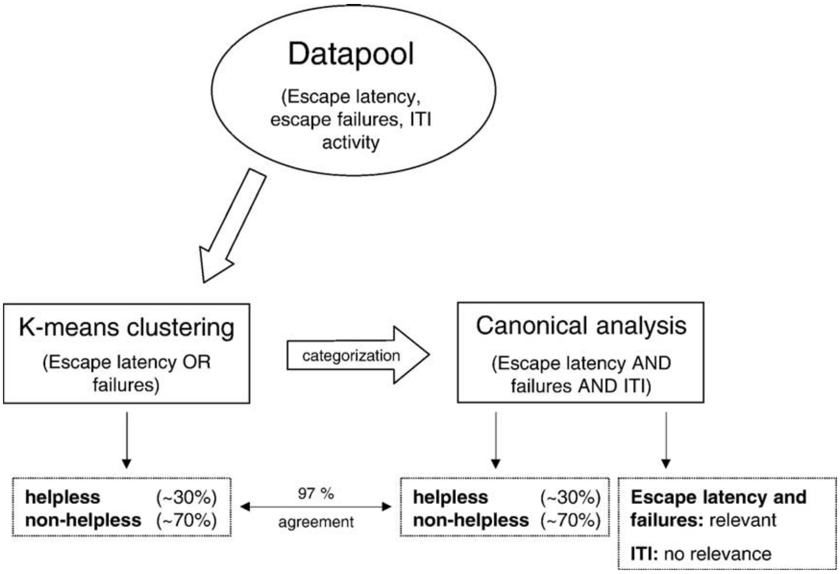

Fig. 2. Statistical characterization of helplessness. Statistical analysis was applied to a data pool comprising relevant parameters of the paradigm, e.g. escape latency or escape failures. *k*-means clustering indicates a classification of helpless and non-helpless individuals. For confirmation of appropriateness of the classification, a canonical analysis was performed, including intertrial interval activity in the parameters evaluated, in order to determine its relevance for the categorization of helplessness. Canonical analysis verified a proper classification and subsequent defined criteria for helplessness with a probability of 97%.

these indices are not mutually independent and they are redundant towards others already included (i.e. the number of escapes is an algebraic combination of failures and avoidances). Results indicated that only the number of failures and, to a lesser extent, the escape latencies defined both groups ([$F_{(2,214)} = 7.634$, $P < 0.001$] and [$F_{(2,214)} = 4.728$ $P < 0.01$], respectively). The factors "intertrial activity" and "initial activity" failed to enter in the model. Therefore, this model is exclusively based on the number of failures and the latency to escape, because these indices accounted for 100% of the variance (Eigenvalue: 3.200). Thus, the activity parameters can be ruled out as a confounding factor in this paradigm. Although it differed according to the group, this model presented an average concordance of 97% (see Table 1B for detailed results).

In a second step, canonical coefficients (data not shown) and classification equations (adjusted to the sample size) were obtained. While these results are presented in Table 2, we would like to highlight that these equations can be used to calculate the classification of any subject tested in the avoidance task described in Section 4.3 as the probability of being classified as "helpless" or "non-helpless". To estimate the possible incidence of "spontaneous helplessness" as a confounding factor, 93 control (non-shocked) mice were tested in this procedure. As expected, all these subjects were classified as "non-helpless" according to the given equations. Figs. 3A and B show the frequency histograms of the distribution of number of failures and escape latencies for these three groups.

To ensure helplessness as an effect of uncontrollability of the stress that was applied in our paradigm, two additional test groups of animals ($n = 16$ each), which were exposed to controllable and uncontrollable conditions before learned helplessness testing, were compared with regard to immunization effects. This revealed a statistically significant difference, with a clear immunization in animals that had the possibility to control the stress (failures $P = 0.012$, escape latency $P = 0.0028$, data not shown). Basal changes in locomotion were not detected.

5.2. Time course of helplessness

The average duration of robust learned helplessness was 10 days after the second footshock exposure (Fig. 1). This

Table 1A
k-means clustering revealed two distinct clusters among a total number of 214 animals, which reveals a distribution of 30.37% and 69.26%, respectively

	Cluster 1 "helpless"	Cluster 2 "non-helpless"
Number of subject (% of total sample)	65 (30.37%)	149 (69.26%)
Failures	19.80 ± 0.79	2.55 ± 0.24
Latency to escape (s)	7.51 ± 0.21	2.49 ± 0.09

Means ± SEM of the 2 parameters of relevance, failures, and escape latency.

Table 1B
Two-step discriminant-canonical analysis confirmed the categorization according to *k*-means clustering with a probability of 90.77% in helpless animals, and was in a 100% accordance with the classification of non-helpless animals, respectively

		Predicted		Percent correct
		Helpless ($P = 0.30374$)	Non-helpless ($P = 0.69626$)	
OBSERVED	Non-helpless	0	149	100%
	Helpless	59	6	90.77%
	Total	59	155	97.2%

Table 2
Classification equations obtained after a two-step discriminant-canonical analysis

	Constant	Failures	Escape latency
Non-helpless	−8.19995	−2.26963	8.60186
Helpless	−20.5300	−1.6956	9.7177

Cells display the constant and the weights corresponding to the two parameters relevant for each subject's classification as "helpless" or "non-helpless", respectively. These values are combined in a linear equation: NLH = ¯8.19995 + (¯2.26963 * failures) + (8.60186 * escape latency) or LH = ¯20.5300 + (¯1.6956 * failures) + (9.7177 * escape latency). These equations can be used to perform a priori classifications of any subject tested with the protocol. The case would be classified as belonging to the group for which it has the highest classification score, which reflects a smaller squared Mahalanobis distance towards the centroid of the corresponding group. Also and because we assume a multivariate normal distribution around each centroid, these distances (i.e. the scores of these equations) can be used to calculate the posterior probabilities (i.e. probability of belonging to the group to which each subject has been ascribed).

period was followed by a "critical phase", in which significant improvements of the coping deficits were observed. The given time window of 10 days corresponds closely to the time course of learned helplessness in rats [26]. This time course restricted the pharmacological validation of this model and will also limit future pharmacological experiments to a short treatment interval of 5–6 days.

5.3. Pharmacological validation of the model

The duration of helplessness for approximately 10 days dictated an antidepressive treatment interval of 5–6 days. Thus, the animals underwent 5 days of vehicle (NaCl), 10 or 30 mg/kg b.w. imipramine regimen. 94 male C57BL/6N mice were trained and tested according to the methods described in Sections 4.2 and 4.3. These mice were classified as "helpless" or "non-helpless" according to the classification equations presented in Table 2. The next day, vehicle or imipramine treatments were administered as described in Section 4.5 followed by retesting in the avoidance task on day 6.

The change (after–before treatment difference) in the squared mahalanobis distances to the centroid of the "non-helpless" group was used as main indicator of effects of imipramine treatment (Fig. 4). This is a measure of the distance between two points in the multidimensional space defined by the predictor factors similar to the Euclidean distance, which does not presuppose that the predictors are orthogonal. By taking the centroid of a group as one of these points, and each individual score as the other, the probability of belonging to that group can be estimated, and considering the difference after–before, the impact of the imipramine treatment in the two criteria involved in the helpless definition can be quantified simultaneously. Thus, a two-way ANOVA revealed a significant effect of both factors, group (helpless vs. non-helpless) and treatment (vehicle (NaCl), 10 or 30 mg/kg b.w. imipramine) [$F_{(1,88)} = 35.49$, $P < 0.0001$] and [$F_{(2,88)} = 5.25$, $P < 0.01$], respectively. As the interaction between both factors failed to reach statistical significance, appropriate Bonferroni corrections were performed in the subsequent mean comparisons. These comparisons revealed a significant ($P < 0.05$) dose-dependent reduction of the squared mahalanobis distances to the centroid of the "non-helpless" group in helpless animals treated with imipramine. Interestingly, the same pharmacological treatment did not show any significant effect in the same variable in non-helpless mice, thus discarding an indiscriminate improving effect of imipramine in learning/memory processes. Therefore, as it is selectively reverted by antidepressant treatment, these results seem to confirm that poor avoidance performance after uncontrollable shock exposure is indicative of helplessness.

We also compared the individual's classification provided by the equations presented in Table 2 before and after pharmacological treatment. Pharmacological treatment only affected the classification of "helpless" mice, thereby confirming the results of Fig. 4. Thus, in the cohort of "helpless" mice treated pharmacologically, imipramine produced not only a significant average improvement of latencies and failures, but also in more than half of the animals a reversal from "helpless" to "non-helpless". As expected, these changes were also related to the imipramine dose, a higher dose being more effective (percentage of subjects becoming "non-helpless": 57.1, 50.0, and 0.0% of subjects after 30, 10, and 0 mg/kg of imipramine, respectively, see also Table 3).

Alternatively, it has to be considered that the pharmacological treatment produced a variable improvement of depression-like symptomatology within each group. Thus, as an example of another possible application of the herein proposed operational definition of helplessness, the classification equations in Table 2 can be used to provide a qualitative index of the response to antidepressant (i.e. "responsive" vs. "resistant") or vehicle (i.e. "spontaneous remission") treatment.

5.4. Assessment of potential artifacts

Statistical evaluation of the activity in-between the shuttle-escape trials showed that helplessness represents a true shock effect, which is not related to general changes in activity that could falsify the results by assessing spontaneous shuttling. Furthermore, alterations in pain sensitivity were excluded to correlate with the level of helplessness ($P = 0.998$ and $R^2 = 0.0000000108$), which could be responsible for escape deficits because of a lack of sensitivity to the unconditioned aversive stimulus.

6. Discussion

6.1. Setup of the protocol

In order to establish a valid and reliable mouse model of depression as it has been described in rats [25], we designed

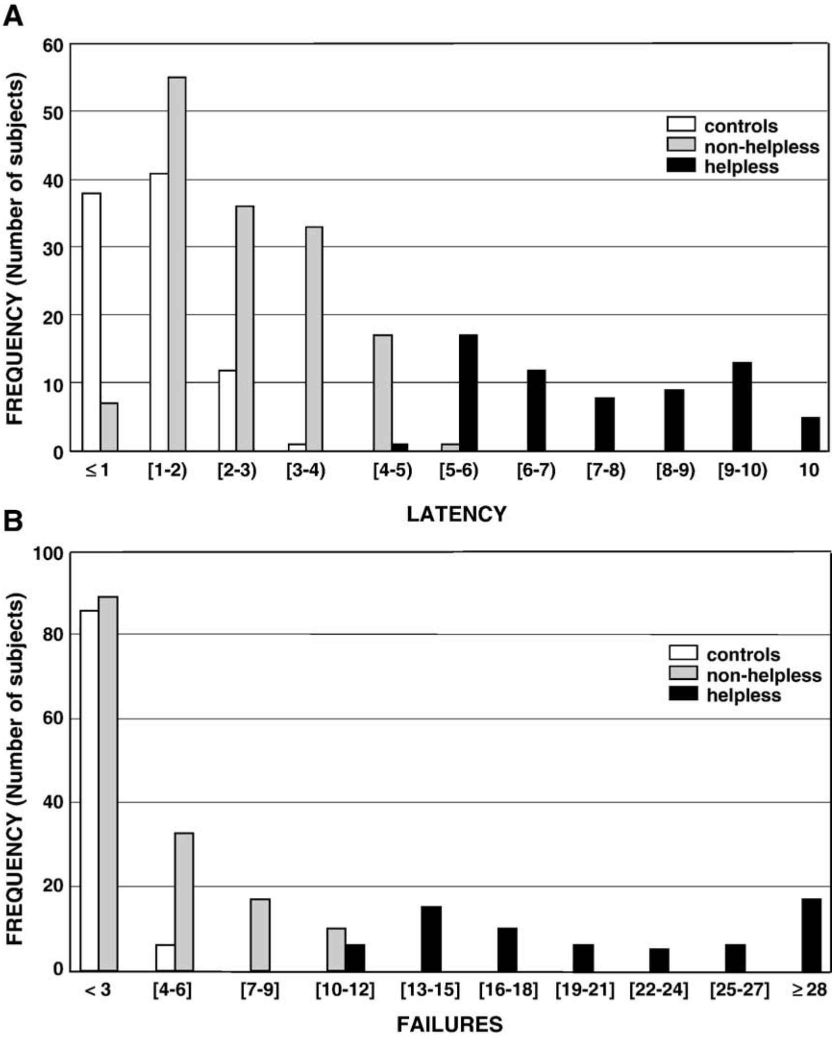

Fig. 3. Distribution of critical parameters for helplessness. (A) Escape latencies and (B) number of failures observed in our learned helplessness paradigm. Mice (n = 307) were tested in a shuttle box for escape deficits as described in Section 4.3 after uncontrollable shock exposure (n = 214) or sham-treatment without footshocks (n = 93). The distribution curves demonstrate that shock-exposed mice, which are classified as "non-helpless" by k-means clustering (n = 155, see text for details), and "control" (*i.e.* non-shocked) animals display the lowest values in both variables. The overlapping distribution of these values in both groups also suggests that all these mice fit into a unique population. Conversely, mice classified as "helpless" (n = 59) show a different (i.e. non-overlapping) distribution and display high values in both parameters, confirming the interpretation that these mice belong to a different population with different behavioral features.

a learned helplessness procedure, in which two series of unpredictable and uncontrollable footshocks applied over 2 days under dark conditions evoke helpless performance in a shuttle box, while controllable shock "immunizes" against helplessness. This is in line with most rodent depression models that use stress as a tool to induce depression-like symptoms.

6.2. Time course

Supporting the face validity of our paradigm (i.e. the similarity to the symptoms of depression), time course experiments revealed a helpless period of about 10 days, which seems comparable to the duration of a depressive episode in humans if set in relation to the lifespan of a mouse (however, one has to keep in mind that a direct transfer from animals to humans is always problematic). This time window should also be considered, when the learned helplessness paradigm is embedded in a behavioral test battery, which includes stressful handling or testing, because this may prolong the period of helplessness.

6.3. Categorization: definition of learned helplessness

According to our criteria for the definition of learned helplessness, approximately 30% of the stressed mice become helpless after shock exposure. This rather large fraction allows the investigation of several relevant biological factors, because the procedure yields enough animals for several cohorts, e.g. with different antidepressant treatment. Furthermore, a robust fraction of 30% is not prone to floor or ceiling effects.

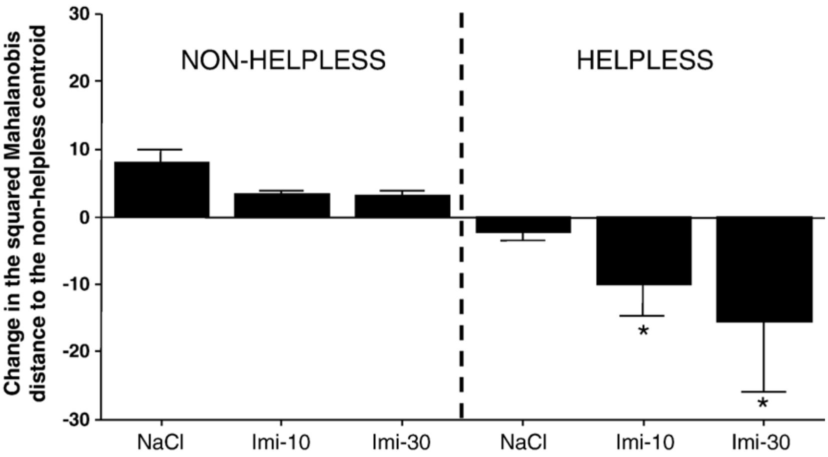

Fig. 4. Pharmacological validation. Effects of imipramine on murine helplessness: bars depict mean + SEM of the difference before–after pharmacological treatment in the squared mahalanobis distances from the "non-helpless" cluster centroid (see Detailed procedure section for details). Mean comparisons revealed that imipramine does not modify this distance in "non-helpless" animals but it significantly ($P < 0.05$) reduces it in "helpless" mice. Repeated injection of vehicle solution (0.9% saline) does not modify this parameter in any group. Taken together, these results suggest that imipramine produces a selective and specific reduction of helplessness.

6.4. Pharmacological validation of the model

The pharmacological validation of our model confirms its specificity by demonstrating a statistically significant improvement of helplessness following antidepressive treatment, while NaCl was not effective. Consequently, helplessness, as defined by our experiments, seems to represent a specific depression-like syndrome, since it can be antagonized by pharmacological treatment (57.1% after 30 mg/kg imipramine) with a similar efficiency as in humans, which have an approximate remission rate of 60% [1].

6.5. Potential artifacts and troubleshooting

To confirm a depressive-like phenotype, several artifacts due to general changes in behavior have to be considered, i.e. general changes in activity, which may alter the performance in the shuttle box, or pain sensitivity, which may alter the impact of footshock stress. In our setup, using C57BL/6N mice, artifacts due to alterations in locomotion (i.e. ITI activity) or pain (i.e. hotplate) could be excluded, again supporting the validity of this model. It is crucial that these potential confounding aspects are investigated and always included in the experimental design, especially when phenotyping mutants, in which general changes in baseline behavior could account for "false positive effects" in the learned helplessness paradigm and other behavioral depression models.

Table 3
Effects of pharmacological treatment with imipramine (10 or 30 mg/kg b.w. vs. saline) on the classification as helpless or non-helpless

	Pharmacological treatment		
	Saline	Imipramine 10	Imipramine 30
Before	Failures: 24.4 ± 0.82 Latency: 8.74 ± 0.45 (8)	Failures: 24.0 ± 1.58 Latency: 8.63 ± 0.41 (8)	Failures: 23.6 ± 2.8 Latency: 9.03 ± 0.42 (7)
After	Failures: 21.7 ± 3.54 Latency: 7.68 ± 1.03 (8)	Failures: 13.6 ± 4.48 Latency: 5.4 ± 1.2 (4)	Failures: 16.4 ± 5.47 Latency: 5.97 ± 1.70 (3)

Cells display mean ± SEM of failures (F) and latency to escape (L) before and after treatment. Numbers in brackets contain the number of helpless animals before/after treatment in each group.

6.5.1. The animals are hyperactive

If a large proportion of animals shows hyperlocomotion, which could minimize the read-out of the learned helplessness, it is suggested to decrease stress levels by (i) reducing noise pollution in the housing facilities, (ii) supplying nesting material [17,18], and (iii) separating the genders, in order to reduce olfactory stimulation. It goes without saying that adequate and standardized handling (i.e. with gloves) is fundamental. Prior to the learned helplessness procedure, an openfield can be performed to identify and differentiate between hyperactive and normal animals. Since hyperactivity may be a confounding factor in models of anxiety and depression, it is suggested to solve this problem by a covariance analysis, thereby excluding the effects attributed by the hyperlocomotion. Nonetheless, severe hyperactivity will cause a floor effect, which cannot be compensated, and will therefore limit the suitability of the learned helplessness paradigm, and any other model that implicates locomotion.

6.5.2. Too few or too many animals are helpless

If, after inescapable shock treatment, too few or too many animals turn out to be helpless, this may indicate that the paradigm is not sufficiently stressful or includes too much stress, respectively. It is essential that the protocol allows a broad spectrum of behavioral responses, therefore floor and ceiling effects have to be avoided, possibly by changing current intensity (see [14]) or the length of the unconditioned stimulus. Additionally, a fecal bolus can lead to short-circuiting of the shock generator, resulting in only minimal current delivery or even an absence of the electric stimulus to the mouse. Permanent monitoring is therefore crucial to exclude those animals from the experiment. In general, housing conditions in terms of predispositions for stress-susceptibility (i.e. group or single, enriched or impoverished), should be considered for a successful experimental design [3].

6.5.3. Animals avoid the shocks

Animals that climb to the walls (approximately 1%, no statistical data available) of the apparatus to avoid the footshocks, are excluded from the experiment.

6.6. Conclusions and preview

To investigate depressive-like behaviors in mice, especially the C57BL strain, which is a commonly used background strain for mouse mutations, we designed a learned helplessness protocol with good face, construct, and predictive validity. A reliable and valid protocol in mice is particularly important with respect to the investigation of transgenic animals, representing potential genetic models of affective disorders. Apart from the analysis of mutant mice, this model can be generally used as a test for emotional behavior, to investigate neurobiological mechanisms in mice that exhibit helplessness or good coping performance in the shuttle box, respectively. Last but not the least, this paradigm may turn out to represent a valuable and responsive tool for the assessment of new antidepressant strategies.

7. Quick procedure

(a) Expose the mice to 360 scrambled footshocks (intensity: 0.150 mA) on two consecutive days.
(b) 24 h later, perform a two-way avoidance paradigm (30 shuttle escape trials) in a shuttle box.
(c) Analyze the data by considering "failures" and "escape latency" as the most relevant parameters for the identification of helpless animals.

8. Alternative protocols

Training procedures reported previously, based on the paradigm by Shanks and Anisman (1988) and Caldarone et al. (2000) may represent alternative protocols [2,21]. In these protocols, learned helplessness is induced by administering 120 inescapable 4 s footshocks (0.30 mA) with a random interval (range 3–50 s) over a 1 h session. Training is given in two sessions that are spaced approximately 24 h apart. Mice are placed on either side of the shock chamber, so that the shock is administered either to one or two mice simultaneously. Mice of the same gender are always shocked together. A control group does not receive shock but is exposed to the apparatus for an equal period of time. Shuttle escape testing is conducted approximately 24 h after the second LH training session. The side of the chamber, on which each mouse is placed at the start of the test session, is alternated. Mice are given 30 shuttle escape trials with 30 s intervals between the start of each trial. A gate between the two chambers opens when the shock turned on and the trial terminated when the mouse crosses through the gate into the adjacent compartment. Shock termination is delayed by 1 s after crossing. If an escape response is not made, the trial is terminated 24 s after shock onset.

MacQueen et al. [9] make use of another modification, in which the animals receive 360 shocks, at an intensity of 0.150 mA, lasting 2 s with intershock intervals of 9 s. Testing in a 30 shuttle escape trial paradigm is conducted immediately after application of the footshocks [9]. The chambers, in which testing takes place, are equipped with a hurdle that opens 4 s after onset of the shock. Shock is terminated as soon as the mouse crossed to the other chamber. The gate is closed immediately and reopens until 4 s after initiation of the next escape trial.

All protocols described here represent alternative exceptional procedures to generate helplessness in mice. However, since the exact reproduction due to diverse restrictions and internal and external factors seems to be a major problem, it is recommendable to carefully consider all aspects, which are presented in the particular versions of learned helplessness induction and modify the model according to the requested criteria.

Alternatively, to our proposed canonical analysis, the subjects could be classified by using the 95% confidence intervals. This classifies animals, which have scores higher than the mean ±2 standard deviations of the non-helpless population, as defined by *k*-means clustering, as helpless. In our case, animals with an escape latency $\geq$4.75 s and failures $\geq$6 qualified as helpless. This method, though, has the caveat of splitting the classification into two independent variables. Thus, an animal, which qualifies as helpless by having a high escape latency may still display a low number of failures, and consequently has to be excluded from the study.

9. Essential literature references

[2,4–6,10,12,13,16,20,22,25,27]

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References

[1] T.K. Birkenhager, W.W. van den Broek, P.G. Mulder, J.A. Bruijn, P. Moleman, Comparison of two-phase treatment with imipramine or fluvoxamine, both followed by lithium addition, in inpatients with major depressive disorder, Am. J. Psychiatry 161 (2004) 2060–2065.

[2] B.J. Caldarone, T.P. George, V. Zachariou, M.R. Picciotto, Gender differences in learned helplessness behavior are influenced by genetic background, Pharmacol. Biochem. Behav. 66 (2000) 811–817.

[3] S. Chourbaji, C. Zacher, C. Sanchis-Segura, R. Spanagel, P. Gass, Social and structural housing conditions influence the development of a depressive-like phenotype in the learned helplessness paradigm in male mice., Behav. Brain Res. 164 (2005) 100–106.

[4] J.F. Cryan, C. Mombereau, In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice, Mol. Psychiatry 9 (2004) 326–357.

[5] E. Edwards, J. Johnson, D. Anderson, P. Turano, F.A. Henn, Neurochemical and behavioral consequences of mild, uncontrollable shock: effects of PCPA, Pharmacol. Biochem. Behav. 25 (1986) 415–421.

[6] L. Greenberg, E. Edwards, F.A. Henn, Dexamethasone suppression test in helpless rats, Biol. Psychiatry 26 (1989) 530–532.

[7] W.R. Klecka, Discriminant analysis, Sage University Paper Series on Quantitative Applications in the Social Sciences, 07-038, Sara Miller McCune Sage Publications Inc. Lewis-Beck, MS. Beverly Hills and London, Thousands Oaks, CA, 1980.

[8] Macintosh, Statistica for the Macintosh, vols. I and II, Statsoft Inc., 1984–1994.

[9] G.M. MacQueen, K. Ramakrishnan, S.D. Croll, J.A. Siuciak, G. Yu, L.T. Young, M. Fahnestock, Performance of heterozygous brain-derived neurotrophic factor knockout mice on behavioral analogues of anxiety, nociception, and depression, Behav. Neurosci. 115 (2001) 1145–1153.

[10] S.F. Maier, Learned helplessness and animal models of depression, Prog. Neuro-Psychopharmacol. Biol. Psychiatry 8 (1984) 435–446.

[11] S.F. Maier, Exposure to the stress environment prevents the temporal dissipation of behavioral depression/learned helplessness, Biol. Psychiatry 49 (2001) 763–773.

[12] S.F. Maier, R.L. Jackson, Learned helplessness: all of us were right (and wrong): inescapable shock has multiple effects, in: G.H. Bower (Ed.), The Psychobiology of Learning and Motivation, 1979, pp. 155–218.

[13] S.F. Maier, M.E.P. Seligman, Learned helplessness: theory and evidence, J. Exp. Psychol. Gen. 105 (1976) 3–46.

[14] S.S. Newton, J. Thome, T.L. Wallace, Y. Shirayama, L. Schlesinger, N. Sakai, J. Chen, R. Neve, E.J. Nestler, R.S. Duman, Inhibition of cAMP response element-binding protein or dynorphin in the nucleus accumbens produces an antidepressant-like effect, J. Neurosci. 22 (2002) 10883–10890.

[15] J.B. Overmier, M.E. Seligman, Effects of inescapable shock upon subsequent escape and avoidance responding, J. Comp. Physiol. Psychol. 63 (1967) 28–33.

[16] F. Petty, A.D. Sherman, Regional aspects of the prevention of learned helplessness by desipramine, Life Sci. 26 (1980) 1447–1452.

[17] M.E. Seligman, Learned helplessness as a model of depression. Comment and integration, J. Abnorm. Psychol. 87 (1978) 165–179.

[18] M.E. Seligman, S.F. Maier, Failure to escape traumatic shock, J. Exp. Psychol. 74 (1967) 1–9.

[19] M.E. Seligman, J. Weiss, M. Weinraub, A. Schulman, Coping behavior: learned helplessness, physiological change and learned inactivity, Behav. Res. Ther. 18 (5) (1980) 459–512.

[20] M.E. Seligman, J. Weiss, M. Weinraub, A. Schulman, Coping behavior: learned helplessness, physiological change and learned inactivity, Behav. Res. Ther. 18 (1980) 459–512.

[21] N. Shanks, H. Anisman, Stressor-provoked behavioral changes in six strains of mice, Behav. Neurosci. 102 (1988) 894–905.

[22] A.D. Sherman, J.L. Sacquitne, F. Petty, Specificity of the learned helplessness model of depression, Pharmacol. Biochem. Behav. 16 (1982) 449–454.

[23] D.J. Sheskin, Handbook of Parametric and Nonparametrical Statistical Procedures, CRC Press, New York, 1997.

[24] M. Ukai, M. Suzuki, T. Mamiya, Effects of U-50,488H, a kappa-opioid receptor agonist, on the learned helplessness model of depression in mice, J. Neural Transm. 109 (2002) 1221–1225.

[25] B. Vollmayr, F.A. Henn, Learned helplessness in the rat: improvements in validity and reliability, Brain Res. Brain Res. Protoc. 8 (2001) 1–7.

[26] B. Vollmayr, H. Faust, S. Lewicka, F.A. Henn, Brain-derived-neurotrophic-factor (BDNF) stress response in rats bred for learned helplessness, Mol. Psychiatry, 6 (2001) 471–474, 358.

[27] J.L. Williams, S.F. Maier, Transsituational immunization and therapy of learned helplessness in the rat, J. Exp. Psychol., Anim. Behav. Processes 3 (1977) 240–253.