

Research report

Rats with congenital learned helplessness respond less to sucrose but show no deficits in activity or learning

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Received 20 June 2003; received in revised form 23 July 2003; accepted 23 July 2003

Abstract

Inbred rat strains for congenital learned helplessness (cLH) and for congenital resistance to learned helplessness (cNLH) were investigated as a model to study genetic predisposition to major depression. Congenitally helpless rats respond less to sucrose under a progressive ratio schedule. This is not confounded by locomotor hypoactivity: in contrast, cLH rats show a slight hyperactivity during the first 5 min of an open field test. cLH rats acquire operant responding to sucrose as readily as cNLH rats and exhibit normal memory acquisition and retrieval in the Morris water maze, thus ruling out general learning deficits as the cause of the decreased response to sucrose. Reduced total responses and reduced breaking points for sucrose in the cLH strain argue for anhedonia, which is an analogue to loss of pleasure essential for the diagnosis of major depressive episodes, and thus confirm the validity of congenitally learned helpless rats as a model of major depression.

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Keywords: Learned helplessness; Animal model; Depression; Anhedonia; Sucrose; Operant behavior; Open field; Morris water maze

1. Introduction

Animal models are needed to investigate the neurobiology underlying major depression [28,29]. Learned helplessness is a well established stress model of major depression with good construct validity [42,43]: uncontrollable and unpredictable stress induces learned helplessness in the rat [33,34]. The model shows good face validity with vegetative changes such as weight loss, loss of libido, and increased REM sleep [1,7,8]. Endocrine changes with decreased dexamethason suppression during learned helplessness and pharmacological specificity for antidepressant drug treatment also argue for an animal model of major depression [15,36]. Moreover, reduced behavioral plasticity of learned helplessness goes along with alterations in monoaminergic systems [9–12,25]. Learned helplessness lasts for more than 1 week which is comparable to the duration of human depressive episodes if the rats' much shorter life span is considered [17,41]. In our paradigm moderate stress induces learned helplessness

only in a portion of susceptible animals, thus modeling the human susceptibility to depression [40]. By selectively breeding learned helpless animals and non-learned helpless, respectively, two lines have been developed: one shows congenital learned helplessness (cLH) the other one exhibits resistance to learned helplessness (cNLH) [18].

A loss of pleasure (anhedonia) and interest in nearly all activities is an essential feature of major depressive episodes. Anhedonia is commonly tested in rats as a decreased consumption of sweet palatable solutions [44]. More specific than total intake of sucrose, operant responding for sucrose under a progressive ratio (PR) schedule can be used to assess the motivational state of the responding animals [23]. The breaking point where an animal stops responding determines how much effort an animal is willing to exert to gain access to an reinforcing substance [24]. The breaking point therefore can be used to assess the effectiveness of the reinforcement which is determined by the reinforcer and the hedonic capacity of the individual, but also to compare the persistence of the animals to exert sustained effort over time [32]. We thus used the PR schedule to measure differences in anhedonia in cLH and cNLH rats.

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2. Materials and methods

2.1. Animals

By selecting for susceptibility for learned helplessness, two lines of rats were generated: cLH (congenitally learned helpless) and cNLH (congenitally non-helpless). Breeding of the helplessness colonies has been described [21,22,37–39]. Briefly, Sprague–Dawley rats were tested in the learned helplessness paradigm [40]. Twenty-four hours after a total of 20 min uncontrollable and unpredictable 0.8 mA footshocks, the rats were tested in an escape paradigm where foot shock could be eliminated with a single lever press: animals with more than 10 failures (out of 15 trials) to eliminate footshock were considered as helpless, animals with less than five failures were considered as non-helpless. Helpless animals and non-helpless animals, respectively, were mated for the subsequent generations avoiding sib crosses and resulting in two selective strains: the congenitally helpless strain (cLH), demonstrating helpless behavior without prior inescapable shock, and the congenitally non-helpless strain (cNLH), resistant to the development of learned helplessness [18]. Rats from the 48th and 50th generation were used for the experiments and underwent learned helplessness testing at the age of 8 weeks. More than 12 weeks later, 22 rats, 11 cLH and 11 cNLH were used for the open field test and Morris water maze test and 16 rats, 8 cLH and 8 cNLH were tested for anhedonia/anergia. All behavioral studies were done between 09:00 and 14:00 h.

Animals were group housed in standard hanging rodent cages under a 12 h/12 h artificial light/dark cycle (lights on at 06:00 h), unless indicated otherwise. Room temperature was kept constant (temperature: 22 ± 1 °C, humidity: $55 \pm 5\%$). Standard laboratory rat chow and water were provided ad libitum. The animals were treated in accordance with the European Communities Council Directive of 24 November, 1986 and German Animal Welfare Act of 25 May, 1998, experiments were approved by the Regierungspraesidium Karlsruhe.

2.2. Anhedonia assessment

Rats were trained and tested in standard operant chambers (28 cm × 21 cm × 21 cm) enclosed in sound attenuated chambers provided with fans for ventilation and background noise (MED associates, St. Albans). The test cages were equipped with a retractable lever on the left side panel and a liquid drop dispenser and receptacle next to the lever. Training and tests were performed using 7% sucrose solution, based upon our pre-experiments and a report that lower concentrations have too little hedonic value and higher concentrations do not further increase the achieved ratios but possibly mask differences between groups [2]. Initially, the rats were 24 h deprived of water and then presented 30 drops (1 drop = 25–30 μ l) of sucrose in the liquid dis-

perser. Rats were liquid deprived for 24 h prior to the first 24-h training session and the subsequent three 1-h training sessions under a fixed ratio (FR) 1 (i.e. one sucrose drop per lever press). To stabilize lever pressing behavior, the animals were allowed three more FR1 training sessions without deprivation or until animals responded with standard deviations of the mean less than 10%. Training sessions were given within 2 weeks. Based upon own pre-experiments and on reports that sucrose consumption is dependent on the feeding status [13,16,26], the rats were then housed individually and kept on a food restriction schedule with 12 g standard laboratory rat chow per day for 1 week resulting in a weight reduction to 90% of their free feeding weight. There were no differences in body weight or weight loss between the two strains: baseline body weight was 663 ± 20 g in the cLH (691 ± 19 g in the cNLH) rats, under restricted calory intake cLH rats lost 64 ± 6 g (cNLH: 60 ± 4 g). Sucrose reinforcements (25–30 μ l) could be earned with the following number of bar presses [31]: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 36, 40, 44, 48, 52, 56, 64, 72, 80, 88. The final ratio achieved represented the break point value. Significance of differences was determined using one-sided Student's *t*-test for unpaired samples.

2.3. Open field test

Rats were introduced into the open field (150 cm diameter) and their activity was recorded for 15 min by a video camera and analyzed in 5-min intervals (Noldus Ethovision, Wageningen, Netherlands). Light intensity was 20 lx. Locomotor activity was assessed by the total distance traveled (m). Statistical significance of differences among strains was tested with analysis of variance (ANOVA) for repeated measures followed by Bonferroni post hoc analysis.

2.4. Morris water maze

Rats were introduced into the black maze (150 cm diameter) filled with 21 °C water. A black platform (12 cm × 12 cm) was mounted 0.5 cm below the water surface, invisible for the animals. The light intensity measured at the water surface was 20 lx, there were extramaze cues at the walls around the maze. Training comprised two trials per day with a 1-h intertrial interval. If an animal had not found the platform within 2 min, it was tracked to the platform. During the first trial on the fourth day, the platform was removed and 2 min of probe trial were recorded. Swim paths were recorded by a video camera mounted above the pool and analyzed off-line (Noldus Ethovision, Wageningen, Netherlands). The time to find the platform and the distance traveled were assessed during acquisition trials and visible trial, time spent in the trained, adjacent and opposite quadrants was assessed during the probe trial. Statistical significance of differences among strains was tested with analysis of variance for repeated measures.

3. Results

Animals from both strains learned to press the lever for sucrose during a 24-h session on a FR1 and did not differ in FR1 responses for sucrose during the training sessions for 24 or 1 h, no matter if tested after water deprivation or without water deprivation. After 24 h water deprivation, cLH rats had 214 ± 40 lever presses during a 1-h session, whereas cNLH rats responded 210 ± 36 times. Without water deprivation, lever presses were reduced to $116 \pm 39 \text{ h}^{-1}$ in cLH and $133 \pm 36 \text{ h}^{-1}$ in cNLH. However, during PR testing congenitally helpless rats pressed significantly less the lever for sucrose ($P = 0.02$), they reached only 25% of the lever presses if compared to congenitally non-helpless rats. Likewise, the finally achieved ratio was 18.1 ± 4.9 in cNLH but only 7.3 ± 1.5 in cLH rats ($P = 0.026$) (Fig. 1).

As a possibly confounding variable for learned helplessness or anhedonia, locomotor behavior was tested in an open field. cLH displayed no hypoactivity, instead during the first 5-min interval hyperactivity was observed. Analysis of the data by ANOVA for repeated measures revealed a highly significant interaction of the groups and the time ($F = 14.8$, d.f. = 1.28, $P < 0.001$) showing that only cLH animals were hyperactive in response to novelty (Fig. 2). cNLH demonstrated low activity throughout the whole observation and no initial hyperactivity. For comparison, outbred Sprague–Dawley rats as the paternal strain of the cLH and cNLH colonies, have a locomotor activity between cLH and cNLH with some initial hyperactivity (data not shown).

To control for possible unspecific learning deficits of the cLH strain we examined spatial learning in the Morris water maze. cLH and cNLH rats both showed identical good acquisition of the learning task. Analysis of variance for repeated measures revealed no significant difference and, more importantly, there was no systematic difference in the mean distance to find the platform between the two groups. Likewise, there was no difference in the probe trial, indicating that both strains had the same learning strategy, and all rats

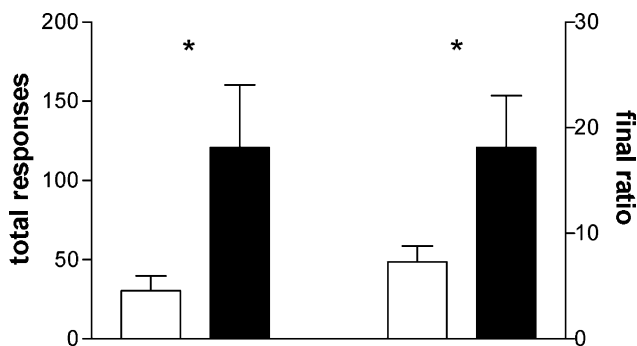


Fig. 1. Congenitally learned helpless (cLH) rats respond less for sucrose under operant conditions. cLH rats achieve less lever presses (left; t -test: $*P = 0.020$) and lower ratios (right; t -test: $*P = 0.026$) for 7% sucrose under a PR schedule than congenitally non-learned helpless (cNLH) rats ($n = 8$ cLH, white bars; $n = 8$ cNLH, black bars). Results are given as mean \pm S.E.M.

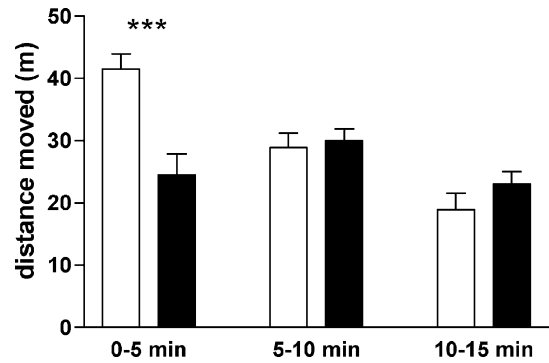


Fig. 2. Congenitally learned helpless (cLH) rats display elevated locomotor activity only during the first 5-min interval in an open field test compared to congenitally non-learned helpless (cNLH) rats ($n = 11$ cLH, white bars; $n = 11$ cNLH, black bars; ANOVA followed by Bonferroni: $***P = 0.001$). Expressed is the mean distance moved \pm S.E.M.

quickly reached the platform during the visible trial suggesting that there is no visual impairment in the strains (Fig. 3).

4. Discussion

Essential features of major depressive episodes are either depressed mood or a marked loss of interest and pleasure in nearly all activities. Communication of changes in mood and thoughts during depressive episodes relies on language and is not testable in animal models. Therefore, anhedonia or the reduced capacity to experience pleasure is an important analogue of major depression in animal models [42,43]. The consumption of sweet palatable solutions or pellets as a measure of hedonia is reduced in the animal models of chronic mild stress and chronic learned helplessness [6,14,44]. The amount of sucrose consumed, however, does not only depend on its reinforcing effects but on a variety of other factors like deprivation, satiety and taste, and the exact relationship between consumption and motivation is not known [3–5,24,27,45]. The PR procedure assesses the amount of effort an animal is willing to exert to gain access to a reinforcing substance, it thus reflects not only reinforcing mechanisms but also the individuals capacity to exert a sustained effort over time [23,24,32]. Reduced persistence under a PR schedule is the analogue to loss of interest, fatigue and loss of energy during depressive episodes.

We show here that cLH rats respond with less lever presses to sucrose under a PR schedule and achieve lower break-points. This can either be caused by a decreased capacity of the cLH rats to experience pleasure (anhedonia) or could also be secondary to a deficit in persistence (anergia). Further experiments are needed to differentiate between the two possibilities. However, anhedonia and anergia are both core symptoms of human depression. The finding that cLH rats show essential symptoms of major depression is a validation of the congenitally helpless strain as a useful model to study mechanisms predisposing to depression. Willner and

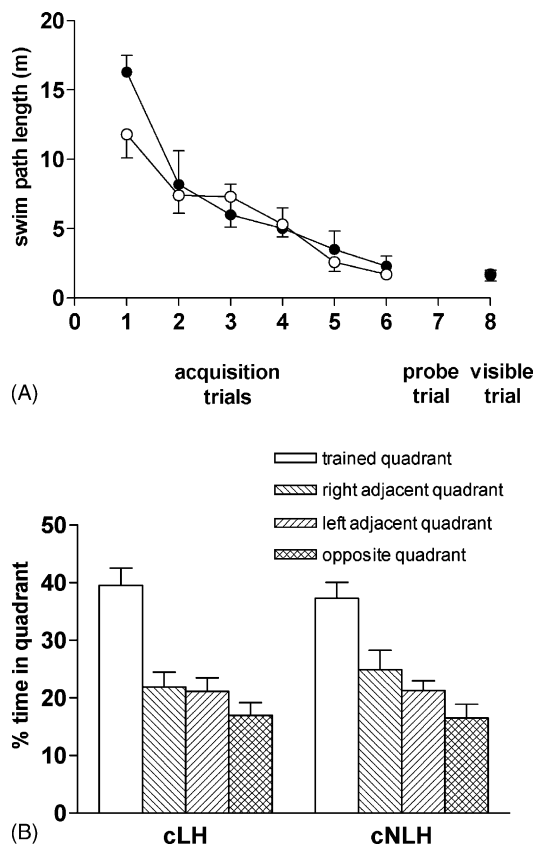


Fig. 3. Congenitally learned helpless (cLH) rats have no general learning deficit in the water maze task compared to congenitally non-learned helpless rats (cNLH). (A) cLH and cNLH rats show similar acquisition of the learning task and identical distances during the visible trial (mean distance moved \pm S.E.M.; $n = 11$ cLH, open circles; $n = 11$ cNLH, closed circles). (B) Comparison of percent time spent in the trained vs. adjacent left or right and opposite quadrants during the 30 s probe trial (mean \pm S.E.M.). cLH and cNLH rats show the same preference for the trained quadrant.

Mitchell [46] discriminate two types of animal models to study the predisposition for depression: those increasing the ease with which an analogue of major depression can be evoked and those with a presentation analogous to chronic mild depression (dysthymia). According to this definition, selective breeding of learned helpless and non-learned helpless rats has resulted in a dysthymic phenotype in the strain with cLH. cLH rats show learned helplessness without experiencing uncontrollable shock and, as we demonstrate here, exhibit anhedonia and/or anergia under baseline conditions. In contrast, rats from Flinders Sensitive Line (FSL), an alternative genetic animal model of depression, consume more saccharin under baseline conditions than control rats from Flinders Resistant Line (FRL) and thus do not exhibit a dysthymic phenotype, but show pronounced anhedonia in response to chronic mild stress [30].

It has been argued that learned helplessness may be an artifact of learned inactivity [35]. Although inaccurate choices with normal speed of responding in the Y-maze after in-

escapable shock argue against a general inactivity causing the deficits of learned helplessness [19], inactivity or hypoactivity would lead to a deficit to initiate operant responses, thus mimicking learned helplessness behavior. However, normal FR1 responding argues against motor deficits of the cLH rats or differences in basal activity. Furthermore, there is no general hypoactivity of the cLH rats in an open field test, we thus can exclude impairments in activity of the cLH strain as a confounding factor for the failure to perform the escape response during learned helplessness testing or during the PR procedure. On the contrary, there was an initial hyperactivity of the cLH strain in the open field. This can be interpreted as a increased response to novelty and is consistent with the report of King et al. [20] that cLH exhibit a more pronounced response to novelty.

In the Morris water maze, cLH and cNLH show a similar acquisition of the task and similar memory retrieval during the probe trial. Moreover, cLH animals acquire the FR1 task for sucrose as readily as cNLH animals. Therefore, the failure of the cLH to perform contingencies during the test for learned helplessness or under a PR schedule for sucrose cannot be explained by a general learning deficit but has to be attributed most likely to difficulty sustaining performance in the aversely motivated as well as in the appetitively motivated task.

In summary, rats bred for high susceptibility to develop learned helplessness show an increased sensitivity not only to foot shock stress but also to novelty. Reduced breaking points for sucrose under a PR schedule argue for anhedonia and/or anergia in the congenitally learned helpless strain and confirm the validity as a model of major depression. Learning and memory in the Morris water maze and under continuous reinforcement are normal, which excludes a general learning deficit of the cLH rats and argues for a specific deficit to sustain responses under operant conditions.

Acknowledgements

The authors thank Dr. B. Krumm for help with statistical analysis, Dr. C. Sanchis Segura for helpful comments on the manuscript, and C. Zacher and H. Schamber for excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft to B.V. (VO 621 3-1) and P.G. (GA 427 4-1).

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