Reduced sensitivity to sucrose in rats bred for helplessness: a study using the matching law

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Anhedonia is a core symptom of depression. As it cannot be directly assessed in rodents, anhedonia is usually inferred from a reduced consumption of, or preference for, a reinforcer. In the present study we tried to improve the measurement of anhedonia by performing a detailed preference analysis based on the generalized matching law and tested its sensitivity in rats congenitally prone (cLH) or resistant (cNLH) to learned helplessness. According to the current interpretation of learned helplessness as a model for depression, a reduction in the rewarding properties of sucrose in cLH rats was hypothesized. Our results revealed that the 'preference allocation' index provided by this test, but not the traditional measures of sucrose consumption or preference over water, was significantly lower in cLH rats, and was correlated with the helpless behaviour as measured in an escape procedure. Therefore, it is clear that more subtle preference measures provided by the analysis of choice using the matching law principles are more sensitive and discriminative than those based on consumption of, or preference for, a single concentration of sucrose over water. Moreover, our data are in agreement with the proposed relationship between helplessness and sucrose preference, and support the usefulness of the cLH and cNLH rats as a model of depression. ***Behavioural Pharmacology*** **16:267–270 © 2005 Lippincott Williams & Wilkins.**

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Introduction

After uncontrollable stress exposure some organisms display a subsequent reduction of their motivation and abilities to escape from painful or stressful stimuli. This syndrome is called helpless behaviour (or helplessness) and it has been proposed as a depression model showing good construct, face and predictive validity (Willner, 1990, 1995). Interestingly, exposure to uncontrollable stressors of moderate intensity produces learned helplessness in only a proportion of animals, thereby modelling the human variations in predisposition to depression (Willner and Mitchell, 2002). By selective breeding, we have developed two lines: rats showing a deficit in an escape procedure without prior exposure to inescapable shock (cLH) and rats showing good escape performance even after exposure to inescapable shock (cNLH). The strains differ in neurochemical and behavioural (Henn and Vollmayr, 2005) parameters that are related to depression. In this regard, if impaired escape behaviour observed in cLH rats reflects helplessness, following current views concerning depression one should expect other depressive-like features in these animals, such as a differential hedonic value of reinforcing stimuli, such as sucrose (Willner 1990, 1995; Papp *et al.*, 1991; Vollmayr *et al.*, 2004).

In the present study we show that cLH rats display a mismatch between their preference allocation and the relative magnitude of two reinforcement sources simultaneously available at the same cost, as measured in a procedure based on the behavioural matching law (Herrnstein, 1970). This procedure is similar to the classic two-bottles free-choice test, but incorporates two special features: (1) it is independent of the total volume consumed; and (2) it incorporates not only a measurement of preference for sucrose over water but also more subtle comparisons between different sucrose concentrations.

Methods

Subjects

Eight male cLH and seven male cNLH rats from the 52nd generation were used in the present study. The origin and selective breeding of both strains have been described previously (Vollmayr *et al.*, 2001; Henn and Vollmayr, 2005). Rats were housed singly in standard laboratory conditions ($21 \pm 0.5^{\circ}C$ and 50% relative humidity; 12-h light/dark cycle; free access to water and food). The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body and performed following the German Law on the Protection of Animals.

Procedure

At the age of 9 weeks, rats were tested for escape behaviour as described previously (Vollmayr and Henn,

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2001). The test (in boxes from TSE, Bad Soden, Germany) consisted of 15 trials in which an electric footshock (0.8 mA, 60 s) could be stopped by pressing a bar. Trials not stopped after 20 s were considered as escape failures.

To prevent any interference of the helplessness testing, sucrose consumption was assessed using a two-bottles procedure 23 weeks after the test for helplessness. Ball-tipped caps were used on 250 ml polycarbonate bottles (Ehret, Emmendingen, Germany) to prevent spillage (Sanchis-Segura *et al.*, 2004). Thus, completing 5 sessions (duration: 1 h) per week and in a total time of 8 weeks, 14 different conditions (listed in Table 1) testing sucrose preference were implemented in the rats' homecages. Data were analysed using the 'generalized matching law equation'. This equation states that $\log B1/B2 = a(\log r1/r2) + \log c$, where B represents the allocation of the behavioural responses to alternatives 1 and 2, and r represents the rate or relative reinforcing magnitude of the two alternatives, and a and c are empirically obtained parameters which illustrate the individual sensitivity to the ratio reward and bias for one or the other alternatives, respectively (Anderson and Woolverton, 2000). This equation is usually used in an operant setting, but when applied to a two-bottles free-choice procedure (for a discussion of caveats see Martinetti *et al.*, 2000), the consumed volume (V_X) of each bottle provides the index of the relative behavioural allocation, whereas the concentration of the available solution (C_X) represents the magnitude of the reinforcer. Thus, for each rat, the ratio of the consumed volume from the bottle located on the left over that located on the right (V_L/V_R) as well as the concentration of the respective solutions (C_L/C_R) was calculated. When any of the terms was zero, 0.1 was substituted for this value. This value represents the accuracy limit of our measurement method, and by this substitution, division by 0 is avoided with minimal distortion of the subsequent calculations. In a second step, the logarithms (to the base e) of these ratios were plotted on arithmetic coordinates and, by using the method of the least squares, the best-fit regression line was estimated.

Table 1 Summary of the experimental conditions tested for sucrose choice analysis

Condition	Sucrose concentration (%) left bottle	Sucrose concentration (%) right bottle
1	2	0
2	0	2
3	7	2
4	2	7
5	7	0
6	0	7
7	1	2
8	2	1
9	7	1
10	1	7
11	0	1
12	1	0
13	7	7
14	0	0

Experimental conditions tested for the sucrose choice analysis. A total of 14 different choice situations were implemented to compare the relative preference for different sucrose concentrations in a pseudo-random order. All sessions were conducted over a total period of 8 weeks, 5 sessions per week (session length: 1 h). Each condition was tested for 2–6 sessions.

Results

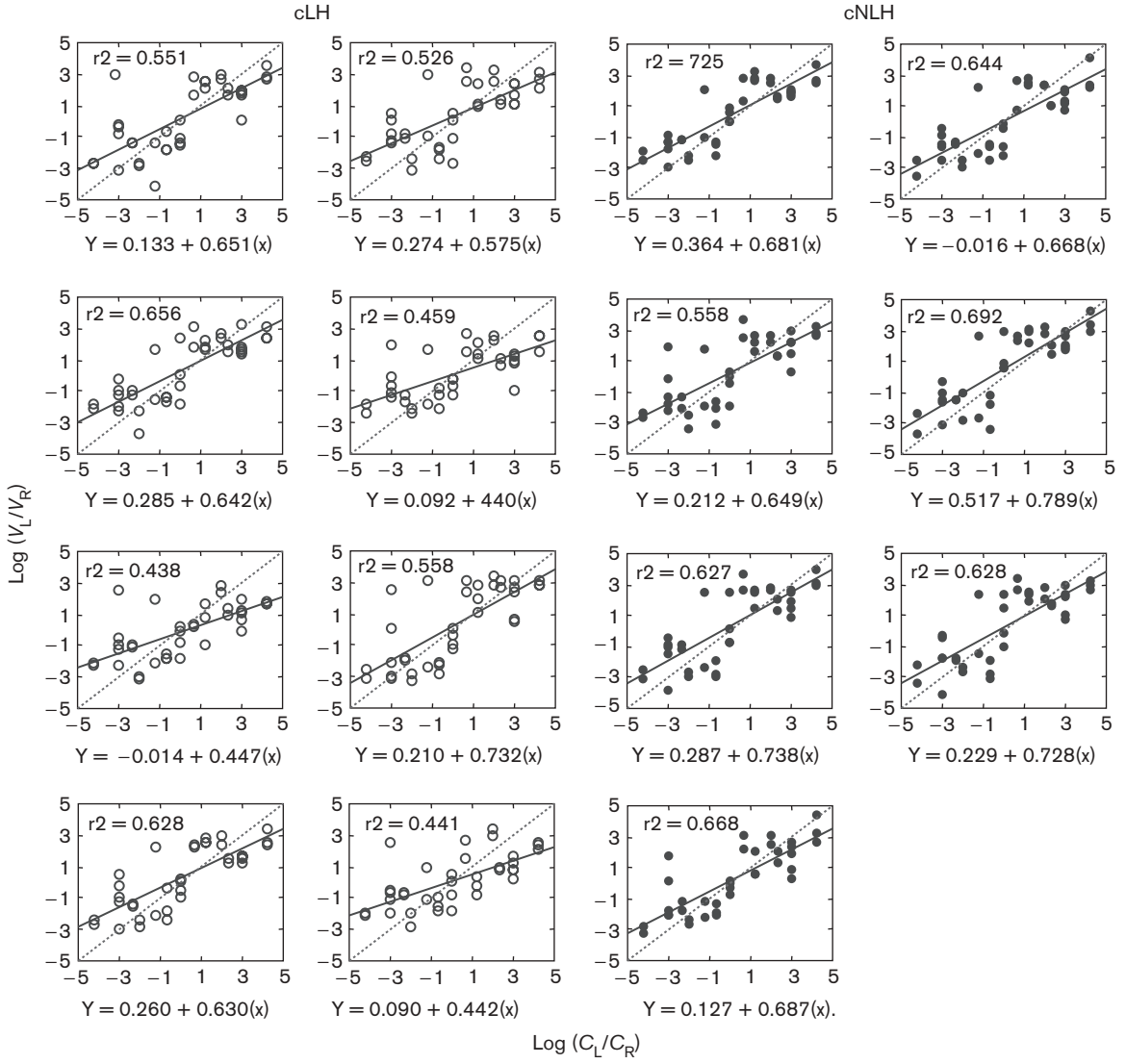
As expected, cLH rats showed a poor performance in the escape task, as expressed in a significantly higher number of escape failures [$t(13) = 8.34$; $P < 0.001$] than cNLH rats (mean ± SEM were 9.5 ± 0.68 and 1.85 ± 0.59 for cLH and cNLH, respectively), despite the fact that cLH had no inescapable shock exposure.

Data on sucrose choice are presented in Fig. 1, including the individual regression equations and goodness of the fitting procedure (r^2). All coefficients of determination (r^2) were statistically significant ($P < 0.05$). cLH showed poorer fitting functions than cNLH rats, although the possible functional significance of this finding remains unclear. Interestingly, the slope value, but not the bias (intercept value), index was inversely correlated with the number of failures to escape in the test for learned helplessness ($r = -0.64$; $P < 0.01$, and $r = -0.38$; NS, respectively), illustrating the inverse relationship between helplessness and sensitivity to sucrose reward.

This association was also observed when genotype differences were considered. Thus, the calculated individual intercepts and slopes were averaged by genotype and compared by two Student's t-tests for independent samples. These tests show that whereas the averages of the intercepts were not different [$t(13) = -1.09$; NS], the slopes markedly differed [$t(13) = -2.92$; $P < 0.02$] between genotypes (slope means ± SEM were 0.57 ± 0.04 and 0.71 ± 0.02 for cLH and cNLH, respectively). Because we observed a significant correlation between the slope and intercept values ($r = 0.62$; $P < 0.01$), the genotype-based differences in the slope values were confirmed by means of a one-way analysis of variance (ANOVA) using the intercept values as co-variates [$F(1,12) = 6.74$; $P < 0.05$]. Conversely, a one-way ANOVA comparing the intercept values using the slope values as co-variates confirmed that the bias was not related to the genotype [$F(1,12) = 0.32$, NS].

From the same data, the average volume of a sucrose solution consumed (1, 2 or 7% w/v) and its preference over water were calculated. Thus, after individually averaging the consumption of sucrose observed under the conditions of Table 1 involving concurrent availability of water and a sucrose concentration (conditions 11–12, 1–2 and 5–6 and 13–14 for 1, 2 and 7% w/v, respectively), the possible genotype-based differences were assessed. A two-way

Fig. 1



Individual analysis of sucrose choice in rats that exhibit congenital helplessness (cLH; open circles) or resistance to learned helplessness (cNLH; filled circles). As described in the Methods section, for each rat the ratio of the consumed volume (V_L/V_R) as well as the concentration of the respective solutions (C_L/C_R) were calculated (see Table 1 for details about the testing conditions). Then, the logarithms (to the base e) of these ratios were plotted on arithmetic coordinates and, by using the method of the least squares, the best-fit of regression line was estimated. Individual parameters of the empirical regression lines (plain line) and wellness of fitting (r^2) are included in the corresponding scattergrams. For a better comparison, the theoretical perfect matching behaviour is shown (dashed line).

repeated-measures ANOVA (genotype × concentration) revealed that the sucrose concentration factor [$F(2,26) = 164.11$; $P < 0.001$], but not the genotype [$F(1,13) = 1.50$; NS] or their interaction [$F(2,26) = 0.51$; NS] yielded a significant effect over sucrose consumption. Thus, whereas cLH rats consumed 5.25 ± 0.72, 5.1 ± 0.84 and 16.6 ± 1.96 ml of a 1, 2 and 7 % (w/v) sucrose solution, cNLH rats consumed a little more, i.e. 6.31 ± 0.5, 7.44 ± 0.53 and 17.72 ± 0.46 ml of the same sucrose concentrations. Similar results were found when preference instead of consumption was considered. Again the sucrose concentration factor [$F(2,26) = 61.12$; $P < 0.001$], but not the genotype [$F(1,13) = 2.80$; NS] or the interaction [$F(2,26) = 1.23$; NS], resulted in a significant effect. In this case the average preference for a sucrose solution (1, 2 or 7% w/v) over water was 79.28 ± 2.36, 68.70 ± 2.84 and 91.07 ± 0.83 for cLH and 79.89 ± 1.11, 75.12 ± 2.78 and 93.55 ± 0.67 for cNLH. Furthermore, and again in contrast to the slope values of the matching law, the number of avoidance failures was not correlated with the sucrose consumption or preference over water at any tested concentration (1, 2 or 7% w/v).

Discussion

Depressed mood and loss of interest or pleasure in nearly all activities are essential features of major depression. In animals this translates to the incapacity to initiate and sustain behaviours leading to the access of reinforcing stimuli (anergia) as well as a devaluation of their emotional value (anhedonia). Learned helplessness has been proposed as a rodent model of depression. We have previously reported that rats bred for learned helplessness (cLH) exhibit anergia, as demonstrated by lower break-points for sucrose under a progressive ratio schedule (Vollmayr *et al.*, 2004). The results of the present study are understood as indicative of congenital anhedonia in cLH rats.

We observed reduced matching between preference and the relative magnitude of two reinforcers simultaneously available in cLH rats, as reflected in their lower slope values of the generalized matching law equation (Herrnstein, 1970) as compared to those of the cNLH rats. Although, strictly speaking, these slope values reflect the sensitivity to the changes in the ratio of two reward sizes, this index could be a better index of the rewarding properties of a stimulus, and consequently of anhedonia, than traditional measures of preference. Indeed, it has been previously labelled as 'sensitivity to reward'. (Martinetti *et al.*, 2000). This measure identified a genotype difference and showed a significant correlation with the previously established helplessness criterion (individual number of failures in an escape procedure), in contrast to raw measures of sucrose consumption or preference over water. These data are in agreement with the fact that cLH and cNLH rats do not differ in operant responding for sucrose (fixed-ratio 1) (Vollmayr *et al.*, 2004), thus confirming that highly sensitive measures are needed to detect subtle differences in sensitivity to sucrose reward (i.e. hedonia) in non-deprived animals. However, as for any measure based on preference, the procedure described here is not independent of the ability to discriminate the available reward sources. Future efforts are needed to circumvent this problem and to try to isolate the perceived reward value in this paradigm.

In summary, two main findings can be highlighted. First, it is clear that the more subtle preference measures provided by the analysis of choice using the matching law principles are more sensitive and discriminative than those based on raw consumption or preference of a single concentration over water comparisons. Second, these results also show that cLH rats are less sensitive to the rewarding properties of sucrose. This fact not only reaffirms the relationship between helplessness and sucrose preference, but also the usefulness of the cLH and cNLH rat strains as an animal model of depression.

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