

Trait sensitivity to negative feedback determines the intensity of compulsive alcohol seeking and taking in male rats

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Background: Alcohol use disorder is one of the most common psychiatric disorders, and it is a leading cause of mortality worldwide. It has been demonstrated previously that people with alcohol use disorder are less sensitive to the negative outcomes of their actions and less able to use negative feedback to guide and adjust their ongoing behaviour. However, far less is known about the aberrant processing of negative feedback before the onset of alcohol use disorder. In this study, we investigated the theoretical claim that sensitivity to negative feedback — as a stable and enduring behavioural trait — can predict vulnerability to the development of compulsive alcohol consumption in rats. **Methods:** We trained and tested rats in a series of probabilistic reversal learning tests, and based on this “negative feedback sensitivity screening,” we classified each rat as more or less sensitive to negative feedback. Then, in the intermittent-access 2-bottle choice paradigm, we measured alcohol consumption in the animals classified above. In the next step, using the instrumental second-order chained schedule of alcohol reinforcement task, we examined the influence of sensitivity to negative feedback on the development of compulsive alcohol seeking behaviour. Finally, we measured how trait sensitivity to negative feedback affected the extinction and reinstatement of alcohol seeking after a period of abstinence. **Results:** Trait sensitivity to negative feedback predicted the vulnerability of rats to the development of compulsive alcohol seeking and consumption. We also found significant differences between the more sensitive and less sensitive groups in their propensity to extinguish alcohol seeking behaviours when the alcohol was no longer available. **Limitations:** The findings from our study did not answer the question of whether individual differences in sensitivity to negative feedback have a genetic basis or develop in response to postnatal experiences. **Conclusion:** The results of our study suggest that negative feedback sensitivity screening could be used to evaluate individual vulnerability to the development and maintenance of alcohol use disorder.

Introduction

Alcohol use disorder is one of the most common psychiatric disorders, and it is a leading cause of mortality worldwide, contributing to 3 million deaths each year, and to disabilities and comorbidities.¹ It is a chronic, complex, relapsing disease, characterized by progressive escalation from moderate to excessive alcohol consumption and accompanied by cognitive, social and occupational impairments. According to the *Diagnostic and Statistical Manual for Mental Disorders*,² alcohol use disorder is a pattern of alcohol consumption with co-occurring symptoms such as high alcohol intake, uncontrollable seeking of alcohol and drinking despite adverse consequences. The latter symptom, a hallmark phenotypic characteristic of alcohol use disorder, is thought to be associated with deficient processing of negative feedback. Indeed, accumulating experimental evidence supports this

idea. Several studies have demonstrated that people affected by chronic alcoholism recurrently make decisions that favour drinking, even in the face of mounting adverse consequences.^{3–5}

It has been hypothesized that people with alcohol use disorder are less sensitive to the negative outcomes of their actions⁶ and less able to use negative feedback to guide and adjust their ongoing behaviour,⁶ suggesting a deficient feedback processing system.⁷ However, despite an abundance of data linking alcohol use disorder to impaired decision-making, far less is known about the aberrant processing of negative feedback before the onset of alcohol use disorder. In fact, no study thus far has directly shown that biased sensitivity to feedback affects the transition from recreational to compulsive alcohol abuse, largely because it is difficult to obtain information about people’s sensitivity to feedback before they develop an addiction.

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In humans, sensitivity to feedback can be investigated using a framework of neurophysiological^{8–10} and neuropsychological measures.^{11–15} One measure that offers an effective and fully translational way of assessing an individual's sensitivity to feedback is the probabilistic reversal learning test.¹⁶ In this behavioural paradigm, participants are presented with 2 (and sometimes more) stimuli in each trial; using trial-and-error feedback after each response, they learn to select the stimulus that is usually correct (i.e., rewarded in more trials or unrewarded in fewer trials) and to avoid the stimulus that is usually incorrect (i.e., unrewarded in more trials or rewarded in fewer trials). This rule reverses intermittently, so that the stimulus that was usually rewarded becomes unrewarded, and vice versa, and responses must be adjusted to make favourable choices. Lose-shifts (i.e., unrewarded outcomes followed by a decision to change the choice) constitute a measure of sensitivity to negative feedback. Win-stay ratios (number of rewarded outcomes after which the subject repeated the choice divided by the total number of rewarded trials on a given stimulus) represent a measure of sensitivity to positive feedback. The use of probabilistic reinforcement increases the complexity of the task so that the information from any given choice is insufficient to guide future behaviour; participants must engage their cognitive function to track the outcome history for both types of stimuli to determine the stimulus that is more beneficial overall.¹⁶

The probabilistic reversal learning paradigm has been applied successfully in a number of studies that investigated the neuroanatomical and neurochemical correlates of reinforcement sensitivity in humans and animals.^{11,16,17} It has also been used to demonstrate that sensitivity to feedback can be measured in animals as a stable and enduring cognitive trait.^{18–21} These studies have opened a new and fascinating avenue of preclinical research that provides an opportunity to investigate the interplay between sensitivity to feedback and other cognitive processes and mental disorders. However, none of these studies has investigated sensitivity to feedback in the context of vulnerability to alcohol use disorder.

One of the main challenges in modern studies of alcohol addiction is the development of animal models that can be characterized by high ethanol intake and mimic the transition from controlled alcohol use to excessive alcohol abuse that occurs in human alcohol use disorder. A common method of achieving voluntary alcohol consumption in rats involves intermittent access to alcohol in the intermittent-access 2-bottle choice paradigm. Exposure to repeated cycles of free choice between 2 bottles (ethanol solution and water) and subsequent withdrawal causes a gradual increase in preference and voluntary alcohol consumption, reaching levels of 3–9 g/kg body weight per 24 hours, depending on the strain used (reviewed by Carnicella and colleagues²²).

However, this procedure does not reflect all of the motivational and reinforcement processes responsible for alcohol seeking and consumption in humans. Therefore, animal models of high ethanol intake achieved through repeated cycles of alcohol intake and withdrawal must be complemented by procedures that involve instrumental training, such as the recently developed instrumental second-order chained schedule

of alcohol reinforcement (ISOC SAR) task described by Giuliano and colleagues.²³ This model allows for measurement of the hallmark symptoms of alcohol use disorder, such as compulsive preparatory and consummatory behaviours, motivation for alcohol and persistence of alcohol intake in the face of aversive consequences.²³

Because relapse is one of the important components of alcohol use disorder, it is also important to address an individual's predisposition to reinstating alcohol seeking behaviour after a period of forced abstinence. Although current animal models do not mimic self-imposed abstinence, the use of periods of forced abstinence followed by restoration of the alcohol-related environment has been shown to reflect the relapse observed in humans.

In the present study, we investigated the theoretical claim that sensitivity to negative feedback — as a stable and enduring behavioural trait — can predict subsequent vulnerability to the development of compulsive alcohol consumption in rats. For this purpose, we initially trained and tested the animals in a series of probabilistic reversal learning tests. Based on this “feedback sensitivity screening,” we classified each rat as more or less sensitive to negative feedback. Then, using the 2-bottle choice paradigm, we measured alcohol consumption in the animals classified above. In the next step, using the ISOC SAR task,²³ we examined the influence of sensitivity to negative feedback on the development of compulsive alcohol seeking behaviour. Finally, we measured how trait sensitivity to negative feedback affects the extinction and reinstatement of alcohol seeking after a period of abstinence.

Methods

Animals and housing

We used 20 male Sprague Dawley rats (Charles River) weighing 176–200 g upon arrival at our facility. The rats were group-housed (4 animals per cage) in an enriched environment with controlled temperature ($21 \pm 1^\circ\text{C}$) and humidity (40%–50%) and using a 12-hour light–dark cycle (lights on at 7:00 am). Throughout the experiment, rats were mildly food-restricted to 85% of their free-feeding weight (according to the normal growth curve recommended by the laboratory rodent supplier) by providing 15 g of food pellets per rat per day (standard laboratory chow). Water was available *ad libitum*. All behavioural procedures and tests were performed during the light phase of the light–dark cycle.

Apparatus

The probabilistic reversal learning tests were conducted in operant conditioning chambers (Med Associates) enclosed in sound-attenuating boxes. Each chamber was equipped with a fan (which also served to eliminate extraneous noise), a house light, a speaker, a food dispenser set to deliver a sucrose pellet (Dustless Precision Pellets, 45 mg; Bio-Serv), a fluid receptacle and 2 retractable levers at the sides of the feeder.

Tests examining alcohol seeking behaviour in the seeking-taking task were conducted in the same operant chambers,

except that the levers were on the wall opposite to the liquid dispenser to create a new experimental setup that would not interfere with any habits the animals had acquired in the probabilistic reversal learning paradigm.

Measuring sensitivity to negative feedback with the probabilistic reversal learning test

After the initial instrumental training described in detail elsewhere,¹⁹ and upon reaching the initial training criterion of fewer than 7.5% omissions on each lever (i.e., fewer than 15% total omissions but equally distributed between the 2 levers) for 3 consecutive training days, the rats were trained in the probabilistic reversal learning paradigm.

Briefly, each probabilistic reversal learning training session lasted until the completion of 200 trials, and each trial lasted for a maximum of 22 seconds. The start of a trial was signalled by the house light, which remained on until the end of the trial. Two seconds after the trial had started, both levers were presented; 1 lever was randomly assigned as the “correct” one, which delivered a reward (1 sucrose pellet) 80% of the times it was pressed. A press on the other lever (the “incorrect” lever) would result in a rewarding outcome only 20% of the times it was pressed. If the rat made no response in 10 seconds, the intertrial interval was triggered and the trial was counted as an omission. During the intertrial interval, both levers remained retracted and the house light was turned off. The same intertrial interval directly followed an unrewarded outcome (i.e., no reward on 20% of the “correct” and 80% of the “incorrect” lever presses). After every 8 consecutive “correct” lever presses (regardless of outcome), the criterion for the reversal of the outcome probabilities was reached; at that point, the previously “correct” lever became the “incorrect” lever, and vice versa. This pattern was followed until the end of the session. The probabilistic reversal learning training phase was repeated daily until the rats achieved sufficient performance levels (i.e., a minimum of 3 reversals in 3 consecutive training sessions, with fewer than 15% omissions per session).

Parameters measured in the probabilistic reversal learning test

To measure rats’ sensitivity to negative feedback (as their ability to ignore an infrequent and misleading lack of reward), we monitored their decisions trial by trial. Unrewarded outcomes for the “correct” lever that were followed by the animal’s decision to switch levers (probabilistic lose-shifts) were scored and expressed as a ratio of all unrewarded outcomes for that lever.

To measure rats’ sensitivity to positive feedback, all rewarded outcomes (true and misleading) followed by a decision to stay with the lever that delivered them (win-stays) were counted jointly for the “correct” and “incorrect” levers and expressed as a ratio of all rewarded outcomes for that lever. This means of analyzing sensitivity to positive feedback follows the method described by Bari and colleagues¹⁷ and was dictated by the fact that win-stay behaviours after misleading rewards on the incorrect lever were too uncommon to undergo robust analysis.

We used the number of reversals completed during the test as a measure of the animal’s performance.

Feedback sensitivity screening

After the rats had achieved stable performance in the probabilistic reversal learning test (a minimum of 3 reversals in 3 consecutive sessions, with fewer than 15% omissions per session), they were then tested in 10 consecutive probabilistic reversal learning tests over 10 days. Based on this “sensitivity screening,” the rats were classified (using the median as a cut-off) as more or less sensitive to negative feedback. We made the classification based on each animal’s average ratio of lever changes after misleading unrewarded outcomes (probabilistic lose-shifts) across all 10 screening tests. The results of our previous studies clearly indicated that a dichotomous categorization based on median split was well suited for investigating negative feedback sensitivity as a stable and enduring cognitive trait in rats;^{18–21} therefore, we extended this means of data analysis to the present study.

Intermittent-access 2-bottle choice paradigm

To induce drinking behaviour and determine the level of alcohol consumption in the rats, we conducted 18 sessions of the intermittent-access 2-bottle choice test every second day. During the 2-bottle choice test, rats were separated into individual cages for 24 hours, where they were presented with 1 bottle of 10% ethanol (wt/wt) and 1 bottle of water. We chose the percentage of the ethanol solution based on the findings of Giuliano and colleagues.²³ To avoid the potential effects of a side preference, we changed the position of the bottles after 12 hours. We weighed the bottles before and after each session to determine alcohol consumption (g ethanol per kg body weight). We calculated the volume of liquids consumed as the difference in bottle weights from the beginning and end of each session, subtracting volume lost as a result of dripping from bottles in empty cages.

Characterization of compulsive alcohol seeking behaviour

Taking task

Initially, the rats were trained to associate the pressing of the taking lever with alcohol delivery under a fixed-ratio-1 schedule of reinforcement. Each trial began with insertion of the randomly assigned taking lever and the house light on. Pressing on the lever resulted in presentation of the dipper on the opposite side of the box, delivery of 0.1 mL of 15% ethanol (wt/wt) and retraction of the taking lever. We chose the percentage of the ethanol solution based on the findings of Giuliano and colleagues.²³ Failure to respond to the lever within 10 seconds was considered an omission. Regardless of the result, each trial was followed by a 10-second intertrial interval, during which the taking lever was retracted and alcohol was not available. Rats were limited to a maximum of 60 rewards for a 30-minute training session. After they had achieved the performance criterion of at least

20 taking responses in 3 consecutive sessions, the animals were shifted to the seeking-taking phase of the training. The position of the taking lever was counterbalanced across animals.

Seeking-taking task

During this task, each trial began with insertion of the seeking lever next to the previously assigned taking lever (which remained retracted). Pressing on the seeking lever led to the extension of the taking lever after a random interval of 1–15 seconds. Pressing on the taking lever resulted in presentation of the dipper on the opposite side of the box, delivery of 0.1 mL of 15% ethanol (wt/wt) and retraction of both levers. Each trial was followed by a 10-second intertrial interval, during which both levers were retracted and alcohol was not available. Rats were limited to a maximum of 100 rewards for a 45-minute session. After they had achieved the performance criterion of at least 20 taking responses in 3 consecutive sessions, the animals were ready for the seeking-taking-punishment task.

Seeking-taking-punishment task

To measure the persistence of seeking behaviour in the face of aversive consequences, we used the seeking-taking-punishment task. In this paradigm, each trial began as described for the seeking-taking task — with the insertion of the seeking lever. The seeking lever response resulted in the extension of the taking lever after a random interval of 1–15 seconds, or in a 1 second electric shock (0.10–0.50 mA) administered through a grid floor. Each session consisted of 25 trials, of which 8 (30%) were punished with foot shock and 17 (70%) were reinforced by the delivery of 0.1 mL 15% ethanol after the taking lever response. When animals were punished after a seeking lever response, the taking lever and dipper were not presented, and no alcohol was available. The intensity of the shock increased gradually over consecutive test sessions according to the following pattern: 0.10, 0.20, 0.30, 0.30, 0.40, 0.40, 0.50 and 0.50 mA. Although punishment occurred randomly in each session, never more than 2 consecutive trials resulted in a foot shock, and the first trial of the session was always reinforced.

Extinction of alcohol seeking and taking behaviours

After the rats completed seeking-taking-punishment testing, they underwent 5 additional seeking-taking tests (baseline). They then underwent daily extinction sessions (lasting 15 minutes), during which the seeking lever response resulted in the extension of the taking lever (random interval of 1–15 seconds), but pressing the taking lever had no programmed consequences, and alcohol was not available. After 10 seconds of exposure, the lever was retracted and a 10-second intertrial interval began. After reaching the extinction criterion (fewer than 5 seeking responses in 3 consecutive sessions), the rats underwent 30 days of alcohol abstinence, during which they were not tested.

Reinstatement of alcohol seeking and taking

The reinstatement of alcohol seeking after extinction is one of the most common animal models for studying relapse and its

underlying neural mechanisms.²⁴ The rate of operant responding (i.e., reinstatement) on the lever that was associated with alcohol delivery is taken as a measure of the animal's urge to obtain alcohol — a model of craving in patients.²⁵ After the extinction phase and 30 days of abstinence, the rats underwent a series of seeking-taking tests to measure how quickly they reinstated their alcohol seeking behaviour and brought their performance up to the pre-extinction baseline levels. The animals were not alcohol-primed, and apart from the context, no specific cue induced the seeking behaviour. The animals received response-contingent alcohol during the reinstatement sessions, and they were tested until they reached an average number of seeking responses from 5 tests that was equal to or higher than the average number of seeking responses from their 5 baseline seeking-taking tests.

Experimental schedule

The experimental schedule is summarized in Figure 1.

Statistical analysis

We analyzed the data using SPSS (version 25.0; SPSS Inc.). We verified the normality of the sensitivity to feedback data using the Kolmogorov–Smirnov test. We analyzed the data for the negative feedback sensitivity screening, 2-bottle choice, seeking-taking, seeking-taking-punishment and reinstatement tasks using 2-way repeated-measures analyses of variance; the within-subject factor was test day or session, and the between-subjects factor was feedback sensitivity.

To analyze the differences between the less sensitive and more sensitive groups in terms of average quantity of alcohol consumed and number of tests needed to achieve extinction and reinstatement criteria, we used *t* tests or, for nonparametric data, Mann–Whitney *U* tests. For pair-wise comparisons, we adjusted the values using Sidak correction for multiple comparisons.²⁶ We also computed a Pearson correlation coefficient to assess the relationship between negative feedback sensitivity and investigated measures of alcohol seeking and taking in rats.

All tests of significance were performed at $\alpha = 0.05$. We tested homogeneity of variance using a Levene test, and for repeated-measures analyses, we confirmed sphericity using a Mauchly test. Data are presented as mean \pm standard error of the mean.

Results

Probabilistic reversal learning training and testing

All animals fulfilled the probabilistic reversal learning training criteria and qualified for the probabilistic reversal learning screening. On average, they reached the criteria after 6.8 ± 0.58 probabilistic reversal learning tests. The groups that were more or less sensitive to negative feedback did not differ significantly in terms of the number of probabilistic reversal learning tests needed to reach the criterion ($t_{18} = 0.338, p = 0.74$).

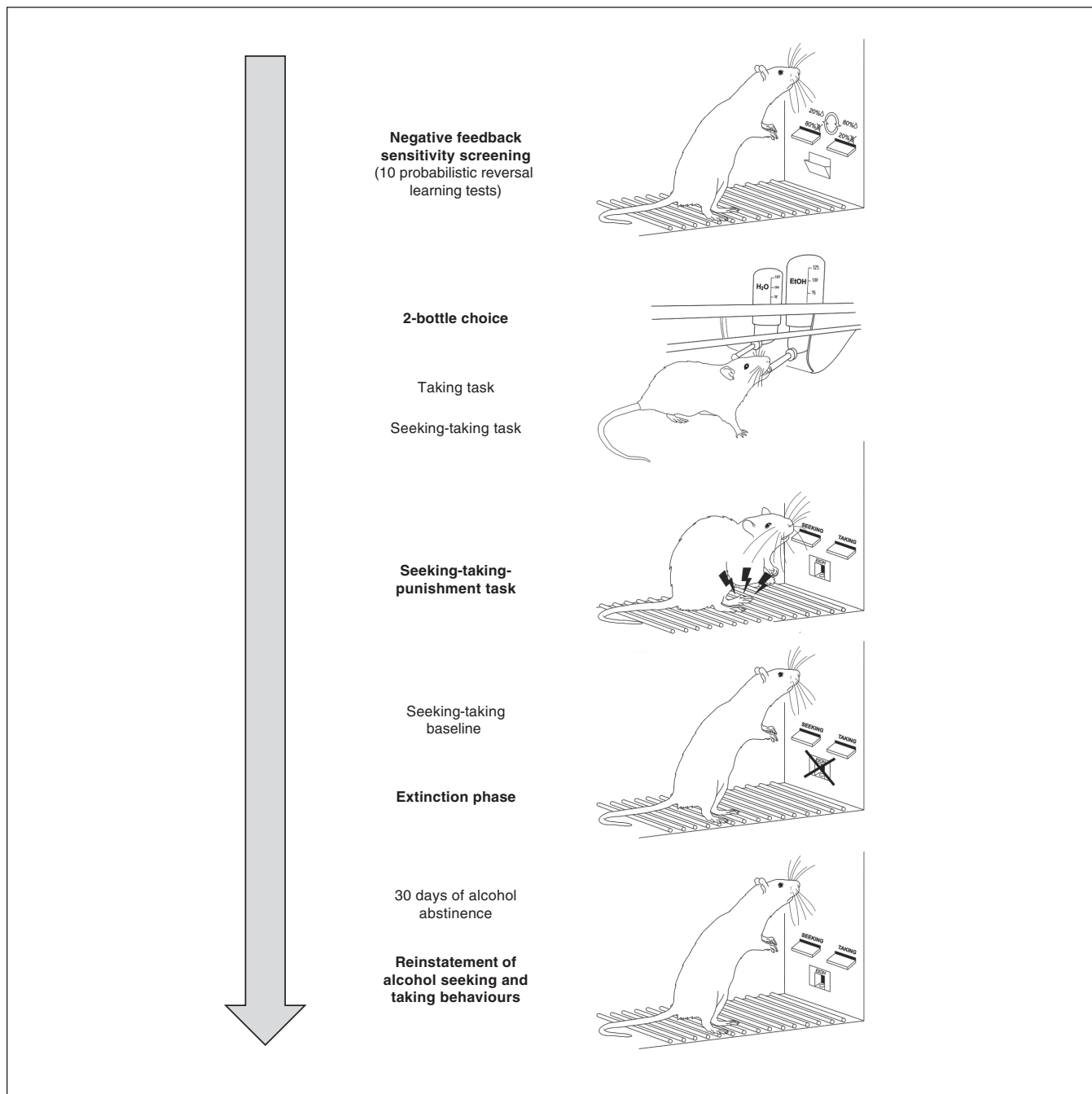


Figure 1: The experimental schedule. Initially, we trained and tested the animals in a series of probabilistic reversal learning tests. Based on this “negative feedback sensitivity screening,” we classified each rat as more or less sensitive to negative feedback. Then, in the 2-bottle choice paradigm, we measured the consumption of alcohol in the animals classified above. In the next step, using the alcohol seeking-taking-punishment task, we examined the influence of sensitivity to negative feedback on the development of compulsive alcohol seeking behaviour. We then measured how trait sensitivity to negative feedback affected the extinction of alcohol seeking and the reinstatement of this behaviour after a period of abstinence.

Negative feedback sensitivity screening

For the animals classified as less sensitive to negative feedback, the average proportion of probabilistic lose-shift behaviours after misleading negative feedback

ranged from 0.358 to 0.532, with an average of 0.453 ± 0.018 . For the animals classified as more sensitive to negative feedback, the average proportion of probabilistic lose-shift behaviours ranged from 0.537 to 0.698, with an average of 0.583 ± 0.015 .

The between-group difference in sensitivity to negative feedback was stable across the screening period (i.e., no significant interaction between screening day and sensitivity to negative feedback; $F_{9,162} = 0.566$, $p = 0.82$) — a significant sensitivity effect ($F_{1,18} = 31.19$, $p < 0.001$; Figure 2A). The more and less sensitive groups did not differ significantly in terms of average sensitivity to positive feedback ($F_{1,18} = 1.149$, $p = 0.30$; Figure 2B) or average number of reversals made during the screening tests ($F_{1,18} = 1.984$, $p = 0.18$; Figure 2C).

Induction and assessment of drinking behaviour

During the 18 intermittent-access 2-bottle choice sessions, the rats significantly ($p < 0.05$) increased their alcohol intake (Figure 3). Average intake in the first session was 3.49 ± 0.58 g/kg per 24 hours, increasing to an average of 4.95 ± 0.41 g/kg per 24 hours in the last session (significant main effect of session; $F_{17,221} = 2.774$, $p < 0.001$). We observed no significant differences in alcohol consumption between the less and more sensitive groups (nonsignificant effect of sensitivity; $F_{1,13} = 0.1661$, $p = 0.69$) and found a nonsignificant session \times sensitivity interaction ($F_{17,221} = 1.016$, $p = 0.44$).

Because only 15 of the 20 rats achieved the criteria for the taking and seeking-taking tasks, we analyzed alcohol consumption during the 2-bottle choice sessions in only these animals.

Characterization of compulsive alcohol seeking behaviour

In the next step, the animals were trained to associate the pressing of the taking lever with alcohol delivery under a fixed-ratio-1 schedule of reinforcement.

The number of sessions needed to achieve the taking task criterion ranged from 4 to 39, with an average of 16.3 ± 3.9 . The animals from the less sensitive group reached the taking task criterion after 11.9 ± 4.6 sessions; animals from the more sensitive group needed 22.8 ± 6.3 sessions.

The number of sessions needed to achieve the seeking-taking task criterion ranged from 4 to 31, with an average of 17.5 ± 1.7 . To achieve the seeking-taking task criterion, animals from the less sensitive group needed 17.6 ± 2.9 sessions, and animals from the more sensitive group needed 17.3 ± 0.8 sessions.

We observed no significant differences between the 2 groups in terms of number of sessions needed to achieve the taking test criterion ($U = 17.50$, $p = 0.24$) or the seeking-taking test criteria ($t_{13} = 0.061$, $p = 0.95$).

After the taking and seeking-taking training, the rats were tested in the seeking-taking-punishment task. Completion of trials during the seeking-taking-punishment task was an indicator of the animals' persistence in seeking alcohol in the face of aversive consequences. Two-way repeated-measures analysis of variance revealed a significant sensitivity \times shock intensity interaction ($F_{7,77} = 3.427$, $p = 0.003$,

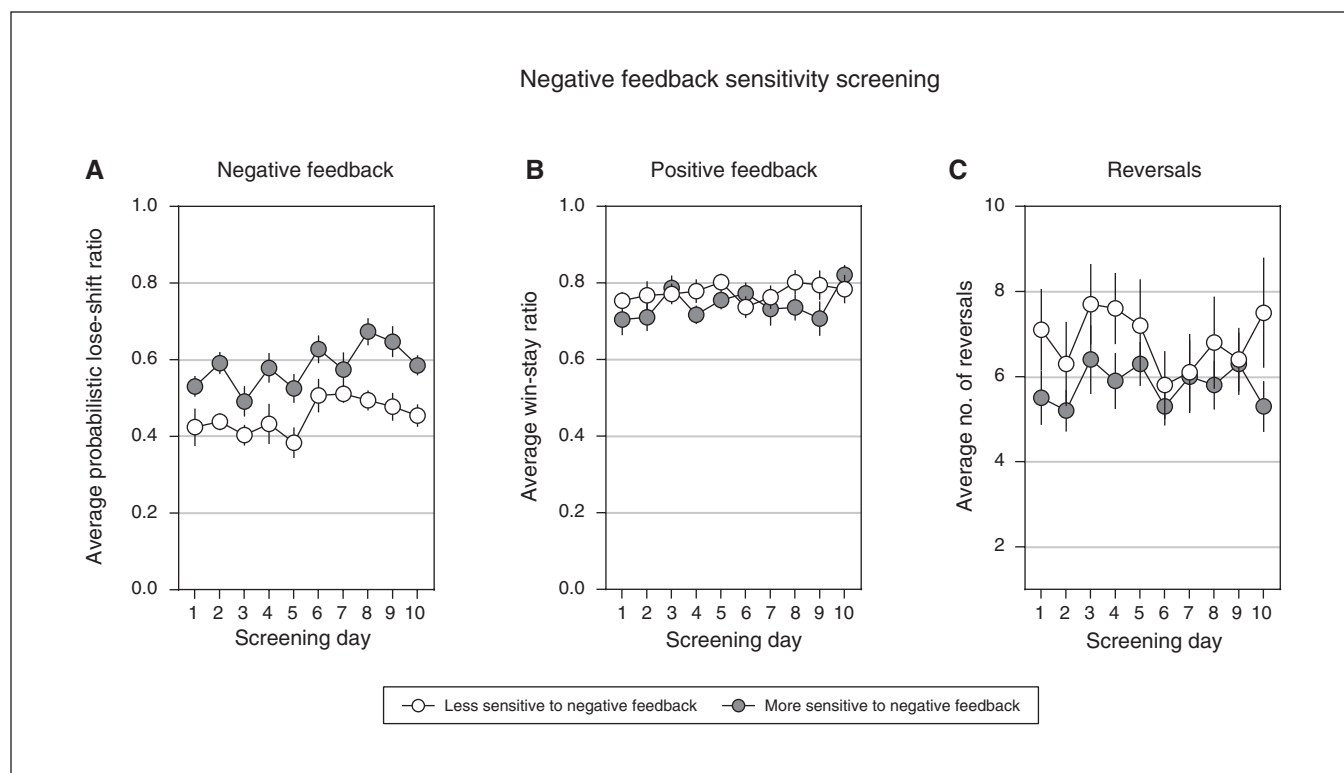


Figure 2: Results of negative feedback sensitivity screening. (A) Average proportion of lose-shift behaviours after misleading unrewarded outcomes; (B) average proportion of win-stay behaviours after a reward; and (C) average number of reversals in animals classified as less sensitive (open circles, $n = 10$) and more sensitive (filled circles, $n = 10$) to negative feedback during the 10 screening probabilistic reversal learning tests. Data are presented as the mean \pm standard error of the mean.

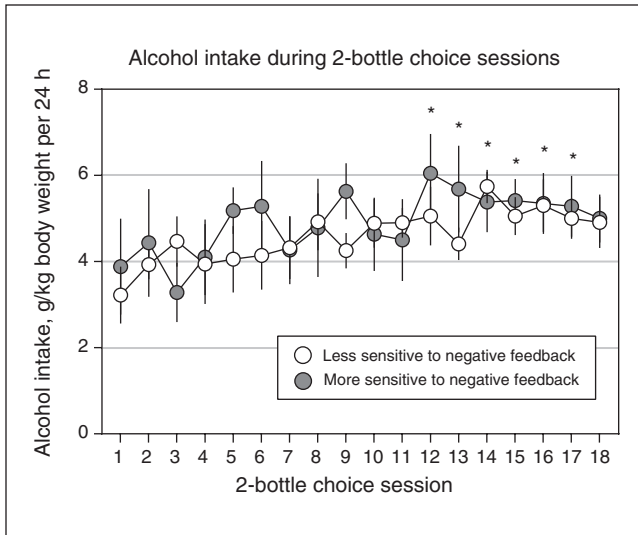


Figure 3: Alcohol intake during the intermittent-access 2-bottle choice sessions. Average daily alcohol intake (g/kg of body weight) in groups of rats classified as less sensitive (open circles, $n = 9$) and more sensitive (filled circles, $n = 6$) to negative feedback. Data are presented as mean \pm standard error of the mean. *Significant ($p < 0.05$) difference in average alcohol consumption (for the entire cohort) between a given 2-bottle choice session and the first 2-bottle choice session.

and $F_{7,77} = 2.494$, $p = 0.023$ for seeking responses and completed trials, respectively).

Two rats (1 less sensitive to negative feedback and 1 more sensitive) that showed a significantly different pattern of behaviour on the seeking-taking-punishment task were excluded from the analysis based on the Grubbs test for outliers. Because the behaviour of these 2 rats differed only during the seeking-taking-punishment tests, their data were excluded for those tests only, and included in the analyses for other parts of the study.

As the shock intensity increased from 0.10 to 0.50 mA during consecutive sessions, the rats classified as more sensitive to negative feedback significantly decreased their number of seeking responses (Figure 4A) compared to their initial performance ($p = 0.012$ at 0.4 mA and $p < 0.001$ at 0.50 mA) and compared to their less sensitive counterparts ($p = 0.045$ and $p = 0.002$ at 0.5 mA). We observed similar differences between the less and more sensitive groups in the number of completed trials (Figure 4B). As the shock intensity increased from 0.10 to 0.50 mA over consecutive sessions, the rats classified as more sensitive to negative feedback significantly lowered their number of completed trials compared to their initial performance ($p < 0.001$ at 0.5 mA) and compared to their less sensitive counterparts ($p = 0.035$ at 0.5 mA).

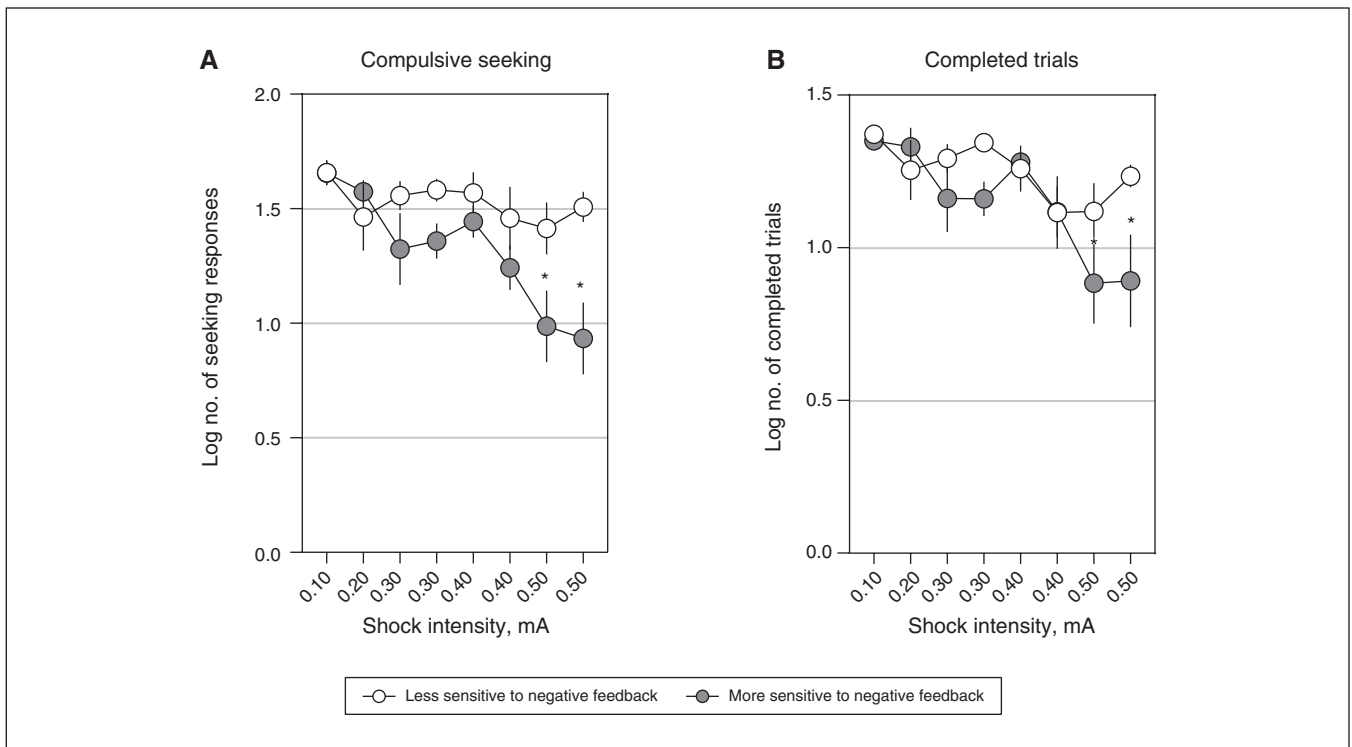


Figure 4: Trait sensitivity to negative feedback determines compulsive alcohol seeking and taking in rats. Rats were trained on an instrumental second-order chained schedule of alcohol reinforcement task to work for alcohol, and then their seeking responses were punished by mild electric foot shocks of increasing intensity (from 0.1 through 0.2, 0.3 and 0.4 up to 0.5 mA). As the shock intensity increased, the rats classified as more sensitive to negative feedback (filled circles, $n = 5$) significantly decreased (A) their number of seeking responses and (B) their number of completed trials compared to their baseline performance and to the less sensitive cohort (open circles, $n = 8$). Data are presented as mean \pm standard error of the mean. *Significant ($p < 0.05$) difference between the less sensitive and more sensitive groups.

After seeking-taking-punishment testing, all animals underwent 5 baseline seeking-taking tests before the start of the extinction phase. We found no significant differences between groups in the average number of seeking and taking responses during the baseline seeking-taking tests ($t_{13} = 0.695$, $p = 0.49$, and $U = 25$, $p = 0.84$, respectively).

Extinction and reinstatement of alcohol seeking behaviour

The number of sessions needed to achieve the extinction criterion ranged from 4 to 20, with an average of 11 ± 1.32 . All rats extinguished their seeking lever responses, but those more sensitive to negative feedback needed significantly fewer sessions than their less sensitive counterparts to cease their seeking behaviour (7.67 ± 1.17 sessions v. 13.22 ± 1.71 sessions; $t_{13} = 2.39$, $p = 0.033$; Figure 5A and inset).

We assessed the effect of sensitivity to negative feedback on the reinstatement of alcohol seeking after a 30-day abstinence interval. Over the course of 10 seeking-taking tests, most of the animals (apart from 2 less sensitive rats and 1 more sensitive rat) reinstated their pre-extinction baseline level of seeking responses. We observed no significant differences in the number of seeking responses across the reinstatement phase between those less and more sensitive to negative feedback (nonsignificant effect of sensitivity; $F_{1,13} = 0.1928$, $p = 0.67$), and we found a nonsignificant session \times

sensitivity interaction ($F_{9,117} = 0.6824$, $p = 0.72$; Figure 5B). The average number of sessions needed to achieve the reinstatement criterion was 6.1670 ± 0.5752 . The rats from the less and more sensitive groups did not differ significantly in the number of sessions needed to reinstate the baseline levels of seeking responses ($U = 13$, $p = 0.39$).

Correlation between negative feedback sensitivity and measures of alcohol seeking and taking

We computed a Pearson correlation coefficient to assess the relationship between negative feedback sensitivity and measures of alcohol seeking and taking in rats. We found a negative correlation between negative feedback sensitivity and the log number of seeking responses during the seeking-taking-punishment task at 0.30 mA ($r_{13} = -0.6576$, $p = 0.015$) and in both 0.50 mA trials ($r_{13} = -0.6701$, $p = 0.012$; $r_{13} = -0.7043$, $p = 0.007$). We also found a trend toward statistical significance for the negative correlation between negative feedback sensitivity and number of seeking responses during the trial of the extinction criterion ($r_{13} = -0.4878$, $p = 0.06$). The analysis revealed no significant correlations between sensitivity to negative feedback and other investigated measures of alcohol seeking and taking. Findings are presented in Appendix 1, Table S1, available at www.jpn.ca/lookup/doi/10.1503/jpn.210220/tab-related-content.

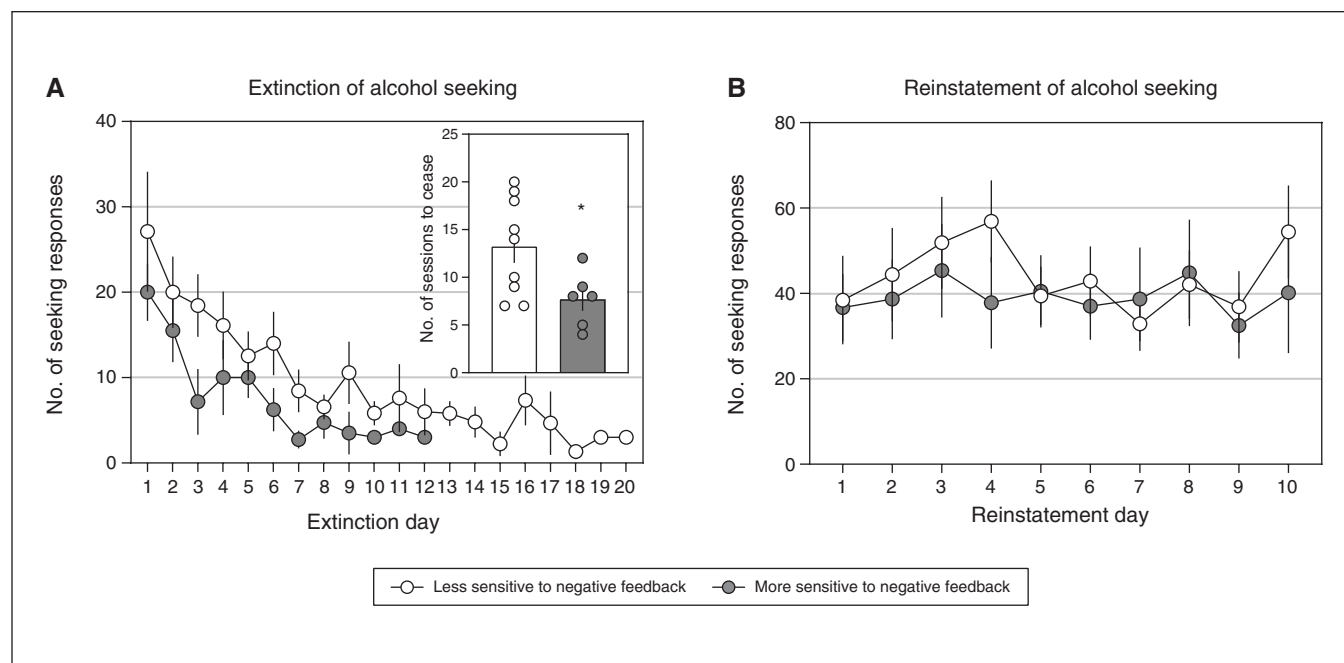


Figure 5: The effects of trait sensitivity to negative feedback on the length of extinction and reinstatement of compulsive alcohol seeking in rats. (A) After the punishment tests, the rats underwent 5 baseline seeking-taking tests and then underwent the extinction phase, during which seeking responses did not result in alcohol delivery. All rats extinguished their seeking lever responses, but animals more sensitive to negative feedback (filled circles, $n = 6$) needed significantly fewer sessions to cease their seeking behaviour than their less sensitive counterparts (open circles, $n = 9$; inset). *Significant ($p < 0.05$) difference between the more sensitive and less sensitive groups. (B) After a 30-day abstinence interval, rats from the less sensitive (open circles, $n = 9$) and more sensitive (filled circles, $n = 6$) groups underwent 10 seeking-taking tests to measure how quickly they reinstated their baseline level of alcohol seeking responses. Data are presented as mean \pm standard error of the mean.

Discussion

The results of the present study demonstrated that trait sensitivity to negative feedback predicts the vulnerability of rats to the development of compulsive alcohol seeking and consumption in a situation when these behaviours are punished. Our findings also showed significant differences between animals classified as less and more sensitive to negative feedback in their propensity to extinguish alcohol seeking behaviours after the termination of alcohol availability. Finally, our findings complement the existing literature, proving that the development of compulsive alcohol seeking and taking behaviours in Sprague Dawley rats can be achieved with intermittent free access and instrumental alcohol drinking paradigms.

Apart from excessive drinking to the point of intoxication, people addicted to alcohol also devote much time and effort to compulsively seeking alcohol, in spite of the consequences. Although several preclinical studies have reported on procedures mimicking the persistence of alcohol consumption in the face of aversive consequences,^{27–29} none of them directly addressed the compulsive nature of alcohol seeking, which occurs before drinking and is mechanistically dissociable from the acute intoxicating effects of the drug.

A breakthrough occurred in 2015, together with the development of a behavioural procedure allowing for the temporal separation of seeking and taking instrumental responses for alcohol. In their study, Giuliano and colleagues³⁰ introduced a new behavioural paradigm allowing for the above-mentioned separation and demonstrated for the first time that in rats, a propensity to consume and spontaneously prefer alcohol is dissociable from the propensity to compulsively seek it. This observation suggested that in rats, individual vulnerability to compulsive seeking of alcohol may depend on cognitive mechanisms other than a simple preference.

The experiments in the present study have confirmed the above assumption. Although all rats displayed similar initial alcohol consumption, those with lower sensitivity to negative feedback were more vulnerable to compulsive alcohol seeking than their more sensitive counterparts. This increased vulnerability was demonstrated by their weaker reaction to the unpredictable punishment of seeking responses (i.e., foot shock intensity increasing from 0.1 to 0.5 mA over repeated sessions) and their prolonged extinction of instrumental alcohol seeking responses when alcohol was no longer available.

In contrast, rats classified as being more sensitive to negative feedback progressively decreased their alcohol seeking, significantly reducing it at shock intensities of 0.4 to 0.5 mA, and they needed significantly fewer alcohol-free instrumental sessions to extinguish their alcohol seeking behaviours. These intuitive results were in line with studies in humans showing that individuals with a high sensitivity to adverse outcomes tend to use less alcohol than those who are less sensitive.³¹ Our results were also consistent with those of studies in students; among those who drank heavily and received an infraction for their alcohol use, those with a higher sensitivity to punishment were more likely to reduce their drinking.³² To the best of our knowledge, this is the first direct evidence that

in an animal model of alcohol dependence, sensitivity to negative feedback interacts with the development of compulsive intake of alcohol.

Although further studies are needed to directly pinpoint the neurobiological correlates of the interaction we observed, our results may be at least partially explained using the framework of psychobiological models of motivation, reinforcement sensitivity theory being one of the most influential.^{33,34} According to these models, input from the basal ganglia, mesolimbic dopamine projections from the ventral tegmental area to the ventral striatum, the nucleus accumbens, and mesocortical dopamine projections to the prefrontal cortex (constituting the neural circuit of the behavioural activation system) mediate the rewarding effects of alcohol and the reactions associated with seeking it. In turn, differences in sensitivity to negative feedback, which interacts with compulsive alcohol seeking, could account for differences in the activity of the behavioural inhibition and fight-flight-freeze systems, which are neuroanatomically bound to the septohippocampal system, periaqueductal grey matter, medial hypothalamus, amygdala, cingulate cortex, and dorsal and ventral prefrontal cortices.³⁵

Limitations

Based on the data from the present study, we could not unequivocally infer whether the differences in the length of extinction of alcohol seeking were parallel or secondary to the differences in persistent drinking despite negative consequences, but this second-level validation confirms the role of trait sensitivity to negative feedback in the development of compulsive alcohol consumption. Because the extinction was based on a lack of reward, the results from this phase also exclude the unlikely possibility that the differences we observed in the seeking-taking-punishment task could have resulted from the various sensitivities to electric foot shocks.

In contrast to previous studies,^{23,30} the present study used an outbred Sprague Dawley strain rather than inbred, alcohol-preferring rats. This strain has been reported to demonstrate moderate alcohol consumption in the intermittent-access 2-bottle choice paradigm (reviewed by Carnicella and colleagues²²), and to our knowledge, it has never been tested with the ISOCAR task. The use of a strain without a genetic predisposition to alcohol preference demonstrated naturally occurring differences in alcohol consumption, but also accounted for the fact that almost a quarter of the tested animals failed to meet task criteria. The fact that most of the excluded rats came from the more sensitive group lent strength to the results of our experiments, suggesting that a high sensitivity to negative feedback could be associated with a generally weaker vulnerability to the effects of alcohol. However, this concept calls for further investigation.

Several other issues should be investigated further. The findings from our study did not answer the question of whether individual differences in sensitivity to negative feedback have a genetic basis, develop in response to postnatal experiences, or both. We also do not know whether a similar difference in sensitivity to negative feedback could be observed in females,

or the relationship between sensitivity to negative feedback and social hierarchy. Finally, considering the caloric value of alcohol, we do not know if food restriction affected alcohol consumption in rats. Although alcohol has a fairly high caloric value, these are so-called “empty calories” with no hunger-quenching potential, and the literature suggests mixed effects: a 2001 study found that alcohol decreases the level of leptin, a hormone involved in the regulation of energy balance by inhibiting hunger,³⁶ but in 2005, Calissendorff and colleagues³⁷ found that alcohol inhibits appetite-stimulating ghrelin secretion.

Conclusion

Using multiple, consecutive probabilistic reversal learning tests, we confirmed our previous observation that sensitivity to negative feedback in rats is a stable and enduring behavioural trait. We also showed that this trait may determine the rats’ vulnerability to the development of compulsive alcohol seeking, maintained despite the risk of punishment. Trait sensitivity to negative feedback was also associated with a better ability to cease alcohol seeking behaviour when it was no longer available. Our results call for further investigation of the neurobiological mechanisms involved. Future studies should also determine whether trait sensitivity to negative feedback interacts with molecular and physiologic correlates of compulsive alcohol intake. Finally, it is possible that negative feedback sensitivity screening could be used to evaluate individual differences in response to the therapeutic effects of drugs used in alcohol use disorder.

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