

Decrease of vanillin sucrose intake by victorious and defeated mice: development of anhedonia?

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Abstract

Hedonic reactions to various rewards play a key role in various forms of motivated behavior. The influence of repeated experience of social victories or defeats in daily agonistic interactions between male mice on voluntary consumption of vanillin sucrose solution used as hedonic reinforcer was studied. Intake of vanillin sucrose solution was shown to decrease in the winners and losers exposed to social confrontations as compared with the controls. Three days of deprivation failed to restore the intake of vanillin sucrose solution to the control level in the losers and did so in the winners. The results obtained imply that similar reaction of animals to a hedonic non-drug reinforcer may have different motivational origin depending on positive or negative social experience.

Key words: Anhedonia, social defeats, social victories, depression, aggression, social stress, vanillin sucrose solution, agonistic interactions

Introduction

Hedonic or affective reactions to food, water, sex, and other rewards play a vital causal role in the motivated behavior [6]. Different forms of stress can render animals more susceptible to positive reinforcers such as drugs and ethanol [15, 20], but also can lead to the development of anhedonia toward non-drug reinforcers - sucrose or saccharin solution [2, 16, 23, 25]. Little is known, however, about the significance of social experience and social status for the development of addiction and hedonic processes. Subordinates and losers were shown to consume more ethanol than dominants and winners, respectively [8, 14]. Dominant rats take less drugs than subordinate ones, but, in contrast to the latter, increase drug consumption after social disturbances [26]. Chronic social defeat stress in animals was shown to be able to evoke the development of depression [7, 13, 21], and to decrease voluntary sucrose intake [21, 27]. The latter is considered as the development of anhedonia, which, is one of the core symptoms of major depression in humans [1]. However, nothing is known about the hedonic processes in aggressive animals with repeated experience of victories in daily agonistic interactions – in the winners.

The present study aims to compare the influence of chronic positive and negative social experience accompanied by social victories or defeats, respectively, on voluntary consumption of vanillin sucrose solution (used as hedonic reinforcer) by male mice. After a period of 21 days the victorious and defeated mice were subjected to three days interruption of vanillin sucrose supply. It was hypothesized that the losers drink less vanillin sucrose solution than the winners.

Materials and methods

Animals

Adult male mice of the C57BL/6J strain (26-28g) from stock maintained in Animal House at the Institute of Cytology and Genetics SD RAS, Novosibirsk were used. The animals were housed under standard conditions (12:12 h light/dark regime; food (pellets) and water available *ad libitum*). Mice were weaned at one month of age and housed in groups of 8-10 in plastic cages (36 x 23 x 12 cm). Experiments were performed on mice 10-12 weeks of age. All procedures were in compliance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Winners and losers

Winners and losers were generated using the sensory contact model [11]. Pairs of animals were placed in cages (28x14x10 cm) divided in two compartments by a perforated transparent partition allowing the animals to see, hear and smell their neighbor, but not to contact them physically. Test sessions commenced 2 days after adaptation of the animals to these new housing conditions and sensory contact. Every afternoon (between 2.00 p.m. and 5.00 p.m.) the steel cover of the cage was replaced by a transparent one, and 5 min later (the period needed for adaptation to the lighting condition and activation) the partition was removed for 10 min to allow agonistic interactions. Superiority of one of the partners was evident within 3 daily test sessions with the same partner. One partner attacked, bit, and chased the other, who displayed defensive behavior only (sideways, upright postures, withdrawal, lying on the back or freezing). Then, every day after the test session, each defeated mouse was placed in another two-compartment cage with a partition, in which another winner was present in the other compartment. The winners remained in their own compartments. Agonistic interactions were discontinued by lowering the partition if intensive attacks lasted more than 3 min. The procedure yielded equal numbers of males with experience of aggression, evidenced by victories (aggressors, winners) and with social defeats (defeated mice, losers) in agonistic interactions. As controls were used male mice after 5 days of individual housing. They were thought to be the most adequate controls, because the submissiveness of grouped males has been removed, and the effects of social isolation have not been established [for details see 11].

Experimental procedure

Vanillin sucrose solution supply

1% sucrose solution supplemented with 0,2% vanillin was used in this experiment. After 7 consecutive days of agonistic interactions, the winners and losers were subjected to a period of forced overnight vanillin sucrose solution intake. For that, after agonistic interactions bottles containing vanillin sucrose solution only were supplied to the animals in the night-time (5.00 p.m. to 10.00 a.m.). In the morning a bottle with water was added. Then a two bottles free choice was offered to mice for 21 days. In half of the cages, the losers were offered vanillin sucrose

solution and water, while the winners – only water. In the other half of the cages, the winners received vanillin sucrose solution and water, while the losers – only water. Animals and the bottles with vanillin sucrose solution and water were weighed to estimate liquid consumption per day/per body weight at day 1 (1st measure), 6 (2nd measure), 11 (3d measure), 16 (4th measure), 21 (5th measure). The position of each bottle was changed daily after agonistic interactions to prevent side preference. Daily agonistic interactions continued during the whole experimental period. Then the animals were deprived of vanillin sucrose solution for 3 days. Agonistic interactions between the winners and losers were stopped during the deprivation period. After this period the two bottles free choice was given again to the winners and losers to estimate consumption of vanillin sucrose solution per day. Mean values of vanillin sucrose solution intake by the controls during four days beginning from day 2 were used as control consumption. Each experimental group consisted of 12–15 animals.

Total liquid (water and vanillin sucrose solution) intake in grams per body weight, g/g, water intake/body weight, g/g, vanillin sucrose solution intake/body weight, g/g, vanillin sucrose solution preference (% of the consumed vanillin sucrose solution relative to the total amount of liquid intake) were analyzed as parameters of hedonic (anhedonic) behavior.

Statistics

Repeated measures analysis of variance (ANOVA) was used to determine the influence of social status, repeated measures and interaction on water and vanillin sucrose solution intakes, total liquid intake and vanillin sucrose solution preference. A paired sample t-Student test was used to compare measures over time. Data are presented as means \pm standard errors. Differences between experimental groups were considered to be significant if $P < 0.05$.

Results

The data on vanillin sucrose solution and water consumption are presented in Fig. 1 and Fig. 2. Winners and losers after 7 days of agonistic interactions on the first day of vanillin sucrose solution supply preferred to drink vanillin sucrose solution similarly to the single-housed controls (about 70% of total liquid intake) (Fig.1). Repeated measures ANOVA revealed no

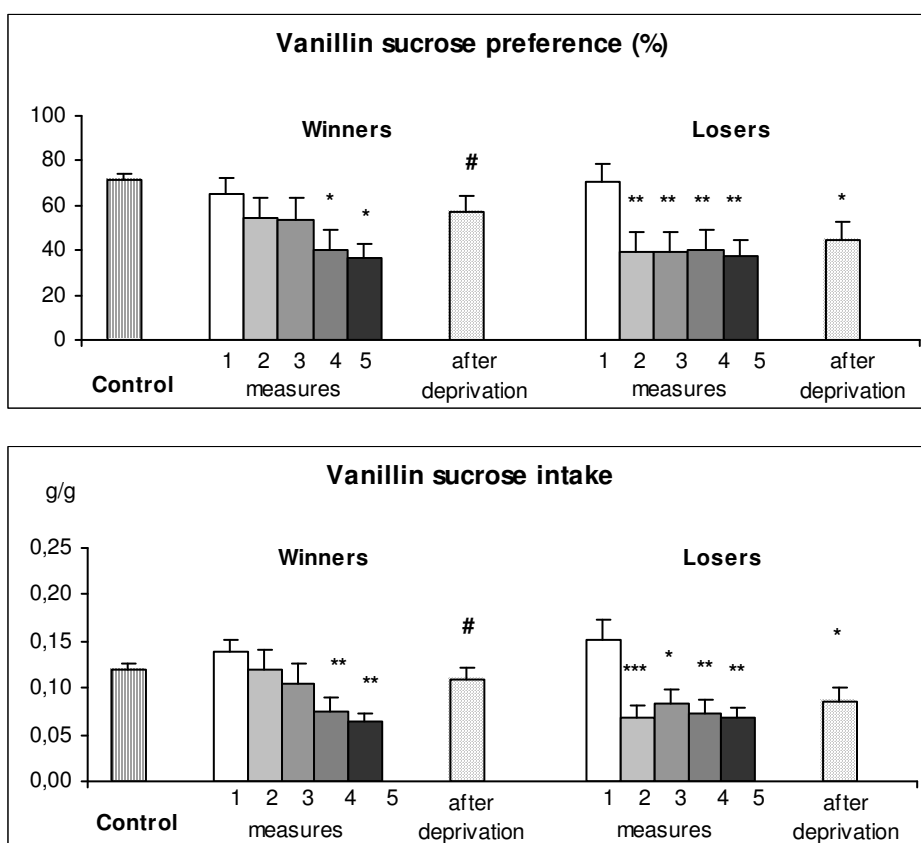


Fig. 1. Vanillin sucrose solution intake (g/g) and vanillin sucrose solution preference (%) by the winners and losers during agonistic interactions. * $P < 0.05$; ** - $P < 0.01$, *** - $P < 0.001$ vs 1st measure, # - $P < 0.05$, vs 5th measure, paired T-Student test.

significant influence of social status (winners and losers) on vanillin sucrose solution intake [$F(1,22) = 0.58$, $P > 0.05$] and vanillin sucrose solution preference [$F(1,22) = 0.35$, $P > 0.05$]. Effect of repeated measures on the vanillin sucrose solution intake [$F(4,88) = 9.92$, $P < 0.001$] and vanillin sucrose solution preference [$F(4,88) = 6.18$, $P < 0.001$] were found. There was no interaction effect of social status and repeated measures on vanillin sucrose solution intake [$F(4,88) = 1.74$, $P > 0.05$], and vanillin sucrose solution preference [$F(4,88) = 0.91$, $P > 0.05$]. Paired t-Student test showed that in comparison with 1st measure, on the 4th and 5th measures the winners decreased vanillin sucrose solution intake ($P < 0.01$, both) and preference ($p < 0.05$, both). As compared with 1st measure, losers were shown to decrease vanillin sucrose solution intake ($P < 0.001$ for 2nd; $P < 0.05$ for 3d, $P < 0.01$ for 4-5th measures) and preference ($P < 0.01$, for 2d-5th measures). No significant differences were found in vanillin sucrose solution intake by the winners and losers and single-housed controls ($P > 0.05$).

In the winners, vanillin sucrose solution intake and preference grew after a 3-day deprivation period as compared with those parameters before deprivation (5th measure) ($P < 0.05$). After deprivation no significant differences were found in the winners in comparison with the control and the 1st measure ($p > 0.05$ for both parameters). In the losers vanillin sucrose solution intake and preference did not differ significantly before and after deprivation (for both $P > 0.05$).

Repeated measures ANOVA revealed no significant influence of social status (winners and losers) on water intake [$F(1,22) = 0.36$, $P > 0.05$] and total liquid intake [$F(1,22) = 0.004$, $P > 0.05$] (Fig. 2). Effect of repeated measures on the water intake [$F(4,88) = 3.10$, $P < 0.05$] and total liquid intake [$F(4,88) = 6.02$, $P < 0.001$] were found. There was no interaction effect of social status and repeated measures on water intake [$F(4,88) = 0.73$, $P > 0.05$]. Significant interaction effect of social status and repeated measures was revealed for total liquid intake [$F(4,88) = 6.2$, $P < 0.001$].

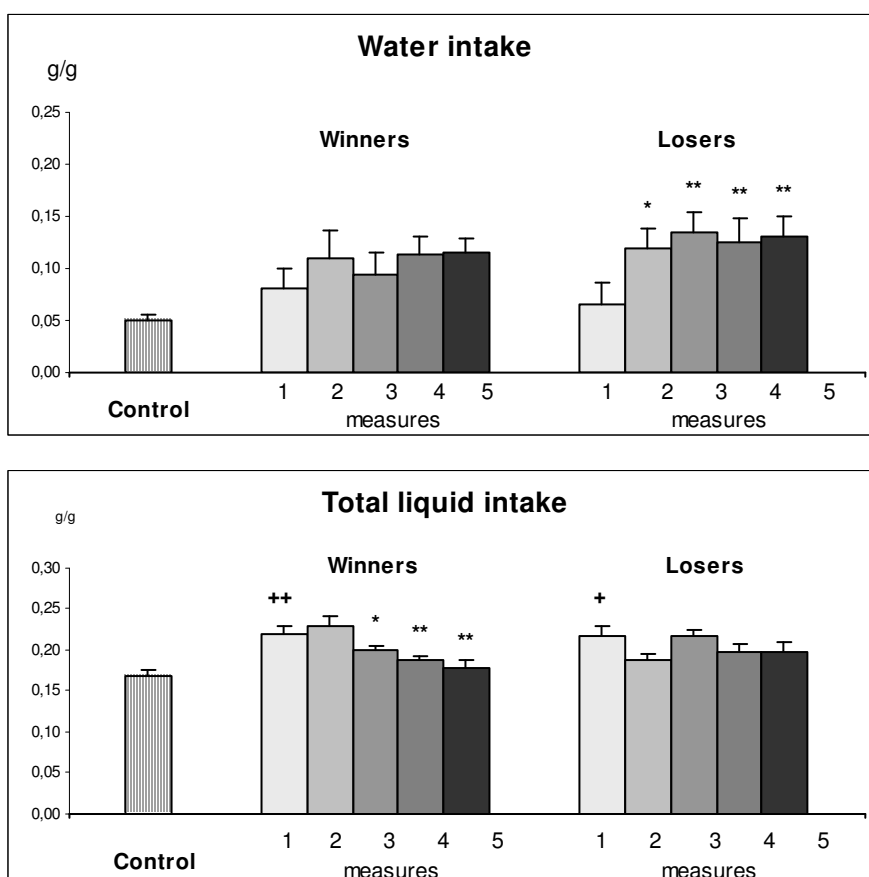


Fig. 2. Total liquid (water and vanillin sucrose solution) intake and water intake by the winners and losers during agonistic interactions. * $p < 0.05$; ** - $p < 0.01$, vs 1st measure; + $p < 0.01$; ++ - $p < 0.001$, vs control, paired T-Student test.

No significant differences were found in water intake by the winners and losers and single-housed controls ($P > 0.05$). Total liquid intake on the 1st measure was significantly higher in the winners and losers in comparison with the control ($P < 0.001$ and $P < 0.01$, respectively). No differences in water consumption was found in the winners on all measures ($P > 0.05$). In the losers, an increase in water intake was revealed on the 2d-5th measures ($P < 0.05$ for 2d; $P < 0.01$ for 3d-5th measures) as compared with the 1st measure in contrast to total liquid intake, which remained the same on all measures ($P > 0.05$). In comparison with the 1st measure a decrease in total liquid intake was revealed on the 3d-5th measures ($P < 0.05$, for 3d, $P < 0.01$, for 4-5th measures) in the winners.

Discussion

On the first day of vanillin sucrose solution supply, which is the 8th day of social confrontations, upon the formation of stable alternative social statuses under repeated experience of social victories or defeats [11], the

winners and losers preferred to drink vanillin sucrose solution (70% of the total amount of liquid intake) similarly to the control mice. In 5 days vanillin sucrose solution preference and intake decreased significantly on all measures in the losers. In the winners these parameters were found to be significantly lower on the 4th and 5th measures - after two weeks of vanillin sucrose solution supply. Thus, consumption of vanillin sucrose solution was shown to decrease in both the victorious and defeated mice.

Decrease in vanillin sucrose solution intake under chronic social defeat stress shows the best correlation with the development of depression in the losers. As shown earlier [13], social defeats repeatedly experienced in agonistic confrontations lead to dramatic changes in social and individual behaviors as well as welfare. A severe behavioral deficit was developed in the losers: after experiencing social defeats for 20 days they could only demonstrate passive defence and immobile postures, whereas at the beginning they preferred active defence and withdrawal. The losers displayed reduced

ambulation in the open-field, increased immobility time in the Porsolt's test and demonstrated no aggression in any situation, no matter how provoking. Pronounced anxiety has been revealed in the plus-maze test. Losers demonstrated disturbed social behavior – social withdrawal in the partition test as well as a loss of weight and decreased stress reactivity. In the losers, repeated treatment with the antidepressants, imipramine or tianeptine, decreased the level of depressiveness estimated by the Porsolt's test. Chronic treatment with anxiolytics (ethanol, ipsapirone, buspirone) reduced anxiety, but did not prevent depression in the losers. Chronic unavoidable social stress is believed to be a pathogenic factor that leads to a mixed anxiety/depression state in mice. Decreased brain serotonergic activity was shown in the losers [13]. Thus, the aetiology, response to treatment, symptomatology and brain neurochemical changes are analogous to those of human depression. Decrease in vanillin sucrose solution consumption may be regarded as development of anhedonia under chronic social defeat stress in depressive mice.

However, the finding that vanillin sucrose solution consumption by the winners also decreases was totally unexpected, since they did not demonstrate any signs of depression after repeated experience of aggression accompanied by victories. Instead, the winners demonstrated enhanced aggressivity and impulsivity, increased motor activity, hostility, malignancy etc. [12]. Therefore, a decrease in vanillin sucrose solution intake as a result of repeated experience of victories and aggression challenges the generally accepted thesis that anhedonia measured by decrease in sucrose intake in rats and mice [2, 16, 23, 25] is a key symptom of depression at least in animal models. Of course, a decrease in vanillin sucrose solution consumption in both the winners and losers might be the consequence of common processes developing under social confrontations: for example, enhanced anxiety exhibited by animals with alternative social status [3] or stress of agonistic interactions. However, careful analysis of total liquid consumption and water intake by the winners and losers allows a different interpretation of the results obtained. In the losers total liquid intake did not change during the experiment. However, beginning from 2d measure, vanillin sucrose solution intake abruptly decreased and water intake increased in the losers, who then started developing depression. In depressive state the

losers preferred water to vanillin sucrose solution. Since the senses of smell and taste are known may be affected in depressive patients [10, 19], decrease in vanillin sucrose solution intake in the losers may reflect the development of aversion to hedonic stimuli and anhedonia.

In the victorious mice water consumption did not change during the entire experimental period. That means that the decrease in total liquid solution intake found in the winners is due to the decrease in vanillin sucrose solution intake. Two suppositions are possible. In the winners the decrease in vanillin sucrose solution intake might be explained by *satiety of novel*, even if tasty, solution and consequently the loss of interest to it. It is also possible that reduced vanillin sucrose consumption by the winners is due to the substitution of one source of positive reinforcement (vanillin sucrose solution) by another one – experience of aggression and victories. According to many authors [9, 17, 22], aggression is rewarding in laboratory rodents and in humans and any positive reinforcement can be used the tendency to behave aggressively [5, 17].

Opposite effect of deprivation on vanillin sucrose solution intake in the winners and losers indicates the involvement of different motivation components of hedonic behavior toward this reinforcer in the animals with alternative social status. After 3 days of deprivation without social confrontations, the level of vanillin sucrose solution intake in the winners recovered to that on the first day, which was similar to vanillin sucrose solution intake by the control. It has been shown that deprivation of non-drug reinforcer saccharin might not be associated with depression-like behavior [24]. In the losers the reduced vanillin sucrose solution intake and preference did not change significantly after the deprivation and was similar to that before the deprivation. This is in agreement with persistence of depressive state in defeated animals shown earlier [4, 7]. Opposite effect of deprivation on vanillin sucrose solution intake and preference in the winners and losers confirms the data [18] demonstrating high context-dependency of deprivation effect in relation to saccharin solution.

Thus, reduced vanillin sucrose solution intake under agonistic interaction might be the result of totally different processes in animals with alternative social status - the winners and losers. Anhedonia is formed in animals exposed to social defeats stress accompanied by the development of depression. Anhedonia is

manifested by an abrupt reduction in vanillin sucrose solution consumption and by failure to recover vanillin sucrose consumption after deprivation, indicating the persistence of the developed pathology. Reduced vanillin sucrose solution consumption by aggressive males, who exhibit no signs of depression, might be evoked by social stress, anxiety, satiety of the stimulus and substitution of one reinforcing stimulus by

another one. After deprivation aggressive males recover interest to the stimulus.

The experiment allows the following general conclusion: interpretation of similar behavioral or physiological changes arising under agonistic interactions in animals with alternative social statuses may differ and depends on the subject of interpretation.

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