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Research report

Anti-anxiety self-medication in rats: Oral consumption of chlordiazepoxide and ethanol after reward devaluation

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HIGHLIGHTS

- Rats exposed to a reward devaluation show signs of emotional distress.
- Oral consumption of the anxiolytic chlordiazepoxide increases after devaluation.
- Ethanol consumption also increases, but water consumption does not increase.
- Anti-anxiety self-medication may underlie the initial stages of addiction.

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ABSTRACT

Rats increased preference for ethanol after sessions of appetitive extinction, but not after acquisition (reinforced) sessions (Manzo et al., 2014). Drinking was not influenced by appetitive extinction in control groups with postsession access to water, rather than ethanol. Because ethanol has anxiolytic properties in tasks involving reward loss, these results were interpreted as anti-anxiety self-medication. The present experiment tested the potential for self-medication with the prescription anxiolytic chlordiazepoxide, a benzodiazepine with an addictive profile used in the treatment of anxiety disorders. To test this hypothesis, Wistar rats exposed to a 32-to-4% sucrose devaluation received a two-bottle, 2-h preference test immediately after consummatory training. One bottle contained 1 mg/kg of chlordiazepoxide, 2% ethanol, or water for different groups (the second bottle contained water for all groups). Three additional groups received the same postsession preference tests, but were exposed to 4% sucrose during consummatory training. Rats showed suppression of consummatory behavior after reward devaluation relative to unshifted controls. This effect was accompanied by a selective increase in preference for chlordiazepoxide and ethanol. Downshifted animals with access to water or unshifted controls with access to the anxiolytics failed to exhibit postsession changes in preference. Similar results were observed in terms of absolute consumption and consumption relative to body weight. This study shows for the first time that a prescription anxiolytic supports enhanced voluntary consumption during periods of emotional distress triggered by reward loss. Such anti-anxiety self-medication provides insights into the early stages of addictive behavior.

There is abundant evidence of a correlation between emotional stress and the consumption of psychoactive substances, such as alcohol, opiates, and anxiolytics [1,2]. War veterans who developed posttraumatic stress disorder consume more psychoactive substances and are more likely to experience use relapse after an acute stressful event than veterans who did not develop such disorder [3–6]. Similar effects were described in relation to physical and sexual abuse, early neglect, natural catastrophes, loss of a spouse, family conflicts, poverty, and chronic pain [7–9].

These findings suggest that the onset and maintenance of substance use disorders (SUDs) are related to the short-term benefits of consuming substances that can attenuate aversive emotions – the self-medication hypothesis [10–14]. The present research is concerned with the role of anti-anxiety self-medication in the initial consumption of psychoactive substances, rather than in the maintenance of an established SUD [15]. The Diagnostic and Statistical Manual for Mental disorders (DSM-V; [16]), which provides detailed criteria for SUDs, could serve as a guide. However, the DSM-V criteria for SUDs do not apply because the initial use of a

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psychoactive substance should not be characterized by sustained excessive consumption, cravings, tolerance, and withdrawal symptoms, all included in the DSM-V for diagnosing an SUD. Therefore, a new set of criteria has to be postulated for the special case of animals that are consuming a substance with addiction potential, but do not yet show any of the symptoms associated to an SUD. The present research is based on the following hypotheses:

- Significant levels of anxiety are necessary for the enhanced voluntary consumption of anxiolytics.
- Increased consumption is restricted to periods of increased anxiety.
- Increased consumption is selective to solutions containing substances that reduce anxiety.

Relevant evidence requires an induction task generating an aversive emotion followed by voluntary consumption of substances that reduce such emotion. Most of the available evidence has limitations. For example, (1) studies focus on recreational substances, ignoring prescription drugs with addictive potential; (2) the response to the stressor is not usually reported, thus making it difficult to determine whether there was evidence of anxiety before substance consumption; (3) controls for consumption are not usually included, thus it is not possible to determine whether the induction task affects the consumption of the target substance or just consumption per se; and (4) anxiety is induced by presenting aversive stimuli, rather than by withdrawing reward, thus ignoring the potential consequences of reward loss to induce anti-anxiety self-medication (see Discussion for references).

The present experiment overcame these limitations. The induction task was consummatory successive negative contrast (cSNC), involving a devaluation from 32% to 4% sucrose (compared to controls always exposed to 4% sucrose). cSNC involves a transient reduction in sucrose consumption during downshift sessions [17] and it is known to induce negative emotional states via reward loss [18,19]. For example, reward loss increases the release of stress hormones [20], is modulated by opioid receptors [21], and supports escape conditioning [22]. This task also allows a behavioral assessment of anxiety levels during induction in terms of the degree of consummatory rejection of the downshifted solution. The postsession preference test involved access to chlordiazepoxide (CDP) and water. CDP, a benzodiazepine anxiolytic, is frequently prescribed for anxiety disorders [9,23], has addictive potential [24], and reduces the cSNC effect [25–29]. A CDP concentration approximating a typical clinical dose was chosen using the dose-translation formula from humans to rats [30]. Groups receiving ethanol–water (positive control) and water–water (negative control) were included. We predicted anti-anxiety self-medication effects for CDP (and ethanol) groups during the reward devaluation phase of the induction task.

1. Method

1.1. Subjects

The subjects were 48 male Wistar rats, experimentally naïve, purchased from Harlan Laboratories (Barcelona, Spain). Rats were housed individually in polycarbonate cages with water continuously available, in a room with constant temperature (18–22 °C) and humidity (50–60%), and lights on between 08:00 and 20:00 h. At the start of the experiment, rats were approximately 90 days old. The mean weight was 327.4 g (SEM = 1.8; range: 304–360 g). Animals were food deprived and maintained within 80–85% of their ad lib weight by supplemental food at least 30 min after the end of their daily protocol (details below). All rats were handled for 3 days before the start of the experiment. During the 15 days of the experiment, the mean weight of all animals varied between 263.3–280.2 g (SEMs varied between 1.5 and 1.7). This experiment followed the European Union directive guidelines for the use of animals in research (2010/63/EU) and Spanish Law (6/2013; R.D. 53/2013).

1.2. Apparatus

Consummatory training (induction task) involved 6 Plexiglas boxes, each measuring 30 cm × 15 cm × 30 cm ($L \times W \times H$). The front wall had a hole through which the sipper tube of a graduated cylinder was inserted. The 32% (4%) sucrose solution was prepared w/w by mixing 32 g (or 4 g) of sucrose for every 68 g (or 96 g) of distilled water. A magnetic mixer (Nahita Magnetic Stirrer 680–9, Beriain, Spain) was used to dissolve the sucrose. Session length was measured with a manual stop watch (Extech, model 365510, Madrid, Spain).

Access to CDP, ethanol, or water was provided in polycarbonate home cages measuring 32 cm × 15 cm × 30 cm ($L \times H \times W$). The floor was covered with saw dust. Each cage contained two plastic bottles (50 ml) and an area to store food pellets on a wire lid. Fluid consumption was measured by weighing the bottles before and after each 2-h preference test (Cobos, JT-300C Digital Scale, Barcelona, Spain). All sipper tubes used in this experiment were stainless steel, 1 cm in diameter, and equipped with a ball. CDP (chlordiazepoxide hydrochloride, Sigma Aldrich, Madrid, Spain) and ethanol (from 96%, Panreac, Castellar del Vallés, Spain) were diluted in tap water on a v/v basis. CDP (1 mg/kg) was weighted with a precision scale (Cobos, Precisa 125A, Barcelona, Spain). This CDP dose for rats was derived using the standard formula for calculating the equivalent dose from clinical studies with humans [30]. This dose (1 mg/kg of CDP) refers to the concentration of the drug inside the bottle containing the solution. These bottles contained 12 ml of diluted CDP. Thus, the stated dose would be applicable to an animal that drank the entire amount in the bottle. If, for example, an animal drank 10 ml of the solution, the corresponding dose would 0.83 mg/kg of CDP. The solution volume was selected on the basis of previous studies in which the amount of water consumed in a period of 2 h was registered [14]. The 2% dose of ethanol was selected on the basis of a previous study [31]. Animals were weighed daily (Baxtran, Model BS3, Girona, Spain).

1.3. Procedure

1.3.1. Induction task

On Days 1–4, two bottles containing tap water were placed in the animal's home cage. On Day 5, animals were placed in the consummatory box for a 5-min habituation session, without fluids. This session served to familiarize the animals with the consummatory box. On Days 6–15, 10 preshift sessions were administered in the consummatory box. In each session, animals received free access to 32% or 4% sucrose, depending on the experimental condition, and the amount of sucrose consumed was registered from the graduated cylinder. On Days 16–20, 5 postshift sessions were administered exactly as during acquisition, except that all animals received 4% sucrose. Each session lasted 5 min starting from the first contact with the sipper tube. Rats were transported in squads of 6 animals, all from the same group. The order of squads was randomized across days. Home cages were cleaned and the saw dust replaced every other day.

1.3.2. Preference test

Immediately after each session of consummatory training, animals were placed back in their home cage with two bottles. For one set of groups (CDP), one bottle contained tap water and the