



## Incentive relativity in middle aged rats

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### HIGHLIGHTS

- Aged rodents exhibit alterations in cognitive functions.
- The goal was to evaluate the effect of age in the incentive' assessment.
- We use an emotional-cognitive protocol and only a cognitive one.
- Aged rats had a mild cognitive impairment in both procedures.

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### ABSTRACT

Response to a reinforcer is affected by prior experience with different reward values of that reward, a phenomenon known as incentive relativity. Two different procedures to study this phenomenon are the incentive downshift (ID) and the consummatory anticipatory negative contrast (cANC), the former is an emotional-cognitive protocol and the latter cognitive one. Aged rodents, as also well described in aged humans, exhibit alterations in cognitive functions. The main goal of this work was to evaluate the effect of age in the incentive' assessment using these two procedures. The results indicated that aged rats had an adequate assessment of the rewards but their performance is not completely comparable to that of young subjects. They recover faster from the ID and they had a cognitive impairment in the cANC. The results are discussed in relation to age-related changes in memory and emotion.

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### 1. Introduction

To act efficiently in the environment mammals continuously compared the value of the stimuli to which they are exposed. If there is a mismatch between what is expected and what actually occurs, emotions emerged, such as frustration or anxiety. Hence, response to a reinforcer is affected by the animal' previous experience and the reward value assigned to it, a phenomenon known as incentive relativity [6,11,16,17,29].

This phenomenon can be studied using the consummatory successive negative contrast (cSNC) and incentive downshift (ID). In cSNC animals that received a 4% sucrose solution after several trials in which they were exposed to 32% sucrose solution exhibit an abrupt decrease in sucrose consumption compared to an unshifted

control group that always receives access to the 4% solution. The cSNC requires a comparison between the current downshifted solution and the reactivated memory of the preshift solution. Such comparison induces an approach–avoidance conflict that results from the competing tendencies to approach the sipper tube and consume the downshifted solution because of the food deprivation state and to avoid the sipper tube because of anticipated emotional rejection of the downshifted solution. This conflict makes cSNC susceptible to anxiolytic, opioid and cannabinoid drug treatment [3,12,14,18,25,31].

The ID procedure employed groups that experienced the incentive downshift but not utilized the control group that always consumed the devaluated solution. Downshifted animals exhibit an abrupt decrease in sucrose consumption on the 1st day of a concentration shift [23,24]. It has been suggested that the behavioral reaction to a low valued reinforcer in the presence of signals previously paired with a larger reward is aversive in nature and elicits negative emotional responses [2], this procedure serves as a model situation to study the intersection between learning, motivation, and emotion [21].

Another way to study the incentive relativity without the emotional component is through the consummatory anticipatory

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negative contrast (cANC). In cANC each daily trial involves a magnitude transition. Each trial consists of two successive components separated by a brief midtrial interval. In the experimental group, animals have access to a small reward in the first component followed by access to the large reward in the second component. In the control group, animals have access to the small reward in both components. cANC is observed in the first component (low incentive for both groups) when the performance of the experimental animals is significantly below controls.

Different pharmacological profiles were found between cANC and cSNC [11,22]. In this sense, the benzodiazepine anxiolytic chlordiazepoxide, which reduces downshift, has no effect on cANC [13]. On this basis, Flaherty [11] argued that cANC, unlike incentive downshift, “has nothing in common with animal models of anxiety.”

Aged rodents, as also well described in aged humans, exhibit alterations in cognitive functions in several tasks, e.g. the performance in different learning tasks, sexual behavior and grooming [15,19,30]. For example, related to incentive paradigm, Bentosela, D'Ámbros, Mustaca and Papini [5] assess the performance of middle-aged (14-month old) and young (3-month old) rats in a cSNC situation. The retention interval between the last preshift trial and the first postshift trial was either 1 day or 5 days in different groups. cSNC was generally similar in middle-age and young rats in the 1-day retention interval condition. However, middle-age rats recovered faster than young rats from cSNC when a 5-day retention interval was used. These results suggest that the faster recovery of the aged rats on cSNC implicated cognitive deficits. Another way to compare the involvement of memory deficit in middle-aged and adults rats is generating a weak memory of the preshift phase with fewer trials with the high appetitive solution of ID procedure. This was the goal of Experiment 1 in which the incentive downshift was performed after only two trials. The Experiment 2 used a cANC paradigm to evaluate the effect of age in incentive assessment in a cognitive procedure without the emotion involved in the ID procedure.

## 2. Material and methods

### 2.1. Subjects

The subjects were 71 female, experimentally naive Wistar rats, about 3 and 14 months old at the start of the experiments. One week before the start of each experiment, animals were placed in individual cages with free access to water and food. The average weight was 249 g (range: 210–323 g) for the young animals and 302 g (range: 272–357 g) for the aged subjects. The amount of food was gradually reduced across days until the animals reached 85% of their weights. This level of deprivation was maintained throughout the duration of the experiment by posttraining supplementary food administered at least 20 min after the end of the daily trial. Animals were kept in a daily light-dark cycle of 12 h (lights on at 07:00 h). Experiments were performed between 12 h and 15 h PM. The housing and testing rooms were maintained at constant temperature (around 22 °C) and humidity (around 60–70%). All efforts were made to minimize animal suffering and to reduce the number of animals employed.

### 2.2. Behavioral procedures

Rats were trained in 4 conditioning boxes (MED Associates, Fairfax, VT). Each box measured 24.1 cm in length, 29.2 cm in width, and 21 cm in height. The floor was made of aluminum bars (0.4 cm in diameter, 1.1 cm apart from center to center). In the center of a lateral wall, there was a 5-cm hole, 3.5 cm deep, 1 cm above the floor

level, through which a sipper tube could be manually introduced from the outside. When fully inserted, the sipper tube protruded 2 cm into the box. A photocell was located just in front of the tip of the sipper tube, inside this hole. Goal-tracking time (measured in 0.01-s units) was automatically recorded by a computer that measured the cumulative amount of time that the photocell was activated during the trial. This measure correlates with fluid intake for the two sucrose concentrations used in this experiment [20], and it has been used concurrently with fluid intake yielding the same results [22,26]. Each box was enclosed in a sound and light-attenuating cubicle equipped with a source of white noise and diffused house light.

#### 2.2.1. Incentive downshift procedure (ID)

One way to weaken the pre shift memory is increasing the interval between the pre and post shift phases [5]. Another way is generating a weak memory of the pre shift phase, with fewer trials with the high appetitive solution. To achieve this goal a short ID procedure was used, it had 5 trials, the performance of middle aged rats ( $n=7$ ) was compared with adult young rats ( $n=8$ ). During the first 2 trials (Pre shift phase) the animals had access to a 32% sucrose solution and then they received 3 trials with the 4% sucrose solution (Post shift phase). Each trial was separated by 24 h and lasted 5 min starting from the first interruption of the photocell located by the sipper tube and trials interval were of 24 h. On each trial, the sipper tube was manually introduced into the box before rats were placed in the conditioning box. An enhanced consummatory behavior in the aged rats in comparison with the young ones is expected.

#### 2.2.2. Consummatory anticipatory negative contrast procedure (cANC)

In cANC, young and aged rats were evaluated in a cognitive procedure. For all the rats, the first solution was 4% sucrose. This component (called first bottle) lasted 3 min, counting after the first interruption of the photocell. The second component (called second bottle) started after a midtrial interval of approximately 20 s. Animals were randomly assigned to one of four groups. In the second bottle, two groups received access to 32% sucrose: 4-32/Aged ( $n=15$ ), 4-32/Young ( $n=15$ ), whereas the remaining groups received access to 4% sucrose: 4-4/Aged ( $n=15$ ), and 4-4/Young ( $n=11$ ). The second bottle also lasted 3 min, starting with the first interruption of the photocell. There were 6 trials throughout the experiment. The main dependent measure was the goal-tracking time during the first bottle. cANC is observed when there is lower consummatory response in the first bottle in Group 4-32 than in Group 4-4, across days. Thus, anticipation of 32% sucrose suppresses performance directed at 4% sucrose. cANC is usually interpreted as a special case of pavlovian conditioning in which the initial trial acts as a conditioned stimulus that signals the presentation of the second trial, which could be viewed as the unconditioned stimulus [11]. Therefore, suppression of consummatory behavior relates to the anticipation of a preferred sucrose solution. If the aged rats had a cognitive deficit the prediction would be a delay in acquisition of contrast effect, i.e. an enhanced consume of the sucrose solution in the first bottle during more trials than young animals.

Sucrose solution (w/v) was prepared by mixing 40 g or 320 g of commercial sugar in 1 L of tap water. Animals were tested in squads of four. The order of the squads was randomized across days. Each box was swept with a damp towel after each training trial.

### 2.3. Data analysis

Goal-tracking times (recorded in 0.01-s units) were examined by analysis of variance (ANOVA). The loci of significant main effects

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