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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.

1. Introduction

To act efficiently in the environment mammalians 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, DíÁmbros, 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

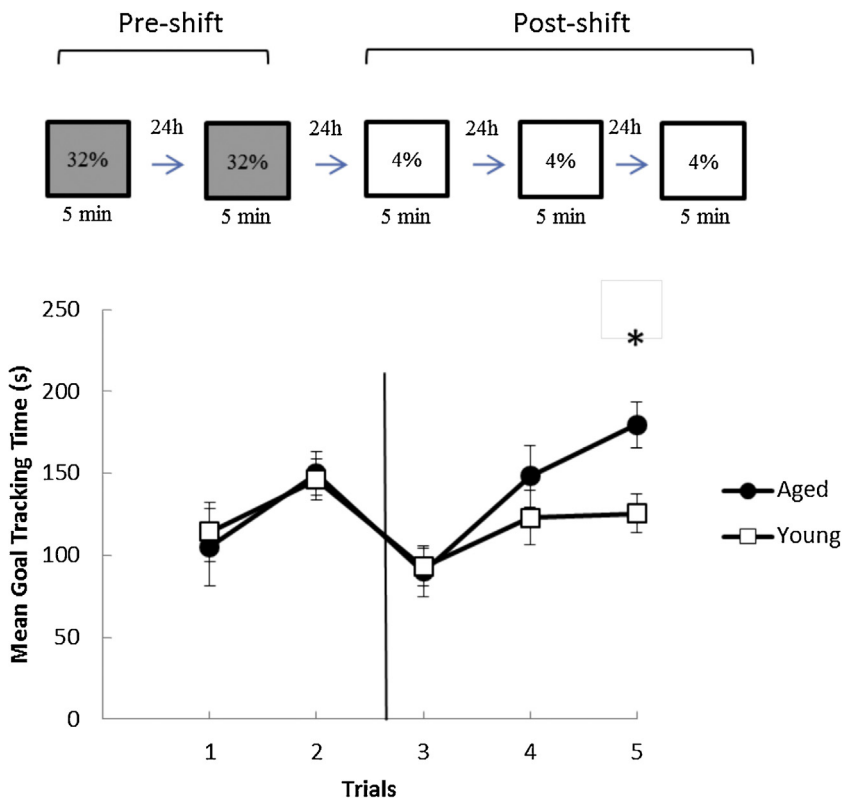

Fig. 1. Mean goal-tracking times of rats trained in the incentive downshift procedure in Experiment 1. All rats had access to 32% sucrose solution during 2 trials and then 3 trials with 4% sucrose solution. * $p < 0.05$ significative differences between aged and young rats.

or significant interactions were subsequently analyzed using pairwise comparisons (Fisher's Least Significant Difference post hoc test). Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Incentive downshift

The results obtained in this experiment are depicted in Fig. 1. In the Pre-shift phase an Age (Aged vs. Young) × Trials (1–2, repeated measures) ANOVA yielded a significant effect of Trials, $F(1, 13) = 11.05$, $p < .01$. All groups gradually increased their consumption throughout this phase. No significant main effects of Age or significant interactions between the factors were found ($p > 0.05$).

In the Post shift phase an Age × Trials ANOVA yielded a significant effect of Trials, $F(1, 13) = 43.56$, $p < .001$ and the interaction Age × Trials $F(1, 13) = 7.79$, $p < .01$. To further analyze the source of this interaction post hoc comparisons were employed. *Post hocs* tests revealed that the Aged animals exhibited significant more sucrose acceptance than the Young ones in the last post shift trials ($p < 0.001$). These results indicated that the aged animals recover faster from the incentive downshift situation than the young subjects.

3.2. Consummatory anticipatory negative contrast

Fig. 2 shows the results of this experiment. A cANC effect developed over trials in both groups, as seen in the top panel (first bottle). These results were subjected to a Contrast (4-32, 4-4) × Age (Young, Aged) × Trial (1–6) analysis. First-bottle goal-tracking times for 4% sucrose were significantly lower in animals exposed to 32% sucrose in the second bottle than in animals exposed to 4% sucrose again, $F(1, 52) = 10.63$, $p < 0.002$. A significant main effect for Age was also found, $F(1, 52) = 5.19$, $p < 0.05$. There was also a main effect of Trial, $F(5, 260) = 34.22$, $p < 0.001$. Also significant were the Trial × Contrast interaction, $F(5, 260) = 5.01$, $p < 0.001$. None other analysis indicate significant differences.

To further analyze the interaction Trial × Contrast, and elucidate from which trial begins the anticipatory behavior in the experimental groups pairwise comparisons were analyze. This analysis indicated that the 4-32/Young and 4-4/Young groups showed significant differences from trial 5 ($p < 0.001$), and the 4-32/Aged vs 4-4/Aged groups showed differences from the trial 6 ($p > 0.05$). Also, the experimental groups are statistically different in the 5th trial ($p < 0.001$). Although the observation of the figure suggest that controls groups are different in some trials, the pairwise comparison didnít show significant differences in any of the trials ($p > 0.05$).This results indicated that the aged group needed 1 further trial to achieve the anticipatory behavior, i.e. the aged experimental group showed a cognitive impairment in comparison with the young experimental group.

4. Discussion

The main goal of this work was to study the effect of age on incentive assessment. For this purpose two procedures that evaluate the incentive relativity were employed, the incentive downshift and the anticipatory contrast. The results suggested that middle aged rats had an adequate assessment of the rewards but their performance is not completely comparable to that of young subjects.

The outcomes of the first experiment indicated that aged animals recover faster from the incentive loss situation. This is in line with the results found in a cSNC [5]. Typically, animals reduce their consummatory behavior of the downshifted solution because they have the expectation of receiving the higher value sucrose solution. Previous work indicates that mnemonic components are

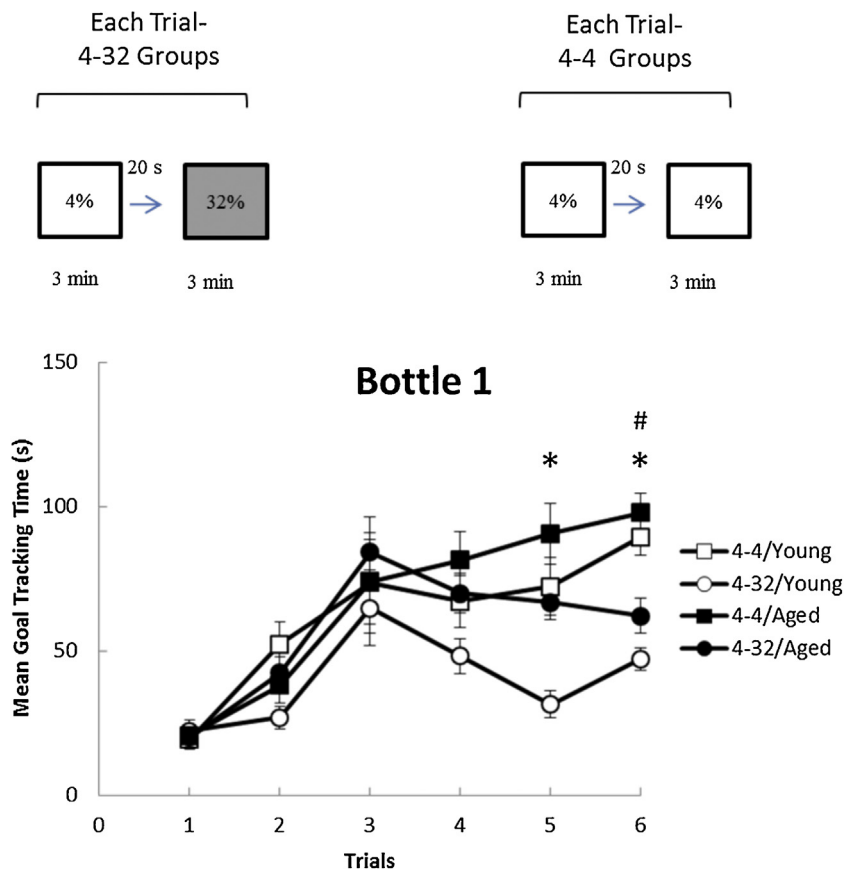

Fig. 2. Mean goal-tracking times of rats trained in an anticipatory negative contrast situation with two daily components in Experiment 2. In the first component, all rats had access to 4% sucrose. In the second component, rats in different groups had access either to the same 4% sucrose or to 32% sucrose in independent groups. Group labels refer to the sucrose concentration received in the first component. * $p < 0.05$; significative differences between control and experimental young rats. # $p < 0.05$; significative differences between control and experimental aged rats.

involved in the memory consolidation of reward' information of the downshift phase [5,29]. There are several explanations for this result. In first instance, faster adjustment of consummatory behavior to the downshifted reward could be related to a mnemonic deficit in aged rats, due to either from a failure to consolidate and/or retrieve the memory of the pre shift incentive magnitude, or from a failure to consolidate and/or retrieve the memory of the downshifted experience.

Several findings indicate that animals experience an emotional reaction to incentive value shifts [21,29]. Besides the cognitive deficit indicated above, it is also possible that aged rats exhibit a reduced emotional reaction to the incentive downshift, thus the faster recovery observed would indicate an interaction between emotional and cognitive components.

It' well documented that, in the incentive downshift events, the amygdala is highly involved [28]. Electrolytic lesions of the central area of the amygdala complete block the cSNC, and basolateral lesions reduce the contrast effect [4]. Besides, there is extensive research that indicates that this brain area deteriorates with age [1,7,8]. Accordingly it is tempting to suggest that the observed deficit on the downshift procedure could be attributable to an amygdala dysfunction due to aging; however, studies are in progress in order to confirm the connection between aging, amygdala deficit and the incentive downshifted impairment hypothesis.

The second experiment provides a new paradigm that evidences a cognitive deficit associated to age in a reward anticipation procedure. Middle age rats exhibited a cANC effect on trial 6, while the adult rats on trial 5. Several studies showed cognitive impairments in aged animals [5,8,27]. Middle and aged rats showed impairment in contextual fear conditioning [9], active and passive avoidance conditioning [10], spatial memory [32], etc.

Would be interesting to evaluate what would happen with older animals, since in our study we employed middle age ones. It is likely that the older animals will have worse deficits.

The behavioral experiments presented here provided evidence for behavioral cognitive impairment in middle aged rats. Future experiments should address neurochemical mechanisms implicated in the kind of procedures used in these studies.

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