Consummatory successive negative contrast in young and middle-aged rats

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ABSTRACT

The performance of middle-aged (14-month old) and young (3-month old) rats was assessed in the consummatory successive negative contrast (cSNC) situation. Rats received 10 daily preshift trials of access to a 32% sucrose solution followed by 5 postshift trials of access to 4% sucrose solution. Unshifted controls had access to the 4% solution in every trial. 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. This finding is discussed in relation to age-related changes in memory and emotion.
Keywords: Incentive Contrast; Age; Frustration; Memory

RESUMEN

Contraste negative sucesivo consumatorio en ratas jovenes y maduras. Se evaluaron las respuestas de ratas maduras (14 meses) y jóvenes (3 meses) en una situación de contraste sucesivo negativo consumatorio (cSNC). Los animales experimentales tuvieron acceso a una solución azucarada al 32% (Fase de pre-cambio) durante 10 ensayos diarios de 5 min. seguidos de 5 ensayos de acceso a la misma solución con una concentración al 4% (Fase de post-cambio). Los animales del grupo control recibieron la solución al 4% durante los 15 ensayos. El intervalo de retención entre el último día de pre-cambio y el primero de post-cambio fue de a 1 o 5 días. El cSNC fue similar entre las ratas maduras y jóvenes en la condición de un día de retención. En cambio, las ratas maduras se recuperaron más rápido del cSNC cuando el intervalo de retención fue de 5 días. Estos resultados se discuten en relación con los cambios emocionales y mnésicos de las ratas maduras en comparación con las jóvenes.
Palabras clave: contraste sucesivo, edad, frustración, memoria.

        Consummatory successive negative contrast (cSNC) is a behavioral effect resulting from a sudden and unexpected downshift in reinforcer magnitude or quality (Flaherty, 1996). Subjects exposed to incentive downshifts show abrupt reductions in behavior beyond the response level of unshifted controls. In the consummatory procedure, animals show a suppression in consummatory behavior (licking or time of contact with the drinking spout) when the reinforcer consisting of 32% sucrose solution is replaced by 4% sucrose solution, compared to animals that always receive 4% sucrose solution (Flaherty, 1996). 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 (Amsel, 1992; Papini & Dudley, 1997). Consistent with this interpretation, cSNC is correlated with an increase in plasma corticosterone (Flaherty, Becker, & Pohorecky, 1985; Mitchell, & Flaherty, 1998) and is reduced by anxiolytic drugs such as diazepam (Mustaca, Bentosela, & Papini, 2000).

        Cognitive mechanisms are also involved in frustration. SNC implies that the animal evaluates the value of the present reinforcer against the reactivated memory of the previously experienced reinforcer. A comparison between current and remembered incentives affects consummatory behavior (Papini & Pellegrini, in press). Despite the potential importance of memory processes in SNC, empirical evidence is scarce. In the runway situation (instrumental SNC, or iSNC), intraamygdala infusions of the muscarinic cholinergic agonist oxotremorine immediately after the first session of reward downshift produced a delay in the recovery from contrast (Salinas, Introini-Collison, Dalmaz, & McGaugh,1997). In the case of cSNC, however, systemic administration of scopolamine, a cholinergic antagonist, has no effect when administered 20 min before the first downshift trial (Flaherty & Meinrath, 1979). Cholinergic drugs (atropine and scopolamine) administered immediately after the first downshift trial also failed to affect cSNC (Bentosela, D'Ambros, Altamirano, Muzio, Baratti, & Mustaca, 2005). An iSNC study with 6 trials per session in a runway situation showed that glucose administration immediately after the first postshift session improved retention of the downshifted memory in 24-month old Fischer-344 rats, whether the retention interval was 1 or 7 days long (Salinas & Gould, 2005). The saline groups showed no evidence of retention of the downshifted memory in either retention interval. Unfortunately, no unshifted controls were included in this study, so these conclusions are based on comparisons with the final preshift level.

        Another way to study the contribution of memory to cSNC is by using aged rats. Aging is known to correlate with memory impairment. Memory loss is one of the most frequent complications associated with aging. The subjective complaint concerning mnemonic lessening is present in about 70% of old persons (Laurent, Allegri, & Thomas-Anterion, 1998). Animal studies have demonstrated that a major characteristic of age-related memory impairments is that aging is accompanied by rapid rates of forgetting, relative to those observed in young animals. Changes due to aging include a deficit both in the acquisition of new information and in event recall. For example, results obtained using Morris water maze showed differences in the spatial learning of a submerged hidden platform location between young and older rats, while no differences

were observed when the platform was visible (Gallagher & Colombo, 1995; Markowska & Savonenko, 2002). Other studies with aged rats have also demonstrated prefrontal deficits; a persistent and progressive deterioration of executive functions, especially in the ability to select, discriminate and process relevant stimuli, relations, and simultaneous cognitive associations. Poor performance in tasks requiring a sustained attention processing, such as complex discrimination and reversal learning, is observed in aged rats compared to young subjects (Burk, Herzog, Porter, & Sarter, 2002; Schoenbaum, Nugent, Saddoris, & Gallagher, 2002). Given that the prefrontal cortex has also shown to be associated to the processing of incentive value of clues necessary for goal-directed behavior, as well as with changes in the reward value (Bentosela & Mustaca, 2003), it might be interesting to study the effects of aging on this processing function.

Tasks used to study recognition memory are generally based on paired events. Typically, such experiments involve three stages: stimulus presentation, retention interval, and a final test when the subject can demonstrate recognition of the stimulus presented original usually in a choice situation. As the retention interval increases, recognition memory during the test tends to fail (Steckler, Saugal, Aggleton, & Drinkenburg, 1998).

The aim of the present experiment was to assess the involvement of memory in cSNC by comparing the performance in middle-aged and young rats under two retention interval conditions. These experiments will also provide information about ontogeny of cSNC. Several findings indicate that iSNC emerges in postweanling rats (Amsel, 1992). However, little is know about incentive contrast in aged subjects.

METHOD

Subjects

The subjects were 56 naive female Wistar rats. Twenty nine of these rats were 3-month old at the start of the experiment (called "young" and assigned to groups labeled "Y"), whereas 27 were 14-16-month old at the start of the experiment (called "middle age" and assigned to groups labeled "M"). Ten days before the start of training, rats were transferred to individual cages with water freely available. The daily amount of food was gradually reduced until their weights were lowered to an 80-85% of individual ad libitum weights. During the course of the experiments, rats were fed daily at least 20 min after the training trial. The animal colony was under a 12:12 h cycle of light and darkness (lights on at 07:00 h). Temperature and humidity levels in the testing rooms and animal colony were kept relatively constant throughout the experiment.

Apparatus

Rats received training in four similar conditioning boxes (MED Associates, East Fairfield, VT) enclosed in a sound-attenuating cubicle. Each box measured 24.1 cm in length, 29.2 cm in width, and 21.0 cm in height. The floor was made of aluminum bars (0.4 cm in diameter, 1.1 cm apart). A diffuse house light was located in the front wall, 18 cm above the floor. In the center of one of the lateral walls there was a 5-cm hole,

3.5 cm deep, 1 cm above the floor level, through which a sipper tube could be introduced from the outside. When fully inserted into the box, the sipper tube protruded 2 cm. A photocell located within 0.2 cm of the tip of the sipper tube detected licking behavior. The cumulative amount of time per trial (in 0.01-s units and labeled goal-tracking time) when the photocell was interrupted was the main dependent variable. Goal-tracking time yielded results similar to more conventional dependent measures, including licking rate (Riley & Dunlap, 1979), and amount of fluid consumption (Papini, Mustaca, & Bitterman, 1988). Furthermore, goal-tracking time measure under the same conditions used in this experiment was shown to correlate positively and significantly with amount of fluid intake for both 32% and 4% sucrose solutions (Mustaca, Freidin, & Papini, 2002). Under the conditions used in this experiment, goal-tracking time yields data with less individual variability than the more typical licking frequency measure.

Procedure

Individuals were matched in pairs for weight and randomly assigned to one of eight groups. The group were labeled according to the sucrose concentration during preshift trials (4%, 32%), their age (M, Y), and the retention interval, in days, between the last preshift trial and the first postshift trial (1, 5). Group names were the following: 4/M/1 (n= 5), 32/M/1 (n= 7), 4/Y/1 (n= 6), 32/Y/1 (n = 8), 4/M/5 (n= 7), 32/M/5 (n= 8), 4/Y/5 (n= 7), and 32/Y/5 (n= 8). The four groups assigned to each of the two retention-interval values were run at different times, but under otherwise equal conditions of training. A single trial per day, seven days per week, was administered throughout the experiment. During the preshift trials (1-10), rats received 5 minutes of free access to either 4% or 32% sucrose solution; during the postshift trials (11-15), all animals received access to the 4% solution. Sucrose solutions were prepared w/v by adding tap water to 40 g (or 320 g) of sucrose until one liter of solution was obtained. The 5-min duration of each trial was counted from the first activation of the photocell. Animals were run in squads of four. The running order of the squads was randomized across days. Each box was swept with a damp towel after each training trial. Goal-tracking times were subject to analysis of variance. Planned pairwise comparisons were calculated using Fisher's test. In all the statistical tests, the value for alpha was set at the 0.05 level.

RESULTS

All animals consumed sucrose solution during the first trial, exhibiting no clear evidence of taste neophobia. The overall preshift performance (trials 1-10) was similar for rats across both ages, but rats with access to 32% sucrose exhibit higher average goal tracking times (M= 164.4 s, Y= 147.0 s) than those with access to 4% sucrose (M= 119.0 s, Y= 129.9). A mixed-model analysis with Contrast (32%, 4%), Age (M, Y), and Trial (1-10) as factors indicated significant main effects for contrast, $F_{(1, 52)}= 11.52$, and trial, $F_{(9, 468)}= 59.15$; none of the other factors or interactions reached significance, $Fs< 2.77$.
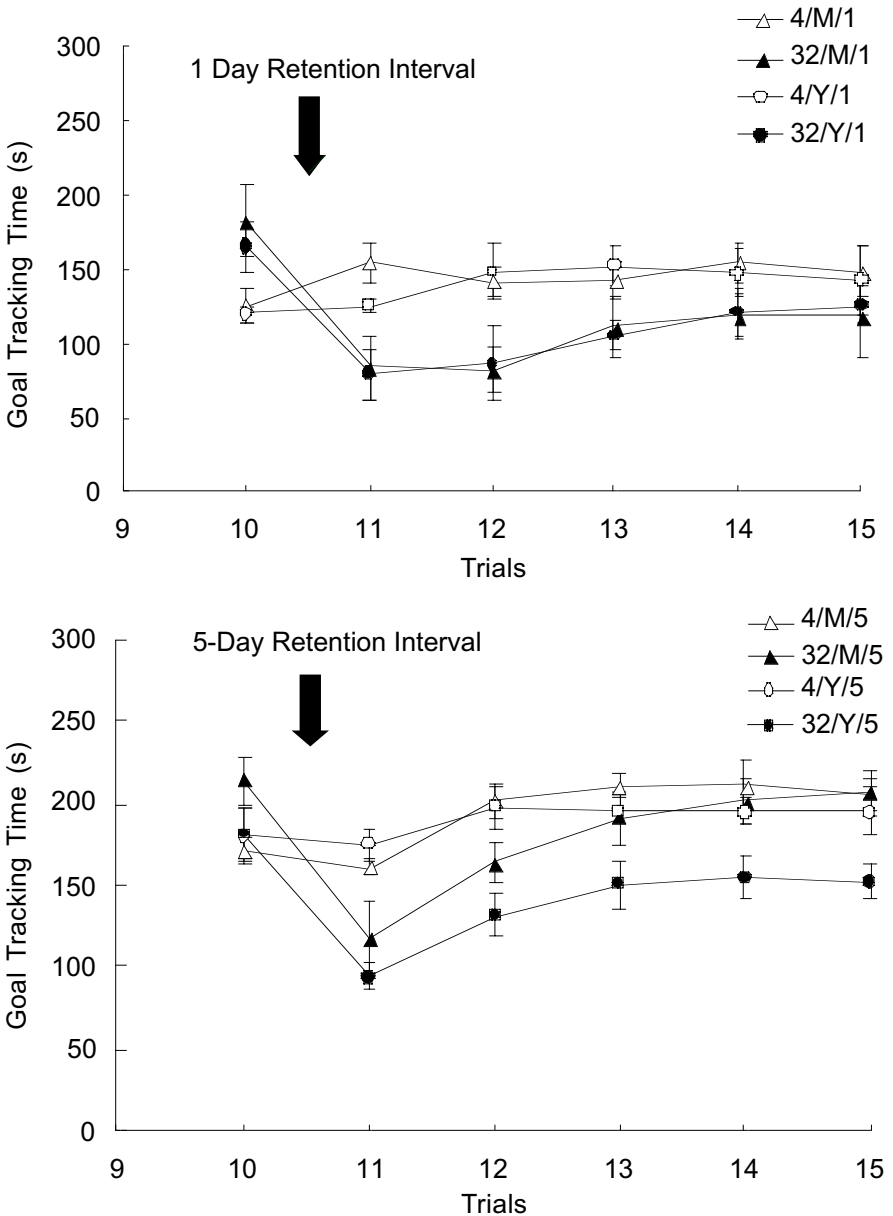
Figure 1. Mean goal tracking time (± SEMs) in middle-age (M) and young (Y) rats exposed either to an incentive downshift from 32% sucrose to 4% sucrose (32) or to an unshifted control condition always trained with 4% sucrose (4), and to a retention interval between the last preshift trial (trial 10) and the first postshift trial (trial 11) of either 1 or 5 days. The retention interval is marked with an arrow.

A mixed-model analysis with Contrast (32%, 4%), Age (M, Y), Retention Interval (5, 1), and Trial (11-15) as factors indicated significant main effects of contrast, $F(1, 48)= 24.24$, age, $F(1, 48)= 37.76$, and trial, $F(4, 192)= 25.73$. Additionally, the interactions between trial and contrast, $F(4, 192)= 6.97$, and between trial and retention interval, $F(4, 192)= 4.66$, also achieved a significant level. All other effects were nonsignificant, $Fs< 2.29$.

To further analyze the effects of these retention intervals on cSNC, separate analyses were computed for the groups exposed to 1- or 5-day retention interval between trials 10 and 11. As suggested in Figure 1, top panel, trial-10 performance for the 1-day retention interval was significantly higher for 32% than for 4% sucrose, $F(1, 22)= 10.09$. The same analysis indicated nonsignificant effects for age and for the age by contrast interaction, $Fs< 1$. The postshift results were analyzed with a mixed-model design with Contrast, Age, and Trial (11-15) as factors. Consummatory behavior in the 1-day retention interval groups was significantly lower in the downshifted groups than in the nonshifted controls, $F(1, 22)= 13.07$, and the change across postshift trials was significant, $F(1, 22)= 4.77$. There was also a significant interaction of contrast conditions and trials, $F(4, 88)= 2.65$. No evidence was found of significant effects for age, for the interaction between contrast and age, and for the interaction between contrast, age, and trial, $F< 1$.

In the 5-day retention interval condition (Figure 1, bottom panel), an analysis of trial-10 performance revealed nonsignificant differences for sucrose, age, and their interaction, $Fs< 2.33$. The consummatory behavior of downshifted groups in the 5-day retention interval groups was significantly lower than that of nonshifted controls, $F(1, 26)= 11.31$. More interestingly, middle-aged rats behaved significantly above young rats, $F(1, 26)= 4.01$. There was also a significant interaction between contrast and trial, $F(4, 104)= 4.80$, and a significant change across trials, $F(4, 104)= 31.02$. All other factors were nonsignificant, $Fs< 2.04$.

Four additional Contrast by Trial (11-15) factorial analyses were carried out on pairs of groups exposed to either downshifted or unshifted contrast conditions, and to either 1- or 5-day retention intervals. The results indicated that Group 32/M/5 displayed significantly higher consummatory behavior during postshift trials than Group 32/Y/5, $F(1, 14)= 10.93$. All other group effects were nonsignificant, $Fs< 1$. Consummatory performance changed significantly across postshift trials in the downshifted groups with either 1-day retention interval, $F(4, 52)= 10.93$, or 5-day retention interval, $F(4, 56)= 24.12$. In the unshifted controls, the trial effect was nonsignificant for the 1-day retention interval, $F< 1$, but significant for the 5-day retention interval, $F(4, 48)= 9.65$. None of the interactions between sucrose and trials achieved significance, $Fs< 1.56$.

Finally, a one-way analysis of variance was calculated on all eight groups, followed by Fisher LSD pairwise tests for the performance on trial 11. The group effect was significant, $F(7, 55)= 9.22$, and the following pairwise comparisons also achieved significance. First, all downshifted groups performed significantly below their respective unshifted controls, $ps< 0.02$, demonstrating evidence of cSNC in young and middle-aged rats, and after 1- or 5-day retention interval. Second, downshifted groups exposed to either 1- or 5-day retention interval did not differ from each other. Third, the downshifted

group of middle-aged rats exposed to a 1-day retention interval performed significantly below the downshifted middle-aged rats exposed to a 5-day retention interval, p< 0.05. In contrast, the two downshifted groups of young rats exposed to different retention intervals did not differ from each other, p< 0.40. Pairwise tests extended over the rest of the postshift trials (all of which yielded a significant group effect, Fs> 5.43) allowed an estimation of the strength of the cSNC in a comparison of each downshifted group with its own unshifted control. Thus, Groups 32/Y/1 and 4/Y/1 were significantly different on trials 11, 12, and 13 (ps< 0.02), whereas Groups 32/M/1 and 4/M/1 were significantly different on trials 11 and 12 (ps< 0.01). Groups 32/Y/5 and 4/Y/5 exhibited cSNC in trials 11, 12, 13, and 15 (ps< 0.05), whereas Groups 32/M/5 and 4/M/5 were significantly different only on trials 11 and 12 (ps< 0.05). Therefore, although all groups exhibited evidence of cSNC, middle-aged rats showed a cSNC effect that was one trial shorter than their young counterparts for a 1-day retention interval, but 2 trials shorter than their young counterparts for a 5-day retention interval.

DISCUSSIÓN

These results provide the first evidence of cSNC in middle-age rats. Middle-aged rats showed a similar cSNC effect to that of young rats under the usual conditions of training, except that it was a trial shorter. However, a 5-day retention interval between the last preshift trial and the first postshift trial revealed an age effect relative to the performance of young rats. Whereas cSNC was observed also under these conditions, the performance of middle-aged rats during the postshift phase was significantly above that of the young rats. The cSNC effect was also shorter in middle-aged rats than in young rats trained with a 5-day retention interval. A small but reliable age effect was detected in downshifted rats during the first postshift trial for the 5-day retention period, but not for the 1-day retention period. These results suggest at least two possible interpretations, one based on age-related memory deficits and the other on age-related emotional reactivity.

The age-related memory deficit posits that the enhanced recovery from cSNC exhibited by middle-aged rats, especially after a 5-day retention interval, follows either (1) from a failure to consolidate and/or retrieve the memory of the preshift incentive magnitude acquired on trials 1-10, or (2) from a failure to consolidate and/or retrieve the memory of the downshifted experience acquired on trial 11. Concerning the memory of the preshift solution, there is no available literature on its underlying mechanism. It can be plausibly argued that experience with the preshift solution establishes a long-term memory via Pavlovian conditioning with the solution itself or some feature of the context proximate to the sipper tube acting as a signal for the sucrose consumption that follows (see Wood, Daniel, & Papini, 2005). A similar view involving early cues as signals for later consumption has been suggested in the case of alcohol consumption (Cunningham, 1998). This mechanism would then establish the basis for a memory recognition discrepancy on trial 11 that leads to cSNC (Papini & Pellegrini, in press). An age-dependent memory deficit for the preshift solution would predict preshift differences in consummatory behavior, which were not detected in the present experiment.

As far as the physiological basis for this mechanism, the conventional cholinergic hypothesis does not seem apply to cSNC. In one experiment (Bentosela, D'Ambros, Altamirano, Muzio, Baratti, & Mustaca, 2005), the administration of physostigmine and atropine after trial 10, the last preshift trial, did not interfere with the normal course of cSNC. However, the extensive exposure to the preshift solution in the 9 trials before drug adminstration suggests that the memory was well-established by the time physostigmine and atropine were delivered.

Concerning the memory of the downshifted experience, a procedure that has been used in several experiments involves the posttrial 11 administration of pharmacological agents. Previous studies using drugs that target the cholinergic system and modulate memory in other learning situations have found deteriorating effects on iSNC (Salinas *et al.*, 1997), but not on cSNC (Bentosela *et al.*, 2005). However, successful enhancement of the cSNC effect was demonstrated after posttrial 11 administration of corticosterone (Bentosela, Ruetti, Muzio, Mustaca, & Papini, 2006) and the k-opioid receptor agonist U-50,488H (Wood, Norris, Daniel, & Papini, 2006). In both cases, the effect is present when the drug is administered immediately after trial 11, but not if the drug is administered 3 h after trial 11. Since there are known interactions between corticosterone and the k opioid system (Taylor, Wu, Soong, Yee, & Szeto, 1996), it seems plausible that the opioid system may mediate the establishment of the downshift memory. The issue remains as to whether age-related changes in the opioid system can explain the enhancement of recovery from cSNC observed in middle-aged rats, especially when exposed to a 5-day retention interval.

A second possibility lies with age-related deficits in emotional reactivity. This hypotesis posits that incentive downshift induces a less intense aversive internal state (frustration; see Wood *et al.*, 2005) in middle-age rats than in young rats. As a result, the aversive learning that takes place during trial 11 is attenuated and the rats recover faster. Although the involvement of the opioid system in cSNC is beyond dispute (Papini, Wood, Daniel, & Norris, 2006), it is still unclear whether it facilitates the consolidation and/or retrieval of the aversive memory of the downshift, or whether it simply modulates the intensity of the aversive internal state induced by incentive downshift. Further research involving opioid manipulations in aged rats is required to clarify these issues.

NOTES

Note 1: Wood MD, Norris JN, Daniel AM & Papini MR (2006). *Bidirectional opioid effects on consummatory successive negative contrast*. Manuscript in preparation.

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