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Corticosterone and propranolol's role on taste recognition memory

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ABSTRACT

Taste recognition is a robust procedure to study learning and memory processes, as well as the different stages involved in them, i.e. encoding, storage and recall. Considerable evidence indicates that adrenal hormones and the noradrenergic system play an important role in aversive and appetitive memory formation in rats and humans. The present experiments were designed to characterize the effects of immediate post training corticosterone (Experiment 1) and propranolol administration (Experiment 2 and 3) on taste recognition memory. Administration of a high dose of corticosterone (5 mg/kg, sc) impairs consolidation of taste memory, but the low and moderate doses (1 and 3 mg/kg, sc) didn't affect it. On the other hand, immediate post-training administration of propranolol (1 and 2 mg/kg, ip) impaired taste recognition memory. These effects were time-dependent since no effects were seen when drug administration was delayed 3 h after training. These findings support the importance of stress hormones and noradrenergic system on the modulation of taste memory consolidation.

1. Introduction

Animals organize their past experience as "memories", all that they learn is encoded and stored in their brains, and they preserve the environmental information in order to enable better adaptation to future situations (Ruetti et al., 2009a, 2009b). One of the most adaptive learning that the animals had is related to the intake, since consumption guarantees the subsistence, and certain errors in the selection of food or flavors that they taste could cause irreparable damages and even cause death. From an evolutionary perspective taste memory had a significant relevance since it increases the survival of animals, allowing them to recognize, due to past experiences, what and what is not safe to eat or taste. The subjects had to discriminate among the familiar stimuli and the novel ones to conclude what information requires more attention to be encoded in their long term memory (Bures et al., 1998; Domjan, 1976; Mickley et al., 2000). Fear of novel stimuli is usually observed during the first encounter with a novel stimulus; for example, a novel food with a taste and/or odor-relevant component is usually ingested in significantly lower amounts than a familiar one; after several presentations of the originally novel food, consumption increases, which is interpreted as attenuation of neophobia or habituation to the novel taste and also as appetitive memory for that food (e.g., Núñez-Jaramillo et al., 2010).

Taste recognition is a robust procedure to study learning and memory processes, as well as the different stages involved in it, i.e. encoding, storage and recall (Bérmudez-Rattoni, 2004). Considerable evidence indicates that the noradrenergic system plays an important role in aversive and appetitive memory formation in rats and humans (Cohen and Gotthard, 2011; Do-Monte et al., 2010; McGaugh, 2000; McGaugh and Roozendaal, 2009). In particular, noradrenergic agonists enhance, whereas noradrenergic antagonists impair, learning for many kinds of aversive experiences (Gallagher et al., 1977; Introini-Collison et al., 1996; Izquierdo et al., 1992; LaLumiere et al., 2003).

On other hand, studies performed in our laboratory reported evidence about the role of corticosterone on appetitive reward memory, using a negative contrast paradigm in which animals were exposed to different sucrose solutions. For example, Bentosela et al. (2006) reported that administration of corticosterone (3 mg/kg, sc) immediately (but not 3 h after training) after the change in sucrose solutions concentrations (e.g. 32% a 4%), led to an increase in the size and duration of the contrast effect. Thus, temporal contiguity between the downshift experience and corticosterone administration is necessary for peripheral and/or central glucocorticoids to influence memory consolidation.

Glucocorticoid receptor (GR) agonist administered bilaterally into the nucleus accumbens (NAc) shell after ingestion of an appetitive saccharin drinking solution enhanced long-term retention, in a dose- and time-dependent fashion, of the safe taste experience. Moreover, GR agonist administration into the NAc shell after pairing of the saccharin taste with a malaise-inducing agent enhanced retention of the aversive taste learning experience. Furthermore, concurrent antagonism of β-adrenoceptor activity within the NAc blocked the GR agonist induced retention enhancement on both tasks. Altogether, these findings

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suggest that GR activation interacts with the noradrenergic arousal system within the NAc to enhance memory consolidation of emotionally arousing training experiences regardless of valence (Wichmann et al., 2012).

Besides, it has been demonstrated that propranolol administration (β-adrenergic antagonist) in the insular cortex (IC) and in the basolateral amygdala (BLA) previously to the presentation of a novel flavor impairs memory in attenuation of neophobia paradigm or habituation to novel taste (e.g. the animals do not increases the intake of the novel solution in the following trials; Miranda et al., 2008). This study shows that noradrenergic activity is required in acquisition of a novel taste, but didn't indicates how the administration of the drug is involved in the consolidation of the information.

In line with this background, it is expected that adrenal hormones and the antagonist of its receptors modulate the consolidation of taste memory. For that reason the aim of the present experiments was to evaluate the role of corticosterone and propranolol administration in the consolidation of a taste recognition test (TRT).

2. Material and methods

2.1. Subjects

The subjects were 131 male, experimentally naive Wistar rats, about 3 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 ad libitum weight was 338 g (range: 239–463 g). The amount of food was gradually reduced across days until the animals reached 85% of their ad libitum 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). Training trials were conducted between 10:00 and 15:00 h to avoid the peak of the circadian release of corticosterone, which occurs at the onset of the dark period (Romero, 2002). 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 used.

2.2. Behavioral procedures

Rats were trained in 4 conditioning boxes (MED Associates, Fairfax, VT). Each box was 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 (Mustaca et al., 2002) and it has been used concurrently with fluid intake yielding the same results (Papini et al., 1988; Riley and Dunlap, 1979). Each box was enclosed in a sound and light-attenuating cubicle equipped with a source of white noise and diffused house light.

The TRT procedure had 2 trials, during training (trial 1), all rats received access to 4% sucrose solution; in this trial the animals acquired the information of the taste, this is the acquisition or encoding stage, after this trial ends starts the consolidation process. 24 h later the test trial was conducted to evaluate the taste recognition memory, by exposing to the animals to the sucrose solution again (trial 2). In this last trial the recovery of the first trial information is retrieved, this stage is considered as the recognition (or recall) one. On each trial, the sipper tube was manually introduced into the box before rats were placed in the conditioning box. Training and test trials lasted 5 min starting from the first interruption of the photocell located by the sipper tube. Sucrose solution (*w*/*v*) was prepared by mixing 40 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. Drug administration

To prepare corticosterone (from Sigma-Aldrich Laboratories, Saint Louis, MO), ethanol 100% was diluted in 0.9% isotonic saline to a 5% ethanol concentration. Corticosterone was then diluted in this vehicle to the target dose. Controls received the same volume of 5% ethanol in isotonic saline. This drug was administered subcutaneously (sc).

Propranolol (from Sigma-Aldrich Laboratories, Saint Louis, MO) was diluted in isotonic saline to the target dose. Controls received the same volume of isotonic saline. This drug was administered intraperitoneally (ip). The doses of both drugs were selected from previous research and preliminary experiments (Bentosela et al., 2006; Campbell et al., 2008; Crowe et al., 1991; Ruetti et al., 2009b).

2.4. Experimental designs

Three experiments were performed, each of them with an inter-subject design. The Experiment 1 evaluate the effect of corticosterone on the TRT, the Experiment 2 the role of propranolol in the TRT, and the Experiment 3 discarded possible unspecific effects that propranolol could have and bias the results of the second experiment. The following paragraphs detailed the rationality of each design.

High circulating levels of corticosterone during and immediately after an emotionally arousing event, but not after relatively neutral events, are known to modulate memory consolidation and/or retrieval (Okuda et al., 2004). In taste recognition of saccharin corticosterone enhances the retention of the safe taste (Wichmann et al., 2012). According to this research, the goal of the first experiment was to investigate the effect of several doses of corticosterone on a taste recognition test. Immediately after trial 1, different groups of animals were injected with corticosterone 1 mg/kg (group named C1; $n = 10$) or 3 mg/kg (group named C3; $n = 10$) or 5 mg/kg (group named C5; $n = 10$) or vehicle (group named VEH; $n = 10$), in an inter-subject design. With this administration the goal was to affect the consolidation of the taste memory.

The administration of β-adrenergic antagonist also modulates memory consolidation, causing an amnesic effect in animals (McGaugh, 2000; Sun et al., 2011). Consistent with this, the goal of the second experiment was to evaluate the propranolol's effect in the taste recognition test. Immediately after the first trial, different groups of animals were injected with propranolol 1 mg/kg (group named P1; $n = 10$) or 2 mg/kg (group named P2; $n = 10$) or vehicle (group named VEH; $n = 10$), in an inter-subject design. With this administration the goal was to affect the consolidation of the taste memory.

To confirm the propranolol's effect on memory consolidation, the 3rd experiment was carried out. This last design tested possible unspecific effects that the drug administration should have. There were four groups, the first one received the administration of propranolol immediately after the first trial, in a dose of 1 mg/kg (group named P immed, $n = 15$); the second group of animals received the administration of the vehicle immediately after the first trial (group named VEH immed, $n = 15$), the third group was administered with propranolol 1 mg/kg but 3 h after the first trial was ended to test possible unspecific effects of the drug (group named P 3 h, $n = 15$) and the last group received the application of vehicle after 3 h (group named VEH 3H, $n = 16$).

2.5. Data analysis

Goal-tracking times (recorded in 0.01-s units) for training and test trials were subject to one way analysis of variance (ANOVA). Post-hoc least-significant difference (LSD) pairwise comparisons of selected trials were included when necessary. For estimation of effect size the partial Eta square was utilized (η^2p). The value of alpha was set at the 0.05 level.

3. Results

3.1. Experiment 1

Fig. 1 shows the goal-tracking time (s) of all groups in training and test trials. It is observed that in the first trial all groups of animals consumed the same amount of solution. This observation was confirmed by the one way ANOVA for training trial which fail to show significant differences between the groups $F(3, 23) = 2.12$ ($p > 0.05$), this outcome was the expected since no drug administration was performed. However, in the test recognition trial the ANOVA indicates a significant effect of group $F(3, 23) = 6.64$, $p < 0.003$ (η^2p $= 0.499$). *Post hoc* test shows that in the test trial the C5 group is different from the group VEH, also it is different from group C1 and the group C3, $p < 0.01$; the groups VEH, C1 and C3 did not differ between them ($p > 0.05$). These results indicate that high dose of corticosterone (5 mg/kg) impaired recognition of the novel solution, since the animals had a lower consummatory behavior than the other groups.

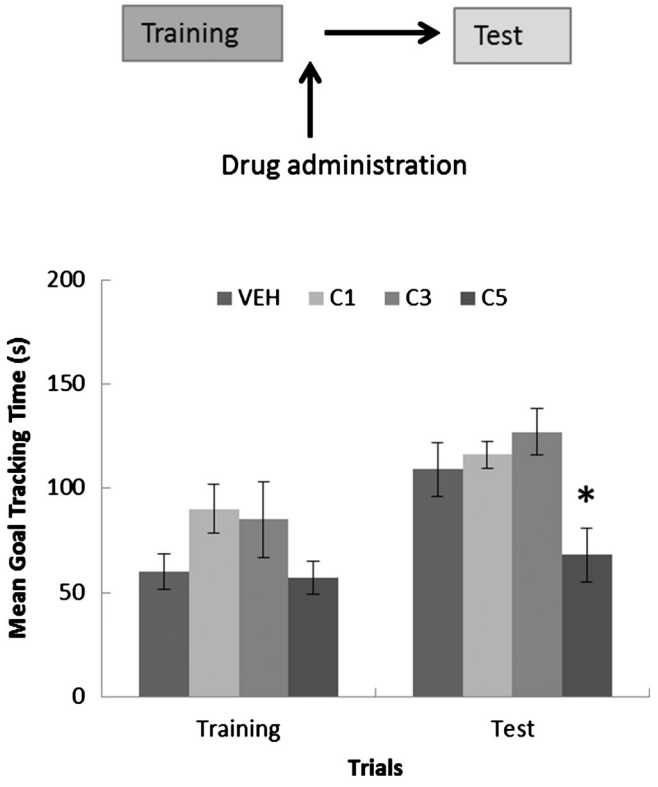

Fig. 1. Mean goal tracking time (s) and standard error of the groups that received the administration of several doses of corticosterone (1, 3 and 5 mg/kg, sc) or vehicle, immediately after a trial with a 4% sucrose solution. 24 h later the test trial was conducted to evaluate the taste recognition memory, by exposing to the animals to the sucrose solution again. The * indicated significant differences between groups. In the training trial there were no significant differences between groups. In the test trial the group that received the administration of corticosterone on a 5 mg/kg dose (C5) is different from the other three groups that did not differ between them (C1, C3 and VEH).

3.2. Experiment 2

Fig. 2 shows the results of this experiment. Similar to experiment 1 there were no significant differences between the groups in the first trial, which was confirmed by the one way ANOVA for the training trial [$F(2, 19) = 1.92$ ($p > 0.05$)]; again this outcome was the expected one. However, in the test trial, the one way ANOVA indicates significant differences among the groups $F(2, 19) = 3.65$, $p < 0.048$ (η^2p $= 0.301$). Post hoc test shows that the VEH group consumed more sucrose solution than the group P1 and the group P2 ($p < 0.05$), which in turn did not differ between them ($p > 0.05$). These results indicate that both doses of propranolol deteriorated the recognition of the novel solution.

3.3. Experiment 3

Fig. 3 shows the results of this experiment. In the first trial the groups did not differ between them; this was confirmed by the one way ANOVA which indicated that there were no significant differences between the groups for the training trial [$F(3, 78) = 0.18$, ($p > 0.05$)]. Nonetheless, the one way ANOVA for the test trial showed significant differences among groups $F(3, 78) = 5.26$, $p < 0.002$ (η^2p $= 0.174$). Post hoc test indicates that the P immed group is different from the other three groups, i.e. VEH immed; VEH 3 h and P 3 h ($p < 0.01$). The last three groups did not differ between them ($p > 0.05$). These results indicate that the propranolol deteriorated the taste recognition in the group that received the immediate administration of the drug, but wasn't effective when the administration was delayed 3 h later of the exposition to the reward, confirming the result of the previous experiment, dismissing non-specific effects of the drug and suggesting that propranolol's effects on taste recognition were time-dependent.

4. General discussion

The main goal of this research was to study the role of systemic administration of corticosterone and propranolol on taste memory recognition. The results of the experiments performed indicated

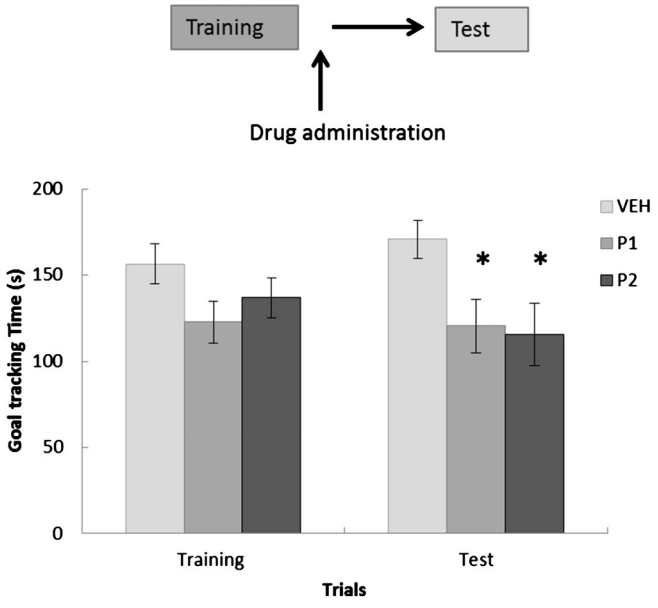

Fig. 2. Mean goal tracking time (s) and standard error of the groups that received administration of two doses of propranolol (1 and 2 mg/kg, ip) or vehicle, immediately after a trial with a 4% sucrose solution. 24 h later the test trial was conducted to evaluate the taste recognition memory, by exposing to the animals to the sucrose solution again. The * indicated significant differences between groups. In the training trial there were no significant differences between groups. In the test trial the group that received the administration of vehicle (VEH) is different from the other two groups that were administered with propranolol (P1 and P2); these last groups did not differ between them.

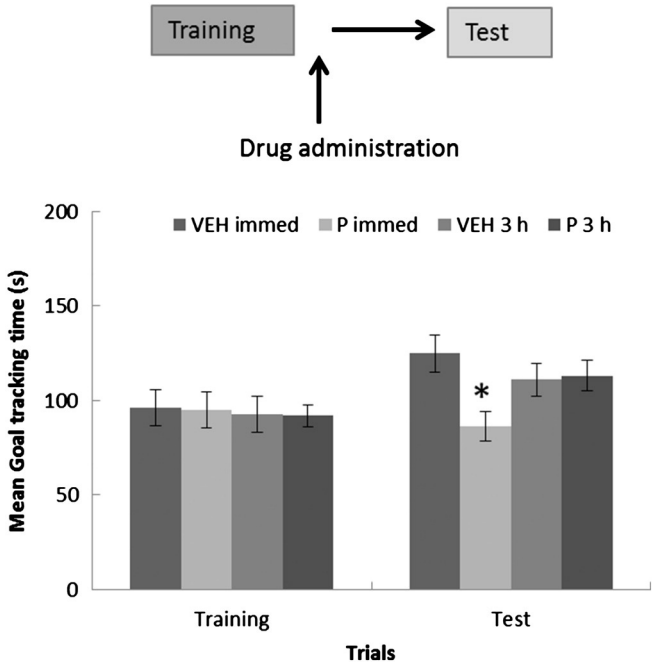

Fig. 3. Mean goal tracking time (s) and standard error of the groups that received propranolol administration (1 mg/kg, ip) or vehicle, immediately or after 3 h of a trial with a 4% sucrose solution. 24 h later the test trial was conducted to evaluate the taste recognition memory, by exposing to the animals to the sucrose solution again. The * indicated significant differences between groups. In the training trial there were no significant differences between groups. In the test trial the group that received the administration of propranolol immediately after the first trial (P immed) is different from the other three groups which did not differ between them (P 3 h, VEH Immed, VEH 3 h).

that administration of a high dose of corticosterone (5 mg/kg) impaired consolidation of taste memory (e.g., C5 consumed less sucrose solution in the recognition test), but the low and moderate doses (1 and 3 mg/kg) didn't affect it. On the other hand, several doses of propranolol, a β-adrenergic antagonist, were administered to evaluate the role of noradrenergic system on reward memory modulation. The administration of propranolol (1 and 2 mg/kg) immediately after presentation of a novel taste deteriorates the memory consolidation of it. Nonetheless, the administration of the drug 3 h later, when the information was consolidated, didn't affect the reward memory.

Consequently the main finding of this research is that systemic administration of corticosterone and propranolol modulates the taste recognition memory. Propranolol modulation of reward memory showed to be dose and time-drug dependent, meanwhile corticosterone effects on taste recognition were dose-dependent. On the one hand, in this experimental paradigm, the moderate dose of corticosterone didn't produce an enhancement of memory, like in previous work (Bentosela et al., 2006; Ruetti et al., 2009a, 2009b). On the other hand, an elevated dose of this hormone (5 mg/kg) impaired animals' taste memory. It's well known that the effects of the stress hormone change as a function of the levels of corticosterone, either in an endogenous or exogenous way, and may enhance or impair memory. Propranolol administration showed an "amnesic" effect on reward memory. The post training administration of this drug impaired memory consolidation of the novel flavor. This effect was observed when propranolol administration occurred immediately training but was no longer seen when injection was delayed 3 h. These results suggest that propranolol could be affecting memory taste consolidation. At a neurobiological level, there are studies that point to the amygdala as the responsible structure of taste aversion memory modulation. In this sense, Miranda, LaLumiere et al. (2003) study the involvement of the noradrenergic system on a conditioned taste aversion paradigm. These authors administered propranolol (β-adrenergic antagonist) or clembuterol (β-adrenergic agonist) into the BLA before the presentation of lithium chloride, and they found that propranolol impairs the taste memory and the agonist didn't have an effect on the conditioning.

In the same sense, propranolol administration into BLA blocked the attenuation of neophobia but didn't have an effect on conditioned taste aversion (Miranda et al., 2008). In other work, propranolol was administered after the presentation of the novel flavor in the central and basolateral region of the amygdala (Bahar et al., 2003). The authors found dissociation between these regions; the antagonist administration impairs the learning of taste aversion when the drug was administered in the central region but not in the BLA. In line with these evidences, the β-adrenergic receptors in amygdala are involved in taste memory consolidation, both appetitive and aversive ones, as well as in the flavor-malaise visceral association. The amygdala participation it's not only important to process the sensorial stimuli but also it's a fundamental structure to the development of the taste learning. The role of areas of memory, such as the insular cortex and hippocampus, will not be discussed in this paper. (Bérmudez-Rattoni, 2004).

To summarize, post training administration of corticosterone and propranolol modulates taste recognition memory. This modulation of the reward memory depends not only of the dose used but also in the time in which the drugs are administered. These findings support the importance of stress hormones and noradrenergic system on the modulation of appetitive taste memory.

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