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Association of stimuli at long intervals in conditioned odor aversion

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ABSTRACT

Rats learn to avoid a tasteless odorized solution if they experience visceral malaise after consuming it. This phenomenon is referred as Conditioned Odor Aversion (COA). It is widely accepted that an odor can only be associated with illness if the inter-stimulus interval (ISI) is shorter than 15 min. However, this conclusion is based on long-term memory tests usually made 48 h after conditioning, thus precluding the possibility to discriminate between a specific failure to make the odor–malaise association rather than the failure to consolidate the short-term association into long-term memory. In the present study, we compared the short-term and long-term memories for COA in rats trained with long ISIs.

Independent groups of male rats were conditioned using 5, 15, 30, 60 or 90 min ISIs and tested either 4 or 48 h after conditioning. We found a reliable odor aversion at 5, 15, 30 and 60 min, but not at 90 min ISIs, when tested 4 h after conditioning. In contrast, odor aversion was only found at 5 and 15 min ISIs in the groups tested 48 h after training. Our results show that COA can be acquired when malaise follows the odor CS by at least 60 min. This finding indicates that the lack of aversion at long ISIs is not due to an association failure, but rather to a limitation in consolidating short-term memory into long-term memory of COA.

1. Introduction

Conditioned Odor Aversion (COA) is a form of classical conditioning in which rats learn to avoid the consumption of an odorized tasteless solution (conditioned stimulus, CS) that was previously paired with visceral malaise (unconditioned stimulus, US) [1–4]. Most forms of classical conditioning yield optimum results only when the CS precedes the US by a few seconds [5,6]. Conditioned taste aversion (CTA) and COA are notable exceptions, since both of them can be obtained even with a long inter-stimulus interval (ISI) of several hours for CTA [5,7–9] and up to 15 min for COA.

Since the classic research of Garcia [2] and coworkers a reliable COA has been observed using ISIs that range from 0 to 5 min [3,10], following a steep gradient for odor-illness conditioning, in which ISIs of 15 min produced an attenuated COA and longer ISIs (i.e., 30 min) were ineffective in inducing odor aversion [2]. More recent papers confirmed these earlier reports and extended them by showing that COA can be acquired at longer ISIs only after particular manipulations that facilitate the CS–US association, i.e., lesions of entorhinal cortex, blockade of the basolateral amygdala $GABA_A$ receptors, and proximal presentation of the odor diluted in the drinking water [1,4,11–13]. The standard account for facilitated as well as simple COA is that memory for the odor CS is only associated with the malaise US during a limited time interval lasting less than 15 min [1,11,12]. According to this idea, the odor memory trace decays rapidly, which prevents its association with the US at longer ISIs. Consequently, the facilitation effects correspond to lengthening the persistence of the odor memory trace so it can associate with the delayed US [1,11–13].

There is however, an alternative account for all the COA results mentioned here, because these interpretations were based on retention tests given 48 h after conditioning that evaluated long-term memory (LTM) for the odor association [1–4,10,11,14]. According to the standard interpretation, there is a failure of the odor association at long ISIs, but an alternative is that the association failed to be consolidated into LTM, which is why it could not be detected 48 h after conditioning.

Since the lack of association between the olfactory CS and visceral US has been the key argument to explain both the failure to achieve delayed COA and also the facilitation of COA by different manipulations such as odor aversion potentiated by taste, it is important to evaluate the validity of this standard interpretation. We therefore investigated whether odor and illness can be associated after long ISIs by testing short-term memory (STM) and comparing it with LTM of COA. For the purpose of the present study STM and LTM refer to conditioned response retention 4 h and 48 h after the presentation of the CS, respectively. These testing times were chosen on the basis of pilot experiments and previous reports showing that between 2 and 3 h is a suitable interval for reliable retrieval testing without interference of the US aftereffects [15–17], whereas 48 h is the standard for LTM tests.

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2. Materials and methods

2.1. Animals

One hundred and twenty nine adult male Wistar rats (250–300 g) obtained from the breeding colony of the Faculty were used. Animal care and experimental procedures were in accordance with the rules established by the ethics committee of the Faculty of Medicine, Universidad Nacional Autónoma de México according to the Mexican Laws for Animal Care, which comply with the Guide for the Care and Use of Laboratory Animals (NIH publication 80–23, revised 1996). Rats were kept two per cage in the vivarium of the laboratory under a 12–12 h light–dark cycle (lights on at 0800 h), with *ad libitum* access to food and tap water until experiments began.

2.2. Apparatus

Experiments were carried out in a plastic box (47×32×20 cm) equipped with two 30 ml pipettes mounted on the internal lateral walls of the box. The tip of each pipette was positioned 10 cm above the floor and 1 cm apart from a 1.5-cm circular hole made in both lateral walls. A 3-cm filter paper disk was placed inside a plastic bottle cap ("essence cap"), which was externally attached to each hole.

2.3. Behavioral testing

Independent groups of rats were deprived of water for 24 h and submitted to a COA training paradigm for five consecutive days in which they were allowed to drink water for 10 min as follows: On Day 1, rats were placed in the conditioning box and permitted to drink water from both pipettes. On Day 2, 0.2 ml of McCormick's vanilla odor extract (innocuous stimulus, IS) was placed in both essence caps and rats drank in the presence of the odor. On Day 3, the rats were allowed to drink again and experience the CS, which was the same amount of McCormick's almond odor extract applied to both essence caps. After 5, 15, 30, 60 or 90 min rats received the US, an IP injection of 0.15 M LiCl 2% BW. Control rats received an IP injection of physiological saline instead of LiCl 5 min after the CS. Four hours after the presentation of the CS, rats assigned to the STM test were returned to the conditioning box and allowed to choose between the two pipettes, one scented with the IS the other with the CS. On Day 4, rats assigned to the LTM test drank water again in the conditioning box to rehydrate them, and on the fifth day (i.e., 48 h after CS), they were permitted to drink from the two pipettes, one scented with the IS and the other with the CS. In order to rule out the possibility that preference for the non-conditioned IS might enhance the aversion to the CS during retrieval testing, two additional control groups were performed: rats were conditioned using a 5 min ISI, but were allowed to chose between tap water (without any odor) and the CS during the STM (4 h) and LTM (48 h) tests.

2.4. Data analysis

Water consumption was recorded for each rat and averaged for groups. Data were analyzed with GraphPad Prism 5 statistical software (San Diego, CA, USA) by using a one-way ANOVA and a Newman–Keuls multiple comparisons post-hoc test. A $p<0.05$ was considered statistically significant.

3. Results

Fig. 1 illustrates the results of the short and long term memory tests. Among the groups tested for STM 4 h after training, those conditioned with 5, 15, 30 and 60 min ISIs exhibited a clear aversion as indicated by very low water intake of the CS. The 90-min ISI group could not be distinguished from the saline-injected control group,

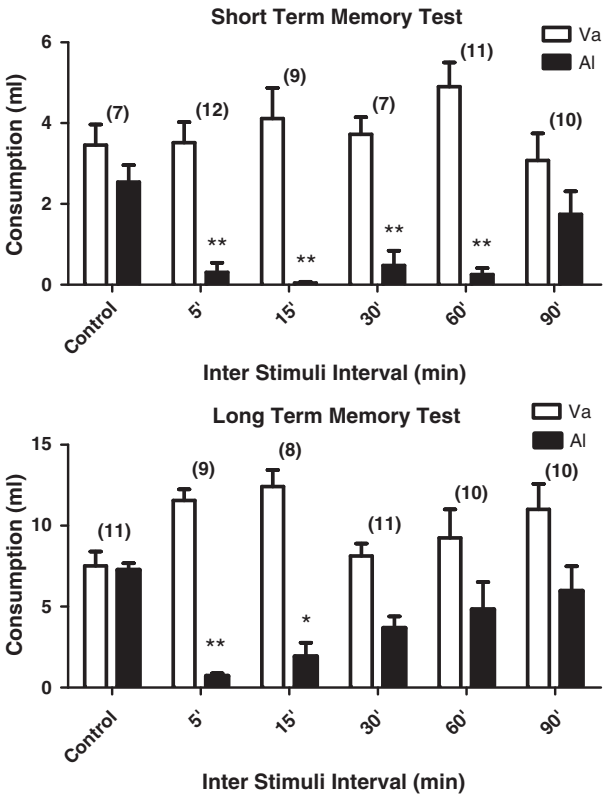

Fig. 1. Mean ± SEM water consumption during STM and LTM retrieval tests, measured with 0.2 ml precision. Water intake of the IS (vanilla, Va) and the CS (almond, Al) are plotted together to show differences between consumption within each group. Neuman–Keuls post hoc test: $^{*}p<0.05$ and $^{**}p<0.01$ vs. Al control group. Values in parentheses are the number of rats per group.

indicating lack of aversion. Conversely, among the groups tested for LTM 48 h after training, only those conditioned with 5 min and 15 min ISIs displayed a strong and a mild aversion respectively, while the remaining groups (30, 60 and 90 min ISIs) showed no aversion. A one-way ANOVA confirmed a significant effect of the ISI on the preference for the CS ($F_{(11, 100)}=15.5$, $p<0.001$ and $F_{(11, 106)}=9.68$, $p<0.001$, for STM and LTM, respectively). A post-hoc Newman–Keuls multiple comparison test revealed significant differences between the saline injected control group and all the ISI groups (5, 15, 30 and 60 min; $p<0.01$) except the 90 min group for the STM test. Moreover, the 5 and 15 min ISI groups differed from the control group ($p<0.05$) for the LTM test.

Finally, rats showed a clear-cut aversion to the CS when tested against odorless water (Fig. 2). Furthermore, they drank a comparable amount of the odorless water than of the non-conditioned IS indicating that a possible preference for this stimulus did not contribute to the aversion found to the CS.

4. Discussion

The aim of the present study was to investigate whether odor and illness can be associated at long ISIs by comparing STM and LTM of COA. Our main finding is that the STM test revealed that odor and illness can be reliably associated at ISIs as long as 60 min, which is at least three times longer than previously reported for orthonasal (distal) odor presentation. This finding demands a reconsideration of the standard understanding of the strength of the odor memory trace and its relative importance in food-related aversion learning.

We tested LTM to verify that in our hands COA is comparable to that of other researchers [1–3,10]. Confirming prior work, we did not find COA at ISIs longer than 15 min when tested 48 h after conditioning. Even