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Research report

Taste-dependent sociophobia: When food and company do not mix

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ABSTRACT

Using a combination of the paradigm of conditioned taste aversion (CTA) and of the paradigm of social interactions, we report here that in the rat, eating while anxious may result in long-term alterations in social behavior. In the conventional CTA, the subject learns to associate a tastant (the conditioned stimulus, CS) with delayed toxicosis (an unconditioned stimulus, UCS) to yield taste aversion (the conditioned response, CR). However, the association of taste with delayed negative internal states that could generate CRs that are different from taste aversion should not be neglected. Such associations may contribute to the ontogenesis, reinforcement and symptoms of some types of taste- and food-related disorders. We have recently reported that a delayed anxiety-like state, induced by the anxiogenic drug meta-chlorophenylpiperazine (mCPP), can specifically associate with taste to produce CTA. We now show that a similar protocol results in a marked lingering impairment in social interactions in response to the conditioned taste. This is hence a learned situation in which food and company do not mix well.

1. Introduction

In conditioned taste aversion (CTA), the subject learns to associate a tastant (the conditioned stimulus, CS) with delayed toxicosis (an unconditioned stimulus, UCS) to yield taste aversion (the conditioned response, CR) [8,15,27,28]. In some versions of this paradigm, the context in which the subject consumes the food may also come to serve as a cue to elicit subsequent taste aversion [26]. Not all UCSs are, however, effective in inducing CTA. For example, cutaneous pain is ineffective. Although malaise-inducing UCSs are widely used in CTA, the association of taste with delayed negative internal states that could generate CRs different from taste aversion [6,23] should not be neglected. Particularly, this is the case of anxiety and anxiety-like states [18,30]. Among several methodologies which can be used in order to induce experimentally anxiety-like state in animals, the utilization of pharmacological agents is particularly useful. Thus, injections of the serotoninergic agent meta-chlorophenylpiperazine (mCPP), which acts as a 5-HT_{2C} receptor agonist, has been shown to produce in human and in animal emotional states isomorphic to anxiety-like state [3,4,9,10]. We have recently reported that a delayed anxiety-like state induced by intraperitoneal injection of mCPP was able to specifically associate with taste to produce long-term CTA [18]. This association has been shown to be rather specific to anxiety itself, and not to pharmacological side effects of the drug [18].

Using a combination of CTA and social interaction measurements, we report here that a similar conditioning procedure results in a marked lingering impairment in social behavior in response to the conditioned taste. This probably represents a component of the anxiety that generates a social CR in a type of protocol that commonly quantifies only gustatory CRs. This is hence a learned situation in which food and company do not mix well. Such associations may potentially contribute to the ontogenesis, reinforcement and symptoms of some types of eating and digestive disorders.

2. Materials and methods

2.1. Animals

Rats (Wistar males, ~60 days old, 220–370 g) were caged individually at 22 ± 2 °C under a 12 h light/dark cycles regime. Water and food were available *ad libitum* unless otherwise indicated.

2.2. CTA training

CTA was induced as previously described [18,27]. Briefly, rats were trained over 3 days to obtain their daily water ration from two pipettes, each containing 10 ml. The time of pipette exposure was of 30 min the first day and 10 min on the 2 subsequent days. On Day 4, the rats were presented with glycine (ICN Biomedicals, Aurora, OH, 1%, w/v) instead of water. Fifteen minutes later, they received the UCS, which was i.p. injection of a solution of either mCPP (Sigma, St. Louis, MO, 0.5 mg/kg) or LiCl (Merck, Darmstadt, 0.15 M, 2% body weight). The rats were then allowed 10 min access to 20 ml water on Days 5 and 6. Animals were tested either in a *Taste-Choice*

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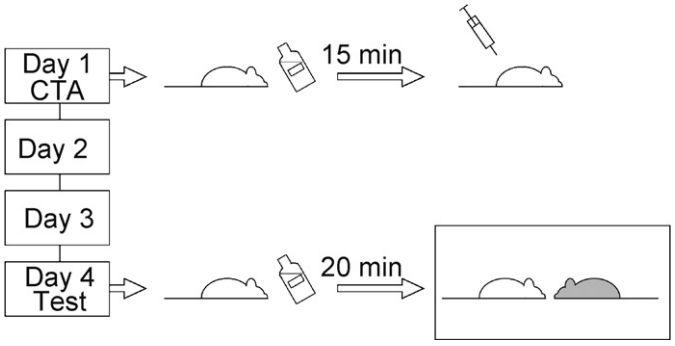

Fig. 1. Experimental protocol. During CTA training (Day 1), animals were exposed to the unknown taste. Fifteen minutes later, animals received an i.p. injection of saline, mCPP, or LiCl. Test took place 3 days after the CTA training (Day 4), animals received anew the conditioned taste. Twenty minutes later, animals were presented with an age-matched, unfamiliar, naïve conspecific, in an experimental arena. Social interactions were then quantified during 20 min.

(*TC*) situation or in *No Taste-Choice* (*NTC*) situation on Day 7, as specified under Section 3. In the *TC* test, the rats were allowed free access for 10 min to an array of 6 pipettes, 3 × 5 ml glycine and 3 × 5 ml water. The aversion index (AI) was defined as [(water consumed)/(water + tastant consumed) × 100] [27]. In non-conditioned rats, glycine is preferred over water (AI < 50). In the *NTC* test, the rats were presented for 10 min with 2 pipettes containing 10 ml glycine each. In the experiments in which social interaction was measured, all the rats were presented on Day 7 for 10 min with 2 pipettes containing 10 ml glycine each.

2.3. Social interaction test

Three days after CTA conditioning the rats were presented for 10 min with the non-reinforced taste CS. Twenty minutes later each rat was submitted to a social interaction test, performed in a transparent plastic arena (21 cm × 37 cm × 26 cm). Pairs of age-matched male rats, unfamiliar with each other, were placed in the unfamiliar test arena for an observation period of 20 min (Fig. 1). Their social behavior [29] was scored simultaneously by two independent observers. The interaction time was defined as the time spent in active social interactions (*sniffing*, *following*, *grooming* the partner, *wrestling*, *crawling* over or under). In addition, the number of behavioral events was quantified for each rat in the following categorizes: number of social events (*sniffing*, *following*, *grooming* the partner, *crawling* over or under), number of aggressive events (*wrestling* or *biting*), number of conspecific-*following behavior*, and number of conspecific-*escapes*. After each trial, the rats were returned to their home cages and the arena was washed with 70% ethanol followed by water, and thoroughly dried to remove odor cues.

2.4. Experimental groups

Two sets of rats were used. The first set ($n = 16$) was used to quantify mCPP-induced CTA under different test conditions. These rats were conditioned using glycine as CS and mCPP as the UCS, as detailed above. Three days after the CTA training, half of the rats were presented with the CS in the *TC* test, and the other half in the *NTC* test.

The second set included 8 groups of 16 rats each. In each group, half of the animals were "naïve" and half "experimental", as detailed below. In the *naïve* group, the remaining 8 rats were also naïve. In the *LiCl* group, the experimental animals received a classical LiCl-induced CTA training. In the *mCPP* group, the experimental animals received in training mCPP i.p. instead of LiCl i.p. as the UCS. In the *backward conditioning* group, the experimental animals were submitted to a backward conditioning procedure, in which the mCPP UCS preceded the exposure to the glycine CS by 3 h. In the *noCS* group, the experimental animals received mCPP i.p. in training but the glycine CS was replaced by water. In the *4 h* group, the experimental animals were trained as in the *mCPP* group, but tested for social interaction 4 h rather than 20 min after the exposure to the CS in the test (time at which effects of the drug have been shown to be strongly decreased [14]). In the *different taste* group, the rats were conditioned with as in the mCPP group but tested for social interactions after exposure to saccharine in the CTA taste. In the *mCPP*(*s*) group, the rats were trained as in the mCPP group but with saccharine as the CS, and tested for their social interactions after receiving saccharine in the test.

2.5. Statistical analysis

Results are expressed as mean ±S.E.M. Non-parametric statistical analysis was applied to treat the behavioral data. Significance was assessed Mann–Whitney non-parametric *U* test.

3. Results

3.1. Effect of mCPP-induced CTA training on liquid consumption

The serotoninergic agonist mCPP (0.5 mg/kg i.p.) administered 15 min after the offset of drinking of an unfamiliar taste (glycine 1%), induced long-term CTA (AI = 63 ± 3.05 vs. AI = 28.97 ± 4.1 for mCPP-treated and control animals, respectively, $p < 0.01$). This mCPP-induced CTA, quantified in a *TC* situation (see under Section 2), is however weaker than that induced by the conventional UCS, LiCl i.p. (on LiCl-induced CTA (AI = 96.7 ± 0.7). Furthermore, the mCPP-induced taste aversion can be masked in the *NTC* situation, in which no alternative thirst quencher is available (Table 1).

3.2. Effect of CTA training on post-retrieval social interactions

The total time spent in active social behavior over the 20 min of the test was 624 ± 30 s in the *naïve* group (Fig. 2A). The time spent in active social behavior was not significantly different in any of the other groups, with the notable exception of the *mCPP* and *mCPP*(*s*) groups. In these two groups, the total time spent in active behavioral interaction between the two animals, was significantly lower than in any of the other groups (438 ± 42 and 465 ± 23 s, for *mCPP* and *mCPP*(*s*), respectively, $p < 0.001$). In all the groups, except the *mCPP* and *mCPP*(*s*) groups, the total number of social events (*sniffing*, *following*, *grooming the partner*, *wrestling*, and *crawling over or under*) was highly conserved across groups and similar for the experimental and naïve animals in any given group (71.9 ± 6.4 and 73.4 ± 7.5 for animals from the *naïve* group, Fig. 2B). However, a totally different situation was observed in the *mCPP* and *mCPP*(*s*) groups. Experimental animals from the *mCPP* and *mCPP*(*s*) groups displayed markedly less social events than any of the experimental animals of the other groups, and less social events than the co-tested naïve animals (26.3 ± 3.4 for experimental animals from the *mCPP* groups and 34.5 ± 3.0 for experimental animals from the *mCPP*(*s*) group, $p < 0.001$, compared to co-tested naïve animals, Fig. 2B). Further, when compared to naïve animals from other groups, naïve animals from the *mCPP* and *mCPP*(*s*) groups performed significantly less social acts (58.5 ± 4.2 for naïve animals from the *mCPP* groups and 57.9 ± 5.4 for naïve animals from the *mCPP*(*s*) group, respectively, compared to the naïve animals from other groups, $p < 0.01$). This makes sense, because if the experimental animal displays aberrant social behavior, this is likely to affect the social behavior of the naïve partner as well.

In all groups except the *mCPP* and *mCPP*(*s*) groups, the number of followings was relatively stable and not statistically different between experimental and naïve animals (Fig. 2C). However, in the *mCPP* and *mCPP*(*s*) groups, the number of followings of naïve animals was remarkably high compared to any other group of animals,

Table 1
Consumption of glycine solution after CTA training

	Control	LiCl	mCPP
No Taste-Choice	9.9 ± 0.5	0.7 ± 0.1	9.2 ± 0.6
Taste-Choice	G = 10.3 ± 0.8 W = 4.3 ± 0.7	G = 0.2 ± 0.03 W = 6.3 ± 0.8	G = 4.3 ± 0.4 W = 7.5 ± 0.6

Consumption of glycine solution after CTA training in which glycine was used as the CS, under two test conditions: *No Taste-Choice* (*NTC*), in which the rats were provided with the CS solution only, and *Taste-Choice* (*TC*), in which the rats were presented with the CS and water simultaneously. Data are in ml; $n = 8$–20 each. Separate groups were used for each condition. *LiCl*, CTA training using LiCl i.p. as the US; *mCPP*, CTA training using mCPP i.p. as the UCS; G, glycine; W, water. The LiCl groups displayed strong aversion of the CS under both the *NTC* and the *TC* conditions, whereas the mCPP groups displayed significant aversion only under the *TC* conditions. G = glycine consumption; W = water consumption.