Anxiety-like State Associates with Taste to Produce Conditioned Taste Aversion

Matthieu J. Guitton and Yadin Dudai

**Background:** The interactions among experience, emotion, and memory are considered to be instrumental in the ontogeny and maintenance of acquired emotional and behavioral disorders (e.g., phobias). Here we address the question whether an anxiety-like state can associate with taste to produce conditioned taste aversion (CTA).

**Methods:** We have used an anxiogenic agent, the 5-HT$_2C$ receptor agonist meta-chlorophenylpiperazine (mCPP), to induce an anxiety-like emotional state in rats after consumption of an unfamiliar tastant.

**Results:** The anxiogenic agent induced CTA. The mCPP-induced CTA could be prevented by concomitant administration of ethanol, which is known to reverse mCPP-induced anxiety-like behavior, at a concentration that had no effect on CTA memory. In contrast, ethanol did not prevent LiCl-induced CTA. Administration of mCPP before the consumption of the tastant had no effect on the preference for that tastant.

**Conclusions:** Taken together, these results indicate that anxiety-like state can serve as the unconditioned stimulus in CTA training. This finding may be relevant to the ontogeny of pathologies involving food aversion.

**Key Words:** Anxiety, CTA, mCPP, memory, taste

---

**Methods and Materials**

**Animals**

Rats (male Wistar, ~60 days old, 250–350 g) were caged individually at 22 ± 2°C in a 12-hour light–dark cycle. Water and food were available ad libitum unless otherwise indicated. All experiments were approved by the Weizmann Institute of Science animal care and use committee.

**Behavioral Procedures**

Conditioned taste aversion was induced and tested as previously described (Rosenblum et al 1997). Briefly, rats were trained over 3 days to obtain their daily water ration within 10 min from two pipettes, each containing 10 mL. On day 4, the rats were presented with glycine instead of water. Fifteen minutes later, they were injected intraperitoneally (iP) with the solution used as US. On day 5, a 10-min multiple-choice test was performed to determine the acquired aversion. In the test, rats were allowed free access to an array of six pipettes, three with 5 mL glycine and three with 5 mL water. The aversion index (AI) was defined as [(water consumed)/(water + tastant consumed) × 100] (Rosenblum et al 1997). Under normal conditions, glycine and saccharin are known to be strongly preferred to water (aversion index AI < 50).

Open field activity was used to quantify the anxiety-like behavior (Campbell and Merchant 2003). Animals were placed in the center of a squared arena divided into 16 quadrangles (12 in periphery and 4 in center) 45 min after the injection of saline or mCPP. Both the total number of crossing and the number of crossing in central quadrangles were then measured during a 10-min period.

Twelve groups were used in this study (Table 1). Glycine was from ICN Biomedicals (Aurora, Ohio), saccharin and mCPP from Sigma (St. Louis, Missouri), ethanol (analytic grade) from Bio Lab (Jerusalem, Israel) and LiCl from Merck (Darmstadt, Germany). Drugs were dissolved in physiologic saline. Mann–Whitney U test was used to determine statistical significance.

**Results**

The serotoninergic agonist mCPP induces anxiety in humans and anxiety-like behavior in animals (reviewed in Bourin et al 1998). Its anxiogenic effect was verified by us in this study in the open field test, widely employed to assess state anxiety in rats.
Campbell and Merchant (2003). The dose used (0.5 mg/kg) markedly diminished crossing of center-field squares in the open field (11.5 ± 3.4 vs. 1.5 ± 5 for control [Group 1] and treated [Group 2] animals, respectively; n = 4 each, p < .05), without affecting the total number of crossing (Group 1: 50.5 ± 19.8 vs. Group 2: 49.25 ± 19.2). Similarly, the time spent in the central part of the arena was of 114.8 ± 15.9 sec for the control animals (Group 1) versus 4.0 ± 1.1 sec for the treated animals (Group 2; Figure 1).

The same dose of mCPP, when administered IP 15 min after drinking an unfamiliar tastant (glycine), induced CTA (AI = 29 ± 4 vs. AI = 60 ± 4 for saline- [Group 3] and mCPP-injected rats [Group 4], respectively, p < .001; Figure 2). Ethanol (2 mg/kg), administered concomitantly with mCPP completely prevented the mCPP-induced CTA (AI = 24 ± 4, Group 7; Figure 2) but had no effect on LiCl-induced CTA (AI = 96.7 ± 7 for LiCl alone, Group 9 vs. AI = 97 ± 1 for LiCl + ethanol, Group 10; Figure 2). Ethanol per se also had no effect on glycine consumption (AI = 32 ± 3, Group 6; Figure 2). Although ethanol can serve as a solvent for mCPP (Sigma Chemical Data Sheet), we wished to verify that it is not the concomitant administration of mCPP and ethanol in the same injection that exerts the effect of ethanol on

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Effect of an injection of meta-chlorophenylpiperazine (mCPP; 0.5 mg/kg) on open field behavior 40 min after the injection. (Left) mCPP markedly diminished crossing of center-field squares in the open field between control animals injected with saline (Group 1) and animals injected with mCPP (Group 2). (Right) Similarly, mCPP treatment drastically decreased the time spent in the central quadrangles between control (Group 1) and treated animals (Group 2). * p < .05.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Aversion indices for glycine after intraperitoneal injection of different compounds, 15 min after the offset of glycine consumption. Animals preferred glycine over water when injected with saline. In contrast, meta-chlorophenylpiperazine (mCPP) injection led to a significant aversion for glycine. Ethanol did not induce any aversion for glycine; however, it totally reversed the mCPP-induced aversion when administered concomitantly with mCPP. LiCl injection induced marked aversion for glycine, even when coinjected with ethanol. * p < .05.
mCPP. We therefore tested an additional group of rats (Group 8) that received two subsequent injections, one of mCPP (.5 mg/kg), the other with ethanol (2 mg/kg), 15–30 sec apart, 15 min after glycine consumption. In this case, the effect on CTA was the same as with the concomitant administration (AI = 24 ± 4 for Group 7 vs. AI = 26.9 ± 6.2 for Group 8).

To ensure that this effect of mCPP and its blockade by ethanol was not confined to a particular taste, we replicated the experiment with another taste, saccharin (.1% W/V). Again, an mCPP US–induced aversion (AI = 62 ± 7, Group 11; Figure 3). As in the case of glycine, ethanol prevented the mCPP-induced CTA to saccharin (AI = 35 ± 6, Group 12, p < .05, Figure 3).

Finally, to rule out the possibility that mCPP induces a generalized long-lasting taste aversion, we injected mCPP to rats in the absence of prior presentation of the conditioned stimulus (CS; Group 5). The response to the first presentation of the CS was then measured 24 hours later. No acquired aversion for the novel taste was detected (AI = 36 ± 3 for the first exposure to glycine), indicating that mCPP did not induce sensitization that modifies taste preference.

**Discussion**

Emotion and memory interact intensively to modify behavior (e.g., Akirav and Richter-Levin 1999; Bahar et al 2003; LeDoux 1993). In this study, we focused on one aspect of emotion, anxiety. Specifically, we wished to determine whether a drug-induced anxiety-like state can substitute for the US in CTA, that is, whether an anxiety-like state following gustatory experience may lead later on to long-lasting, specific rejection of the consumed food.

Systemic application of mCPP has been shown to induce anxiogenic-like responses in animal models, including social interaction and light–dark choice behavior (Bilkei-Gorzo et al 1998; Bagdy et al 2001). Local microinfusion of mCPP into the basolateral amygdala has also been shown to induce acute fear-like responses in rats (Campbell and Merchant 2003). In humans, mCPP exacerbates symptoms of clinical anxiety in numerous anxiety disorders, including panic disorder, acute anxiety, obsessive–compulsive disorder, and agoraphobia (Charney et al 1987; Bourin et al 1998; Erzegovesi et al 2001). Most of these effects have been attributed to 5-HT$_{2C}$ receptors (Bagdy et al 2001; Kennett et al 1994). mCPP has also been reported to induce hypolocomotor and hypophagic effects in rats, but only at doses much higher than those used to induce anxiety-like behavior (Kennett et al 1989). Anxiety-like behavior in the animals was assessed using open field, which is a well-established test to measure state anxiety (i.e., situational and transitory manifestation of anxiety-like state). The behavior in the open field strongly supports the notion that mCPP induced rats an anxiety-like state isomorphic to state anxiety. The effects of mCPP injection on trait anxiety (i.e., individual tendency to undergo anxiety-like state) as well as on fear were not tested in this study. The smaller magnitude of the aversion induced by mCPP compared with the classical LiCl-induced CTA could be related to the heterogeneity of complex emotional states like anxiety-like state and thus argue against a direct action of mCPP and for a role of anxiety-like state as a reinforcer.

At sufficiently high concentrations, many types of drugs could induce malaise that might result in CTA (Bures et al 1998). Hence, although we used a low concentration of mCPP, it was pertinent to verify that the effect observed was indeed likely to be due to the anxiogenic effects of the drug rather than to general malaise. Toward that end, a proper approach is to block the anxiogenic-like effects with an anxiolytic agent. Antagonists of the 5-HT$_{2C}$ receptor (e.g., SB 200646A, mianserin, metergolotine, or cyproheptadine) reverse mCPP-induced anxiety-like behavior in animal models (Kennett et al 1989, 1994), but because all these drugs interact with the same molecular receptor, they may still, in addition to their anxiolytic effect, block other nonanxiogenic effects of mCPP and hence cannot serve as an appropriate control. The anxiogenic effect of mCPP is also antagonized by anxiolitics that do not significantly interact with the 5-HT$_{2C}$ receptor. Particularly effective are the 1,4-benzodiazepines, which interact with GABA$_A$ receptors. The marked sedation by benzodiazepines itself might, however, affect the behavioral measure of anxiety-like behavior in animals (Bilkei-Gorzo et al 1998; File 1985).

We thus decided to use another nonserotonergic anxiolytic drug to verify that mCPP-induced CTA is indeed due to the anxiety-like state. Toward that end, ethanol seemed to be the appropriate choice: it does not directly act on the molecular target of mCPP yet is known to have anxiolytic effects (Costall et al 1988) and reverses the anxiety-like state induced by mCPP at a range of concentration low enough to avoid motor or cognitive side effects (Bilkei-Gorzo et al 1998).

We used the lowest dose of ethanol known to reverse mCPP-induced anxiety-like state in rats (2 mg/kg) to avoid other side effects of ethanol. Such a concentration of ethanol has been shown to leave unaffected memory in adult rats (Land and Spear 2004). At this dose, ethanol totally abolished the CTA induced by

![Figure 3. Aversion indices for saccharin after intraperitoneal injection of meta-chlorophenylpiperazine (mCPP) alone or mCPP + ethanol. When injected with mCPP, animals developed a strong aversion for saccharin. Concomitant administration of ethanol reversed this phenomenon. * p < .05 between the two groups.](image-url)
mCPP, suggesting that the aversive stimulus in this case is anxiety-like state and not a direct 5-HT2C-mediated effect of mCPP. Strikingly, ethanol failed to reverse the CTA induced by LiCl, suggesting that the effect of ethanol was not a general effect on learning or on taste behavior, but rather a specific anxiolytic effect. Similarly, ethanol alone given as US did not induce any detectable change in aversion index. This result is in accordance with previous literature showing that ethanol can induce CTA only at very high concentrations (Blair and Amit 1981).

The blocking by ethanol of mCPP's ability to serve as a US in CTA renders it highly likely that what is associated in the mCPP-CTA training is the anxiety-like state with the perception or consumption of the tastant. This finding may be of interest in the context of the ability of anxiety or anxiety-like states to function as a reinforcer in general, and to induce or augment taste or food aversion in particular. Demonstration that a behavioral induction of an anxiety-like state could also generate significant learned taste aversions would be of interest. It is noteworthy that CTA is unique among other types of associative learning, in tolerating a long CS–US interstimulus interval. This suggests that anxiety that occurs long after food consumption may still tag this food as aversive.

This work was supported by a grant from the Israel Science Foundation, Jerusalem. We thank Shoshi Hazvi and Danya Elyashiv for assistance and Irith Akirav, Tali Koboli, Efrat Forst, Orit Furman, Mark Eisenberg, Nimrod Dorfman, and Jimmy Stebbins for valuable comments.


Bagdy G, Graf M, Anheuer ZE, Modos EA, Kantor S (2001): Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT2C receptor antagonist SB-242084 but not the 5-HT1A receptor antagonist WAY-100635. Int J Neuropharmacol 4:399–408.


LeDoux JE (1998): Fear and the brain: where have we been and where are we going? Biol Psychiatry 44:1229–1238.


www.elsevier.com/locate/biopsych