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Brief Report

Muscarinic transmission in the basolateral amygdala is necessary for the acquisition of socially transmitted food preferences in rats

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ABSTRACT

We examined the involvement of muscarinic receptors in the basolateral amygdala (BLA) in the social transmission of food preference (STFP) learning by assessing the effects of scopolamine (20 μg/side), injected prior to social training, on a 24-h food-choice test. Muscarinic receptor blockade in the BLA significantly impaired STFP, as shown by the rats' chance preference for the odorized trained food. The present results are consistent with the suggestion that intact cholinergic transmission in the BLA is necessary for acquisition and/or initial consolidation and provide evidence that BLA integrity is part of the underlying circuit of STFP learning.

Social transmission of food preferences (STFP) is a naturalistic learning paradigm widely used in rodents to study nonspatial relational memory (Alvarez, Wendelken, & Eichenbaum, 2002). In the STFP, a subject (observer) associates two olfactory stimuli on the breath of a conspecific (demonstrator) that has recently eaten odorized food (Galef, Mason, Preti, & Bean, 1988), expressing its memory in a subsequent choice test through a substantial enhanced preference for whatever food was ingested by the demonstrator (Galef, Kennett, & Stein, 1985). There is substantial evidence that the integrity of several brain regions such as the hippocampus and related areas, the basal forebrain or the prefrontal cortex is necessary for a good STFP performance (for a review see Carballo-Marquez, Vale-Martinez, Guillazo-Blanch, & Marti-Nicolovius, 2008). A modulatory role for acetylcholine (ACh) in STFP learning has been proposed, since pre-training selective cholinergic damage of the nucleus basalis magnocellularis (NBM) or the orbitofrontal cortex (OFC) produces deficits in the expression of the task (Ross, McGaughy, & Eichenbaum, 2005; Vale-Martinez, Baxter, & Eichenbaum, 2002). Moreover, muscarinic receptor (mRs) blockade with scopolamine in another NBM cortical target area, the prelimbic cortex (PLC), also disrupts STFP acquisition (Boix-Trelis, Vale-Martinez, Guillazo-Blanch, & Marti-Nicolovius, 2007).

Another region also involved in STFP learning is the basolateral amygdala (BLA). The BLA participates in olfactory and taste associative learning (for a review see Wang, Fontanini, & Katz, 2006), as shown, for example, in the conditioned taste aversion (CTA) paradigm (Reilly & Bornovalova, 2005). Thus, lesions or pharmacological manipulations of the BLA severely impaired CTA acquisition (for a review see Miranda, Ferreira, Ramirez-Lugo, & Bermudez-Rattoni, 2003). However, its precise role in STFP acquisition still remains unclear. Wang et al. (2006) showed that BLA inactivation during STFP training abolished acquisition whereas other studies have failed to report STFP learning disruption after pre-training ibotenic lesions (Burton, Murphy, Qureshi, Sutton, & O'Keefe, 2000) and have found no c-fos activation following STFP acquisition (Smith, Countryman, Sahuque, & Colombo, 2007). As the BLA receives strong cholinergic inputs from the NBM (Mesulam, Mufson, Levey, & Wainer, 1983), in the present study we sought to elucidate the involvement of BLA mRs in STFP learning. In particular, this experiment determines the effects of bilateral injections of 20-μg scopolamine in the BLA, immediately before the social training with demonstrators, on a memory test 24 h later. The muscarinic transmission in BLA may make an essential contribution to the acquisition of this olfactory relational task since it relies on an odor-odor association (Galef et al., 1988) that may be influenced by BLA and ACh.

Forty-eight male Wistar rats (mean weight 440.45 g, *SD* 33.12; mean age 88.87 d, *SD* 3.58) were used as observer subjects. Sixteen juvenile male Wistar rats (mean weight 122.24 g, *SD* 21.21; mean age 31.5 d, *SD* 2.97) served as demonstrators. Young demonstrator rats were used in order to avoid fighting and to favor social interaction (see also Alvarez et al., 2002; Vale-Martinez et al., 2002).

All materials, procedures, drug dosage and timings were carried out in a similar way to previous work, where they are explained in

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detail (Boix-Trelis et al., 2007), and performed in compliance with the European Community Council Directive for care and use of laboratory animals (86/609/ECC) and with *Generalitat de Catalunya* authorization (DOGC 2450 7/8/1997, DARP protocol number 3211). Observers underwent stereotaxic bilateral implantation of chronic guide cannulae in the BLA (AP, −2.6 mm from bregma; ML, ±4.9 mm from midline, and DV, −7.5 mm from cranium surface) (Paxinos & Watson, 1997). Prior to surgical procedures, the observers were habituated for 3 d to powdered chow from glass jars. Seven days after post-surgical recovery they were rehabituated for 2 days and adapted to a mock infusion protocol (no solutions injected).

The behavioral task began when a demonstrator ate food with 2.2% cocoa (Oxfam Fairtrade, Belgium) or 1% cinnamon (Carmencita, Alicante, Spain) for 30 min. Before the 30-min period was complete, observers received a bilateral intracerebral infusion of PBS (VEH) or 20 μg of scopolamine (SCOP) (volume 0.5 μl/hemisphere for 2 min) in the BLA. The drug dosage was based on previous studies in which scopolamine in the PLC disrupted STFP acquisition (Boix-Trelis et al., 2007) and another olfactory associative task (Carballo-Marquez, Vale-Martinez, Guillazo-Blanch, Torras-Garcia, Boix-Trelis, & Marti-Nicolovius, 2007). Immediately after the infusion, a demonstrator that had just eaten flavored chow was placed into the observer's cage and the two rats were allowed to interact with no barriers for 30 min. To control for a potential social interaction performance or olfactory deficit, we scored the number of times each observer sniffed the muzzle, body or anogenital region of the demonstrator during the social training. All observers were tested 24 h after STFP training by placing two jars filled with odorized food, one containing the chow given to demonstrators (trained food) and the second jar a different scented chow (untrained food). The observers were allowed to eat for 45 min and a preference score for the trained food was calculated as follows: 100 × (weight of trained food eaten/weight of all food eaten). To determine whether scopolamine produced changes in neophobia, we compared the amount of regular food eaten during post-surgery habituation (unodorized ground food) and the amount of new food eaten during the test (total odorized food, trained + untrained). The number of times the observer was on top of the jar with both forepaws during the first 20 min of the test (*jar climbs*) was also scored to evaluate whether scopolamine produced changes in locomotor activity or exploration (Boix-Trelis et al., 2007).

After behavioral testing, observers' brains were sectioned at 40 μm thickness on a cryostat (Shandom Cryotome FSE, Thermo Electron Corporation) and the sections were processed for acetylcholinesterase histochemistry, essentially as described elsewhere (Paxinos & Watson, 1997).

Rats having cannula tips in the dorsal region of BLA showing no tissue damage resulting from the rate or volume of the infusions were included in the final sample (Fig. 1), SCOP ($n = 14$) and VEH ($n = 12$). Incorrectly implanted cannulae (located in other amygdalar nuclei, $n = 19$) or the existence of technical problems during infusion ($n = 3$) were considered grounds for exclusion. Statistically significant between group differences with an ANOVA analysis were revealed in the preference for the trained food ($F_{(1,25)} = 16.062$, $p = 0.001$). Accordingly, SCOP rats showed a preference similar to chance level (50%) ($t_{(13)} = -0.252$, $p = 0.805$), whereas control rats performed above chance ($t_{(11)} = 10.742$, $p < 0.0001$) (Fig. 2). The analysis (ANOVA) of the variables scored during social interaction (Fig. 3A) showed no significant differences between SCOP and VEH groups in any of the measures (muzzle: $F_{(1,24)} = 1.816$, $p = 0.191$; body: $F_{(1,24)} = 1.349$, $p = 0.257$; anogenital: $F_{(1,24)} = 0.003$, $p = 0.957$). Nor were there any statistically significant between group differences observed in the total amount of food (trained + untrained) consumed during testing ($F_{(1,25)} = 0.013$, $p = 0.911$) (Fig. 3B). As for possible changes in neophobia (Fig. 3B), a mixed ANOVA analysis showed no significant effect of food ($F_{(1,24)} = 0.967$, $p = 0.335$), group ($F_{(1,24)} = 0.016$, $p = 0.900$) or group × food interaction ($F_{(1,24)} = 0.004$, $p = 0.950$. As for jar climbs, there were no statistically significant differences between groups ($F_{(1,25)} = 0.021$, $p = 0.886$).

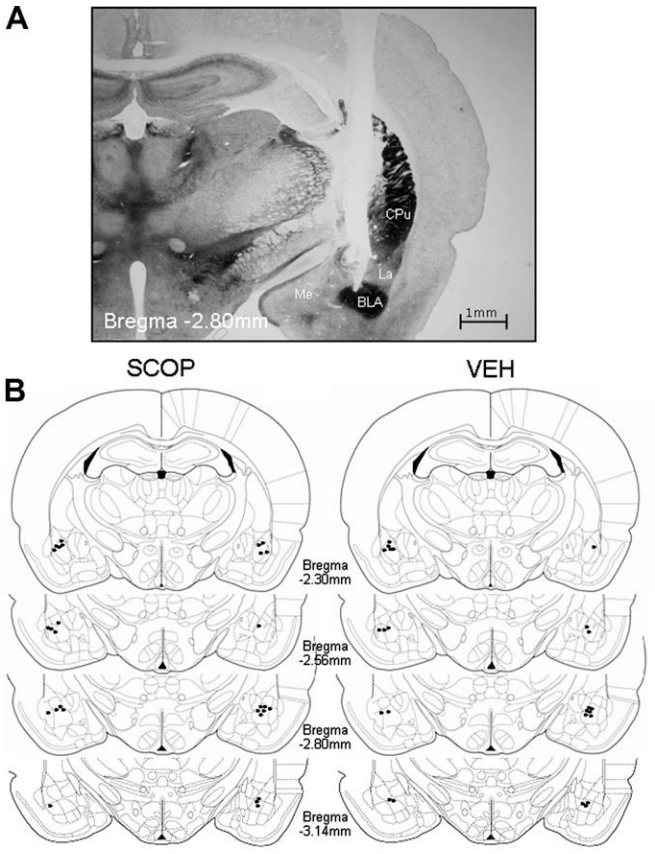

Fig. 1. (A) Photomicrograph of acetylcholinesterase staining at the level of the BLA area (AP, −2.80 mm posterior to bregma) showing the cannula track and the micro-injector tip of a representative subject. (B) Micro-injector tip placements throughout the rostral-caudal extent of the BLA in scopolamine- and vehicle-injected rats. The cannulae were located along different brain coordinates from −2.30 to −3.14 mm posterior to bregma according to the stereotaxic atlas. Reprinted with permission from Elsevier©1997, Paxinos and Watson (1997).

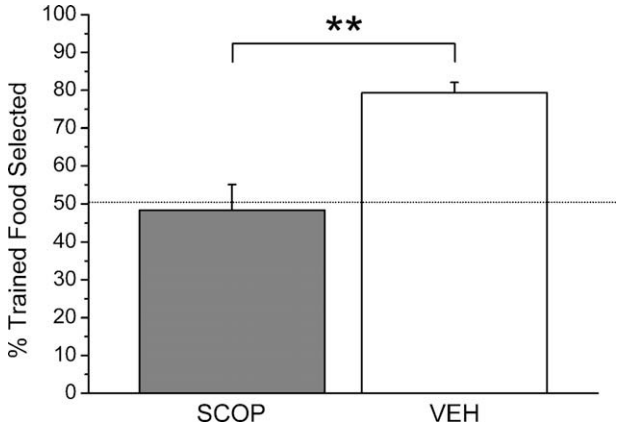

Fig. 2. Percentage of trained food selected, expressed as the mean percentage (±SEM) of the total amount of food consumed at the STFP test. Rats injected with scopolamine showed a lower preference for the trained food (similar to chance) than control rats (**p = 0.001).