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Olfactory fear conditioning paradigm in rats: Effects of midazolam, propranolol or scopolamine ☆

Juliana A.V. Kroon, Antonio Pádua Carobrez *

Departamento de Farmacologia, CCB, Universidade Federal de Santa Catarina, Florianópolis, SC 88040-900, Brazil

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ABSTRACT

In rodents, fear conditioned responses are more pronounced toward olfactory stimulus, since olfaction is a dominant sense in these subjects. The present study was outlined to investigate if the association between coffee odor (CS1) and electrical footshock (US) would be an effective model for the study of fear-induced behavior and whether compounds used in humans for emotional-related disorders such as midazolam, propranolol, or scopolamine, applied during the different stages of fear conditioning (acquisition, consolidation and expression), affect the defensive responses to both, the olfactory CS1, and the context (CS2) where the CS1 had been presented (second order conditioning). The results revealed that five pairings between coffee odor (CS1) and electrical footshock (US) were able to elicit consistent defensive responses and a second order conditioning to the context (CS2). Midazolam (0.375–0.5 mg/kg; i.p.) treatment was able to interfere with the CS1–US association and with the consolidation of the aversive information. The propranolol (5–10 mg/kg; i.p.) treatment interfered with the CS1–US association, with the retention of fear memory and with the CS1–CS2 association. Propranolol also attenuated the expression of conditioned fear responses when applied before the CS1 test session. Scopolamine (0.6–1.2 mg/kg; i.p.) treatment impaired the acquisition of CS1–US and CS1–CS2 associations, and also disrupted the expression of conditioned fear responses when injected prior to the CS1 test session. These findings have pointed out the usefulness for the olfactory fear conditioning paradigm to investigate drug effects on the acquisition, consolidation and expression of fear conditioned responses.

1. Introduction

Fear responses are considered evolutionary defensive mechanisms involved in protecting animals or humans against potentially dangerous threats. On the other hand, fear may also represent maladaptive physiological and behavioral responses and, in this case, it can be characterized as a pathological process. Anxiety disorders are the most common type of psychopathologies in the American population, with an incidence of 18.1% and a lifetime prevalence of 28.8% (Kessler, Berlung, & Demler, 2005; Kessler, Chiu, & Demler 2005). Although human studies have evolved to neuropsychological and neuroimaging methodologies (Bishop, 2007; Büchel & Dolan, 2000), Pavlovian or classical fear conditioning, a simple paradigm that has been extensively investigated in animals, has still been considered a useful tool for trying to unravel the process and mechanisms underlying the pathophysiology of fear (Fendt & Fanselow, 1999; Kim & Jung, 2006).

Fear conditioning occurs when a neutral emotional stimulus is paired with a biologically significant aversive event, the unconditioned stimulus (US). After one or a few pairings, the neutral stimulus can acquire the ability to elicit fear conditioning responses (CR1) that typically occurs in the presence of the US, thereby becoming a conditioned stimulus (CS1) (LeDoux, 2000). In addition, the aversive significance acquired by the CS1 can promote a new fear association (second order conditioning), after been paired with a novel neutral stimulus (CS2) that acquires the ability to elicit defensive conditioned responses (CR2) (Gewirtz & Davis, 2000). The vast majority of fear conditioning investigations in rodents has employed auditory (Debiec & LeDoux, 2004; Gravius, Barberi, Schafer, Schmidt, & Danysz, 2006; Roozendaal, Hui, Hui, Berlau, McGaugh, & Weinberger, 2006), visual (Campeau & Davis, 1995; Shi & Davis, 2001; Tazumi & Okaichi, 2002) or contextual (Fanselow, 2000; Resstel, Lisboa, Aguiar, Corrêa, & Guimarães, 2008) conditioned stimuli paired with electrical footshock. However, studies have used odor as a CS, since olfaction is the most important sensory system used to recognize conspecifics, predator, prey, reproductive behavior and feeding in mammals like the rat (Brennan & Keverne, 1997; Restrepo, Arellano, Oliva, Schaefer, & Lin, 2004).

The neural circuits and the modulatory systems mediating Pavlovian fear conditioning have been elucidated and shed light on interactions between emotional and cognitive process in the brain (LeDoux, 2000; Maren, 2001; Otto, Cousens, & Herzog, 2000).

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* Corresponding author. Fax: +55 48 33375479.
E-mail address: adepadua@farmaco.ufsc.br (A.P. Carobrez).

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However, among a variety of fear conditioning eliciting stimulus used as CS, olfactory cues appear to be the only sensory input able to activate the medial hypothalamic defensive circuit, a neural system engaged on natural fear responses (Canteras, 2002; Canteras & Blanchard, 2008; Canteras, Kroon, Do-Monte, Pavesi, & Carobrez, 2008).

Behavioral studies have already established that olfactory stimuli serve effectively as CSs in a fear conditioning paradigm. Otto and colleagues (1997, 2000) have reported that pairings between an olfactory CS and a footshock US result in robust and long-lasting freezing responses. In addition, Richardson, Vishney, and Lee (1999), and later, Paschall and Davis (2002a) have shown that this CS–US association is an effective stimulus for potentiating the startle response in rats. Moreover, a study conducted by Paschall and Davis (2002b) has demonstrated that olfactory cues serve as efficient CS1 and CS2 stimuli in second order fear-potentiated startle paradigms.

The involvement of γ-aminobutyric acid (GABA), noradrenergic and cholinergic neurotransmission systems in learning and memory processes as well as in the mediation of defensive behavior, fear and anxiety have been well documented (Bertoglio & Carobrez, 2004; Cahill, Pham, & Setlow, 2000; De-Mello & Carobrez, 2002; Dielenberg, Arnold, & McGregor, 1999; File & Aranko, 1988; Millan, 2003; Stern, Carobrez, & Bertoglio, 2008). Moreover, it has been shown that the administration of benzodiazepines, β-adrenergic antagonists or anticholinergic agents impairs the acquisition and/or the expression of fear memories (Anagnostaras, Maren, & Fanselow, 1995; Anagnostaras, Maren, Sage, Goodrich, & Fanselow, 1999; Fanselow & Helmstetter, 1988; Pain, Launoy, Fouquet, & Oberling, 2002; Resstel, Joca, Moreira, Corrêa, & Guimarães, 2006; Rudy, 1996; Santos, Gárgaro, Oliveira, Masson, & Brandão, 2005; Walker & Davis, 2002; Young, Bohenek, & Fanselow, 1995).

Taking into account the wide utility of the Pavlovian fear conditioning paradigm and the biological relevance of olfaction for rodents, this study was outlined to investigate if the association between coffee odor (CS1) and electrical footshock (US) would be an effective model for the study of fear-induced behavior. In addition, a further evaluation addressed whether compounds used in humans for emotional-related disorders such as midazolam (Olkkola & Ahonen, 2008), propranolol (Famularo, Kinscherff, & Fenton, 1988; Pitman et al., 2002), or scopolamine (Furey & Drevets, 2006), applied during the different stages of fear conditioning, affect the defensive responses to both, the olfactory CS1, and the context (CS2) where the CS1 had been presented.

2. Materials and methods

2.1. Subjects

Adult, 12–16 weeks, male Wistar rats ($n = 335$) obtained from the Universidade Federal de Santa Catarina, weighing 300–450 g were used in this study. The animals were housed in polypropylene cages (50 cm × 30 cm × 15 cm) in groups of three or four, under a 12 h light: 12 h dark cycle, in a temperature-controlled environment (23 ± 1 °C) and with food and water freely available. The protocols were approved by the Universidade Federal de Santa Catarina, Animal Ethics Committee (23080.006118/2004-36/UFSC) and the experiments were carried out in accordance with the Brazilian Society of Neuroscience and Behavior Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs

Midazolam (Dormonid®, Roche, Brazil), propranolol hydrochloride (Sigma–Aldrich, USA), and scopolamine hydrobromide (Sigma–RBI, USA), were dissolved in 0.9% saline, which alone served as a vehicle control. The solutions were administered intraperitoneally in an injection volume of 1.0 ml/kg. Midazolam, propranolol and scopolamine dose selection were based on previous studies (Bertoglio & Carobrez, 2004; De-Mello & Carobrez, 2002; Dielenberg et al., 1999; Stern et al., 2008). Coffee powder (roasted and ground, Tradicional, Melitta®, Brazil) was used as an odor source.

2.3. Apparatuses and behavioral measures

This experiment comprised two different apparatuses: a conditioning chamber and an odor box (Fig. 1).

The conditioning chamber (50 cm × 26 cm × 35 cm) was constructed with stainless steel walls and a grid floor composed of 1 cm spaced stainless steel bars connected to a shock generator (Insight, Ribeirão Preto, SP, Brasil) that, when appropriate, delivered a 0.4 mA shock for 2 s. A 15 g amount of coffee powder was uniformly distributed in a compartment under the grid floor which served as an olfactory stimulus. The conditioning chamber was housed in a sound-attenuating room with illumination level of 80 lux.

To reduce contextual influence, assessment of conditioned fear took place in a different behavioral chamber, in a distinct room with low illumination (4 lux). The odor box, a laboratory analog of the burrow systems used by rodents in the wild (Dielenberg & McGregor, 2001), was made up of black Plexiglas and consisted of an open compartment (40 cm × 26 cm × 40 cm) and an enclosed (roofed) compartment (20 cm × 26 cm × 40 cm). A 6 × 6 cm open door allowed the rat to move through both compartments. On the opposite wall of the enclosed compartment was placed a cloth. During the CS1 test session, a cloth containing a 15 g amount of coffee powder was used as an odor source. One of the lateral walls of the chamber was made up of clear Plexiglas allowing a video camera and corresponding DVD system to record the animals' behavior for scoring after the experiment was conducted. The following behavioral responses were measured during exposure to the odor box: the amount of time the rats spent near (within 7 cm) the odor source (approach time); the amount of time spent in the enclosed compartment (hide time) and the amount of time spent stretching out from the enclosed compartment toward the open compartment (head-out time).

After each session and between subjects, the apparatuses were cleaned with a 10% alcohol–water solution.

2.4. General procedures

This experimental paradigm consisted of two consecutive phases: the acquisition of olfactory fear conditioning (2 days) and the expression of olfactory fear conditioning (3 days), as illustrated in Fig. 1. All sessions were spaced 24 h apart.

The acquisition of olfactory fear conditioning was performed in the conditioning chamber and the sessions were carried out at variable times, ranging from 2 to 3 min 20 s. On day 1, the rats were placed in the apparatus and were allowed to explore it freely, in a session called familiarization. On the following day, conditioning took place in a session in which subjects received pairings of coffee odor (CS1) and electrical footshock (0.4 mA/2 s; 40–60 s inter-trial period) (US).

The expression of olfactory fear conditioning was performed in the odor box and consisted of three consecutive sessions (10 min duration), each spaced 24 h apart: familiarization (Day 3), CS1 test (Day 4) and CS2 test (Day 5). In the familiarization session, the subjects were habituated to the novel apparatus and baseline levels of behavioral parameters were measured in the absence of any odor stimulus, since a neutral cloth was used in this session. On the following day, during CS1 test session, a cloth containing a 15 g