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Acetaldehyde self-administration by a two-bottle choice paradigm: Consequences on emotional reactivity, spatial learning, and memory

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A B S T R A C T

Acetaldehyde, the first alcohol metabolite, is responsible for many pharmacological effects that are not clearly distinguishable from those exerted by its parent compound. It alters motor performance, induces reinforced learning and motivated behavior, and produces different reactions according to the route of administration and the relative accumulation in the brain or in the periphery. The effective activity of oral acetaldehyde represents an unresolved field of inquiry that deserves further investigation. Thus, this study explores the acquisition and maintenance of acetaldehyde drinking behavior in adult male rats, employing a two-bottle choice paradigm for water and acetaldehyde solution (from 0.9% to 3.2% v/v), over 8 weeks. The behavioral consequences exerted by chronic acetaldehyde intake are assessed by a set of different tests: trials in an open-field arena and elevated-plus maze provided information on both general motor and explorative activity, and anxiety-driven behavioral responses. The Morris water maze allowed the exploration of cognitive processes such as spatial learning and memory. Determination of acetaldehyde levels in the brain was carried out at the end of the drinking paradigm. Our results indicate that rats exposed for the first time to acetaldehyde at 0.9% displayed a regular and stable daily drinking pattern that reached higher values and a "peaks and drops" shaped-trend when acetaldehyde concentration was increased to 3.2%. Accordingly, an increase in acetaldehyde levels in the brain was determined compared to non-acetaldehyde drinking rats. Acetaldehyde intake during the free-choice paradigm exerted an anxiogenic response in the open-field arena and elevated-plus maze, which in turn correlates with an enhancement in cognitive flexibility and spatial orientation skills, when an adaptive response to a stressful environmental challenge was required. These findings further support the idea that acetaldehyde is indeed a centrally active and behaviorally relevant metabolite of alcohol.

Introduction

Animal paradigms of self-administration serve a heuristic function by modeling human alcohol consumption in controlled environments. In particular, free-choice drinking procedures are reliable tools to investigate alcohol preference, acquisition, and maintenance of drug-taking behavior in the rat home cage (Roberts et al., 2001; Sillaber et al., 2002; Wilson, Neill, & Costall, 1997; Wolffgramm & Heyne, 1995). Long-term, 24-h, free-choice alcohol intake is considered a consummatory behavior whose underlying neurobiological processes primarily control the amount of alcohol consumed once drinking has started, and interact with experience of post-ingestional drug effects to alter consumption as well (Samson & Czachowski, 2003). One of the key drivers of central and peripheral effects associated with chronic or acute overconsumption of alcohol is acetaldehyde (ACD), alcohol's first metabolite. It is produced by alcohol oxidative metabolism both in the periphery, via alcohol dehydrogenase (ADH), and in the brain, where catalase and CYP2E1 produce the majority of ACD following alcohol consumption (Zimatkin, Liopo, & Deitrich, 1998; Zimatkin, Pronko, Vasiliou, Gonzalez, & Deitrich, 2006). ACD *per se*, and as a product of alcohol metabolism, is responsible for many pharmacological effects that are not clearly distinguishable from those exerted by its parent

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compound. On the other hand, alcohol activity in the brain strongly depends on doses, time, modalities of administration, and on the onset of withdrawal (Davidson, Hutchison, Dagon, & Swift, 2002; Lukoyanov, Madeira, & Paula-Barbosa, 1999; Pohorecky, 1977). Consequently, the results of studies on the behavioral consequences of chronic alcohol consumption are equivocal. Specifically, memory performance in rodents can be impaired (Farr, Scherrer, Banks, Flood, & Morley, 2005; Matthews & Morrow, 2000), unaffected (Fadda, Cocco, Stancampiano, & Rossetti, 1999; Gál & Bárdos, 1994; Homewood, Bond, & MacKenzie, 1997), or even improved (Krazem, Marighetto, Higueret, & Jaffard, 2003; Robles & Sabriá, 2008), following chronic alcohol exposure. Indeed, rats chronically exposed to alcohol, but not submitted to withdrawal, display an enhancement in their performance in different maze tasks, compared to controls (Cacace, Plescia, La Barbera, & Cannizzaro, 2011; Steigerwald & Miller, 1997).

Similar to alcohol, ACD may alter motor performance, and induce reinforced learning and motivated behavior (Brancato et al., 2014; Cacace, Plescia, Barberi, & Cannizzaro, 2012; Plescia, Brancato, Marino, & Cannizzaro, 2013). Concomitantly, it may produce adverse reactions according to the route of administration and the relative accumulation in the brain or in the periphery (Escrig, Pardo, Aragon, & Correa, 2012). Actually, skepticism still arises when oral ACD is said to have the ability to affect brain functions, and the real and intrinsic activity of ACD represents an interesting and unresolved field of inquiry that deserves further investigation. Thus, our research was aimed at: 1) the observation of rat consummatory behavior in the home cage, using a two-bottle choice paradigm with water and ACD at 0.9 and 3.2% concentrations, during an 8-week period, 2) the quantification of brain ACD concentration as a consequence of the free-choice paradigm, 3) the investigation of the effects of ACD consumption during the two-bottle choice paradigm, on novelty-induced explorative behavior in the open-field arena and in the elevated-plus maze, as a measure of animals' behavioral reactivity and emotional state, and 4) the evaluation of the impact of ACD free access on spatial learning and memory using the Morris water maze. During the behavioral assessments, ACD free access was never interrupted, to prevent interferences due to withdrawal. As far as we know, the results of ACD self-administration in a free-access and free-choice paradigm have not been reported yet. Thus, this is the first study that provides information on rat ACD preference, regulation of self-administration in the home cage, and potential consequences on different dimensions of rat behavior.

Material and methods

Animals and housing conditions

Twenty-four adult male Wistar rats (Harlan, Udine, Italy), weighing 300–330 g, were housed individually in standard rat cages (40 cm × 60 cm, 20 cm in height) and maintained in a temperature (22 ± 2 °C) and humidity (55 ± 10%) controlled room, with free access to water and food. The colony was maintained on 12-h light–dark cycle (8:00 to 20:00 h), and rats were gently handled for 3 min per day for a week before the experimental procedures. The animals were randomly assigned to one of the following two groups: ACD free access (ACD, $n = 12$) and controls (CTR, $n = 12$). Rat body weight, food, and liquid intake were recorded daily. On the test days, the animals were brought into the laboratory 60 min before the experimental sessions to acclimatize. The experiments were carried out in a sound-isolated room between 9:00 and 14:00. Animal performance was recorded on videotape and monitored in an adjacent room. To ensure that a rat's behavior was not affected by the detection of another rat's scent, all the devices were thoroughly cleaned 10 min before the animal's entry into the cages. All the experiments were conducted in accordance with the regulations of the Committee for the Protection and Use of Animals of the University of Palermo, following the current Italian Law on animal experimentation (D.L. 116/92) and the European directives (2010/63/EU).

ACD free-access paradigm

ACD chronic consumption was carried out for 8 weeks as illustrated in Fig. 1. ACD-exposed rats were single housed and daily had the free choice between tap water and ACD solution, 24 h/day. For the first 4 weeks, rats were habituated to a low concentration ACD solution (0.9% v/v), to favor the acceptability of the substance. When the drinking pattern was established, ACD concentration was increased to 3.2% v/v, until the end of the paradigm. Control rats received two bottles of water. In all the cages, the location of the two bottles was alternated every morning to prevent side preference. Every morning, ACD solutions were freshly prepared and, before their replacement, daily intake was carefully recorded between 8:00–9:00 AM. During the paradigm, rats were monitored daily for food and water intake and weight.

ACD solution: preparation

ACD (Sigma–Aldrich SRL, Milan, Italy) was stored at −20 °C, and the solution was prepared by diluting it with tap water. During the first 4 weeks, ACD concentration was 0.9% v/v (0.450 mL in 50 mL of solution) and in the following 4 weeks, it was increased to 3.2% v/v (3.2 mL in 100 mL of solution).

ACD solution: stability assay

Repetitive, quantitative determination of ACD concentration was carried out on two consecutive days, from samples prepared with distilled and tap water, at 0.9% and 3.2% v/v, stored at 25 ± 0.5 °C. ACD concentration was measured spectrophotometrically using a

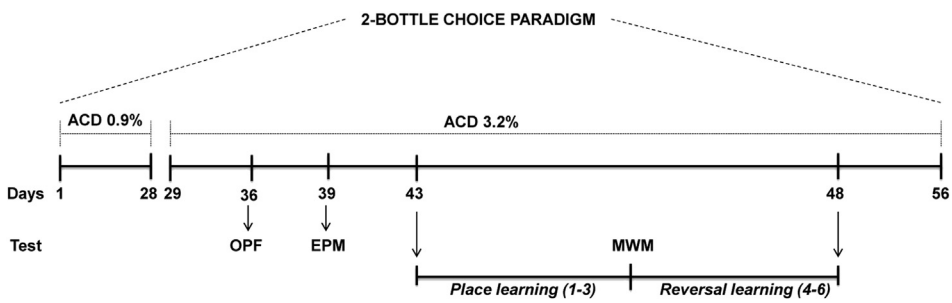

Fig. 1. Timeline of the experimental procedures. ACD: acetaldehyde; OPF: open field; EPM: elevated-plus maze; MWM: Morris water maze.