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Research report

Rats taste-aversive learning with cyclosporine a is not affected by contextual changes

Akin Tuerkmen, Katharina Bösche, Laura Lückemann, Harald Engler, Manfred Schedlowski, Martin Hadamitzky*

Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, 45122 Essen, Germany

HIGHLIGHTS

- The immunosuppressant CsA has direct effects on CNS functioning.
- Extinction of a CsA-induced taste aversion is not affected by contextual changes.
- CTA extinction depends, at least in part, on the compound used as US.

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ABSTRACT

In conditioned taste aversion (CTA) rats associate a novel taste (conditioned stimulus; CS) with a treatment (unconditioned stimulus; US) that induces symptoms of malaise. During retrieval, animals learn that the CS no longer predicts the US, with the consequence that the behavior elicited by the CS extinguishes. Importantly, CTA data with lithium chloride (LiCl) as US indicate that extinction learning is affected by changing the physical context. However, if this is also the case in different taste-aversion paradigms employing compounds other than LiCL as US is unknown. Against this background the present study investigated in a CTA paradigm with saccharin as CS and the immunosuppressant cyclosporine A (CsA) as US the influence of contextual changes on CTA extinction. Our results show, that extinction of a learned CS-US association with CsA is not prone to contextual changes. Due to the direct effects of CsA on CNS functioning, CTA with this immunosuppressant apparently operates under different mechanisms compared to other drugs, such as LiCl. These data indicate that taste aversive learning and its extinction are not necessarily specific to the context in which it is learned but also depends, at least in part, on the physiological and neuropharmacological effects of the drug employed as US.

1. Introduction

From an evolutionary perspective associative learning processes, such as conditioning of immune functions, may have evolved as adaptive mechanism to protect the organism from potentially harmful immune responses by avoiding ingestion or contact with immune status modulating substances [1,2]. Conditioned taste aversion (CTA) is a well-established learning paradigm in rodents where animals learn to associate a novel taste, mainly a saccharin solution (CS) with a drug treatment that produces symptoms of poisoning or illness (US; e.g. lithium chloride; LiCl) [3–5]. As conditioned response animals avoid consuming the saccharin solution during retrieval [6]. The neurobiological mechanisms mediating CTA are relatively well understood. Thus, it is suggested that cholinergic and noradrenergic mechanisms as well as N-methyl-D-aspartate (NMDA)-receptors and cellular processes such as mitogen-activated protein (MAP)-kinase cascades in the insular cortex and the amygdala steering acquisition and retrieval of the CTA [7–11].

As every other learning process the conditioned response or memory trace of the CTA will progressively extinguish when subjects are repeatedly exposed to the CS in the absence of the US [12–15]. Compared to the initial learning process, our knowledge about the mechanisms responsible for the course of this extinction process is far more limited. However, already Pavlov revealed that extinction is not simply unlearning of initially acquired associations but rather a form of inhibition and new learning [15]. Many more recent experimental data confirmed that extinction is a form of

* Corresponding author.
E-mail address: martin.hadamitzky@uk-essen.de (M. Hadamitzky).

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re-learning rather than just erasure or degradation of the memory trace [12,16–22]. This hypothesis is further supported by observations indicating that extinction is specific to the context in which it is learned [9,11]. For instance, in a CTA paradigm where a saccharin-LiCl trace is conditioned in a certain context and later extinguished in a different context, the return to the original conditioning context is usually accompanied by a CS-induced recurrence of the conditioned response, known as *renewal effect* [23]. Likewise, changes in physical context together with changing the retention interval shows additive effects on the relapse of an extinguished CTA [11,24].

However, most studies of taste-aversion employed a LiCl-saccharin trace and a very unique experimental designs for analyzing context-dependency in extinction learning, making a generalizability of these results to other CTA paradigms and drugs used as US difficult. Our laboratory established a CTA paradigm in rodents employing saccharin as CS and the calcineurin inhibitor and potent immunosuppressive drug cyclosporine A (CsA) as US [2,10,25]. In contrast to LiCl, CTA with this immunosuppressant is not associated with nausea in rats since CsA *per se* does not induces symptoms of poisoning or illness. Here the immunosuppressive effects of CsA and its direct impact on brain functioning are probably responsible for the conditioned aversion against the CS (saccharin). In order to test whether contextual influence on taste-aversive learning and its extinction is a general effect, the present study investigated in a CTA paradigm with saccharin as CS and the immunosuppressant CsA as US the impact of context change on CTA extinction.

2. Materials and methods

2.1. Subjects

Adult male Dark Agouti rats (DA/HanRj, 200–230 g) purchased from Janvier (Le Genest-Saint-Isle, France) were single housed with ad libitum access to food and tap water. The vivarium was temperature (20 °C) and humidity (55 ± 5%) controlled and maintained on a reversed 12-h dark (7:00 a.m.–7:00 p.m.), 12-h light cycle to enable performance of the experiments during the normal active phase of the rats' awake/sleep cycle. Rats were allowed to acclimate to the new surroundings for two weeks before initiation of the experiments. All animal facilities, as well as experimental procedures were in accordance with National Institutes of Health and Association for the Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by the Institutional Animal Care and Use Committee (LANUV Düsseldorf, North Rhine-Westphalia).

2.2. Drugs

Based on previous experiments [25] a stock solution (100 mg/ml) of CsA (LC Laboratories, Woburn, USA) containing 100 μl ethanol (96%), and 900 μl Miglyol (Caelo, Germany) was diluted with sterile saline (0.9% NaCl, Braun, Germany) to gain the required drug dose of 20 mg/kg body weight at a final injection volume of 1 ml.

2.3. Behavioral conditioning protocol

Behavioral conditioning was performed as described elsewhere [10,26,27]. Specifically, rats were subjected to a water deprivation regime for five days with two drinking sessions per day, allowing them to drink for 15 min at 8:45 a.m and again at 5 p.m. each day. During the morning session on the sixth day, the acquisition phase started and a drinking solution containing 0.2% (w/v) sodium saccharin (Sigma–Aldrich, Schnelldorf, Germany) as CS was paired with an injection of 20 mg/kg CsA as US in the to-be-conditioned animals, while controls (*VEH*) received saccharin together with a saline injection (Fig. 1A, B). This pairing was repeated three times with a 72 h separation between the CS–US learning trials. All groups received water during the evening session of the three acquisition trials. Before and after each drinking session, the drinking bottles were weighed to measure fluid consumption. Individual mean water consumption in the morning sessions over these five days was taken as baseline level (100%) for "normal" fluid intake [8,28–30]. During six retrieval days both, conditioned rats (*CsA-groups*) and vehicle controls (*VEH*-groups) received the CS (saccharin) in the morning and water in the afternoon. However, to avoid any effect of temporal factors all experimental sessions were conducted starting at the same time each day (8:45 a.m).

2.4. Behavioral conditioning in the context experiment

Beside the animals' home cages –H– a new context A was used in this experiment which was established in a room, other than the vivarium, in the stlye of a recent study [31]. Specifically, in this context A the cages were equipped with black stripes and contained corn as a very differnet type of bedding material, as well as a toy brick. For conditioning six groups of rats were employed.

2.4.1. Preexposure

To analyze and exclude possible novelty effects, which could affect context dependency during conditioning, two groups of animals of the were preexposed to the new context A on the last three consecutive days during water deprivation [23] prior to conditioning (HAH-CsA-pre; HAH-VEH-pre). Preexposure to the new context always started 30 min before the morning drinking sessions. After these drinking sessions, animals remained in the new context for another 30 min and were then transferred back to their home cages (H).

2.4.2. Acquisition

By choosing a HAH-design, acquisition of CTA was conducted in the animals' home cages (H) as described in the behavioral conditioning protocol. In addition to the two pre-exposed groups (HAH-CsA-pre; HAH-VEH-pre), two more groups (HAH-CsA; HAH-VEH) were employed. As control two other groups were conditioned in a HHH-design HHH-CsA; HHH-VEH. All six groups were conditioned in their home cages (H).

2.4.3. Retrieval 1

To investigate a possible impact of context on extinction of the conditioned response (CTA) with CsA, retrieval was performed for six consecutive days. In all groups the general procedure of retrieval was identical as described in the behavioral conditioning protocol only differing in the contextual surroundings. For the HHH groups (HHH-CsA; HHH-VEH) testing was conducted in the same context as had been used for conditioning. For groups HAH (HAH-CsA; HAH-VEH) and HAH-pre (HAH-CsA-pre; HAH-VEH-pre), testing took place in the experimental context (A) different from that of the acquisition phase (H).

2.4.4. Retrieval 2

In order to analyze a possible spontaneous recovery of the conditioned response (CTA), following the six days of retrieval in the designated contexts, all groups were again tested in their home cages (H) after a retention interval of two days.

2.5. Data analyses

The descriptive statistics are based on means, and variance as indicated by the standard error of the mean (SEM). Statistical analyses were conducted using SigmaStat version 12.3 software (SPSS,