Neuroscience 303 (2015) 34–41

EXTINCTION OF CONDITIONED TASTE AVERSION IS RELATED TO THE AVERSION STRENGTH AND ASSOCIATED WITH C-FOS EXPRESSION IN THE INSULAR CORTEX

M. HADAMITZKY, [a]* **K. BÖSCHE,** [a] **A. ENGLER,** [b] **M. SCHEDLOWSKI** [a] **AND H. ENGLER** [a]

[a] *Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, University of Duisburg-Essen, 45122 Essen, Germany*

[b] *Clinic for Anesthesiology and Intensive Care Medicine, University Hospital Essen, University of Duisburg-Essen, 45122 Essen, Germany*

Abstract—Taste aversion learning is a type of conditioning where animals learn to associate a novel taste (conditioned stimulus; CS) with a stimulus inducing symptoms of poisoning or illness (unconditioned stimulus; US). As a consequence animals later avoid this taste, a reaction known as conditioned taste aversion (CTA). An established CTA extinguishes over time when the CS is repeatedly presented in the absence of the US. However, inter-individual differences in CTA extinction do exist. Using a model of behavioral conditioning with saccharin as CS and the immunosuppressant cyclosporine A as US, the present study aimed at further elucidating the factors underlying individual differences in extinction learning by investigating whether extinction of an established CTA is related to the strength of the initially acquired CS–US association. In addition, we analyzed the expression of the neuronal activation marker c-fos in brain structures relevant for acquisition and retrieval of the CTA, such as the insular cortex and the amygdala. We here show that animals, displaying a strong CS–US association during acquisition, maintained a strong CTA during unreinforced CS re-exposures, in contrast to animals with moderate CS–US association. Moreover, the latter animals showed increased c-fos mRNA expression in the insular cortex. Our data indicate that CTA extinction apparently depends on the strength of the initially learned CS–US association. In addition, these findings provide further evidence that the memory for the initial excitatory conditioning and its subsequent extinction is probably stored in those structures that participate in the processing of the CS and the US.

Key words: behavioral conditioning, extinction, insular cortex, CTA, c-fos.

INTRODUCTION

Conditioned taste aversion (CTA) is a established learning paradigm where rats learn to associate a novel taste (conditioned stimulus; CS) with a drug or other treatment (unconditioned stimulus; US), that induces symptoms of poisoning or illness (Garcia et al., 1955; Ader, 1985; Bures et al., 1998). During the learning phase (acquisition), the CS is presented once or multiple times together with the US, leading to the establishment of a CS–US engram. Re-exposure to the CS alone leads to retrieval of the newly acquired memory (Bermudez-Rattoni and McGaugh, 1991; Bermudez-Rattoni, 2004). On the behavioral level, the conditioned response is characterized by avoidance of the CS, evident by a reduced consumption of the gustatory stimulus (Garcia et al., 1955; Bermudez-Rattoni, 2004; Wirth et al., 2011). A number of anatomical and pharmacological studies have shown that the neural network involved in taste-visceral associative learning is mediated via a basic neural circuit comprising sensory and hedonic pathways (Yamamoto, 1993; Sewards, 2004). These include the nucleus tractus solitarius, the parabrachial nucleus, the medial thalamus, the amygdala, and the insular cortex (Yamamoto et al., 1994; Sewards, 2004). Specifically, the insular cortex is essential for acquisition and retention of associative learning (Bermudez-Rattoni and McGaugh, 1991; Cubero et al., 1999), and has been shown to be involved in integration of gustatory and visceral stimuli (Sewards and Sewards, 2001; Pacheco-Lopez et al., 2005).

The initially strong avoidance of the CS is gradually reduced and almost completely eliminated upon subsequent non-reinforced re-exposure to the gustatory CS, a process called extinction (Pavlov, 1927; Berman and Dudai, 2001; Dudai, 2002; Bermudez-Rattoni, 2004). Extinction is defined as a form of learning in which associations between cues (CS) and the events (US) they predict are weakened by exposure to the cues in the absence of those events (Myers and Carlezon, 2010). Berman and Dudai (2001) found that extinction of CTA memory depends on protein synthesis and β-adrenoceptors in the insular cortex. Moreover, it has been shown that extinction of CTA leads to pronounced changes in c-fos expression in the medial prefrontal- and the gustatory cortex (Mickley et al., 2004; Mickley et al., 2005).

*Corresponding author. Address: Institute of Medical Psychology and Behavioral Immunobiology, University Hospital Essen, Hufeland-strasse 55, 45122 Essen, Germany. Tel: +49-201-723-85516; fax: +49-201-723-5948.

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

Abbreviations: ANOVA, analysis of variance; c-FLI, c-fos-like immunoreactivity; CS, conditioned stimulus; CsA, cyclosporine A; CTA, conditioned taste aversion; LiCl, lithium chloride; MTA, moderate taste aversion; NTS, nucleus of the solitary tract; US, unconditioned stimulus.

http://dx.doi.org/10.1016/j.neuroscience.2015.06.040

Pre-exposure to the CS, reduced CS intensity, and longer CS–US delays are well-known factors influencing the strength of CTA learning (Bernstein and Koh, 2007). In addition, inter-individual differences in CTA extinction do exist (Exton et al., 1998b). Against this background, the present study investigated in rats whether extinction of a CTA is related to the strength of the initially acquired CS–US association, using a established model of behavioral conditioning with saccharin as CS and the immunosuppressive drug cyclosporine A (CsA) as US (Exton et al., 2001; Wirth et al., 2011) (Wirth et al., 2011). CsA inhibits the protein phosphatase calcineurin and leads to a suppression of essential T cell functions mainly through inhibition of interleukin (IL)-2 and interferon (IFN)-γ production (Halloran et al., 1999). In experiments of short and long retrieval, animals were divided into groups with either a strong (STA) or a moderate taste aversion (MTA) based on their overall CTA performance. To analyze whether the observed differences in taste aversion learning were related to differences in neuronal activation, we then measured expression of the immediate early gene c-fos as a marker of neuronal activity (Sagar et al., 1988; Herbert et al., 1990; Morgan and Curran, 1991) in brain structures that previously have been shown to be relevant for acquisition and retrieval of the CTA (Yamamoto, 1993; Yamamoto et al., 1994; Pacheco-Lopez et al., 2005).

EXPERIMENTAL PROCEDURES

Subjects

A total of 86 male Dark Agouti rats (DA/HanRj, 200–230 g; Janvier, France) were used for the experiments described. After arrival, animals were allowed to acclimate to the new surroundings for two weeks before initiation of any experimental procedure. Subsequently, rats were single-housed with *ad libitum* access to food, and tap water was available *ad libitum* until the water deprivation regimen started. The vivarium was temperature (20 °C) and humidity (55 ± 5%) controlled and animals were maintained under a 12/12-h reversed light/dark cycle (lights off at 7:00 a.m.) to conduct the experiments during the activity phase of the awake/sleep cycle. All experimental procedures were conducted under red light conditions. The animal facilities and 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).

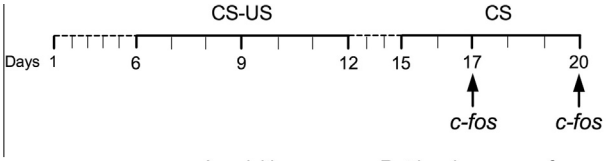
Drugs

A stock solution (100 mg/ml) of CsA (LC Laboratories, Woburn, MA, USA) containing 900 µl Miglyol (Caelo, Germany) and 100 µl ethanol (96%) was diluted with sterile saline to gain the required drug dose of 20 mg/kg body weight at a final injection volume of 1 ml (Exton et al., 1998a).

Behavioral task

The behavioral conditioning protocol has previously been described in detail (Pacheco-Lopez et al., 2005; Niemi et al., 2007; Pacheco-Lopez et al., 2009). Briefly, rats were placed on a water deprivation regime for 5 days, allowing them 15 min of drinking at 8:45 a.m and again at 5 p.m. each day. Individual mean water consumption in the morning sessions over these days was taken as baseline level of "normal" fluid intake. On the 6th day acquisition started in the morning session by presenting the CS, a drinking solution containing 0.2% (w/v) sodium saccharin (Sigma–Aldrich, Schnelldorf, Germany) followed by an intraperitoneal (i.p.) injection of CsA (20 mg/kg), used as US in the conditioning groups (Fig. 1). During the evening sessions of the acquisition animals in all groups received water. The conditioning protocol consisted of three learning trials (CS–US pairings) separated by 72 h. Recent studies have shown, that in taste aversion with CsA extinction of the CTA most commonly occurred between four to seven CS re-exposures (Exton et al., 1998b). Thus, for retrieval, animals were subjected to either three (Experiment 1; n = 8–14 per group) or six (Experiment 2; n = 8–14 per group) non-reinforced CS exposures. Retrieval trials were separated by 24 h while animals were re-exposed to saccharin in the morning sessions and to water in the afternoon sessions. A pharmacological control group (*US*) received water during both occasions (for group allocation see Fig.1). To monitor CTA, drinking bottles were weighed before and after each drinking session and fluid consumption was assessed.

After performing taste aversion experiments with three or six CS re-exposures (each experiment was performed twice with different sets of rats), the initial strength of the CTA during acquisition was compared with the level of CTA during CS re-exposure. Animals with a constantly strong taste aversion (< 20% of the water baseline) during the last acquisition trial and all subsequent extinction trials were assigned to one group (strong taste aversion, *STA*) and compared to those animals



		Acquisition		Retrieval		c-fos
Groups		**Morning**	**Evening**	**Morning**	**Evening**	**60min**
Conditioned	**MTA**	Sac/CsA	Wat	Sac	Wat	Sac
Conditioned	**STA**	Sac/CsA	Wat	Sac	Wat	Sac
CsA treated	**US**	Sac/CsA	Wat	Wat+CsA	Wat	Wat+CsA
Vehicle	**VEH**	Sac/VEH	Wat	Sac	Wat	Sac

Fig. 1. Experimental design and group allocation. Animals in the moderate taste aversion (*MTA*), strong taste aversion (*STA*), and pharmacological control (*US*) groups were conditioned with saccharin (Sac) and cyclosporine A (CsA; 20 mg/kg, i.p.) during acquisition (A). During retrieval, in the morning sessions all groups were re-exposed to Sac, except the *US* group, which received always water together with a injections of CsA (20 mg/kg, i.p.) over all either three (Experiment 1) or six (Experiment 2) retrieval days (B). Subsequently, c-fos expression was analyzed in the amygdala and insular cortex 60 min after the last morning session on the 3rd or 6th retrieval day, respectively.