Hormones and Behavior 86 (2016) 21–26

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

# Chronic corticosterone treatment enhances extinction-induced depression in aged rats

Joseph P. Huston [a,*], Mara Komorowski [a], Maria A. de Souza Silva [a], Valéria Lamounier-Zepter [b], Susanne Nikolaus [c], Claudia Mattern [d], Christian P. Müller [e], Bianca Topic [a]

[a] Center for Behavioral Neuroscience, Institute of Experimental Psychology, Heinrich-Heine-University of Düsseldorf, Universitätsstr. 1, 40225 Düsseldorf, Germany
[b] Medical Clinic III, University of Technology Dresden, Fetscherstr. 74, 01307 Dresden, Germany
[c] Clinic of Nuclear Medicine, University Hospital Düsseldorf, Düsseldorf, Germany
[d] M et P Pharma AG, 6376 Emmetten, Switzerland and Oceanographic Center, Nova Southeastern University, Fort Lauderdale, FL 33314, USA
[e] Department of Psychiatry and Psychotherapy, University Clinic, Friedrich-Alexander-University of Erlangen-Nuremberg, Schwabachanlage 6, 91054 Erlangen, Germany

ARTICLE INFO

*Article history:*
Received 23 May 2016
Revised 8 September 2016
Accepted 10 September 2016
Available online 12 September 2016

*Keywords:*
Depression model
Extinction
Despair
Corticosterone
ACTH
Chronic stress
Goal tracking

ABSTRACT

Withdrawal and avoidance behavior are common symptoms of depression and can appear as a consequence of absence of reward, i.e. extinction-induced depression (EID). This is particularly relevant for the aged organism subjected to pronounced loss of former rewards. Avoidance of the former site of reward and increased withdrawal into a distant compartment accompany extinction of food-rewarded behavior in rodent models. During extinction, behavioral markers for re-learning dissociate from indicators of extinction-induced depression. Here we examined the effect of a chronic treatment with corticosterone (CORT), a well-known inducer of depression-related behavior, on EID in adult and aged rats. Adult (3–4 months) and aged (18 months) male rats were treated with CORT via drinking water for 3 weeks prior to extinction of a cued food-reward task. CORT treatment increased the distance from the site of reward and decreased goal tracking behavior during extinction, especially in the aged rats. Plasma hormone levels measured before and after restraint stress showed a decline in basal ACTH- and CORT-levels after chronic CORT treatment in aged animals. The treatment significantly impaired the HPA-axis activation after acute stress in both, adult and aged animals, alike. Altogether, these findings show an enhancement of EID after chronic CORT treatment in the aged organism, which may be mediated by an impaired HPA-axis sensitivity. These findings may have special relevance for the investigation of human geriatric depression.



## 1. Introduction

Loss of reward or reinforcement play a crucial role in the psychopathology of major depression and can lead to despair, anhedonia, and withdrawal (Wittchen et al., 2011), resulting in avoidance of former desirable situations (Lewinsohn, 1974). The onset of depression is often preceded by stressors and experiences of loss such as unemployment, disease or parting (Kendler et al., 1999). Experiences of loss affect particularly the aged population and may account for the increasing number of old people suffering from depression (Bruce, 2002).

The absence of formerly delivered reinforcers induces cessation, and thus, "extinction" of behavior that previously led to the reinforcer. Extinction is considered to be an active learning process, rather than passive forgetting or erasure of memories (Todd et al., 2014). Extinction of operant behavior in vertebrates has a behavioral, allocentric component, which involves learning that a particular appetitive behavior no longer leads to reward. However, extinction also has emotional, egocentric components, as the omission of an expected reward can induce emotional reactions, including frustration, despair, and aggression (Huston et al., 2013; Papini and Dudley, 1997; Papini, 2003). Animal models for extinction-induced depression (EID) have been developed only recently. It was shown that 'distance from the food cup' and the cessation of 'goal tracking' during extinction are sensitive to antidepressant treatment, and thus, reliable measures of EID (Huston et al., 2012, 2013, 2016; Komorowski et al., 2012). In aged rats, changes in the amount of striatal neurotransmitters (Schulz et al., 2004), neurotrophins (Topic et al., 2008a) and mineral−/glucocorticoid-receptors in the brain after extinction were reported, which correlated with EID measures upon the withholding of negative reinforcement (Topic et al., 2008b). Since especially the aged are confronted with life events that subsume loss of positive reinforcers (Kraaij et al., 2002), we have set out to examine how EID interacts with a stressor in the aged organism. In the present study, we asked how chronic corticosterone (CORT) stress affects EID during aging. We hypothesized that

* Corresponding author at: Center for Behavioral Neuroscience, University of Duesseldorf, Universitaetsstr. 1, 40225 Duesseldorf, Germany.
*E-mail address:* Huston@uni-duesseldorf.de (J.P. Huston).

http://dx.doi.org/10.1016/j.yhbeh.2016.09.003
0018-506X/

CORT treatment would exacerbate EID by increasing avoidance of the former site of reward-delivery and avoidance of goal tracking during extinction predominantly in aged rats.

## 2. Materials and methods

### 2.1. Subjects

Twenty-four adult (3–4 months) and thirty aged (18 months) naive, male Wistar rats were obtained from Zentrale Einrichtung für Tierforschung und Tierschutzaufgaben (University of Düsseldorf; adults: 253.21 ± 3.6 g, aged: 601.93 ± 11.86 g). Water and food were provided ad libitum until food deprivation started. Animals were subjected to a 12 h light/dark cycle (lights on: 7 am) with room temperature maintained at 22 ± 2 °C in the husbandry room. They were housed in type VI macrolon cages in groups of four (adult) and 2–3 (aged). After one week of familiarization, the animals were weighed, marked and food deprived (15 g/animal/day for aged, 12 g/animal/day for adult). The total daily fluid intake of each cage was measured during food deprivation so that the approximate amount of water intake could be calculated for each rat by division. Three handling sessions were carried out prior to the experiment. Food deprivation and weighing were continued until the end of the study. After the acquisition sessions, animals were randomly assigned to the control- or CORT-group in equal numbers ($n = 15$/group for aged, $n = 12$/group for adult rats). The study was carried out in accordance with the German Law on the Protection of Animals and was approved by the state authority (Bezirksregierung Düsseldorf).

### 2.2. Apparatus and procedure

Elongated operant chambers with walls made of dimmed Plexiglas were used (Komorowski et al., 2012; Huston et al., 2016). The chambers were 72 cm long, 28 cm wide and 34 cm high (Fig. 1). The floor was covered with a black rubber mat and the top of the box was open. Each operant chamber was situated in a dark, sound attenuating box, containing a masking white noise of 60 dB. Infra-red cameras (Conrad Elektronik®) were mounted on the top and side of the chambers. The chambers were segmented by half into two compartments by additional side walls made of Plexiglas. Animals could freely enter both compartments through a transition in the middle of the walls. A triple cue light (green, yellow, red), the food magazine (BioServ® Dustless precision pellets) and the food cup (Coulbourn Industries®) with an integrated photodetector were fastened in the middle of the short wall. A house light offered slight illumination (~1 lx). For the magazine training sessions, an external hand-switch was connected to the apparatus to shape the animals' behavior towards the feeder. All modules were connected via a link box from PanLab® and controlled by the PackWin® 2.0.01 Software. Automated tracking of the animals' behavior was done via center-point detection and manually by an experienced rater with Ethovision XT 8® (Noldus®). The sound attenuated boxes were located within the animals' husbandry room.

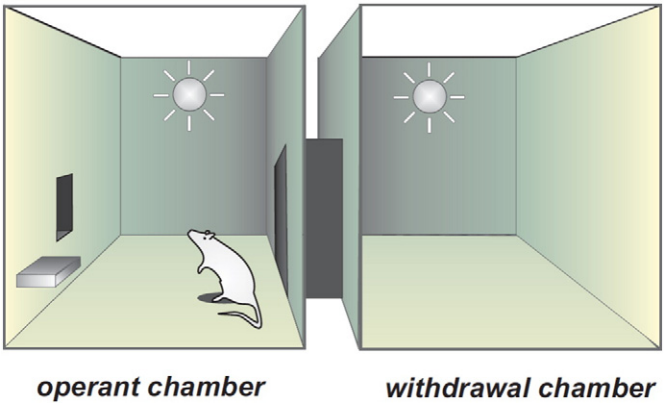


**Fig. 1.** The two-compartment operant chamber.

Three days prior to the experiment, animals received 1 g of the BioServ® pellets daily additionally to their restricted regular rat chow. On the first day of the experiment, all animals underwent a habituation session of 15 min and could freely explore the chamber. Two magazine training sessions of 15 min were performed on the consecutive two days (Komorowski et al., 2012). Hereby, the cue lights were turned on for 5 s each time when reinforcement was delivered. Within the next days, six daily acquisition trials of 15 min were performed with all subjects in random order. A fixed interval cued reward schedule of 30 s (FI 30) was applied whereby the cue lights were turned on every 25 s for 5 s and two food pellets were delivered immediately at the onset. After the last day of acquisition the aged and adult subjects were each assigned to one of two groups, either receiving regular drinking water (vehicle) or CORT-enriched water (CORT; ~6–9 mg/day/kg, depending on the amount of water intake/rat). For the next 23 days, the animals received fresh drinking water daily and the water intake was measured for each 24 h-period to control the intake of CORT. *Re*-acquisition trials were performed on every 3rd day. After 23 days of treatment and re-training, extinction trials were run daily for five days under continuous food deprivation and treatment. Here, the cue lights were presented for 15 min with an FI 30 free reward schedule as well, but without coincident food delivery (Komorowski et al., 2012; Huston et al., 2016). The following variables were recorded as: a.) *EID-related*: mean 'distance to the food cup' and 'goal tracking' (direct contact with the food cup by entering or touching it); b.) *Re-learning-related*: 'number of beam breaks' in the food cup, 'latency between cue light onset and first consecutive beam break', 'sign tracking' (glances/rears towards the cue lights), and residence within the withdrawal compartment of the chamber, and c.) 'horizontal activity' (distance moved) and 'vertical activity' (rearing). The withdrawal compartment was situated in the latter part of the chamber and therefore offered the possibility to withdraw. Goal tracking (direct contact with the food cup by entering or touching it) and sign tracking (glances/rears towards the cue lights) were recorded manually by an experienced and blinded rater.

### 2.3. Restraint stress and blood analysis

In order to test for responsiveness of the hypothalamus–pituitary–adrenal (HPA)-axis, a restraint stress procedure was carried out 3 weeks after extinction. Treatment was continued until then. Immediately after a baseline blood sample ($t = 0$), animals were put into restrainers (Harvard Apparatus®), where they remained for 10 min. The second sample was taken in the restrainer after 10 min ($t = 10$) and further samples in a single cage after 60 ($t = 60$), 120 ($t = 120$) and 180 min ($t = 180$). Blood samples were taken by tail incision (Fluttert et al., 2000). Blood was collected in 300 μl EDTA-coated vials (Microvette® CB 300 Sarstedt®). A red light was mounted over the restrainers to provide warmth. Each subject was taken to the lab individually and remained in a single cage to recover for 2 h after the last sampling. Samples were centrifuged at 4 °C for 10 min with a speed of 4000 rpm. The plasma was separated and stored by −80 °C. For plasma hormone analysis, only animals with sufficient samples could be used, resulting in an $n = 7$–10/group. Adrenocorticotropic hormone (ACTH) plasma concentrations were analysed by chemo-iluminescence immunometric assay (Immulite® 2000 ACTH, Siemens, Erlangen, Germany) and CORT plasma concentrations by ELISA (IBL International, Hamburg, Germany).

### 2.4. Drugs & application

Animals were treated chronically for 28 days until the last day of extinction. Before the onset of the treatment, the daily water intake of the