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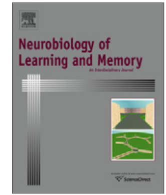

Both mineralocorticoid and glucocorticoid receptors regulate emotional memory in mice

Ming Zhou [a,*], Eveline H.M. Bakker [a], Els H. Velzing [a], Stefan Berger [b], Melly Oitzl [c], Marian Joëls [a,d], Harm J. Krugers [a]

[a] Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, Science Park, 1098 XH Amsterdam, The Netherlands
[b] German Cancer Research Center, Division Molecular Biology of the Cell I, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany
[c] Gorlaeus Lab, Division of Medical Pharmacology, LACDR/LUMC, Leiden University, Einsteinweg 55, 2300 Leiden, The Netherlands
[d] Rudolf Magnus Institute of Neuroscience, Department of Neuroscience and Pharmacology, University Medical Center Utrecht, 3508 AB Utrecht, The Netherlands

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ABSTRACT

Corticosteroid hormones are thought to promote optimal behavioral adaptation under fearful conditions, primarily via glucocorticoid receptors (GRs). Here, we examined – using pharmacological and genetic approaches in mice – if mineralocorticoid receptors (MRs) also play a role in fearful memory formation. As expected, administration of the GR-antagonist RU38486 prior to training in a fear conditioning paradigm impaired contextual memory when tested 24 (but not when tested 3) h after training. Tone-cue memory was enhanced by RU38486 when tested at 4 (but not 25) h after training. Interestingly, pre (but not post)-training administration of MR antagonist spironolactone impaired contextual memory, both at 3 and 24 h after training. Similar effects were also found in forebrain-specific MR knockout mice. Spironolactone also impaired tone-cue memory, but only at 4 h after training. These results reveal that – in addition to GRs – MRs also play a critical role in establishing fear memories, particularly in the early phase of memory formation.

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1. Introduction

Exposure to emotionally arousing events activates the Hypothalamo–Pituitary–Adrenal (HPA) axis. As a consequence, enhanced levels of corticosteroid hormones (corticosterone in most rodents and cortisol in humans) are released into the circulation (de Kloet, Joels, & Holsboer, 2005). Corticosteroid hormones enter the brain and bind to two receptor subtypes; the mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs). MRs are occupied when hormone levels are low and exert their effects classically via transcriptional regulation of responsive genes (de Kloet et al., 2005). However, recent evidence shows that corticosteroid hormones can also exert rapid non-genomic effects via MRs (Karst, Berger, Erdmann, Schutz, & Joels, 2010; Karst et al., 2005; Olijslagers et al., 2008). Compared to MRs, GRs have a 10-fold lower affinity for corticosterone, become activated when hormone levels rise after stress and slowly exert genomic actions (de Kloet et al., 2005; Joels, Pu, Wiegert, Oitzl, & Krugers, 2006) but also non-genomic effects (Di, Malcher-Lopes, Halmos, & Tasker, 2003; Karst et al., 2010).

Corticosteroid hormones alter neuronal activity in areas that play central roles in attention and selection of appropriate behavioral strategies (i.e. hippocampus, prefrontal cortex and amygdala; (de Kloet et al., 2005; Joels, 2010; Roozendaal et al., 2009). As part of behavioral adaptation to stressful events, these hormones via activation of MRs and GRs, in interaction with other hormones and neurotransmitters (Joels & Baram, 2009; Roozendaal et al., 2009), promote the storage of information (McEwen & Gianaros, 2010; Oitzl, Champagne, van der Veen, & de Kloet, 2010).

Corticosteroid hormones in vitro rapidly increase neuronal activity in the hippocampus (Karst et al., 2005; Olijslagers et al., 2008) and amygdala (Karst et al., 2010) via a mechanism that requires MRs activation. Behavioral studies indicate that MRs are involved in appraisal of information and response selection in various tasks (Brinks, van der Mark, de Kloet, & Oitzl, 2007; Oitzl & de Kloet, 1992; Oitzl et al., 2010; Sandi & Rose, 1994a). Moreover, genetic deletion of MRs in the forebrain led to various cognitive impairments, including impaired learning in a Morris water-maze task (Berger et al., 2006). In contrast, via the activation of GRs, a gene-mediated cascade is initiated to slowly restore neuronal activity and suppress synaptic plasticity (Joels & de Kloet, 1989; Kerr, Campbell, Hao, & Landfield, 1989; Kim & Diamond, 2002). At the behavioral level, activation of GRs promotes consolidation of the acquired stressful information at the later phase of memory formation (Lupien & McEwen, 1997; Oitzl, Reichardt, Joels, & de Kloet, 2001; Pugh, Fleshner, & Rudy, 1997a; Pugh, Tremblay, Fleshner, & Rudy, 1997b; Roozendaal, 2003).

* Corresponding author. Fax: +31 20 525 7709.
E-mail address: m.zhou@uva.nl (M. Zhou).

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The existing data on the role of MRs in neuronal activity and cognitive function justifies the question whether MRs also play a role during the early phase of memory formation, in addition to the role of GRs in promoting memory formation at later time points (e.g. up to 1 day after acquisition of information). We therefore tested this hypothesis by examining whether specific blockade of MRs and GRs interferes with contextual and tone-cue memory formation at two different time points (i.e. contextual memory tested either 3 or 24 h after training, tone-cue memory tested 1 h later, i.e. either 4 or 25 h after training). These time points (3–4 h versus 24–25 h) presumably reflect different learning phases (early encoding versus long-term memory) and cellular events that may underlie the learning process (Zhou, Conboy, Sandi, Joels, & Krugers, 2009). Corticosteroids have been reported to particularly modulate synaptic plasticity evoked by weak stimulation paradigms (Alfarez, Wiegert, Joels, & Krugers, 2002; Pu, Krugers, & Joels, 2009) and promote transition from short- to long-term memory in a relatively weak aversive learning paradigm (Cordero & Sandi, 1998; Sandi & Rose, 1994b). We therefore tested the roles of MRs and GRs both on contextual and tone-cue memory formation using mild (less aversive) and relatively strong (more aversive) learning paradigm, with shock intensities of 0.4 and 0.8 mA respectively.

2. Materials and methods

2.1. Animals

Male C57/BL6 mice (6–8 weeks old, derived from Harlan, The Netherlands) and forebrain-specific male MR-deficient animals ($MR^{CAMKCre}$ mice (Berger et al., 2006), 4–5 months old, bred at Leiden University, Leiden) were individually housed 1–2 weeks before the experiment started. Within one experiment, control and experimental animals were trained in a random fashion. All animals were kept at a light/dark cycle of 12 h (lights on at 8 a.m.; room temperature kept at 22 °C ± 2). Food and water were given without restriction. The experiments were carried out in accordance with and approved by the local Animal Committees of the University of Amsterdam and Leiden University.

2.2. Drugs

To examine the roles of GRs and MRs in fear conditioning, we pharmacologically targeted these receptors using the GR-antagonist RU38486 (mifepristone, Sigma) and the MR antagonist spironolactone (Sigma) respectively. RU38486 or its vehicle (DMSO) was administered intra-peritoneally (i.p.) 1 h before training at a dosage of 10 mg/kg which is sufficient to prevent GR mediated effects (Pugh et al., 1997a). Spironolactone or its vehicle (propylene glycol) was injected subcutaneously (s.c.) 1 h before or immediately (within less than 5 min) after training at a dosage of 50 mg/kg which blocks MR mediated effects (Herman & Spencer, 1998; Kumar et al., 2007). The dose of spironolactone was taken as an effective and well-tolerated dose with little effects on spontaneous behavior as documented before (Adamec, Muir, Grimes, & Pearcey, 2007; Koenig & Olive, 2004).

2.3. Immunocytochemistry

Brains from $MR^{CAMKCre}$ and wild type mice were immersion-fixed for 24 h in 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) and stored in PB (+sodium azide) at 4 °C. Before sectioning, the brains were washed and cryoprotected by overnight incubation in 20% sucrose in phosphate buffered saline (PBS, pH 7.4). Frozen coronal sections (30 μm thick) were cut using a sliding microtome and stored in PB (+sodium azide) until needed. Slices were washed thoroughly with PB to get rid of sodium azide, then rinsed with 1% H_2O_2 in 0.1 M Tris Buffered Saline (TBS, pH 7.4) to block endogenous peroxidase activity, followed by washing with TBS, TBS + Triton-X100 (TBS–TX) and TBS. Then, sections were incubated with the first antibody against MR (1D5, 1:500, Alfarez et al., 2009) in TBS for 48 h in a cold room, kept for another 30 min at room temperature and washed in TBS. No blocking step prior to primary antibody application was applied since background staining was virtually absent. Next, sections were incubated for 2 h with the secondary antibody (biotinylated Sheep-anti-Mouse 1:200 in TBS–TX, Amersham Biosciences) at room temperature, followed by washing with TBS and 2-h incubation in ABC-elite (1:800) in TBS–TX at room temperature. Then, sections were washed in TBS and 0.05 M TB (pH 7.6), followed by a DAB reaction. The duration of the reaction was established specifically for this staining; for negative controls always the same duration was kept as for the experimental slices. Specificity of the first antibody was confirmed by omitting the first antibody, which revealed in no staining. The reaction was stopped with three washes in 0.05 M TBS and two washes in TBS before slices were mounted.

2.4. Fear conditioning

Procedures were comparable to those described before (Zhou et al., 2009). The grid floor of the fear conditioning chamber (30 cm × 24 cm × 26 cm; W × L × H) was made of 37 stainless steel rods and connected to a shock generator (Med-Farm LION-ELD) that was developed in-house. During training (between 8:30 and 11:30 am) one mouse at a time was put into the training chamber (cleaned with 1% acetic acid) and was allowed to freely explore the chamber for 3 min before three tone-foot-shock pairs were introduced with an interval of 1 min. Each tone (100 dB, 2.8 kHz) lasted 30 s and was accompanied by a foot-shock of either 0.4 mA or 0.8 mA during the last 2 s. Thirty seconds after the end of the last pairing, the mouse was taken back to its home cage. Three hours or 24 h (in separate experimental groups) later the animal was introduced into the same chamber for 3 min to test contextual memory, followed by tone-cue memory test 1 h later, in a novel chamber with different contextual background. This cage was cleaned with 70% ethanol. After a free exploration period of 3 min the animals were exposed to the same tone (100 dB, 2.8 kHz) only once for 30 s. One minute later the animal was placed back into its home cage. Freezing behavior, defined as no body movements except those related to respiration, was determined every 2 s throughout the training period and during contextual and tone-cue memory testing. The percentage of freezing time was used for statistical analysis. During the training session, freezing behavior immediately after each of the tone-foot-shock pairings was recorded to examine effects on training over time. For the contextual memory test, total freezing behavior over the entire 3 min was compared between groups. In order to examine possible effects over time (Zhou et al., 2009), the whole 3 min was split into two periods, 90 s each. Then, data was averaged per period and between- as well as within-group effects over time were then studied. During the tone-cue memory test, freezing behavior during free exploration and the 30 s tone presentation was recorded and compared between the experimental groups.

2.5. Statistical analysis

Data was analyzed by repeated measures ANOVA or using a two-tailed independent-samples Student's *t*-test. Results are presented as mean ± SEM. *P* values smaller than 0.05 were considered significantly different.

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