Psychoneuroendocrinology (2012) 37, 1676—1687

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# Chronic psychosocial stress results in sensitization of the HPA axis to acute heterotypic stressors despite a reduction of adrenal *in vitro* ACTH responsiveness

**Nicole Uschold-Schmidt, Kewir D. Nyuyki, Andrea M. Füchsl, Inga D. Neumann, Stefan O. Reber***

*Department of Behavioural and Molecular Neurobiology, University of Regensburg, 93053 Regensburg, Germany*

Received 11 January 2012; received in revised form 27 February 2012; accepted 27 February 2012

**KEYWORDS**
Chronic psychosocial stress;
CSC;
Heterotypic stressor;
HPA axis;
Adrenal glands;
ACTH

**Summary** Although chronic psychosocial stress is often accompanied by changes in basal hypothalamo-pituitary-adrenal (HPA) axis activity, it is vital for a chronically-stressed organism to mount adequate glucocorticoid (GC) responses when exposed to acute challenges. The main aim of the present study was to test whether this is true or not for the chronic subordinate colony housing (CSC, 19 days) paradigm, an established and clinically relevant mouse model of chronic psychosocial stress. As shown previously, CSC mice are characterized by unaffected morning and decreased evening plasma corticosterone (CORT) levels despite enlarged adrenals, suggesting a maladaptive breakdown of adrenal functioning.

Plasma CORT levels, determined by repeated blood sampling *via* jugular vein catheters, as well as relative right adrenal CORT content were increased in CSC compared with single-housed control (SHC) mice in response to acute elevated platform (EPF, 5 min) exposure. However, *in vitro* stimulation of adrenal explants with physiological and pharmacological doses of ACTH revealed an attenuated responsiveness of both the left and right adrenal glands following CSC, despite mRNA and/or protein expression of melanocortin 2 receptor (Mc2r), Mc2r accessory protein (MRAP), and key enzymes of steroidogenesis were not down-regulated.

Taken together, we show that chronic psychosocial stressor exposure impairs *in vitro* ACTH responsiveness of both the left and right adrenal glands, whereas it increases adrenal responsiveness to an acute heterotypic stressor *in vivo*. This suggests that an additional factor present during acute stressor exposure *in vivo* rescues left and right adrenal ACTH sensitivity, or itself acts as CORT secretagogue in chronically stressed CSC mice.


* Corresponding author at: Chronic Stress Group, Department of Behavioural and Molecular Neurobiology, University of Regensburg, Universitaetsstrasse 31, 93053 Regensburg, Germany. Tel.: +49 941 9433050; fax: +49 941 9433052.
*E-mail addresses:* stefan.reber@biologie.uni-regensburg.de, stefanreber@gmx.de (S.O. Reber).

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doi:10.1016/j.psyneuen.2012.02.015

## 1. Introduction

Exposure to repeated or chronic stressors has been shown to alter the activity and responsiveness of the hypothalamo-pituitary-adrenal (HPA) axis. For example, adrenocorticotropic hormone (ACTH) secretion from the pituitary gland is down-regulated in response to repeated homotypic stressor exposure (for review see Aguilera, 1994), whereas subsequent exposure to an acute heterotypic stressor results in ACTH responses that match or even exceed those of control animals exposed to the same acute stressor (Berton et al., 1999; Chen et al., 2008). Moreover, repeated or chronic stressors often result in an increased sensitivity of the adrenals to ACTH (Zelena et al., 2003; Engler et al., 2005; Droste et al., 2006; Vining et al., 2007) and, thus, chronically elevated basal plasma glucocorticoid (GC) levels (Dhabhar and McEwen, 1997; Engler et al., 2005). These observations suggest that the attenuated ACTH response during repeated stress reflects habituation to familiar stimuli to prevent excessive levels of deleterious GC, while the resultant sensitized ACTH response to a heterotypic stressor allows a sufficient acute GC response to novel threats (Berton et al., 1999; Chen et al., 2008). Interestingly, in contrast to the level of the pituitary, to our knowledge, nothing is known so far about the role of the adrenal glands with respect to adaptation/sensitization of the HPA axis during chronic and subsequent heterotypic stressor exposure.

Unlike repeated homotypic stressor exposure, chronic subordinate colony housing (CSC, 19 days) represents a mouse model of chronic psychosocial stress. Importantly, it has already been shown that CSC exposure alters HPA axis functionality, particularly at the adrenal level. In detail, despite enlarged adrenals (Reber et al., 2007) CSC mice show normal basal morning GC levels and are incapable of mounting the circadian rise in plasma GC (Reber et al., 2007). In support, the *in vitro* corticosterone (CORT) response to various ACTH doses of isolated adrenal cells (pooled from both left and right glands) was found to be strongly attenuated following CSC (Reber et al., 2007). Although very likely it still has to be proven, whether the latter finding indeed reflects the mechanism underlying CSC-induced dark phase hypocorticism, as cell-to-cell contacts and adrenal medullary cells are missing in isolated adrenal cortical cell preparations. These are known to substantially influence the responsiveness of the adrenal gland to ACTH (for review see Ehrhart-Bornstein et al., 1998; Ehrhart-Bornstein and Bornstein, 2008).

Taken together, these data strongly suggest that CSC exposure causes adrenal insufficiency, mediated by a reduction/loss of adrenal ACTH responsiveness. Therefore, we hypothesize that the CSC-induced physiological changes underlying the attenuated adrenal responsiveness to ACTH represent a maladaptive consequence of, rather than a beneficial adaptation to, chronic psychosocial stress. Consequently, CSC mice should not be able to mount an adequate CORT response to heterotypic stressors. This hypothesis is in line with previous findings showing no attenuation of HPA axis activity and, thus, no adaptation to repeated social stressors (Keeney et al., 2001; Bailey et al., 2004; Engler et al., 2005).

It is important to mention that there are body-side specific differences in adrenal functioning both during basal and stimulated physiological conditions. For example, an increased weight of the left compared with the right adrenal has been found for instance in both rats (Droste et al., 2007) and mice (Droste et al., 2003, 2006) under unstressed conditions. Moreover, there is evidence from the same two rodent species for a body-side specific weight gain of only the right adrenal during prolonged voluntary wheel running (Droste et al., 2003, 2006, 2007) and an increase in specifically the left adrenal weight has been described in humans who committed suicide (Szigethy et al., 1994; Dumser et al., 1998). Therefore, development of body-side specific differences in the reduction/loss of adrenal ACTH responsiveness between left and right adrenals following CSC is not unlikely. Importantly, this hypothesis is not in contrast to our previous data showing an attenuated, but not totally abolished, ACTH responsiveness of pooled left and right adrenal cells of CSC mice (Reber et al., 2007).

Possible mechanisms underlying the reduction/loss of adrenal ACTH responsiveness could be a general or a body-side specific reduction in the expression of the melanocortin 2 receptor (Mc2r), the main receptor for ACTH in the adrenal glands (Xia and Wikberg, 1996; Gorrigan et al., 2011), or of Mc2r accessory protein (MRAP). MRAP is known to play an important role for Mc2r trafficking, cell surface expression and function, and mutations in MRAP have been shown to cause familial glucocorticoid (GC) deficiency type 2 (Metherell et al., 2004, 2005; Clark et al., 2005a,b). Furthermore, effects of CSC on expression levels of steroid acute regulatory protein (StAR), side-chain cleavage enzyme (CYP11A1), 11β-hydroxylase (CYP11B1) and aldosterone-synthase (CYP11B2) in the left and right adrenal have also to be taken into consideration. These enzymes are essential in the progress of CORT synthesis (for review see Miller, 1988; Biason-Lauber, 1998) and their expression and activity is controlled by ACTH signalling (for review see Sewer and Waterman, 2003; Sewer et al., 2007).

Therefore, in the present study we aimed to reveal that (i) there is a lack or at least a reduction of CORT response to an acute heterotypic stressor (elevated platform (EPF), 5 min) after CSC exposure using repeated jugular venous blood sampling in mice. We further aimed to show that (ii) CSC exposure affects adrenal *in vitro* responsiveness to ACTH in a body-side specific manner. To test this we used adrenal explants instead of isolated adrenal cells to keep an intact adrenal architecture. Furthermore, we aimed to show that (iii) possible body-side specific alterations in adrenal ACTH responsiveness seen *in vitro* following CSC are accompanied by respective alterations in the *in vivo* response to EPF exposure. Therefore, we quantified relative adrenal CORT content in the left and right adrenal before and after EPF exposure. Finally, we aimed to elucidate (iv) the body-side specific mechanism underlying CSC-induced adrenal insufficiency. Therefore, we investigated CSC effects on left and right adrenal mRNA and/or protein expression of Mc2r, MRAP, StAR, CYP11A1, CYP11B1, and CYP11B2.

## 2. Material and methods

### 2.1. Animals

Male C57BL/6 mice (Charles River, Sulzfeld, Germany) weighing 19—22 g (experimental mice) or 30—35 g (dominant mice) were individually housed in standard polycarbonate mouse cages (16 cm × 22 cm × 14 cm) for at least one week before