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SOCIAL ISOLATION STRESS REDUCES HIPPOCAMPAL LONG-TERM POTENTIATION: EFFECT OF ANIMAL STRAIN AND INVOLVEMENT OF GLUCOCORTICOID RECEPTORS

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Abstract—Background: Depressive patients show cognitive impairments that are strongly associated with cortisol levels and hippocampus functioning that interact via unknown mechanisms. In addition, a relation between depression and hippocampal synaptic plasticity was described.
Methods: **In the first experiment, strain-dependent effects of 72-h social isolation on long-term potentiation (LTP) in the CA1 area of the *in vitro* hippocampus, was determined. Extracellular field excitatory postsynaptic potentials were recorded and a brief high-frequency stimulation (100 Hz, 1 s) was applied and recording resumed after the high frequency stimulation (HFS) for 30 min to determine the effect of HFS.** ***Methods:*** **In the second experiment we investigated the effect of 72 h of corticosterone treatment and the involvement of glucocorticoid receptors (GRs) in the effect of 72 h of social isolation on LTP in the CA1 area of hippocampus, *in vitro*.**
Results: **Genetic background has a major effect on the level of hippocampal LTP impairment in mice following social isolation. Data showed that the potentiation levels in socially housed (SH) A/J mice were significantly higher than the SH C57BL/6J mice (224.88 ± 16.65, 131.56 ± 6.25% of the baseline values, $t(9) = 2.648$, $p = 0.026$). However, both strains showed depressed induction of potentiation when reared in an isolated environment for 72 h, and no significant difference was recorded between the two (112.88 ± 16.65%, and 117.91 ± 3.23% of the baseline values, respectively, $t(10) = 0.618$, $p = 0.551$). Social isolation increased corticosterone levels significantly and chronic corticosterone infusion in SH phenocopied the LTP impairments observed in socially isolated mice. Infusion of the GR antagonist RU38486 rescued the LTP-impairments following social isolation.**
Conclusions: **These findings support the notion that increased levels of stress hormone act via the GR on hippocampal functioning and that, in this way, the cognitive deficits in mood disorders may be restored.**

Key words: depression, cognitive deficits, activity-dependent synaptic plasticity.

INTRODUCTION

Cognitive deficits, especially those closely related to hippocampus function, appear to be correlated to secreted cortisol levels in depressed patients (Hinkelmann et al., 2009). The hippocampus, besides its major role in learning and memory, is also a key structure in the neuro-endocrinal circuitry of the stress response (Nugent et al., 2013; Persson et al., 2013). The hippocampal structure and function get heavily taxed by the adverse effects of stress due to dense expression of the two types of corticosteroid receptors: mineralocorticoid receptor (MR) and glucocorticoid receptor (GR), throughout the hippocampus (Eberwine, 1998; Sze et al., 2013). Animal studies have shown that GRs get progressively more occupied when the levels of corticosterone rises due to stress and excessive activation of GRs is associated with changes in hippocampal structure, impairments in hippocampal functioning at the cellular and behavioral levels (Kim and Yoon, 1998; Kim and Diamond, 2002; Lupien and McEwen, 1997). In the hippocampus, the transcriptional regulation triggered by GR activation was associated with an increase in gene products which enhance the membrane conductance to Ca^{2+} and excessive levels of Ca^{2+} are linked to hippocampal excitotoxicity (Kim and Yoon, 1998). One of the challenging questions remains whether increased stress hormone levels, can alter hippocampal functioning via GR activation.

Activity-dependent synaptic plasticity in the hippocampus which is broadly regarded as the cellular substrate of memory also appears to be highly responsive to stressful manipulations. It is well documented that experimentally induced stressful experience just as exposure to high levels of glucocorticoids disrupts long-term potentiation (LTP) (Kim et al., 1996; Alfarez et al., 2002; Yang et al., 2004;

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Abbreviations: aCSF, artificial cerebrospinal fluid; fEPSP, field excitatory post synaptic potential; GR, glucocorticoid receptor; HFS, high frequency stimulation; LTD, long-term depression; LTP, long-term potentiation; MWM, Morris water maze; NMDA, *N*-methyl-D-aspartate; SH, socially housed; SI, socially isolated.

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Wiegert et al., 2005; Krugers et al., 2006, 2010). Interestingly the GR receptor blockade as well as *N*-methyl-D-aspartate (NMDA) receptor antagonism prior to stress exposure prevented the adverse effect of stress on synaptic plasticity suggesting that corticosterone targets NMDA receptor-dependent plasticity (Wiegert et al., 2005; Krugers et al., 2006). Stressful experience has been shown to alter the synaptic connections in the hippocampus in such a way that the outcome is not only occlusion of expression of LTP, but also facilitation for long-term depression (LTD) induction (Xu et al., 1997, 1998; Howland and Wang, 2008; Cazakoff and Howland, 2010; Macdougall and Howland, 2012). In freely moving rats however; LTD was recorded to be facilitated only during exploration of complex environments that contain novel objects (Kemp and Manahan-Vaughan, 2004; Manahan-Vaughan and Braunewell, 1999). This may suggest that the mechanisms of hippocampal LTD rather underlie some aspects of novel detection (Kemp and Manahan-Vaughan, 2007; Massey and Bashir, 2007). A few reports were published linking short-term synaptic plasticity (as paired pulse facilitation) and stress (Cazakoff and Howland, 2010). Some however, denied such an effect of stress on PPF (Shors and Thompson, 1992).

Strain of choice appears to be one of the main determinants of behavioral and neurochemical profile following stress exposure. Anisman and colleagues indicated that strain determined corticosterone response (Shanks et al., 1990), performance in behavioral paradigms as measured in shuttle-escape, forced-swimming, spontaneous alteration in Y-maze and Morris water maze (MWM) tasks (Shanks and Anisman, 1988; Francis et al., 1995), and turnover rates of norepinephrine, dopamine and serotonin in a brain-site-specific manner (Shanks et al., 1991) following stressful experience. Moreover, Anisman et al. (2001) reported that the strain of the mice interacts with the nature of the stressor. Inbred strains not only differ in terms of behavioral and neurochemical profile after stressful experience but also in a wide array of learning- and memory-related measures including hippocampal synaptic plasticity. Schimanski and Nguyen (2004) noted that inbred strains differ markedly from each other in MWM performance, cued/contextual fear conditioning test and of particular interest in the expression of hippocampal LTP. Recently, it has also been noted that synaptic plasticity-related proteins in the hippocampus also vary across the mouse strains considerably (Pollak et al., 2005, 2010). The investigation of inbred mouse strains by precise phenotyping measurements and application of forward genetic strategies, such as combination of chromosomal substitution strategy (CSS) and quantitative trait loci (QTL) mapping, promise a robust track for determining the underpinnings of complex behavioral traits such as stress responsivity, learning and memory.

72 h of social isolation, which comprises physical separation from conspecifics while maintaining visual, olfactory, auditory contact, was employed as the stressor. As outlined in Hilakivi et al. (1989) social isolation is associated with social deprivation in rodents and shown to provoke behavioral and neurochemical disturbances such as increase in locomotion, aggression. Dopamine and serotonin synthesis/turnover rates in certain brain regions (Lapiz et al., 2003); and rise in plasma corticosterone levels (Bartolomucci et al., 2003) were also reported. Social isolation also argued to potentiate corticosterone response to future stressors (Bartolomucci et al., 2003). Furthermore, recent research shows that social isolation hinders the physical exercise-induced neurogenesis in the dentate gyrus and suppresses cell proliferation in the presence of an additional stressor (Stranahan et al., 2006).

In the current series of experiments the aim was to investigate the effect of psychosocial stressor on mechanisms of learning and memory in a strain-dependent fashion. It was predicted that social isolation would lead to marked reductions in LTP in a strain-dependent manner in C57BL/6J and A/J mice. To this end, initially, hippocampal LTP induction was examined in both strains. The second part of the research aimed to pharmacologically characterize social isolation. For this purpose, the involvement of corticosterone was examined by treating the socially housed (SH) mice chronically with subcutaneous solid corticosterone or cholesterol pellets. Additionally, socially isolated (SI) animals were treated with GR-antagonist, RU 38486 (mifepristone, RU-486), in order to see whether it is possible to block the effect of social isolation on LTP. Overall, the results provided support for our hypotheses that 72 h of social isolation suppresses hippocampal LTP in the CA1 area in a strain-dependent fashion.

EXPERIMENTAL PROCEDURES

Two experiments were done. In the first experiment we investigated the strain-dependent differences of 72 h of social isolation on long-term potentiation in the CA1 area of the hippocampus, *in vitro.* Thirteen male C57BL/6 and 10 male A/J mice (Jackson Laboratories, Bar Harbor, ME, USA) between 4 and 6 months of age served as subjects during the experiment. The animals were maintained in 12:12 light–dark cycle (light onset 01:00 h) with the temperature fixed at 21 ± 2 °C. All mice were housed in transparent Plexiglas cages with standard wood-chip bedding and they were given *ad libitum* access to food and water and were provided with a piece of tissue for nest building. Until the initiation of the experiments, all mice were SH.

Three days prior to decapitation, seven C57BL/6J and five A/J mice were randomly subjected to social isolation procedure. For the social isolation procedure, the animals were taken away from their home-cages and were placed individually in separate cages identical to their home-cages in the same room. The animals were provided with fresh wood-chip bedding, with a piece of tissue, and with *ad libitum* food and water access. The isolation procedure started 8 h after the light onset. The remaining six C57BL/6J and five A/J mice were kept in their home-cages SH. All of the handling procedures