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Repeated Neck Restraint Stress Bidirectionally Modulates Excitatory Transmission in the Dentate Gyrus and Performance in a Hippocampus-dependent Memory Task

Jadwiga Spyrka [a]* **and Grzegorz Hess** [a,b]

[a] *Institute of Zoology and Biomedical Research, Jagiellonian University, Krakow, Poland*

[b] *Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland*

Abstract—The consequences of stress depend on characteristics of the stressor, including the duration of exposure, severity, and predictability. Exposure of mice to repeated neck restraint has been shown to bidirectionally modulate the potential for long-term potentiation (LTP) in the dentate gyrus (DG) in a manner dependent on the number of restraint repetitions, but the influence of repeated brief neck restraint on electrophysiology of single DG neurons has not yet been investigated. Here, we aimed at finding the effects of 1, 3, 7, 14, or 21 daily neck restraint sessions lasting 10 min on electrophysiological characteristics of DG granule cells as well as excitatory and inhibitory synaptic inputs to these neurons. While the excitability of DG granule cells and inhibitory synaptic transmission were unchanged, neck restraint decreased the frequency of spontaneous excitatory currents after three repetitions but enhanced it after 14 and 21 repetitions. The consequences of repeated neck restraint on hippocampus-dependent memory were investigated using the object location test (OLT). Neck restraint stress-impaired cognitive performance in the OLT after three repetitions but improved it after 14 and 21 repetitions. Mice subjected to three neck restraint sessions displayed an increase in the measures of depressive and anxiety-like behaviors, however, prolongation of the exposure to neck restraint resulted in a gradual decline in the intensity of these measures. These data indicate that stress imposed by an increasing number of repeated neck restraint episodes bidirectionally modulates both excitatory synaptic transmission in the DG and cognitive performance in the object location memory task. © 2018 Published by Elsevier Ltd on behalf of IBRO.

Key words: repeated neck restraint stress, adaptation, spatial memory, synaptic transmission, depression-like behavior, anxiety-like behavior.

INTRODUCTION

It is well established that exposure to an identical stressor for a prolonged time usually results in a reduction in responses to homotypic stress (Rabasa et al., 2015). For example, repeated exposure to loud noise or restraint results in a successive reduction in the hypothalamic–pituitary–adrenal (HPA) axis response, measured by the secretion of adrenocorticotropin (ACTH) and glucocorticoid hormones (Ma and Lightman, 1998; Babb et al., 2014). Such adaptation is thought to be a protective process since repeated or chronic stress may lead to disease in susceptible individuals (reviewed in: McEwen, 2000). A decreased reactivity to re-exposure to an increasingly familiar stressor is observed at both physiological and behavioral levels and is the effect of habituation (Grissom and Bhatnagar, 2011). Behavioral habituation to repeated stress is manifested, for example, as reduced struggling observed during successive restraint sessions (Grissom et al., 2008).

We have previously investigated the effects of brief neck restraint on synaptic plasticity in the dentate gyrus (DG) of the mouse (Spyrka and Hess, 2010; Spyrka et al., 2011). In the course of these experiments an animal was placed for 10 min in a neck restraint apparatus which allows normal breathing and does not hamper leg or body movement, in contrast to commonly used restrainers. Such repeated neck restraint procedures had been used by several laboratories to habituate animals to the restraining apparatus before applying further treatment involving mechanical stimulation of the whiskers

*Corresponding author. Address: Department of Neurophysiology and Chronobiology, Institute of Zoology and Biomedical Research, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland.
E-mail address: jadwiga.spyrka@uj.edu.pl (J. Spyrka).

Abbreviations: ACSF, artificial cerebrospinal fluid; ACTH, adrenocorticotropin; DG, dentate gyrus; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; EPM, elevated plus-maze test; FPO, familiar place object; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; HPA, hypothalamic–pituitary–adrenal; LTP, long-term potentiation; mEPSCs, miniature EPSCs; mIPSCs, miniature IPSCs; NPO, novel place object; OLT, object location test; RMP, resting membrane potential; sEPSCs, spontaneous excitatory postsynaptic currents; TST, tail suspension test; TTX, tetrodotoxin.

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(Siucinska and Kossut, 1996; Tokarski et al., 2007; Jasinska et al., 2013). Using ex vivo field potential recording from DG slices we have shown that neck restraint, repeated over three successive days, impaired the induction of long-term potentiation (LTP) in the DG and that after seven neck restraint sessions the LTP level returned to normal values, consistent with the occurrence of adaptation. However, in slices prepared from mice subjected to neck restraint repeated over 14 and 21 days a larger LTP than in control preparations obtained from unstressed mice, was evident (Spyrka and Hess, 2010). Repeated restraint stress-induced increase in neuronal activity has also been reported to occur in the amygdala (Zhang and Rosenkranz, 2012) and this effect may be related to repeated restraint stress-induced facilitation of fear conditioning (Conrad et al., 1999).

The present study was aimed at investigating the effects of an increasing number of repeated neck restraint sessions on the excitability of DG granule cells as well as the excitatory and inhibitory synaptic inputs to these neurons using whole-cell recordings from ex vivo brain slices. Since the behavioral consequences of neck restraint repeated over successive days have not yet been determined, in the present study we also investigated the relationship between neck restraint and novelty preference using the object location test (OLT). Object location memory, which is based on rodents' drive to learn about their environment, has been shown to be dependent on the function of the DG (Hunsaker et al., 2007; Warburton and Brown, 2015). Since activity of the DG has been shown to modulate behavioral despair (Wang et al., 2015) and anxiety (Kheirbek et al., 2013) as well, stressed mice were subjected to two tests aimed at determining depression- and anxiety-related behaviors: the tail suspension test (TST) and elevated plus-maze test (EPM).

EXPERIMENTAL PROCEDURES

Animals and treatment

The experiments were carried out using C57/BL6J male mice (Hodowla Zwierzat Laboratoryjnych Maria Staniszewska, Poland) which were 5 weeks old at the beginning of the experiment. Animals were housed in groups under a 12/12-h light/dark cycle (light on 8:00 am–8:00 pm). Standard food and tap water were available *ad libitum*. The experiments were approved by the First Local Ethical Committee on Animal Testing at the Jagiellonian University in Krakow and carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. All efforts were made to minimize the number of animals used and their suffering.

The experimental animals were exposed to an increasing number (1, 3, 7, 14 or 21) of daily neck restraint sessions (between 9 and 10 am). Mice were placed in a custom-made restraining apparatus for 10 min (Siucinska and Kossut, 1996). The apparatus, built from stainless steel, consisted of a stable platform and holding element attached to it. The holding element was divided into two parts, the immobile segment with a semicircle groove where the neck of the animal was placed, and the second, mobile element also with a semicircle groove, which, after locking in place, holds the animal by the neck. The apparatus allowed the animal to stay in a physiological position with four paws placed on the platform. It also allowed the animal to breathe normally and did not hamper leg or body movement. Following restraint, the animals were returned to their home cages. Control animals were subjected to manual handling. All mice were weighed every day just before they were subjected to restraint or handling. Different animals were used for each behavioral test, endocrine measures and electrophysiological recordings. In total, 372 animals were used. For the behavioral tests, each group consisted of 12 animals; for hormone assays, six animals per group were used; for electrophysiological recordings, each group consisted of eight animals.

Brain slice preparation and whole-cell recording

Brain slices (400-μm-thick) were prepared one day after the last restraint or handling procedure. Mice were anesthetized with isoflurane (0.2 ml, Aerrane, Baxter) and decapitated, brains were removed into an ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 92 choline Cl, 30.0$NaHCO_3$, 1.25 NaH_2PO_4, 10.0 $MgSO_4$, 2.5 KCl, 0.5 $CaCl_2$, 20.0 HEPES, 5.0 Na^+ ascorbate, 3.0 Na^+ pyruvate, 2.0 thiourea and 10 glucose, bubbled with the mixture of 95% O_2 and 5% CO_2. Slices were cut in a horizontal plane using a vibrating microtome (VT 1000 Leica Microsystems, Wetzlar, Germany) in ACSF in which choline Cl was replaced by equimolar NaCl. Next, slices were transferred to a preincubation chamber containing modified ACSF of the following composition: (in mM): 124 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 1 $MgSO_4$, 4.5 KCl, 1.8 $CaCl_2$ and 10 glucose. Slices remained stored submerged at 32 ± 0.2 °C for approx. 3 h prior to recordings.

Individual slices were placed in the recording chamber and superfused (3 ml/min) with warm (32 ± 0.2 °C), modified ACSF. DG neurons were visualized using Zeiss Axio Examiner. D1 microscope (Zeiss, Germany) with 40× water immersion lens. Patch micropipettes (6–8 MΩ) were pulled from borosilicate glass capillaries (Sutter Instrument, Novato, CA, USA) using the Sutter Instrument P97 puller. The pipette solution contained (in mM): 125 K-gluconate, 20 KCl, 2 $MgSO_4$, 10 HEPES, 4 Na_2-ATP, 0.4 Na-GTP, and 5 EGTA. Osmolarity and pH were adjusted to 290–300 mOsm and 7.2–7.3, respectively. The calculated liquid junction potential using this solution was 13.1 mV, and data were corrected for this offset. Signals were recorded using the SEC 05LX amplifier (NPI, Germany), filtered at 2 kHz, and digitized at 20 kHz using Digidata 1440A interface and Clampex 10 software (Molecular Devices, USA). Mature dentate granule cells were defined by their well-characterized hyperpolarized resting membrane potential (RMP), low input resistance, and soma location in the outer third of the dentate granular cell layer (Overstreet-Wadiche and Westbrook, 2006).

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