Neuroscience 280 (2014) 121–129

EFFECT OF CHRONIC STRESS ON SHORT AND LONG-TERM PLASTICITY IN DENTATE GYRUS; STUDY OF RECOVERY AND ADAPTATION

M. RADAHMADI, N. HOSSEINI AND A. NASIMI *

Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract—Stress dramatically affects synaptic plasticity of the hippocampus, disrupts paired-pulse facilitation and impairs long-term potentiation (LTP). This study was performed to find the effects of chronic restraint stress and recovery period on excitability, paired-pulse response, LTP and to find probable adaptation to very long stress in the dentate gyrus. Thirty-eight male Wistar rats were randomly divided into four groups of Control, Rest–Stress (21 days stress), Stress–Rest (recovery) and Stress–Stress (42 days stress: adaptation). Chronic restraint stress was applied 6-h/day. Input–output functions, paired-pulse responses and LTP were recorded from the dentate gyrus while stimulating the perforant pathway. We found that chronic stress attenuated the responsiveness, paired-pulse response and LTP in the dentate gyrus. A 21-day recovery period, after the stress, improved all the three responses toward normal, indicating reversibility of these stress-related hippocampal changes. There was no significant adaptation to very long stress, probably due to severity of stress.

Key words: stress, plasticity, paired pulse, LTP, recovery, adaptation.

INTRODUCTION

Stress may produce adverse effects on both behavior and physiology (Blanchard et al., 2001). Even though it is a necessary mechanism for survival, long-term stress alters normal brain structures and functions (McEwen, 2000). Chronic stress affects learning and memory processes (Hymie and Giorgio, 1978; Martinez, 1981) especially by changing the morphology and function of the hippocampus (Kim and Yoon, 1998; De Kloet et al., 1999; McEwen, 1999), a key brain structure for learning and memory (Foy et al., 1987; Foster et al., 1996; Zheng et al., 2004; Carboni et al., 2006). Altered neurochemistry, excitability, neuronal energy, plasticity, neurogenesis, neurotoxicity, neuronal morphology and even cell death (Sapolsky, 1996; Reagan and McEwen, 1997; Nacher et al., 2004) have been implicated in the adverse effects of stress on the brain.

Stress dramatically affects synaptic plasticity in the hippocampus (Kim and Yoon, 1998; De Kloet et al., 1999; McEwen, 1999).

It was shown that acute stress disrupts paired-pulse facilitation (Cazakoff and Howland, 2010). Verkuyl et al. (2004) reported that paired-pulse inhibition was unaffected by chronic stress. Acute stress produced a significant overall enhancement in paired-pulse facilitation in the commissural/associational and medial perforant pathway inputs, but chronic stress failed to change paired-pulse inhibition/facilitation in these pathways (Shors and Thompson, 1992; Pavlides et al., 2002).

Both *in vitro* and *in vivo* electrophysiological studies indicated that stress impairs hippocampal long-term potentiation (LTP) (Foy et al., 1987; Shors et al., 1989; Diamond and Rose, 1994; Shors and Dryver, 1994; Kim et al., 1996; Xu et al., 1997), and produces deficits in hippocampal-dependent learning tasks (Luine et al., 1994; Conrad et al., 1996; Krugers et al., 1997; Ohl and Fuchs, 1999). Stress also facilitates the induction of long-term depression (LTD) (Cao and Leung, 1991; Xu et al., 1997; Manahan-Vaughan et al., 1998; Yang et al., 2005). Joëls et al. (2007) and McEwen (2010) reported that stress not only induces remodeling of dendritic architecture, but also alters synapse formation and expression of various neurotransmitter receptors. Stress affects GABAergic and glutamatergic systems (Karst et al., 2005; Gunn et al., 2011) and causes depletion and reorganization of synaptic vesicles (Magarinos et al., 1997).

There are reports studying the effect of recovery period after stress on the stress-induced changes in various brain structures like amygdaloid (Vyas et al., 2004), prefrontal cortex and dentate gyrus (DG) (Lin et al., 2008), showing that recovery period returns, at least some of the changes, toward normal. Lin et al. (2008) reported that recovery restored the measured parameters in stress condition (e.g. corticosterone (CORT) level and expression of genes) to the normal level

*Corresponding author. Tel: +98-031-3792484; fax: +98-031-36688597.
E-mail address: nasimi@med.mui.ac.ir (A. Nasimi).

Abbreviations: ACTH, adrenocorticotropic hormone; ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; Co, Control group; CORT, corticosterone; DG, dentate gyrus; EPSP, excitatory postsynaptic potential; fEPSP, field excitatory postsynaptic potential; GRs, glucocorticoid receptors; HFS, high-frequency stimulation; HPA, hypothalamic–pituitary–adrenal; I/O, input–output; LTP, long-term potentiation; NMDA, N-methyl-D-aspartate; Re–St, Rest–Stress group; St–Re, Stress–Rest group; St–St, Stress–Stress group.

http://dx.doi.org/10.1016/j.neuroscience.2014.09.005

in male rats. In mice, LTP induction in CA1 was impaired in slices prepared at 1–24 h after acute stress, however LTP induction was completely restored 48 h after stress exposure (Garcia et al., 1997). However, in a similar study in rats, LTP still remained impaired 48 h after cessation of stressors and needed 4 days for complete recovery (Shors et al., 1997).

There are many studies about brain's physiological and behavioral responses to stress which can be either adaptive or damaging (McEwen, 2007), demonstrating that different parts of the brain could show adaptive responses to long-term or repeated stressors. These adaptations may include changes in neural activity, alterations in the levels of circulating hormones, and modifications in behavior (Crosby and Bains, 2012). It was found that microinjection of $GABA_A$ receptor antagonist or glutamate receptor agonist into some regions of the brain resulted in robust elevations in plasma levels of adrenocorticotropic hormone (ACTH) and CORT (Bailey and Dimicco, 2001). In contrast $GABA_A$ receptor agonist, muscimol, attenuated the stress-induced elevations in plasma ACTH. Therefore, GABA may be involved in adaption to long-term or repeated stressors (Morin et al., 2001). Also, endocannabinoid (neuroactive lipids) signaling and changes in its receptor density play a role in adaptation of the hypothalamic–pituitary–adrenal (HPA) axis to repeated restraint stress (Patel and Hillard, 2008; Hill et al., 2010). Repeated activation of the HPA axis by restraint stress also demonstrates habituation as measured by a progressive decrease in plasma CORT level with increasing numbers of restraint episodes (Patel et al., 2004).

However no reports were found addressing the adaptation of excitability, paired-pulse response and LTP in DG to chronic stress. So, the aims of this study were to find the effects of chronic restraint stress (21 days) and a recovery period of 21 days on excitability, paired-pulse response and LTP in DG, as well as finding the probable adaptations to very long stress (42 days).

EXPERIMENTAL PROCEDURES

Materials and methods

Experiments were performed on 38 male Wistar rats, with an initial weight of 250–300 g. Experiments were approved by the Committee of Animal Use Ethics of the Isfahan University of Medical Science Animals were housed under light-(12-h light/dark) and temperature-(22 ± 2 °C) controlled condition, with food and water available *ad libitum*.

Rats were randomly divided into four groups as follows:

1. *Control (Co) group*: rats were transported to the laboratory room and handled similar to the experimental animals throughout the study period with no special treatment.
2. *Stress–Rest (St–Re) or recovery group*: restraint stress was applied 6-h/day for 21 days, and then rats remained undisturbed for 21 days (recovery period).
3. *Rest–Stress (Re–St) group*: rats had no special treatment for 21 days, and then chronic restraint stress was applied, 6-h/day for 21 days.
4. *Stress–Stress (St–St) or adaptation group*: rats were under restraint stress 6-h/day for 42 days.

For chronic stress each rat was placed in a Plexiglas cylindrical restrainer, in which it was not possible for the animal to move or turn around (Pavlides et al., 2002).

Electrophysiology

Twenty-four hours after the 21st day of stress, rats were anesthetized with urethane (1.5-g/kg, i.p.) and placed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA). Rectal temperature was maintained at 36.5 ± 0.5 °C using a homeothermic temperature control unit (Harvard, Holliston, MA, USA).

The skull was exposed and two small holes were drilled over the hippocampus (AP = −3.24, ML = ±2–2.2, and DV = 3.2–3.7 from bregma) and the perforant pathway (AP = −6.96, ML = 4–4.2 and DV = 3.2–3.7 mm from bregma). A monopolar recording electrode (Teflon-coated stainless steel, 0.125 mm diameter, Advent Co., Oxford, UK) was positioned in the region of the granular cells of the DG, and a bipolar stimulating electrode was positioned in the perforant pathway.

Extracellular-evoked field potentials were recorded from the dentate granule cell population following stimulation of the perforant pathway. Signal was amplified (1000×), filtered (1 Hz to 3 kHz) (DAM 80 differential amplifier, WPI, Sarasota, FL, USA), digitized, recorded and analyzed using an electrophysiology software (written by A. Nasimi in this lab). Final electrode location was determined when maximum excitatory postsynaptic potential (EPSP) was obtained with minimal stimulation.

After ensuring a steady-state baseline response, an input–output (I/O) function was obtained by systematic variation of the stimulus current (100–1000 μA) in order to evaluate synaptic potency. Stimulus intensity eliciting ~40% of the maximum response was used for paired and LTP experiments.

Paired-pulse responses were evoked by delivering pairs of stimuli at interpulse intervals (IPIs) of 10, 20, 30, 70, 150, 300, 500 and 1000 ms (five trials at each interval). Paired-pulse index was calculated as percent change of the size of the second field excitatory postsynaptic potential (fEPSP) slope, or PS amplitude compared to the first one.

For LTP experiments, first the baseline response was evoked by applying single pulses of stimulation at 0.1 Hz until a stable baseline was established for 30 min. Then LTP was induced by applying a high-frequency stimulation (HFS, 400 Hz, 10 bursts of 20 stimuli, 0.2-ms stimulus duration, 10-s inter-burst interval) using an intensity eliciting 80% of the maximum response. Following HFS, baseline stimulation frequency and intensity (eliciting 40% of the maximum response) were resumed, and responses were recorded at 30, 60 and 120 min later. For each time-point, 10 consecutive evoked responses were averaged. Slope of the linear