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DEFICITS IN COGNITIVE FLEXIBILITY INDUCED BY CHRONIC UNPREDICTABLE STRESS ARE ASSOCIATED WITH IMPAIRED GLUTAMATE NEUROTRANSMISSION IN THE RAT MEDIAL PREFRONTAL CORTEX

JULIANNE D. JETT, SARAH E. BULIN, LAUREN C. HATHERALL, CARLIE M. MCCARTNEY AND DAVID A. MORILAK*

Department of Pharmacology and Center for Biomedical Neuroscience, University of Texas Health Science Center, San Antonio, TX 78229, USA

Abstract—Deficits in cognitive flexibility, the ability to modify behavior in response to changes in the environment, contribute to the onset and maintenance of stress-related neuropsychiatric illnesses, such as depression. Cognitive flexibility depends on medial prefrontal cortex (mPFC) function, and in depressed patients, cognitive inflexibility is associated with hypoactivity and decreased glutamate receptor expression in the mPFC. Rats exposed to chronic unpredictable stress (CUS) exhibit compromised mPFC function on the extradimensional (ED) set-shifting task of the attentional set-shifting test. Moreover, CUS-induced ED deficits are associated with dendritic atrophy and decreased glutamate receptor expression in the mPFC. This evidence suggests that impaired glutamate signaling may underlie stress-induced deficits in cognitive flexibility. To test this hypothesis, we first demonstrated that blocking NMDA or AMPA receptors in the mPFC during ED replicated CUS-induced deficits in naïve rats. Secondly, we found that expression of activity-regulated cytoskeleton-associated protein (*Arc*) mRNA, a marker of behaviorally induced glutamate-mediated plasticity, was increased in the mPFC following ED. We then showed that CUS compromised excitatory afferent activation of the mPFC following pharmacological stimulation of the mediodorsal thalamus (MDT), indicated by a reduced induction of *c-fos* expression. Subsequently, *in vivo* recordings of evoked potentials in the mPFC indicated that CUS impaired afferent activation of the mPFC evoked by MDT stimulation, but not the ventral hippocampus. Lastly, glutamate microdialysis showed that CUS attenuated the acute stress-evoked increase in extracellular glutamate in the mPFC. Together, these results demonstrate that CUS-induced ED deficits are associated with compromised glutamate neurotransmission in the mPFC.

Key words: attentional set-shifting, chronic unpredictable stress, cognitive flexibility, glutamate, medial prefrontal cortex, mediodorsal thalamus.

INTRODUCTION

Deficits in cognitive function and emotional regulation play an integral role in the pathology of stress-related neuropsychiatric illnesses, such as depression and anxiety disorders. Specifically, impaired cognitive flexibility contributes to the onset and maintenance of these illnesses (Taylor Tavares et al., 2007; Disner et al., 2011; Millan et al., 2012). Cognitive flexibility, the ability to modify patterns of thought or behavior in response to feedback from the environment, is strongly associated with medial prefrontal cortical (mPFC) function. Imaging studies have shown that deficits in cognitive flexibility are associated with hypoactivity in the mPFC of depressed and chronically stressed individuals (Anand et al., 2005; Bermpohl et al., 2009; Koenigs and Grafman, 2009; Liston et al., 2009). Further, those suffering from depression also exhibit a decrease in glutamate/glutamine ratios, glutamate receptor expression, and markers of synaptic plasticity in the prefrontal cortex (Hasler et al., 2007; Feyissa et al., 2009). Moreover, acute low-dose administration of the *N*-methyl-D-aspartate (NMDA) receptor antagonist, ketamine, has been shown to induce rapid antidepressant effects in treatment-resistant patients (Carlson et al., 2006; Zarate et al., 2006; Machado-Vieira et al., 2009). Evidence suggests that this therapeutic effect results in part from ketamine enhancing glutamate transmission in the mPFC, including elevated glutamate levels and increased α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor activation (Moghaddam et al., 1997; Li et al., 2010, 2011). Accordingly, changes in glutamatergic signaling in mPFC may play a key role in the pathology of

*Corresponding author. Address: Department of Pharmacology, MC 7764, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900, USA. Fax: +1 210 567 4303.
E-mail address: morilak@uthscsa.edu (D. A. Morilak).

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Arc/Arg3.1, activity-regulated cytoskeleton-associated protein; AST, attentional set-shifting test; BMI, bicuculline methiodide; CD, compound discrimination; CUS, chronic unpredictable stress; D-AP5, D-2-amino-5-phosphonopentanoate; ED, extradimensional set-shift; ID, intra-dimensional shift; IMB, immobility stress; MDT, mediodorsal thalamus; MPEP, 2-methyl-6-(phenylethynyl)pyridine; mPFC, medial prefrontal cortex; NBQX, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(f)quinoxaline-2,3-dione; NMDA, *N*-methyl-D-aspartic acid; R1, first reversal; R2, second reversal; SD, simple discrimination; TTC, trials to criterion; vHipp, ventral hippocampus.

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stress-related neuropsychiatric disorders, as well as their treatment.

Preclinical studies have demonstrated that acute stress enhances glutamate release in the mPFC, and that this response is neuronally mediated (Moghaddam, 1993; Moghaddam et al., 1994; Lupinsky et al., 2010; Musazzi et al., 2011). Furthermore, acute stress-evoked glutamate activity in the mPFC is associated with enhanced working memory (Yuen et al., 2009, 2011), whereas blocking AMPA or NMDA receptors during behavioral testing impairs cognitive flexibility (Stefani et al., 2003; Stefani and Moghaddam, 2005; Dalton et al., 2011). This evidence suggests that acutely evoked glutamate transmission in the mPFC facilitates cognitive function. In contrast, rodents exposed to chronic stress show reductions in glutamate receptor expression and markers of synaptic plasticity that mirror deficits in the prefrontal cortex of depressed patients (Lee and Goto, 2011; Li et al., 2011; Yuen et al., 2012). Chronic stress induces atrophy of pyramidal cell dendrites in the mPFC of rodents (Cook and Wellman, 2004; Radley et al., 2004; Liston et al., 2006). This detrimental effect of chronic stress may result from excessive stress-evoked glutamate release, as NMDA receptor antagonist treatment during chronic stress prevents changes in dendritic atrophy (Martin and Wellman, 2011). Hence, chronic stress-induced changes in dendritic morphology and glutamate receptor expression may be a compensatory response to protect the mPFC from excessive glutamate signaling, excitotoxicity and cell death (Bruno et al., 1993; Skaper et al., 2001). However, such compensatory modifications in glutamate transmission could have secondary consequences, such as attenuated mPFC activity and deficits in higher order cognitive function (e.g., cognitive inflexibility).

To assess cognitive flexibility and stress-induced prefrontal cortical dysfunction in rats, we have employed the attentional set-shifting test (AST) (Birrell and Brown, 2000). This cognitive assay was reverse translated from a human and non-human primate test of cognitive set-shifting (Keeler and Robbins, 2011). In the AST, rats are trained to dig for a food reward in small pots differentiated by cues in two stimulus dimensions: the material with which the pots are filled, and the odor with which they are scented. Thus, the rats must learn which of the two stimulus dimensions is informative for locating the reward, and which cue within that dimension signals the location of the reward. After mastering a given contingency, indicated by reaching a criterion of six consecutive correct trials, the rules are changed and the rat must then learn a new association in the next task. By proceeding through a series of such changes in which the same stimulus dimension remains informative, the rats form a ‘‘cognitive set”, a higher-order learning strategy by which they can more readily acquire the new rule when faced with a subsequent change. However, in the extra-dimensional (ED) set-shifting task, the informative dimension is switched, so the rat must abandon their cognitive set in order to acquire the new rule. This form of cognitive flexibility, called a cognitive set-shift, depends on the function of the mPFC. Lesioning the mPFC of rats induces a deficit on the ED task, similar to deficits seen with impairments in lateral prefrontal cortex function in humans and non-human primates (Owen et al., 1991; Dias et al., 1996; Birrell and Brown, 2000). Moreover, similar to depressed patients, rats exposed to chronic unpredictable stress (CUS) exhibit deficits in cognitive flexibility on the ED task (Taylor Tavares et al., 2007; Bondi et al., 2008).

In this study we tested the hypothesis that CUS-induced ED deficits are associated with compromised glutamate transmission in the mPFC. First, we administered NMDA, AMPA, or metabotropic glutamate receptor (mGluR5) antagonists locally into the mPFC of naïve rats to test if directly compromising local glutamate transmission during the ED task would mimic the CUS-induced cognitive deficits reported previously. Of the mGluR receptor subtypes, we targeted the mGuR5 receptor because, like NMDA and AMPA receptors, it exhibits reduced expression in the prefrontal cortex of depressed patients, is associated with antidepressant efficacy, and modulates learning and memory (Naie and Manahan-Vaughan, 2004; Witkin et al., 2007; Deschwanden et al., 2011). Secondly, we investigated the effects of CUS on behaviorally induced expression of the immediate early gene, *Arc/Arg3.1* (activity-regulated cytoskeleton-associated protein), a marker of experience-dependent plasticity in glutamatergic (i.e., CaMKII-positive) cortical neurons (Shepherd and Bear, 2011). Induction of *Arc* expression in the mPFC during performance of the ED task was used to assess CUS-induced changes in glutamate-mediated plasticity. Next we evaluated if CUS-induced deficits in cognitive function are associated with changes in excitatory afferent-evoked activation of the mPFC by quantifying *c-fos* induction and local field potentials evoked by stimulation of major glutamatergic afferents to the mPFC, namely the mediodorsal thalamus (MDT) or the ventral hippocampus (vHipp) (Gigg et al., 1994; Pirot et al., 1994; Hoover and Vertes, 2007). Both of these regions are associated with the pathology of depression, and also modulate the stress response, emotional regulation, and cognitive flexibility (Floresco and Grace, 2003; Block et al., 2007; Godsil et al., 2013). Lastly, we used *in vivo* microdialysis to investigate whether the acute stress-evoked glutamate response in the mPFC is changed as a consequence of CUS. Together, the results demonstrate that CUS-induced cognitive deficits are associated with impaired glutamate neurotransmission in the mPFC. Portions of this work have been presented in abstract form (Jett et al., 2015b).

EXPERIMENTAL PROCEDURES

Animals

A total of 138 male Sprague–Dawley rats (Envigo, USA), weighing 220–300 g upon arrival, were used for the present studies. Prior to initiating experimental procedures, rats were individually housed in 25 × 45 × 15 cm cages and maintained on a 12:12-h light/dark cycle (lights on at 07:00). All experimental procedures were conducted during the light phase, and food and water were given *ad libitum* unless rats were