*Neuroscience* 284 (2015) 430–443

# REPEATED RESTRAINT STRESS-INDUCED ATROPHY OF GLUTAMATERGIC PYRAMIDAL NEURONS AND DECREASES IN GLUTAMATERGIC EFFLUX IN THE RAT AMYGDALA ARE PREVENTED BY THE ANTIDEPRESSANT AGOMELATINE

**C. A. GRILLO,[a†] M. RISHER,[a†] V. A. MACHT,[a] A. L. BUMGARDNER,[a] A. HANG,[a] C. GABRIEL,[b] E. MOCAËR,[b] G. G. PIROLI,[a] J. R. FADEL[a] AND L. P. REAGAN[a*]**

[a] *Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, 6439 Garners Ferry Road, Columbia, SC 29208, United States*

[b] *Neuropsychiatry Division, Institut de Recherches Internationales Servier, 6, Place des Pléiades, Courbevoie CEDEX, France*

**Abstract—Major depressive illness is among the most prevalent neuropsychiatric disorders and is associated with neuroplasticity deficits in limbic structures such as the amygdala. Since exposure to stressful life events is proposed to contribute to depressive illness, our recent studies examined the effects of stress on amygdalar neuroplasticity. These studies determined that repeated stress elicits deficits in glutamatergic activity in the amygdala, neuroplasticity deficits that can be prevented by some but not all antidepressants. In view of these observations, the goal of the current study was to determine the effects of repeated restraint stress (RRS) on the dendritic architecture of pyramidal neurons in the rat basolateral nucleus of the amygdala (CBL), as well as glutamate efflux in the CBL and central nucleus of the amygdala (CMX) via *in vivo* microdialysis. We also examined the ability of the antidepressant agomelatine to prevent RRS-induced neuroplasticity deficits. Compared with control rats, rats subjected to RRS exhibited atrophy of CBL pyramidal neurons, including decreases in total dendritic length, branch points, and dendritic complexity index. In addition, glutamate efflux was significantly reduced in the CMX of rats subjected to RRS, thereby identifying a potential neurochemical consequence of stress-induced dendritic atrophy of CBL pyramidal neurons. Lastly, an acute stress challenge increased corticosterone (CORT) levels in the CBL, suggesting that stress-induced increases in CORT levels may contribute to the neuroanatomical and neurochemical effects of RRS in the CBL. Importantly, these RRS-induced changes were prevented by daily agomelatine administration. These results demonstrate that the neuroanatomical and neurochemical properties of glutamatergic neurons in the rat amygdala are adversely affected by repeated stress and suggest that the therapeutic effects of agomelatine may include protection of structural and neurochemical plasticity in limbic structures like the amygdala. Published by Elsevier Ltd. on behalf of IBRO.**

**Key words: depressive illness, dendritic complexity index, *in vivo* microdialysis, corticosterone, basolateral amygdala.**

## INTRODUCTION

Major depressive disorder (MDD) is one of the most common psychiatric disorders, affecting an estimated 12–15% of the general population (Kessler et al., 1994). The symptoms of depression include alterations in mood and perception, as well as physiological changes such as changes in appetite and body weight, as well as disruption in sleep patterns. Beyond these alterations in mood and physiology, depressive illness patients also exhibit neuroanatomical alterations in limbic structures such as the amygdala. For example, imaging studies have revealed that amygdala volumes may be increased (Frodl et al., 2002, 2003; Lange and Irle, 2004; Weniger et al., 2006), decreased (Sheline et al., 1998, 1999; von Gunten et al., 2000; Hastings et al., 2004; Andreescu et al., 2008; Burke et al., 2011; Sheline et al., 2012) or unchanged (Bremner et al., 2000; Caetano et al., 2004; Frodl et al., 2004) in MDD patients. While these discrepancies may be related to a variety of factors including illness duration, the number of depressive illness episodes, gender and therapeutic interventions (Campbell et al., 2004), the results demonstrate that the amygdala is a site for neuroanatomical alterations in depressive illness. Interestingly, rodents exposed to stress exhibit structural and functional deficits in the amygdala that are similar to those observed in patients with depressive illness. This includes studies that have demonstrated that repeated stress paradigms may elicit dendritic hypertrophy (Vyas et al., 2002; Johnson et al., 2009) or dendritic atrophy (Vyas et al.,

*Corresponding author. Address: Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, 6439 Garners Ferry Road, D40, Columbia, SC 29208, United States. Tel: +1-803-216-3515; fax: +1-803-216-3538.
E-mail address: lawrence.reagan@uscmed.sc.edu (L. P. Reagan).
† These authors contributed equally to this manuscript.

*Abbreviations:* CBL, basolateral nucleus of the amygdala; CIS, chronic immobilization stress; CMX, central nucleus of the amygdala; CORT, corticosterone; CUS, chronic unpredictable stress; DCI, dendritic complexity index; HEC, hydroxyethyl cellulose; MDD, Major depressive disorder; NSC, non-stressed control; NSC-A, non-stressed controls given agomelatine; NSC-V, non-stressed controls given vehicle; RRS, repeated restraint stress; RRS-A, repeated restraint stress given agomelatine; RRS-V, repeated restraint stress given vehicle.

http://dx.doi.org/10.1016/j.neuroscience.2014.09.047
0306-4522/Published by Elsevier Ltd. on behalf of IBRO.

2002; Gilabert-Juan et al., 2011) in the basolateral nucleus of the amygdala (CBL), morphological changes that appeared to be stressor and cell-type specific. Some studies also suggest that drugs used in the treatment of stress-related mood disorders, such as the antidepressant tianeptine (McEwen and Chattarji, 2004) and the mood-stabilizing drug lithium (Johnson et al., 2009), prevent stress-induced morphological changes in the rat CBL.

We have previously examined the effects of stress on amygdalar neuroplasticity (Reagan et al., 2007; Reznikov et al., 2009). Our studies demonstrated that the acute stress-mediated activation of glutamatergic pyramidal neurons in the CBL is reduced in rats subjected to prior repeated restraint stress (RRS) (Reznikov et al., 2008). We have also examined the effects of stress on glutamate neurochemistry in the amygdala. These studies demonstrated that the effects of acute stress on glutamate efflux in non-stressed control (NSC) rats are fundamentally different compared to responses in rats with a prior stress history and also determined that the effects of stress can be modulated by antidepressant administration (Reznikov et al., 2007; Piroli et al., 2013), including the antidepressant agomelatine (Reagan et al., 2012). Agomelatine is a novel antidepressant (de Bodinat et al., 2010; Guardiola-Lemaitre et al., 2014) that acts as a melatonergic (MT1 and MT2) receptor agonist (Audinot et al., 2003) and $5HT_{2C}$ antagonist (Millan et al., 2003). Agomelatine produces antidepressant-like effects in various animal models of depression (Papp et al., 2003; Bourin et al., 2004; Barden et al., 2005; Bertaina-Anglade et al., 2006; Paizanis et al., 2010; Rainer et al., 2012; Norman et al., 2012; Schmelting et al., 2013) and anxiety (Millan et al., 2005; Papp et al., 2006) and is an effective antidepressant in patients with MDD (Loo et al., 2002; Kennedy and Emsley, 2006). In view of these observations, the goals of the current study were: (1) to examine the effects of RRS upon the dendritic architecture of pyramidal neurons in the rat CBL; (2) to examine the ability of agomelatine administration to prevent stress-mediated morphological changes in CBL pyramidal neurons; and (3) to determine the ability of agomelatine to prevent RRS-induced alterations in glutamate efflux in the rat amygdala.

## EXPERIMENTAL PROCEDURES

### Animals

Eight-week-old male Sprague Dawley rats (CD strain, Charles River) weighing approximately 200–250 g were provided *ad libitum* access to standard Purina rat chow and water. Animals were maintained in a temperature-controlled room, with a light/dark cycle of 12/12 hours (h) (lights on at 07:00 h) and handled daily for five to seven days prior to experimentation. Rats were housed 2–3/cage and all rats in the same cage were given the same treatment. All experiments were conducted during the light phase, beginning at least 1 h after light phase onset and concluding at least 1 h prior to the beginning of the dark phase. Animal care and use procedures were carried out in accordance with protocols written under the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by The University of South Carolina Animal Care and Use Committee.

### RRS and agomelatine treatment

To examine stress-induced structural and functional changes in the rat amygdala, we employed a 10-day RRS paradigm; rationale for this paradigm is described in Reznikov et al. (2008). In this regard, we have previously reported that rats subjected to RRS exhibit decreased activation of glutamatergic neurons in the rat CBL (Reznikov et al., 2008). We have also reported that rats subjected to this RRS paradigm exhibit decreases in glutamate efflux in the CBL (Piroli et al., 2013). Based on these prior observations, we employed this paradigm to examine the effects of repeated stress on morphological parameters of glutamatergic neurons in the rats CBL. Specifically, animals were placed in flexible wire mesh restrainers with protective rubberized edges and restrained for 6 h/day (d) for 10 d in their home cages as described previously (Reznikov et al., 2008, 2009; Piroli et al., 2013). Restrainers were fastened using clips that allowed enough space to compensate for slight movements of the animal within the restraining apparatus. Stress was initiated at 10:00 h and terminated at 16:00 h each day. All restrainers were properly cleaned following daily stress. One hour following the termination of stress (i.e. 17:00 h) and 2 h before lights off on days 1 through 10, rats were administered either vehicle (1% hydroxyethyl cellulose; HEC) or agomelatine (40 mg/kg) by gavage. Agomelatine was suspended in 1% HEC in purified water with dose chosen based upon previous studies (Bertaina-Anglade et al., 2011; Calabrese et al., 2011; Rainer et al., 2012; Schmelting et al., 2013), including our own studies that examined the ability of agomelatine to inhibit stress-induced increases in glutamate efflux in the rat hippocampus and amygdala (Reagan et al., 2012). NSC rats were housed in a separate room to eliminate the potential effects of auditory or olfactory cues from rats subjected to RRS. NSC rats were handled daily and returned to their home cage and received either vehicle or 40-mg/kg agomelatine by gavage daily for 10 d. Therefore, experimental groups were as follows: non-stressed controls given vehicle (NSC-V), rats subjected to repeated restraint stress and given vehicle (RRS-V), non-stressed controls given agomelatine (NSC-A) and rats subjected to repeated restraint stress and given agomelatine (RRS-A). Upon completion of the RRS paradigm, all rats were handled and received either drug or vehicle for two additional days and then sacrificed on day 13. This treatment paradigm is similar to our previous studies and therefore allows for more direct comparisons between the effects of repeated stress on the morphology of CBL pyramidal neurons and the effects of repeated stress on glutamate efflux in the rat amygdala (Piroli et al., 2013).

### Morphological analysis

Rats were processed for a modification of the Golgi-Cox technique developed by Gibb and Kolb (1998) specifically