Neurobiology of Learning and Memory 142 (2017) 218–229

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Inactivation of basolateral amygdala prevents chronic immobilization stress-induced memory impairment and associated changes in corticosterone levels

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ARTICLE INFO

Article history:
Received 9 September 2016
Revised 9 April 2017
Accepted 6 May 2017
Available online 8 May 2017

Keywords:
Chronic stress
Basolateral amygdala
Inactivation of basolateral amygdala
Hippocampal-dependent learning
Cognitive deficits
Corticosterone

ABSTRACT

Chronic stress causes detrimental effects on various forms of learning and memory. The basolateral amygdala (BLA) not only plays a crucial role in mediating certain forms of memory, but also in the modulation of the effects of stress. Chronic immobilization stress (CIS) results in hypertrophy of the BLA, which is believed to be one of the underlying causes for stress' effects on learning. Thus, it is plausible that preventing the effects of CIS on amygdala would preclude its deleterious cognitive effects. Accordingly, in the first part, we evaluated the effect of excitotoxic lesion of the BLA on chronic stress-induced hippocampal-dependent spatial learning using a partially baited radial arm maze task. The BLA was ablated bilaterally using ibotenic acid prior to CIS. Chronically stressed rats showed impairment in spatial learning with decreased percentage correct choice and increased reference memory errors. Excitotoxic lesion of the BLA prevented the impairment in spatial learning and reference memory. In the retention test, lesion of the BLA was able to rescue the chronic stress-induced impairment. Interestingly, stress-induced enhanced plasma corticosterone levels were partially prevented by the lesion of BLA. These results motivated us to evaluate if the same effects can be observed with temporary inactivation of BLA, only during stress. We found that chronic stress-induced spatial learning deficits were also prevented by temporary inactivation of the BLA. Additionally, temporary inactivation of BLA partially precluded the stress-induced increase in plasma corticosterone levels. Thus, inactivation of BLA precludes stress-induced spatial learning deficits, and enhanced plasma corticosterone levels. It is speculated that BLA inactivation-induced reduction in corticosterone levels during stress, might be crucial in restoring spatial learning impairments. Our study provides evidence that amygdalar modulation during stress might be beneficial for strategic management of stress-related cognitive deficits.

1. Introduction

Stress is a state of perturbed homeostasis, which is initially compensated by adaptive processes (Kim & Diamond, 2002). Chronic uncontrollable stress leads to allostatic load, which have a detrimental effect on physiological functioning, including cognitive processes (Kim & Yoon, 1998; McEwen & Sapolsky, 1995). Studies from several laboratories including ours, show that 21 days of chronic restraint stress impair learning and memory (Bhagya, Christofer, & Shankaranarayana Rao, 2016; Conrad, Galea, Kuroda, & McEwen, 1996; Luine, Villegas, Martinez, & McEwen, 1994; Srikumar, Raju, & Shankaranarayana Rao, 2006) and induces depressive-like behavior (Chiba et al., 2012; Veena, Srikumar, Raju, & Shankaranarayana Rao, 2009). Further, these behavioral changes are associated with impaired neurogenesis (Pham, Nacher, Hof, & McEwen, 2003; Veena, Srikumar, Mahati, Raju, & Shankaranarayana Rao, 2011), dendritic atrophy (Cook & Wellman, 2004; McLaughlin, Gomez, Baran, & Conrad, 2007; Ramkumar, Srikumar, Shankaranarayana Rao, & Raju, 2008) and neurochemical deficits (Mizoguchi et al., 2000). In a severe form of stress such as chronic immobilization stress (CIS), these changes are observed sooner. For example, 10 days of CIS resulted in

Abbreviations: BLA, basolateral amygdala; CIS, chronic immobilization stress; RAM, radial arm maze; HPA axis, hypothalamic pituitary adrenal axis; pERK, phosphorylated extracellular signal-regulated kinases; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; LTP, long term potentiation; RMEs, reference memory errors; WMECs, working memory errors-correct; WMEICs, Working memory errors-incorrect.

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http://dx.doi.org/10.1016/j.nlm.2017.05.005
1074-7427/

upregulation of hippocampal catalytic TrkB mRNA expression (Nibuya, Takahashi, Russell, & Duman, 1999), produced a contrasting effect on the dendritic arborization in the amygdala and hippocampus (Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002) and precipitates anxiety-like behavior (Anuradha, Srikumar, Shankaranarayana Rao, & Lakshmana, 2008).

Several lines of research suggest the role of amygdala in mediating stress-induced effects on hippocampal-dependent learning and synaptic plasticity (Kim, Koo, Lee, & Han, 2005; Kim, Lee, Han, & Packard, 2001). Anatomically, there is a widespread connectivity between amygdala and hippocampus (Felix-Ortiz et al., 2013; Pitkänen, Pikkarainen, Nurminen, & Ylinen, 2000). Additionally, stress differentially modulates the amygdalar and hippocampal structure and function (Lakshminarasimhan & Chattarji, 2012; Vyas et al., 2002). Several studies have evaluated the role of amygdala in the effects of stress on the hippocampus. For example, excitotoxic or electrical lesion of amygdala prevents the restraint repeated tail shock-induced impairment in hippocampal CA1 LTP, spatial learning in the Morris water maze and a reduction in phosphorylated extracellular signal-regulated kinases (pERK) signaling (Jeon et al., 2012; Kim et al., 2001). Further, 8 days of restraint tail shock-induced deficits in recognition and spatial location memory were also prevented by optogenetic inhibition of the BLA (Rei et al., 2015). The lesion of BLA also blocked the deleterious effects of glucocorticoid receptor agonist or adrenalectomy (Roozendaal, Portillo-Marquez, & McGaugh, 1996; Roozendaal, Sapolsky, & McGaugh, 1998). Most of these studies were conducted on short-term or acute stressors such as single or 8 days of restraint tail shock, which is quite different from chronic stressors. Additionally, the stress-induced effects are temporally orchestrated. For instance, acute immobilization stress results in delayed spinogenesis in BLA (Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005). Further, the same stressor, when applied for 10 days causes dendritic hypertrophy in BLA, which was persistent even after 21 days of last stressor (Vyas, Pillai, & Chattarji, 2004). Additionally, chronic stress exerts a differential effect on hippocampal and amygdalar structural plasticity (Shankaranarayana Rao, Madhavi, & Sunanda Raju, 2001; Vyas et al., 2002). These studies highlight the differential structural plasticity in hippocampus and amygdala following chronic stress. However, there are no studies in our knowledge that have evaluated the effect of CIS on learning of the radial arm maze (RAM) task. Further, it is not known if the effects of CIS on learning can be prevented by inactivating the BLA during stress.

It has been reported that circulating corticosterone is a key mediator of stress and has a complex biphasic action on hippocampal dependent learning and plasticity (Diamond, Bennett, Fleshner, & Rose, 1992; Herman et al., 1989; Jacobson & Sapolsky, 1991; Kim & Yoon, 1998; Sapolsky, Zola-Morgan, & Squire, 1991). In addition, CIS has been shown to increase both serum (Lakshminarasimhan & Chattarji, 2012; Yang, Kim, Kang, Lee, & Seol, 2014) and plasma (Govindarajan et al., 2006; Liu et al., 2014) corticosterone levels, which were not restored even after 21 days of recovery period. However, whether silencing the amygdala before or during CIS, would prevent the stress-induced increase in corticosterone levels is not known.

Accordingly, the present study aimed to examine the modulatory role of amygdala during CIS on hippocampal-dependent spatial learning. In the first part, we performed ibotenic acid-induced lesions of the BLA, before subjecting them to CIS and evaluated the effect on RAM learning. In the second part, we evaluated if lidocaine-induced inactivation of the BLA only during the stress would mimic the effect of BLA lesioning. Further, we evaluated the effect of amygdalar modulation on stress-induced increase in corticosterone levels.

2. Materials and methods

2.1. Animals

Experiments were performed on adult male Wistar rats (200–225 g; 2–2.5 months old), obtained from the Central Animal Research Facility, NIMHANS, Bengaluru. Adult rats were group-housed (3 per cage) in a climate-controlled vivarium with a 12:12 h dark/light cycle. All animals had free access to food and drinking water except during the stress procedure or behavioral evaluation. The chronic stress procedure, behavioral assessment and blood collection were performed during the light phase between 10:00 h and 14:00 h. All experiments were approved by the Institutional Animal Ethical Committee and performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India.

2.2. Experimental groups

Fig. 1A and B summarizes the timelines and experimental procedures for excitotoxic lesion and temporary inactivation of the BLA. Animals were randomly assigned to one of the 10 groups: non-stressed control rats (NC – non-stressed naïve animals), phosphate buffered saline (PBS) *per se* (PBS – rats subjected to intra-BLA PBS infusion), ibotenate *per se* (IBO – rats subjected to intra-BLA ibotenate infusion), Stress (ST – naïve rats subjected to immobilization stress for 10 days), PBS infusion followed by stress (PBS + ST – rats subjected to single intra-BLA PBS infusion and then exposed to stress), excitotoxic lesion of BLA (IBO + ST – rats subjected to single intra-BLA ibotenate infusion and then exposed to stress), saline *per se* (SAL – rats subjected to intra-BLA saline infusion, 4 min/day for 10 days), lidocaine *per se* (LI *per se* – rats subjected intra-BLA lidocaine infusion, 4 min/day for 10 days), saline infusion followed by stress (SAL + ST – rats subjected intra-BLA saline infusion prior to each session of stress) and temporary inactivation of BLA (LI + ST – rats subjected to intra-BLA lidocaine infusion prior to each session of stress).

2.3. Surgery

Rats were anesthetized with ketamine hydrochloride (90 mg kg^{-1}; Ketmin® 50, Themis Medicare Ltd., India) and xylazine (10 mg kg^{-1}; Xylaxin®, Indian Immunologicals Ltd., India) combination injected via intraperitoneal route, and placed in a stereotaxic instrument (David Kopf Instruments, USA) after ensuring that they are in the surgical plane of anesthesia.

2.3.1. Excitotoxic lesion of BLA using ibotenic acid

A 30-gauge infusion needle was placed in the BLA. The coordinates were 2.8–3.0 mm posterior and 4.8–5 mm lateral to bregma, and 7.8–8.2 mm ventral to the dura (Paxinos & Watson, 2006). Ibotenic acid solution (10 μg/ml) was prepared freshly in PBS, pH 7.4 and infused bilaterally (0.25 μl/per side) using a microinjector (Harvard Apparatus, Holliston, MA) through an infusion needle connected to a Hamilton syringe (Morris, Frey, Kasambira, & Petrides, 1999; Roozendaal & McGaugh, 1996; Roozendaal & McGaugh, 1997; Roozendaal, McReynolds, & McGaugh, 2004; Roozendaal et al., 1998; Salinas, Parent, & McGaugh, 1996; Shankaranarayana Rao, Govindaiah Laxmi, Meti, & Raju, 2001). The infusion needle was left in place for an additional 2 min for drug diffusion. Following lesion, the rats were allowed to recover for 10 days and then subjected to CIS. Rats of the vehicle control group were bilaterally infused with PBS.