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Short-term environmental enrichment is sufficient to counter stress-induced anxiety and associated structural and molecular plasticity in basolateral amygdala

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ABSTRACT

Moderate levels of anxiety enable individual animals to cope with stressors through avoidance, and could be an adaptive trait. However, repeated stress exacerbates anxiety to pathologically high levels. Dendritic remodeling in the basolateral amygdala is proposed to mediate potentiation of anxiety after stress. Similarly, modulation of brain-derived neurotrophic factor is thought to be important for the behavioral effects of stress. In the present study, we investigate if relatively short periods of environmental enrichment in adulthood can confer resilience against stress-induced anxiety and concomitant changes in neuronal arborisation and brain derived neurotrophic factor within basolateral amygdala. Two weeks of environmental enrichment countermanded the propensity of increased anxiety following chronic immobilization stress. Environmental enrichment concurrently reduced dendritic branching and spine density of projection neurons of the basolateral amygdala. Moreover, stress increased abundance of BDNF mRNA in the basolateral amygdala in agreement with the dendritic hypertrophy post-stress and role of BDNF in promoting dendritic arborisation. In contrast, environmental enrichment prevented stress-induced rise in the BDNF mRNA abundance. Gain in body weights and adrenal weights remained unaffected by exposure to environmental enrichment. These observations suggest that a short period of environmental enrichment can provide resilience against maladaptive effects of stress on hormonal, neuronal and molecular mediators of anxiogenesis.

1. Introduction

Prolonged stress causes predisposition to a variety of emotional disorders including anxiety, post-traumatic stress disorder and depression (McEwen, 1999, 2004). Therefore, it is critical to understand biological effects of stress and ways to circumvent them. Prior extensive work on the facilitatory effect of stress on basolateral amygdala (BLA) has been reported. The BLA is a crucial brain structure for generation and maintenance of fear (Davis et al., 1994). This brain structure also increases stress-induced secretion of adrenal hormones through its efferent connections to bed nucleus of stria terminalis and brain stem (Corodimas et al., 1994; Davis, 1992; Davis et al., 1994; LeDoux, 2003; Rodrigues et al., 2009). Importantly, the BLA itself is affected by stress as well as by glucocorticoids secreted during stress. Both stress and stress hormones increase dendritic arborisation of the principal neurons of BLA, resulting in longer dendrites, more dendritic branches and greater spine density (Mitra et al., 2005; Mitra and Sapolsky, 2008; Vyas et al., 2002). This increase in dendritic complexity correlates with greater anxiety in stressed animals, congruent to the role of BLA in mediating fear and associated emotions. Furthermore, blockade of BLA dendritic plasticity using over-expression of hyperpolarizing SK2 K^+ channels simultaneously rescues rodents from anxiety (Mitra et al., 2009b). These observations suggest that permissive effect of stress on the BLA arbors might underlie stress-induced anxiety.

Cognitively stimulating housing conditions, more commonly referred as environmental enrichment (EE), have anxiolytic consequence in a variety of rodent models (Benaroya-Milshtein et al., 2004; Friske and Gammie, 2005; Huzard et al., 2015; Koe et al., 2016; Kondo et al., 2015; Pietropaolo et al., 2014; Ravenelle et al., 2013; Ravenelle et al., 2014; Roy et al., 2001; Soares et al., 2015). Typically, EE consists of housing animals in larger cages with multiple toys, burrowing tunnels and wire mesh walls for climbing. It remains currently unknown if EE reduces BLA dendritic complex-

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ity in parallel to reducing anxiety. This is a pertinent gap because stress-induced anxiety is concomitant to, and is possibly dependent upon, expansion of BLA dendrites. EE increases dendritic complexity in a variety of cerebral cortex regions (Diamond et al., 1976; Diamond et al., 1966; Diamond et al., 1975; Rema et al., 2006); brain regions that share cortical architecture with the BLA. Thus, effects of EE on similar cortical brain regions suggest a dendritic expansion in the BLA; while anxiolytic effects of EE suggest dendritic retraction. In this report, we experimentally test these predictions.

Brain derived neurotrophic factor (BDNF) supports neuronal survival and dendritic growth of neurons in diverse experimental models (Ahmed et al., 1995; Geremia et al., 2010; Geschwind et al., 1996; Larimore et al., 2009; Lu et al., 2005; Mey and Rombach, 1999; Rabacchi et al., 1999; Spencer et al., 2008; Zhou et al., 2006). Chronic restraint stress causes increase of BDNF in the rat BLA (Lakshminarasimhan and Chattarji, 2012) while also causing dendritic hypertrophy and anxiogenesis (Vyas et al., 2002). A forebrain-specific over-expression of BDNF in mice results in increased anxiety and BLA spine density (Govindarajan et al., 2006). Both of these phenotypes are reminiscent of stress effects in the BLA. These observations suggest, but do not prove, that stress causes increased amount of neurotrophic signaling through BDNF, resulting in dendritic expansion and anxiogenesis. The relationship between stress hormones and neurotrophic effects of BDNF is not well studied in the BLA. Yet adrenal glucocorticoids secreted during stress interact with BDNF signaling (Bennett and Lagopoulos, 2014; Kumamaru et al., 2011; Kumamaru et al., 2008; Schaaf et al., 2000) For example, binding of glucocorticoid to its receptors lead to phosphorylation of the BDNF receptor, TrkB (Numakawa et al., 2013; Numakawa et al., 2010). This step occurs downstream to transcriptional activity initiated by occupied glucocorticoid receptors. In light of these observations along with BLA being one of the primary regions that drive HPA stress axis, we measured the amount of circulating glucocorticoids and abundance of BDNF mRNA in BLA after stress and/or EE. Interestingly EE and stress are known to affect BDNF mRNA abundance in reciprocal manner in the hippocampus (Ravenelle et al., 2014; Turner and Lewis, 2003), a comparable set of observations in the BLA are not presently available.

Briefly, in this report we examine effects of enriched environment and stress on anxiogenesis, BLA dendritic complexity and BDNF mRNA abundance in the BLA. Specifically, we experimentally test if enriched environment can reduce the effects of stress.

2. Materials and methods

2.1. Animals

Male Wistar rats (7 weeks old, 220–250 g, and housed 2/cage) were procured from National University of Singapore and habituated for a week in Nanyang Technological University vivarium. Animals were maintained in light-dark cycle of 12 h (light on 0700 h) with *ad libitum* food and water. The institutional animal care and use committee of the NTU approved all experimental procedures. Body weights were recorded at the start and end of the experiments.

2.2. Stress and enrichment procedures

Stress paradigm consisted of chronic immobilization stress (CIS; 2 h a day between 0930 h to 1130 h, 10 successive days; (Vyas et al., 2002)). During stress, rats were immobilized in plastic restraint cones (Braintree Scientific, MA, USA). Unstressed animals remained unhandled in their home cage during this period. Both stressed and unstressed animals were housed 2/cage.

The enriched environment consisted of larger cages ($72 \times 51 \times 110$ cm for 14 successive days) with more individuals per cage (4/cage) and presence of cognitively stimulating objects (Mitra and Sapolsky, 2009) for 24 h every day till end of the experiment (16 days in total). Enrichment objects included climbing walls made of wire-net, plastic tunnels, plastic and wooden objects of varied color and texture, ample nesting material, gustatory variety in form of fruit loops and sunflower seeds and layered tiers within the cage. Running wheel was not provided in the enriched housing. The arrangement of enrichment objects was changed every fourth day.

2.3. Experimental groups

After one week of acclimatization to the vivarium, rats were randomly assigned to four groups: CN (control, non-stressed and non-enriched), EE (environmentally enriched non-stressed), ST (non-enriched, stressed) and EEST (environmentally enriched and stressed). Number of animals in each group are indicated in results and figure legends. Temporal relation between EE and stress protocols for endpoints (morphology and mRNA) is depicted in inset for each individual figure.

2.4. Elevated plus maze

Anxiety was measured using an elevated plus-maze (EPM, Walf and Frye, 2007). The EPM consisted of a plus-shaped arena with two open (75×11 cm, 1 cm wall, 3–4 lx illumination) and two enclosed arms (75×11 cm, 26 cm wall). The arena was elevated to 60 cm above the ground. Animal was placed at the center at start of the trial. Exploration in open and enclosed arms was quantified for duration of five minutes. Open arm exploration (entries and occupancy time) relative to sum of open and enclosed arm exploration was used as an index for anxiety. Entry in an arm was defined as presence of the whole body including head, four paws and at least base of the tail inside the arm. In addition number of head dips was quantified, defined as downward movement of head toward the floor from the open arms extending at least to intra-aural line (Walf and Frye, 2007). It is a measure of exploratory and goal-directed behavior and its absence indicates passive behavior (Cole and Rodgers, 1993; Fernandes and File, 1996; Fernandez Espejo, 1997). Such a trait representing active-coping serves as a potential marker for stress-resilience. Trials were videotaped and coded before offline analysis.

2.5. Dendritic arborisation

Freshly harvested brains were obtained by sacrifice through decapitation. Blocks of brain tissue containing BLA (Bregma −2.04 to −3.36) were processed for Golgi-Cox stain using commercial kit (FD Neurotechologies, Columbia, USA). After processing, 100 μm thick coronal sections were mounted on glass slides, counterstained with cresyl violet, dehydrated using an ascending series of alcohol and xylene, and coverslipped in non-aqueous medium.

Two-dimensional traces of BLA neurons (10 neurons per animal) were obtained at 400X magnification using a camera lucida attachment on the optical microscope (Olympus BX43, Japan). Randomly chosen stellate/pyramidal neurons were analysed for neuronal complexity and spine analysis. Traces were scanned (300 dpi, 8-bit grayscale tiff) along with a calibrated scale for subsequent computerized estimation of dendritic arbors using custom-designed routine embedded in ImageJ (http://rsb.info.nih.gov/ij/). Dendritic length and number of branch points were quantified as function of radial distance from the cell soma (Sholl's analysis, Shankaranarayana Rao et al., 2001; Vyas et al., 2002). This