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Anhedonic behavior and γ-amino butyric acid during a sensitive period in female rats exposed to early adversity

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ABSTRACT

Early life adversity increases depressive behavior that emerges during adolescence. Sensitive periods have been associated with fewer GABAergic interneurons, especially parvalbumin (PV), brain derived growth factor, and its receptor, TrkB. Here, maternal separation (MS) and social isolation (ISO) were used to establish a sensitive period for anhedonic depression using the learned helplessness (LH) paradigm. Female Sprague-Dawley rat pups underwent MS for 4-h/day or received typical care (CON) between postnatal days 2–20; for the ISO condition, separate cohorts were individually housed between days 20–40 or served as controls (CON2). Anhedonia was defined by dichotomizing subjects into two groups based on one standard deviation of the mean number of escapes for the CON group (< 14). This approach categorized 22% of CON subjects and 44% of MS subjects as anhedonic ($p < 0.05$), similar to the prevalence in maltreated human populations. Only 12.5% of ISO rats met criterion versus 28.5% in CON2 rats. Levels of PV and TrkB were reduced in the amygdala and prelimbic prefrontal cortex (PFC) in MS rats with < 14 escapes, but elevated in behaviorally resilient MS rats (> 13 escapes). The number of escapes in MS subjects significantly correlated with PV and TrkB levels (PFC: $r = 0.93$ and 0.91 and amygdala: $r = 0.63$ and 0.81, respectively; $n = 9$), but not in CON/ISO/CON2 subjects. Calretinin, but not calbindin, was elevated in the amygdala of MS subjects. These data suggest that low levels of PV and TrkB double the risk for anhedonia in females with an MS history compared to normal adolescent females.

1. Introduction

Exposure to early life adversity (ELA) in the form of physical/sexual abuse, neglect, loss of a parent or caregiver or a natural disaster affects approximately 33% of the population. The lifetime prevalence rate of depression in an ELA-exposed population is 67% (Andersen, 2015; Andersen and Teicher, 2008; Teicher et al., 2009; Widom et al., 2007) compared to 20% in the general population. Elevated emotionality has been associated with increased amygdala activity in children younger than 11 years old with an ELA history (Malter Cohen et al., 2013; Marusak et al., 2015; Tottenham et al., 2011). More mature connectivity (e.g., resting state) between the amygdala and frontal cortex following ELA has also been described (Gee et al., 2013). In humans, elevated depressive/anhedonic behavior in adults and adolescents has been associated with less GABA in the PFC (Gabbay et al., 2012; Sanacora et al., 1999). However, the relationship of ELA, GABA, and depression during adolescence in animals on an individual basis is not known.

During the preteen years, abused and non-abused children begin to diverge in developmental trajectories (Widom et al., 2007). Depression emerges *earlier* than the general population, and coincides with increased prevalence of depression during a sensitive period of adolescent development (Andersen, 2015; Andersen and Teicher, 2008). A sensitive period is defined as a maturational stage when experience can exert maximal effect on development, but is not as necessary as it is during a critical period (Andersen, 2003; Greenough et al., 1987). Critical periods have been defined by changes in growth factors, such as brain-derived growth factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB), and the GABAergic interneuron that expresses the calcium-binding protein of parvalbumin (PV) (Huang et al., 1999; Morishita et al., 2015). While critical and sensitive periods are defined differently, sensitive periods of affective development may utilize the same underlying mechanisms. The sensitive period for the amygdala has been characterized as starting during the second week of postnatal (P) development in the rodent and ending at postnatal day (P)25 (Gogolla et al., 2009). Indeed, rats exposed to the ethologically relevant rodent model of maternal separation (MS) (Andersen, 2015; Lehmann and Feldon, 2000) show an increase in PV in the basolateral amygdala

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(BLA) by P35 (Giachino et al., 2007), consistent with an end to the sensitive period.

Defining the sensitive period in the PFC has not received as much attention as the amygdala. Levels of PFC PV are low during juvenility and rise during adolescence into adulthood in normal rats (Brenhouse and Andersen, 2011; Caballero et al., 2014; Leussis et al., 2012). These PV data suggest a closing of the PFC sensitive period during adolescence. An early and protracted sensitive period in individuals with a stress history (Andersen and Teicher, 2008) implies that the brain remains highly sensitive to environmental stimuli, and thus, vulnerable during adolescence and even into adulthood. To better determine whether a sensitive period is present for anhedonia, the current study compared MS to the social isolation (ISO (Lukkes et al., 2009a);) as each of these stressor occur at different developmental stages. Comparison of these two 20-day manipulations may shed light on the role of sensitive periods in depression.

Depressive and GABA changes are found in MS rats. MS increases learned helplessness (LH) and impaired working memory in adolescent rats (Brenhouse and Andersen, 2011; Leussis et al., 2012; Matthews and Robbins, 2003). GABA-A receptors are reduced in the amygdala of rats with a history of MS (Caldji et al., 2003), which is likely to lower GABAergic activity. Lower GABA levels or GABAergic activity in the amygdala is associated with increased anxiety and depressive-like behavior (Caldji et al., 1998; Raineki et al., 2012; Ritov et al., 2016). Finally, human post-mortem studies found low amygdala BDNF and GABA mRNA in depressed women (Guilloux et al., 2012). Reduced GABA activity in the amygdala during development can affect cortical development (Berretta and Benes, 2006). Together, these findings support a role for amygdala GABA in affective dysfunction, but has neither established the relationship between the loss of GABA in the amygdala as a direct consequence of MS nor its influence on behavior.

Significant decreases in PFC PV-immunoreactive interneurons (counted by stereology) or PV expression (Western immunoblot) have been described during adolescence following MS between P2-20 days of age (Brenhouse and Andersen, 2011; Leussis et al., 2012). Sex differences in PV loss following MS occur earlier in females than males (Holland et al., 2014), but PV loss secondary to MS has been found in both sexes in adolescence (Leussis et al., 2012). Notably, one study of MS failed to find changes in PV in *octus degus*, a precocial rodent species (Seidel et al., 2008). Relatively little data is available on GABA in the cortex of ISO rats. Cortical chandelier cells (some expressing PV) decreased in males that underwent ISO (P28-84) (Bloomfield et al., 2008). However, magnetic resonance spectroscopy reported no differences in GABA levels between controls and ISO rats (Napolitano et al., 2014).

The current study examined whether a sensitive period of ELA exists for anhedonic depression by comparing behavior, PV, and TrkB in MS and ISO rats during adolescence. As the prevalence of anhedonia was affected by MS, and by ISO, changes in other GABAergic interneurons that express calbindin (CB) and calretinin (CR) were only investigated in MS subjects. There is less evidence supporting their involvement in mood disorders (Sibille et al., 2011), even though the expression of CB changes during adolescence, but CR expression remains steady (Caballero et al., 2014). BDNF levels in cortical cell culture differentially modulated CB and CR (Fiumelli et al., 2000), raising the possibility that MS exposure will effect their expression. To better understand sensitive period-related changes, the inter-relationships between these GABAergic markers, TrkB, and depressive-like behavior were examined in the amygdala and the PFC across two different manipulations.

2. Methods and materials

2.1. Subjects

Pregnant female multiparous Sprague-Dawley rats (250–275 g) were obtained from Charles River Laboratories (Wilmington, MA) on day 16 of gestation. The day of birth was designated as P0. One day after birth, litters were culled to 10 pups (5 males and 5 females), and randomly assigned to either a MS or animal facility reared control group (CON). Only one pup per litter was assigned to a single condition. We acknowledge that the stress of shipping may have influenced our findings (Ogawa et al., 2007), but was minimized by distributing subjects both CON and MS rats equally across all conditions.

Maternal separation: Pups in the MS group (n = 9) were isolated from their peers and mother for 4 h per day between P2 and P20, and kept at a thermo neutral temperature, following our methodology (Andersen, 2015; Brenhouse and Andersen, 2011; Leussis et al., 2012) and others (Plotsky and Meaney, 1993). Pups in the CON (n = 18) group were not disturbed after day two except for routine weekly cage changes, during which pups were weighed. A small, but significant, reduction in weight occurs in MS animals relative to CON during early development (Freund et al., 2013). Rats were housed with food and water available *ad libitum* in constant temperature and humidity conditions on a 12-h light/dark cycle (light period 07:00–19:00). Rats were weaned on P21, and group-housed in same-sex caging in male- or female-only vivarium until behavioral testing. Only females were used in this study.

Social isolation: Rats were born and housed at McLean, as described above. Between P21-40, subjects were individually housed within the vivarium (ISO; n = 7); only females were used and were housed in a female-only room. No other manipulation occurred during this time. The controls (CON2; n = 7) were group housed (3/cage) under the same conditions.

The experimental timeline is outlined in Fig. 1A. These experiments were conducted in accordance with the 2011 Guide for the Care and Use of Laboratory Animals (Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011), and were approved by the Institutional Animal Care and Use Committee at McLean Hospital.

Learned helplessness: In an initial cohort, CON (n = 9) and MS females (n = 9) were tested for depressive-like behavior on P40 and P41. Our previous studies have shown that females exposed to MS exhibited increased escape latency in the no shock (NS) condition (Leussis et al., 2012; Maier and Watkins, 2005; Pryce et al., 2011), which is associated with motivational deficits (Pryce et al., 2011). Females were gently restrained in the testing apparatus on Day 1 while a rat underwent the escapable shock (ES) condition of LH in their presence. This exposure affects escape behavior relative to rats that were not exposed to an ES rat on Day 1 (Lukkes et al., 2017). On Day 2, rats were individually tested in the shuttle box for 30 trials. Subjects could terminate a one mA foot-shock by shuttling to the other side for trials 1–5, or by shuttling to the other side and back again for trials 6–30. This response was cued by a tone that preceded the shock by 2 s. The shock remained on for 30 s, or until terminated by escape. The number of escapes was measured for trials 6–30. A second cohort of n = 9 females was added to the CON condition to increase our sample size for behavior only to increase statistical power to determine the prevalence of anhedonia in a typical population. These subjects did not differ from the first CON cohort on any measure.

Western Immunoblots: Ninety minutes following the onset of behavioral testing on Day 2, animals were decapitated and tissue was regionally dissected in the amygdala and PFC and stored at −80 °C until processed. Tissue was homogenized in 1% sodium dodecyl sulfate (SDS) solution containing a protease inhibitor cocktail (Pierce, Rockford, IL). Protein concentration was determined by the Bradford method (Bio-Rad Laboratories, Hercules, CA; Bradford, 1976). Proteins from the amygdala and PFC were analyzed for PV, CB, CR, and TrkB with each condition represented on a single blot for the CON/MS cohort of subjects. This approach minimized variability for the correlational analyses. The second cohort CON2/ISO was run on a single blot together for PV and TrkB. Eighty μg of protein was mixed in 6× SDS, centrifuged, and boiled for 3 min prior to separation by 15% SDS-PAGE. Proteins were then transferred to a nitrocellulose membrane (Bio-Rad