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Early life stress accelerates behavioral and neural maturation of the hippocampus in male mice

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ABSTRACT

Early life stress (ELS) increases the risk for later cognitive and emotional dysfunction. ELS is known to truncate neural development through effects on suppressing cell birth, increasing cell death, and altering neuronal morphology, effects that have been associated with behavioral profiles indicative of precocious maturation. However, how earlier silencing of growth drives accelerated behavioral maturation has remained puzzling. Here, we test the novel hypothesis that, ELS drives a switch from growth to maturation to accelerate neural and behavioral development. To test this, we used a mouse model of ELS, fragmented maternal care, and a cross-sectional dense sampling approach focusing on hippocampus and measured effects of ELS on the ontogeny of behavioral development and biomarkers of neural maturation. Consistent with previous work, ELS was associated with an earlier developmental decline in expression of markers of cell proliferation (Ki-67) and differentiation (doublecortin). However, ELS also led to a precocious arrival of Parvalbumin-positive cells, led to an earlier switch in NMDA receptor subunit expression (marker of synaptic maturity), and was associated with an earlier rise in myelin basic protein expression (key component of the myelin sheath). In addition, in a contextual fear-conditioning task, ELS accelerated the timed developmental suppression of contextual fear. Together, these data provide support for the hypothesis that ELS serves to switch neurodevelopment from processes of growth to maturation and promotes accelerated development of some forms of emotional learning.

Introduction

Early life stress (ELS) impacts neural development and significantly increases the lifetime risk for the development of cognitive and affective pathology (Felitti et al., 1998; Anda et al., 2006). The effects of ELS on the development and functioning of the underlying neural circuitry is thought to be a major contributing factor increasing risk for adverse outcomes. Currently, the predominant focus has been on the effect of ELS on the birth, survival, and morphology of cells. In the developing hippocampus and cortex, ELS or stress hormone exposure lead to: diminished cell proliferation and increased cell death (Gould et al., 1991c; Gould et al., 1991b; Tanapat et al., 1998), enhanced turnover and progressive loss of dendritic arbors and spines (Chen et al., 2008; Liston and Gan, 2011; Chen et al., 2013), decreased synaptic density (Teicher et al., 2006), and reduced volume in adolescence and adulthood (Vythilingam et al., 2002; Frodl et al., 2010). These results are consistent with observations in the adult animal, where chronic stress leads to dendritic simplification, spine loss, cell death, and suppressed neurogenesis (Gould et al., 1991a; Radley et al., 2004; Wood et al., 2004; Hajszan et al., 2009). Based upon these findings, it has been argued that ELS serves to truncate the process of neural development, with adverse behavioral outcomes often being characterized as developmental delays.

This early silencing of growth has also been linked with what appears to be precocious behavioral maturation. For example, in rodents, priming of the developing brain with stress hormones leads to an earlier emergence of defensive behaviors (Takahashi, 1995, 1996). ELS in the form of manipulations of maternal care or stress hormone exposure leads to a precocious switch from appetitive to aversive learning in a fear conditioning paradigm (Sullivan et al., 2000; Moriceau and Sullivan, 2004) as well as adult-like forms of fear extinction in juvenile animals (Callaghan and Richardson, 2014). Recent work indicates that ELS may also alter neurobehavioral development in humans, as individuals exposed to institutionalized rearing were found to have an earlier expression of adult-like functional connectivity between frontal and limbic brain regions compared with age matched controls (Gee et al., 2013). While these studies provide elegant demonstrations that ELS contributes to what appear to be more mature patterns of behavior and neural activity, these studies did not directly assess the effects of ELS on the ontogeny of neural maturation. It is unlikely that the earlier halting of neurogenesis or effects on cell death are solely supporting the accelerated profile of behavioral development observed by others. Here, we sought to test if in addition to truncating processes of cell

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birth, ELS impacts the rates of neural maturation, and if so, what mechanisms might be driving such effects.

To test if ELS impacts rates of maturation, we examined a mouse model of ELS, fragmented maternal care (Rice et al., 2008), and focused on the hippocampus, an area that is highly sensitive to stress, and an area that has reliably been implicated in the development of stress-associated pathology. We used a cross-sectional dense sampling approach throughout early development (4 to 50 days of age) in which we measured developmental changes in hippocampus-dependent contextual fear, gene expression, and immunohistochemical markers of development at short intervals (~4–10 days, Fig. 1). This approach allowed us to directly examine the effects of ELS on the ontogeny of both neural and behavioral measures of development within the same circuit.

Here, ELS led to an earlier emergence of the timed developmental suppression of hippocampus-associated fear behavior in a fear-conditioning paradigm. Using gene expression analyses and immunohistochemistry, we found that ELS led to the earlier onset and expression of biomarkers associated with neural maturation in the hippocampus and an earlier silencing of biomarkers associated with cell proliferation and differentiation. Taken together, these data provide strong support for the hypothesis that, in the hippocampus, ELS leads to a precocious switch from processes of growth to earlier neural and behavioral maturation.

Materials and methods

Subjects

Male C57BL/6N mice were bred in house, had ad libitum access to food and water, and were housed on a 12 h:12 h light:dark cycle. All animal procedures were approved by the Brown University Institutional Animal Care and Use Committee and consistent with the guide for the care and use of animals in research.

Fragmented maternal care

Four days following birth of a litter, the dam and pups were transferred from their standard home cage, to a home cage with a wire mesh floor and a 2 × 4 cm cotton nestlet as their only source of bedding (modification from- (Rice et al., 2008)). Mice continued to have ad libitum access to food and water. Dam and litters remained in these modified housing conditions for seven days, and were then returned to standard housing, containing cob bedding and a 4 × 4 cm nestlet. Control mice were left undisturbed throughout these procedures. Litters were composed of both male and female pups and litters ranges in size from 5 to 8 pups per litter. We observed no effect of litter size on growth restriction or other measures of neurodevelopment (data not shown). All pups were weaned and sex segregated at 21 days of age. For each assay (corticosterone, neurodevelopment, gene expression, and behavior), we took care to insure that animals from a minimum of two separate litters were sampled from. In previous studies of both mice and rats, elegant studies find that this manipulation leads to a fragmentation in maternal care and elevations in stress hormones in the dam (Molet et al., 2016; Rice et al., 2008; and Avishai-Eliner et al., 2001). Specifically, in those reports, the authors observed an increase in the number of departures from the nest by the dam, but no change in licking and grooming or arched back nursing (Molet et al., 2016). As maternal behavior is difficult to assess mice, in part due to their small size and lack of stereotyped nursing posture, we did not carry out detailed assessment of maternal behavior, and instead relied on our successful replication of other core features of this paradigm, which includes growth restriction of litters.

Developmental assays

To measure early developmental outcomes in control and ELS mice, we collected whole body weight at multiple time points across development. At postnatal day 17, mice were tested on the inverted wire hang task. Mean hang duration over 5 repeated trials was calculated for each animal and used for statistical comparison. On postnatal day 12 and 21 mice were removed from their home cage and individual mice were placed in an open field. Activity of the mouse was digitally recorded for a period of 7 min and distance traveled was measured with the aid of Ethovision XT 9.0 software (Noldus, Leesburg, VA).

Corticosterone ELISA

Mice were transported from the animal colony to the laboratory space and allowed to acclimate for 1 h. Trunk blood was collected by rapid decapitation. To eliminate potential cohort effects, a minimum of at least 2 different litters were used for each developmental time point. Serum corticosterone levels were measured using a competition-based ELISA (AssayMax, Corticosterone ELISA Kit, AssayPro, St. Charles, MO) using the manufacturers protocol. This kit reports a sensitivity of up to 0.3 ng/mL, with a 5–7% intra-assay reliability, and <2% cross reactivity with steroid and stress-related hormones. With this assay, we have observed an intra-assay reliability of 5.5%.

Contextual fear conditioning

Fear conditioning was carried out in Med Associates (St. Albans City, VT) operant chambers using the procedures described in (Pattwell et al., 2011). Briefly, mice received a single session of contextual fear conditioning (3 × 0.7 mA shocks, 1 s in duration) followed 24 h later by a single context test (5 min). Different cohorts of animals, across multiple litters, were used to test mice at the different developmental time points ($n = 7$ to 18 mice per group/time point). Freezing behavior was scored using the activity tracker module in Noldus Ethovision XT9.0 and verified from video by observers blind to treatment.

Real-time quantitative polymerase chain reaction (RT-qPCR)

For each developmental time point, the hippocampus was collected from animals from at least 2 different litters to eliminate the possibility

Fig. 1. Experimental Design. We collected a wide variety of biochemical, genetic, histological, and behavioral measures across early development. Here we plot the timeline of our experimental manipulations, with bedding restriction occurring between P4 and P11. Both during and following this manipulation, we sampled from multiple separate litters of mice to collect basal serum corticosterone levels (Corticosterone), measures of early motor development (Motor Behavior), mRNA to assess gene expression (Gene expression), fixed brain tissue for immunohistochemistry (IHC), and carried out contextual fear conditioning in separate sets of mice (conditioning) and testing for fear expression 24 h following conditioning (Freezing Behavior). For all manipulations, individual animals contributed to only a single measure with unique cohorts of animals being used for the collection of each time point.