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Differential impact of *Met* receptor gene interaction with early-life stress on neuronal morphology and behavior in mice

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ABSTRACT

Early adversity in childhood increases the risk of anxiety, mood, and post-traumatic stress disorders in adulthood, and specific gene-by-environment interactions may increase risk further. A common functional variant in the promoter region of the gene encoding the human MET receptor tyrosine kinase (rs1858830 '*C*' allele) reduces expression of *MET* and is associated with altered cortical circuit function and structural connectivity. Mice with reduced *Met* expression exhibit changes in anxiety-like and conditioned fear behavior, precocious synaptic maturation in the hippocampus, and reduced neuronal arbor complexity and synaptogenesis. These phenotypes also can be produced independently by early adversity in wild-type mice. The present study addresses the outcome of combining early-life stress and genetic influences that alter timing of maturation on enduring functional and structural phenotypes. Using a model of reduced *Met* expression ($Met^{+/-}$) and early-life stress from postnatal day 2–9, social, anxiety-like, and contextual fear behaviors in later life were measured. Mice that experienced early-life stress exhibited impairments in social interaction, whereas alterations in anxiety-like behavior and fear learning were driven by *Met* haploinsufficiency, independent of rearing condition. Early-life stress or reduced *Met* expression decreased arbor complexity of ventral hippocampal CA1 pyramidal neurons projecting to basolateral amygdala. Paradoxically, arbor complexity in $Met^{+/-}$ mice was increased following early-life stress, and thus not different from arbors in wild-type mice raised in control conditions. The changes in dendritic morphology are consistent with the hypothesis that the physiological state of maturation of CA1 neurons in $Met^{+/-}$ mice influences their responsiveness to early-life stress. The dissociation of behavioral and structural changes suggests that there may be phenotype-specific sensitivities to early-life stress.

1. Introduction

Early-life adversity during childhood is associated with increased risk of anxiety, mood and post-traumatic stress disorders in adulthood (Green et al., 2010; Lang et al., 2008; Widom, 1999). Additionally, family and twin studies show that the heritability of these disorders is approximately 0.3–0.4 (Hettema et al., 2001; Stein et al., 2002; Sullivan et al., 2000). Genome-wide association studies and rare variant and mutation analyses have revealed several genetic risk factors associated with e.g. post-traumatic stress disorder (Almli et al., 2014), but a large fraction of heritability of many mental health disorders is still unexplained by single factors. Epidemiological studies have revealed gene-by-environment (G × E) interactions between early adversity, genetic polymorphisms, and increased risk for affective disorders (Bradley et al., 2008; Duncan et al., 2014; Sharma et al., 2015), but there is a need to discover G × E interactions that identify potential mechanisms of action.

In many early-life stress (ELS) rodent models, early adversity is induced by the disruption of postnatal maternal-pup interactions (Baram et al., 2012; Francis and Meaney, 1999; Heun-Johnson and Levitt, 2016; Raineki et al., 2010), which modulates long-term effects on behavior. ELS generally results in reduced social interactions and impaired fear memory in adult mice (Sachs et al., 2013; van der Kooij et al., 2015; Wang et al., 2011a), whereas reported effects on anxiety-like behaviors are mixed, with increased (Bouet et al., 2011; Levine et al., 2012; Mehta and Schmauss, 2011), decreased (Fabricius et al., 2008; Savignac et al., 2011) or unaffected outcomes (Ivy et al., 2008; Naninck et al., 2015; Sachs et al., 2013; Veenema et al., 2007; Zoicas

Abbreviations: ASD, autism spectrum disorders; BLA, basolateral amygdala; vHC, ventral hippocampus; DSI, direct social interaction; ELS, early-life stress; EPM, elevated-plus maze; G × E, gene-by-environment; P, postnatal day; SNP, single nucleotide polymorphism

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and Neumann, 2016).

The social-emotional behaviors relevant to clinical manifestations associated with early life adversity in humans are regulated by specific neuronal activity in several brain regions in rodents, including the ventral hippocampus (vHC) and amygdala. These two structures are directly and reciprocally connected with each other (McDonald and Mott, 2017). Different experimental strategies have been used to stimulate, inhibit or record directly from CA1 neurons in different behavioral tasks, revealing the central role that vHC CA1 pyramidal neurons play in fear memory, anxiety-like behavior, spatial exploration, and goal-directed navigation (Ciocchi et al., 2015; Maren et al., 1998; Maren and Fanselow, 1995; Okuyama et al., 2016; Padilla-Coreano et al., 2016; Xu et al., 2016). Conversely, neurons projecting *from* the basolateral amygdala (BLA) to the vHC CA1 are involved in various social and emotional behaviors (Felix-Ortiz et al., 2013; Felix-Ortiz and Tye, 2014; Huff et al., 2016). These studies suggest that connectivity between the vHC and BLA, within larger networks of other brain regions, modulates behaviors integral to the effects of early adversity. Concomitantly, morphological changes may be apparent in neurons within this pathway after induction of ELS in mice. Indeed, ELS induces long-term morphological changes in amygdalar and (ventral) hippocampal neurons in mice and rats (Brunson et al., 2005; Ivy et al., 2010; Koe et al., 2016; Monroy et al., 2010), but there are no studies in mice that examined specifically neurons projecting from the ventral CA1 to the BLA in the context of ELS.

The vulnerability of the brain to ELS may be influenced by genes that modulate the timing of neuronal maturation in specific developing circuits, for example *Syngap1* (Clement et al., 2012), *Ahrgap12* (Ba et al., 2016), *Grin3a* (encoding NR3A) (Henson et al., 2012), and *Met* (Peng et al., 2016; Qiu et al., 2014). Yet, none of these genes have been studied in the context of ELS. *Met*, the gene encoding the MET receptor tyrosine kinase, regulates cortical and hippocampal circuit morphology and synaptic maturation. MET is enriched at developing synapses (Eagleson et al., 2013) in hippocampal, neocortical and other forebrain structures during normal circuit formation in mice and non-human primates (Judson et al., 2009, 2011a). Complete or partial deletion of *Met* in the brain alters neuronal morphology in *Met*-expressing pyramidal neurons in CA1 of the hippocampus and the anterior cingulate cortex (Judson et al., 2010; Qiu et al., 2014), cued fear conditioning and anxiety-like behavior (Thompson and Levitt, 2015), intracortical connectivity (Qiu et al., 2011), and leads to precocious hippocampal excitatory synaptic maturation (Peng et al., 2016; Qiu et al., 2014). Studies in humans are consistent with a conserved function for MET in these circuits. A single nucleotide polymorphism (SNP; rs1858830 '*C*' allele) in the promoter of the *MET* gene reduces *MET*/MET expression in the neocortex and in peripheral monocytes (Campbell et al., 2006, 2007; Heuer et al., 2011; Jackson et al., 2009; Voineagu et al., 2011). Whereas the SNP is associated with increased risk of autism spectrum disorders (ASD) (Campbell et al., 2006), data most pertinent to the present studies are the findings of altered structural (Hedrick et al., 2012) and functional connectivity (Rudie et al., 2012). The latter neuroimaging study revealed a striking interaction of ASD diagnosis and '*C*' allele dosage, suggesting that other (environmental) factors may affect functional and diagnostic outcomes.

Here, we have addressed whether reduced expression of *Met* in the central nervous system interacts with ELS to impact more robustly than either alone, social-emotional behaviors and the morphology of vHC-BLA projection neurons in mice. We show that ELS alone reduced the number of social interactions, and in $Met^{+/-}$ mice, anxiety-like behaviors were decreased and contextual fear memory was impaired. In pyramidal vHC-BLA projection neurons, ELS and $Met^{+/-}$ genotype independently decreased dendritic complexity, whereas ELS in $Met^{+/-}$ mice resulted in a paradoxical increase in complexity compared to non-stressed $Met^{+/-}$ mice. We discuss possible unique mechanisms that relate to CA1 neuronal maturation at the time of ELS, and may underlie the dissociation of behavioral and morphological G × E effects.

2. Materials and methods

2.1. Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California, and were carried out in accordance with the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals'. Efforts were made to minimize animal suffering and to reduce the number of mice used for experiments. C57BL/6J mice were housed in 'JAG' mouse cages (Allentown Inc., NJ) in a vivarium on a 12-h light/dark cycle, and temperature and humidity levels maintained at 20–22 °C and 40–60%, respectively. The mice had *ad libitum* access to food and water. Only male mice were analyzed for this study. Litter sizes were standardized across conditions by using second litters of mice. $Met^{fx/fx}$ females were mated with $Nestin^{Cre}$ males, all of which are on a C57Bl/6J background. Litters consist of an approximately similar proportion of $Met^{fx/+}$ (Control) and heterozygous $Nestin^{Cre}/Met^{fx/+}$ ($Met^{+/-}$) pups, the latter expressing approximately 50% of normal MET levels (Thompson and Levitt, 2015). The dams do not express *Cre*, and are no different than wild-type mice behaviorally. Three independent cohorts of mice were used for evaluating MET protein expression, behavioral tests, and morphological analyses. Investigators were blind to genotype and environment experienced by each mouse during tissue collection, behavioral and morphological procedures, and data collection. Weaned mice were genotyped as described in Judson et al. (2009), with a final elongation step of the 320 base pair $Nestin^{Cre}$ PCR product of 7 min, and denaturation steps during the Met^{fx} amplification cycles of 1 min.

2.2. Early life stress paradigm

Details of the paradigm used in the current study are reported in Heun-Johnson and Levitt (2016), based on methods reported in Rice et al. (2008). Briefly, ELS is induced by inserting a wire mesh (#RWF75JMV, Allentown Inc., NJ) into the cage on postnatal day (P)2, and providing the dams with two-thirds (1.8 g) of a nestlet square (Ancare Corp, NY). Control cages lacked the wire mesh insert, and contained standard amount of bedding and one nestlet square. All litters were culled to three males and two females on P2. ELS and Control litters were placed into a fresh control cage environment on P9. Additional cage changes were carried out on P16, and at time of weaning (P21).

2.3. Immunoblot analysis of MET protein expression in hippocampus

Whole hippocampal tissues from P9 mice were homogenized using a glass homogenizer (Wheaton) in ice cold homogenization buffer (10 mM Tris-HCl, pH7.4, 1% SDS, 1% protease inhibitor cocktail (#8340, Sigma)), 1% phosphatase inhibitor 2 (#5726, Sigma). The homogenate was centrifuged for 15 min at 1000 × g at 4 °C, and the supernatant diluted with 5x final sample buffer and centrifuged at 13,000 g. Forty microgram total protein was loaded per lane on a 7.5% acrylamide/bis gel, and transferred to nitrocellulose membrane. After blocking with blotto (5% #9999S Cell Signaling in phosphate-buffered saline), anti-MET antibody (#8057, 1:3000, Santa Cruz Biotechnology), and secondary antibody (#715-035-150, 1:5000, Jackson ImmunoResearch) was used for immunodetection, followed by Femto chemiluminescent substrate (#34095, ThermoFisher). The signal was analyzed using a CCD camera (UVP BioImaging System) and VisionWorksLS software (VisionWorks). The immunostaining process was repeated for anti-α-Tubulin protein (#CP06, 1:200,000, EMD Millipore) to normalize anti-MET signal.

2.4. Behavioral tests

Standard protocols were used for behavioral testing occurring in the