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Early emergence of altered 5-HT_{2A} receptor-evoked behavior, neural activation and gene expression following maternal separation

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ABSTRACT

The early stress of Maternal Separation (MS) contributes to the establishment of adult psychopathology. The serotonergic (5-HT) system is implicated during this temporal window in mediating the development of mood-related behaviors. MS is reported to evoke altered 5-HT_{2A} receptor function in adulthood. However, the ontogeny of altered 5-HT_{2A} receptor responsivity following MS remains unknown. Here, we examined 5-HT_{2A} receptor agonist, DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) (2 mg/kg) evoked responses, namely stereotypical head-twitch behaviors in control and MS Sprague-Dawley rat pups at postnatal day 21 (P21). MS involved a separation of pups from the dam for 3 h daily from postnatal day 2–14. MS pups at P21 exhibited significantly enhanced head-twitch behaviors compared to controls. Using c-Fos cell counting we examined neural activation in control and MS pups following DOI treatment. MS pups exhibited altered DOI-evoked c-Fos expression within all mPFC subdivisions, but not in the hippocampus, lateral septum and hypothalamus, suggesting differential prefrontal neural activation upon 5-HT_{2A} receptor stimulation following early stress. Gene profiling of 5-HT_{2A} receptor-regulated immediate early genes (IEGs) indicated a decline in the expression of *Fos*, *Fra1* and *Egr1* mRNA under baseline conditions in the mPFC of MS pups. MS pups also showed an altered pattern in the regulation of several 5-HT_{2A} receptor-regulated IEGs (*Fos*, *Fra1*, *Bdnf*, *Egr1*, *Egr3*) following DOI treatment. Collectively, these results highlight an early emergence of altered 5-HT_{2A} receptor-evoked behavioral responses and neural activation patterns in multiple brain regions in animals with a history of MS.

1. Introduction

Early life stress is a risk factor for the development of psychopathology (Carr et al., 2013; Lyons et al., 2011; Pechtel and Pizzagalli, 2012). Both preclinical and clinical studies have established the existence of critical periods during neurodevelopment wherein the developing brain is particularly sensitive to environmental perturbations (Ansorge et al., 2007; Carr et al., 2013; Gross and Hen, 2004). Early adverse experience is known to program enhanced anxiety and depressive-like behavior in adulthood, and to evoke perturbed neuroendocrine and behavioral responses to stress (Lyons et al., 2011). Amongst the critical substrates for early stress is the serotonergic neurocircuitry (Lesch and Waider, 2012; Ohta et al., 2014). Serotonin (5-HT) plays an important role during development and maturation of neural circuits, influencing progenitor turnover, differentiation, migration, synaptogenesis and dendritic remodeling (Lesch and Waider, 2012). Perturbations of serotonergic neurotransmission during critical windows of postnatal development either via pharmacological methods such as treatment with selective serotonin reuptake inhibitors (SSRIs) (Ansorge et al., 2007) or through genetic approaches evoke altered anxiety and depressive-like behavior (Ansorge et al., 2007; Gross and Hen, 2004). While several previous reports have implicated the 5-HT_{1A} receptor in the development of anxiety and depressive-like behavior (Gross et al., 2002; Richardson-Jones et al., 2011), more recently studies have also suggested an important role for the 5-HT_{2A} receptor in contributing to the behavioral consequences of early adversity (Benekareddy et al., 2011; Holloway et al., 2013; Wischhof et al., 2015). Recent reports indicate that diverse animal models of early life perturbations, including the classical model of maternal separation (MS) involving a 3 h daily separation of pups from the dam from postnatal day 2–14 (Kalinichev et al., 2002), postnatal exposure to SSRIs, as well as gestational models such as maternal immune activation (MIA), exhibit perturbed cortical 5-HT_{2A} receptor expression/function (Benekareddy et al., 2010; Holloway et al., 2013; Sarkar et al., 2014). Further, pharmacological blockade of 5-HT_{2A} receptors during the period of early life perturbations is reported to prevent the emergence of anxiety and depressive-like behaviors in some of these animal models, suggesting a key role for the 5-HT_{2A} receptor (Benekareddy

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et al., 2011; Sarkar et al., 2014).

Adult animals with a history of the early stress of MS, exhibit both enhanced cortical 5-HT_{2A} receptor-mediated electrophysiological responses, as well as 5-HT_{2A} receptor-evoked head-twitch behaviors (Benekareddy et al., 2010), which are known to be mediated via cortical 5-HT_{2A} receptors (Canal and Morgan, 2012). MS animals also exhibit an alteration in the 5-HT_{2A} receptor-regulated transcriptome in the medial prefrontal cortex (mPFC), in particular pertaining to genes associated with signal transduction, neuronal plasticity and cellular development (Benekareddy et al., 2010). Further, enhanced cortical 5-HT_{2A} receptor expression accompanied by increased head-twitch behavior has been observed in adult animals with a history of MIA (Holloway et al., 2013; Malkova et al., 2014). Interestingly, animal models of schizophrenia and psychosis, based on treatment with psychotomimetics in adulthood also exhibit enhanced responses to DOI, namely increased head-twitch behavior (Chiu et al., 2014; Santini et al., 2013) and neuroinflammatory insults that are linked to psychosis-like behavior also result in enhanced neocortical 5-HT_{2A} receptor expression (Holloway et al., 2013; Savignac et al., 2016). While these previous reports have focused on studies in adulthood, we sought to examine the regulation of 5-HT_{2A} receptor-mediated behaviors, neuronal circuit activation using the marker c-Fos and immediate early gene (IEG) expression within the mPFC at a postnatal time point, soon after peak expression of cortical 5-HT_{2A} receptors. 5-HT_{2A} receptor expression shows a dynamic regulation during the first few weeks of postnatal life. 5-HT_{2A} receptor expression appears to first acquire adult-like levels by the end of the first postnatal week (P7-P8), followed by a transient increase reaching peak receptor levels between P10 and P17, after which it declines and is maintained at adult levels commencing around P18- P20 (Basura et al., 2008; Morilak and Ciaranello, 1993). The ontogeny of 5-HT_{2A} receptors indicates highest expression in cortical brain regions during postnatal development overlapping with critical periods in which early stress can program life-long behavioral consequences (Basura et al., 2008; Morilak and Ciaranello, 1993).

Our results indicate that MS animals exhibit enhanced 5-HT_{2A} receptor-evoked behavioral responses at postnatal day 21 soon after the cessation of the MS paradigm. This enhanced 5-HT_{2A} receptor responsivity is accompanied by an altered pattern of neural activation, based on c-Fos cell counting analysis in response to 5-HT_{2A} receptor stimulation in postnatal MS animals. This is also noted in the altered expression of multiple IEGs within the mPFC of MS animals under baseline conditions and in response to 5-HT_{2A} receptor stimulation. Our results indicate that early stress evokes rapid changes in 5-HT_{2A} receptor-evoked responses, including altered transcriptional responses, patterns of neural activation and 5-HT_{2A} receptor-induced behaviors that emerge during postnatal life.

2. Material and methods

2.1. Animals

Sprague-Dawley rat pups maintained on a 12 h light/dark cycle (lights on at 7:00 a.m.) with *ad libitum* access to food and water were used for all experiments. All experimental procedures were conducted as per the national guidelines of the Committee for Supervision and Care of Experimental Animals (CPCSEA) and were approved by the TIFR Institutional Animal Ethics committee.

2.2. Maternal separation paradigm

The early stress paradigm of maternal separation (MS) was performed as described previously (Benekareddy et al., 2010). Dams and their litters were assigned at random to either control or MS groups on postnatal day (P1). All litter sizes ranged between 10 and 12 pups per litter. Pups in the MS group were separated from the dam for a duration of three hours daily from P2- P14. During the period of separation the entire litter was placed in a beaker lined with bedding material and kept on a heating pad in a different room. The dam was placed in a fresh cage throughout the separation period. At the end of the separation, pups were returned to the home cage first followed by the dam. Control litters were left undisturbed apart from standard animal facility maintenance cleaning every three days, which involved minimal disturbance. For all experiments (behavioral and molecular), rat pups were sacrificed at P21. There were three separate experimental cohorts of animals with the following experimental groups, namely Control, MS, Control + DOI and MS + DOI. Within each experimental cohort, the individual experimental groups had animals derived from a minimum of three litters to avoid any litter-specific effects, and included both male and female pups. Experimental cohort 1 was used for the measurement of head-twitch behavior at P21 following DOI treatment ($n = 5 - 7$/group). Experimental cohort 2 was perfused at two hours post DOI treatment for c-Fos immunohistochemistry ($n = 4 - 5$/group). Experimental cohort 3 was sacrificed two hours post DOI treatment and was used for qPCR analysis ($n = 7 - 9$/group).

2.3. Drug treatment and behavioral analysis

Control and MS rat pups (P21) were intraperitoneally administered the 5-HT_{2A} receptor agonist DOI ((±)-1-(2, 5-Dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride) (2 mg/kg) (Sigma-Aldrich, USA) or vehicle (0.9% saline) [Treatment groups: Control + Vehicle, Control + DOI, MS + Vehicle and MS + DOI, ($n = 5 - 7$ per group)]. DOI evokes stereotypical head-twitch behaviors defined by a rapid radial movement of the head. During behavioral analysis rat pups were individually housed in a separate cage. Head twitch behavior was recorded in rat pups administered vehicle or DOI commencing twenty minutes after drug treatment for a thirty minute duration. The number of head-twitch events was manually scored by an experimenter blind to the experimental treatment groups.

2.4. Immunohistochemistry and cell counting

All animals (P21) were sacrificed two hours post vehicle or DOI treatment by first anesthetizing them with an overdose of sodium thiopentone followed by transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 (0.1 M PB). Free-floating 40um thick coronal sections of the brain were obtained using a vibrating blade microtome (Leica, Germany). Sections were incubated with the blocking solution (0.3% Triton X-100, 10% horse serum, 0.1 M PB) for 2 h at room temperature followed by overnight incubation with the primary antibody (1:1000, rabbit anti c-Fos, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Sections were washed in 0.1 M PB and incubated with the biotinylated secondary antibody (1:250, biotinylated donkey anti rabbit, Vector Labs, USA) for 3 h. Sections were then washed with 0.1 M PB and incubated with avidin-biotin complex (Vectastain Elite ABC HRP kit, Vector Labs, USA) for 2 h at room temperature. Following ABC treatment, sections were treated with diaminobenzidine (SigmaFast DAB, Sigma Aldrich, USA) to visualize the signal.

The number of c-Fos positive cells was counted using a Zeiss Axioskop (Carl Zeiss, Germany) with a 20X objective by an experimenter blind to the experimental treatment groups. c-Fos immunoreactive cells were counted in the following brain regions bilaterally: mPFC (6 sections per animal; 4–6 animals per group), hippocampal subfields (4 sections per animal; 4–6 animals per group), lateral septum (LS; 4 sections per animal; 4–6 animals per group) and the paraventricular nucleus of the hypothalamus (PVN; 3 sections per animal; 4–6 animals per group). Different brain regions were identified using the postnatal rat brain atlas (Khazipov et al., 2015). Only intensely stained cells were considered to be c-Fos positive. The cell counts were expressed as number of c-Fos positive cells per section and results are represented as the mean ± S.E.M.

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