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The transcription factor Npas4 contributes to adolescent development of prefrontal inhibitory circuits, and to cognitive and emotional functions: Implications for neuropsychiatric disorders

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ABSTRACT

The adolescent brain is marked by functional and structural modifications, particularly within the inhibitory system of the prefrontal cortex (PFC). These changes are necessary for the acquisition of adult cognitive functions and emotion regulation, and impairments in these processes are associated with neuropathologies such as schizophrenia and affective disorders. The molecular mechanisms regulating this adolescent refinement of prefrontal inhibitory circuits remain largely unknown. Here we demonstrate that the transcription factor Npas4 plays a major role in this process. Using a series of behavioral, molecular, pharmacological and genetic approaches in mice, we demonstrate that deficiency in Npas4 affects adolescent expression of multiple markers of GABAergic transmission in the PFC, including parvalbumin and GAD67, in a sex-specific manner. This abnormal pattern of expression of GABAergic markers is associated with sex-specific cognitive and emotional impairments that occur only when Npas4 deficiency begins at adolescence but not post-adolescence. Finally, we show that chronic treatment with the GABA enhancing drug sodium valproate during adolescence is sufficient to induce long-lasting recovery of the molecular and behavioral abnormalities observed in Npas4 deficient mice. Altogether, we provide evidence for the involvement of the transcription factor Npas4 to the structural changes that affect prefrontal inhibitory circuits during adolescence. Further investigations of Npas4 role in the adolescent brain might provide new insights on the molecular mechanisms underlying neuropsychiatric disorders that emerge during adolescence.

1. Introduction

The transition from adolescence to adulthood is marked by drastic functional and structural changes in the prefrontal cortex (PFC) (Giedd and Blumenthal, 1999; Giedd, 2004), particularly in the GABAergic system (Hoftman and Lewis, 2011). These changes contribute to the acquisition of inhibitory control through a gain of local GABAergic function (Thomases et al., 2013; Cass et al., 2013). Substantial evidence supports the view that disturbances in these prefrontal GABAergic facilitation processes could contribute to psychiatric illnesses including schizophrenia and affective disorders (Lewis and González-Burgos, 2008; Lewis, 2009; Lewis et al., 2012). The changes that affect GABAergic signaling during adolescence have been well described in rats (Caballero et al., 2014), and non-human and human primates (Hoftman and Lewis, 2011; Catts et al., 2013), and consist for instance in increased expression of parvalbumin (PV), and decreased expression of somatostatin (SST). However, the molecular mechanisms that control this postnatal facilitation of prefrontal inhibitory circuits remain largely unknown. Some studies highlighted the possible contribution of Nrg1 and ErbB4 signaling to this process (Fazzari et al., 2010; Yang et al., 2013), while others implicate brain-derived neurotrophic factor (BDNF) (Yamada et al., 2002; Vandenberg et al., 2015). For instance, mice hypomorphic for the BDNF receptor TrkB display decreased levels of GAD67 (the rate-limiting enzyme necessary for the synthesis of GABA) and PV mRNA (Hashimoto et al., 2005). Interestingly, abnormal expression of GABAergic markers in the PFC has been reported in several psychiatric conditions that tend to emerge during the adolescent period. For instance, reduced mRNA levels of GAD67 and PV have been associated with schizophrenia (Lewis et al., 2012), while low mRNA levels of PV and SST have been observed in the brains of patients with bipolar disorder and major depressive disorder, respectively (Sibille et al., 2011).

Despite these recent findings, knowledge of the molecular mechanisms underlying the structural and functional changes that affect the prefrontal GABAergic system during the transition from adolescence

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to adulthood remains scarce. Our goal was thus to identify a potential novel molecular contributor to the changes that affect prefrontal inhibitory circuits during adolescence, the ultimate goal being to provide new insights on neuropathologies that are characterized by an adolescent onset. Our recent work showed that the brain specific transcription factor Npas4 is highly expressed in the adolescent PFC of mice when compared to adulthood (Coutellier et al., 2015). Furthermore, stress-induced reduction of Npas4 during adolescence but not adulthood impairs prefrontal cortex-dependent cognitive functions (Coutellier et al., 2015), a core symptom of neurodevelopmental disorders characterized by impaired maturation of the prefrontal inhibitory system, such as schizophrenia. In vitro and in vivo studies revealed that Npas4 contributes to inhibitory transmission onto excitatory neurons by regulating the expression of activity-dependent genes (such as BDNF) in hippocampal and cortical neurons (Lin et al., 2008; Bloodgood et al., 2013; Spiegel et al., 2014). Altogether, these findings suggest that Npas4 could be a major molecular regulator of the maturational processes that characterize the adolescent prefrontal GABAergic system. This idea is further supported by the behavioral phenotyping of adult Npas4 deficient mice showing behavioral deficits that resemble those observed in patients with schizophrenia or mood disorders, including social anxiety, despair, deficits in pre-pulse inhibition, and hippocampal- and PFC-dependent cognitive functions (Coutellier et al., 2015; Coutellier et al., 2012; Ramamoorthi et al., 2011; Sun and Lin, 2016; Jaehne et al., 2015). In the present study, we thus aimed at demonstrating that Npas4 is necessary during adolescence for the PFC to acquire full maturity of its inhibitory GABAergic system, which promotes adult cognitive and emotional functions. Our experimental design includes both genders, since evidence suggests that sex differences in neurodevelopmental processes could contribute to sex differences in psychiatric conditions, including schizophrenia and affective disorders (Hammerslag and Gulley, 2016). Based on pharmacological, behavioral and molecular methodologies in constitutive and inducible conditional transgenic mice, we provide evidence that expression of the transcription factor Npas4 during adolescence contributes to aspects of the postnatal reorganization of GABAergic circuitry, which when impaired is associated with sex-dependent behavioral impairments relevant to neuropsychiatric conditions whose symptoms arise in adolescence.

2. Material and method

2.1. Animals

All experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee of The Ohio State University and were performed based on the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

All mice used in the current experiments were obtained from our own breeding. They were housed in a humidity- and temperature-controlled facility, maintained on a 12 h reverse light-dark cycle with access to food and water ad libitum. C57Bl/6J original breeder mice were purchased from Jackson Laboratory (Maine, USA); Npas4 conventional wild-type (WT) and knock-out (KO) were obtained by heterozygotes breeding; Npas4 inducible conditional KO (cKO) mice were obtained by breeding Npas4 homozygous flox mice (originally obtained from Dr. Greenberg laboratory, Harvard University, USA) with Cre-ERT2 mice (B6;129S–Tg(UBC-cre/ESR1)1Ejb/J, Jackson Laboratory, Maine, USA). At postnatal day (PND) 21, pups were weaned and group-housed (2–5 animal per cage) per sex. The period between PND21–30 was considered as the pre-pubertal period, while PND30–60 was considered as adolescence. Previous studies indicated that during this time frame, rodents undergo behavioral and neurobiological transformations (Juraska and Markham, 2004; Markham et al., 2007) similar to those observed in other species during adolescence, including humans (Gogtay et al., 2004).

2.2. Assessment of postnatal Npas4 expression in the PFC of C57Bl/6J mice

We used RT-PCR and immunohistochemistry to assess changes in Npas4 expression at baseline in the mouse PFC during postnatal development. For assessment of Npas4 mRNA levels, C57Bl/6 J mice were anesthetized with isoflurane and rapidly decapitated. Brains were collected and flash-frozen on dry ice. All brains were stored at −80 °C until dissection. Primer sequences and efficiency, and RT-PCR procedures are presented in Supplemental information S1a. Npas4 mRNA levels were assessed in male and female mice at PND21 (weanlings), PND 35 (adolescence) and PND70–75 (adult). For immunohistochemistry, C57Bl/6J mice were anesthetized with isoflurane and perfused with saline, followed by 4% cold paraformaldehyde (PFA). Brains were removed and placed in 4% PFA at 4 °C overnight before cryoprotection in 30% sucrose. Brains were then frozen on dry ice and sectioned at 50 μm using a cryostat. Sections were stored in cryoprotectant solution at −20 °C until immunohistochemistry on free-floating sections was performed. The procedure is described in Supplemental information S2. A total of 8–13 sections containing the PFC from 3 animals per age group (weanling: PND21, adolescent: PND35; adult: PND88) per sex were used.

2.3. Assessment of expression of markers of GABAergic transmission in the postnatal PFC of Npas4 deficient mice

We used RT-PCR techniques to determine whether Npas4 contributes to the events that lead to reorganization of the prefrontal inhibitory system and that aim at promoting facilitation of GABAergic transmission during adolescence. To obtain a thorough understanding of potential changes in GABAergic transmission induced by Npas4 deficiency, we selected (Giedd and Blumenthal, 1999) two markers of GABA interneurons: the calcium-binding protein parvalbumin (PV), and the neuropeptide somatostatin (SST); (Giedd, 2004) presynaptic markers: GAD67 and VGat (vesicular GABA transporter); (Hoftman and Lewis, 2011) postsynaptic markers: GABAAα1 receptor subunit and gephyrin. We assessed mRNA levels of these GABAergic markers in the PFC of male and female WT and KO Npas4 mice at weaning (PND21), mid-adolescence at PND 45–47, and adulthood at PND90–96. Brains were processed as described above and in Supplemental information S1a. We used GAPDH as the housekeeping gene as we showed that its expression in the PFC is not affected by age or genotype (Supplemental information S1b). In addition to mRNA level assessments, we used immunofluorescent techniques to gain further insight on the PV system, since this is one of the major system undergoing changes during the transition from adolescence to adulthood (Caballero et al., 2014). We determined the total number of PV positive neurons in the PFC, as well as their co-localization with GAD67, in WT and KO Npas4 male and female mice at the end of the adolescent period (PND53–56). In addition to the number of PV positive neurons in the PFC, we also measured the intensity of the signal in a subset of PV positive neurons as an indicator of protein levels per PV neurons. Immunofluorescent procedures and analyses are described in Supplemental information S2.

2.4. Assessment of PFC-dependent cognitive and emotional functions in conventional Npas4 deficient mice

Behavioral tests were used to assess PFC-dependent cognitive function, general activity, and emotional-related behaviors. Conventional Npas4 transgenic (WT and KO) male and female mice were tested at 7 weeks old. This age corresponds to late adolescence in humans, when the first symptoms of several neuropsychiatric disorders emerge. Behavioral testing was conducted during the dark phase of the day/night cycle by an experimenter blind to the genotype of the mice. The Object Context mismatch Test (OCT) was used to assess contextual information processing, which rely on the integrity of the PFC (Fogelson et al., 2009; Spanswick and Dyck, 2012) and which has been shown to