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Development of transcripts regulating dendritic spines in layer 3 pyramidal cells of the monkey prefrontal cortex: Implications for the pathogenesis of schizophrenia

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ABSTRACT

Certain cognitive deficits in schizophrenia appear to emerge from altered postnatal development of the dorsolateral prefrontal cortex (DLPFC). Dendritic spines on DLPFC layer 3 pyramidal cells are essential for certain cognitive functions, change in density over development, and are reduced in number in schizophrenia. Altered expression of molecular regulators of actin filament assembly and stability, which are essential for spine formation and maintenance, is thought to contribute to the pathogenesis of spine deficits in the disease. However, the normal developmental expression patterns of these molecular regulators of dendritic spines, which might provide insight into the timing of spine deficits in schizophrenia, are unknown. Therefore, we quantified the expression from birth to adulthood of key transcripts regulating dendritic spine density in monkey DLPFC. Layer 3 pyramidal cells, and tissue samples containing layers 3 or 6, were captured by laser microdissection and selected transcripts were quantified using PCR. In layer 3 pyramidal cells, the expression levels of most of the transcripts studied changed early, and not late, in postnatal development. These developmental shifts in expression were generally not detected in tissue homogenates of layers 3 or 6, suggesting that the changes may be enriched in layer 3 pyramidal cells. The timing of these shifts in expression suggests that early, rather than later, postnatal development may be a vulnerable period for layer 3 pyramidal neurons. Disruption of the normal developmental trajectories of these transcripts may contribute to layer 3 pyramidal neuron spine deficits in individuals who are later diagnosed with schizophrenia.

1. Introduction

The disease process of schizophrenia appears to involve altered development of the dorsolateral prefrontal cortex (DLPFC) (Hoftman et al., 2017; Lewis and Levitt, 2002; Selemon and Zecevic, 2015). The DLPFC mediates certain cognitive functions, such as working memory, which improve substantially throughout postnatal development (Luna et al., 2010) and are impaired in individuals with schizophrenia (Kahn and Keefe, 2013). Thus, disturbances in the normal developmental trajectories of elements of DLPFC circuitry that are critical for working memory could give rise to its impairment in schizophrenia.

Pyramidal cells in DLPFC layer 3 play a critical role in working memory (Goldman-Rakic, 1995), and in schizophrenia, these neurons exhibit a lower density of dendritic spines (Garey et al., 1998; Glantz and Lewis, 2000; Konopaske et al., 2014). In contrast, pyramidal cells in layers 5 and 6 appear to have a normal complement of spines (Kolluri et al., 2005). The apparent specificity of the spine deficit to layer 3 pyramidal neurons is thought to be developmental in nature given the laminar differences in the developmental patterns of spine density, and of the excitatory synapses they receive, in both human (Huttenlocher, 1979; Petanjek et al., 2011) and non-human primate DLPFC (Anderson et al., 1995; Bianchi et al., 2013; Bourgeois et al., 1994). For example, the densities of dendritic spines and excitatory synapses on layer 3 pyramidal cells increase rapidly during the neonatal period, reaching a plateau in early childhood that persists until late childhood, followed by a protracted period of pruning over adolescence until stable adult levels are achieved (Anderson et al., 1995; Bourgeois et al., 1994; Petanjek et al., 2011). In contrast, DLPFC layers 5 and 6 exhibit a more modest or absent postnatal pruning of both excitatory synapses (Bourgeois et al., 1994) and dendritic spines (Petanjek et al., 2011).

Dendritic spine formation and maintenance is influenced by a number of gene products that regulate actin filament assembly and stability

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(Koleske, 2013). Indeed, the spine deficits on layer 3 pyramidal cells in schizophrenia appear to reflect altered expression of some of these gene products (Datta et al., 2015a; Hill et al., 2006; Ide and Lewis, 2010) (Fig. 1). For example, cell division cycle 42 (CDC42) is crucial for spinogenesis (Threadgill et al., 1997), whereas p21 activated serine/threonine kinase 1 (PAK1) regulates the stability of existing spines (Hayashi et al., 2004); expression of both of these transcripts is downregulated in layer 3 pyramidal cells in schizophrenia (Datta et al., 2015a). However, the relationship of the expression of these transcripts with changes in spine density over postnatal development is not clear. Moreover, prior work suggests that schizophrenia is associated with a blunting of normal developmental processes in the DLPFC (Fish et al., 2013; Hoftman et al., 2015; Hyde et al., 2011); yet it is not known if the spine deficits, and the altered expression of spine-related transcripts, reflect a blunting of spinogenesis early in development or an exaggerated pruning of spines during adolescence (McGlashan and Hoffman, 2000). Thus, understanding the normal developmental trajectories in the expression of these transcripts could inform the timing of their alterations, and when spine deficits arise, during the pathogenesis of schizophrenia.

To address these questions, we investigated the developmental trajectories of a subset of spine-related transcripts, which show altered expressed in schizophrenia (Fig. 1), selectively in layer 3 pyramidal cells from the DLPFC of macaque monkeys. Because these transcripts may be involved in other cytoskeletal processes that are not unique to dendritic spines in pyramidal cells, we studied the same transcripts in tissue homogenates of layer 3 and layer 6, which differ in the developmental trajectories of dendritic spines (Petanjek et al., 2011) and axospinous synapses (Bourgeois et al., 1994).

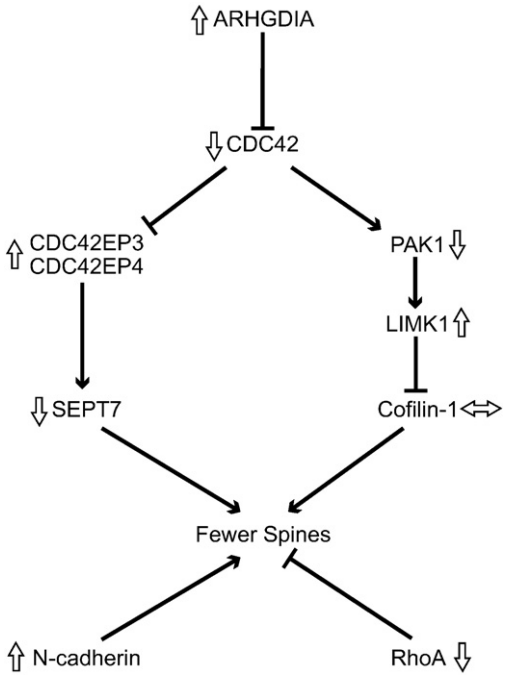

Fig. 1. Signaling pathways for selected transcripts that regulate dendritic spine density. Lines with arrowheads indicate activation and those with blunted ends indicate inhibition. Open arrows indicate the direction of altered expression of each transcript in layer 3 pyramidal cells in schizophrenia (Arion et al., 2015; Datta et al., 2015a; Ide and Lewis, 2010). The protein products of these transcripts contribute to spine stability through regulation of the assembly and stability of actin filaments (Koleske, 2013). Rho GTPases are dendritically translated, are active in the spine, and have well elucidated roles in the morphogenesis and maintenance of dendritic spines, including molecular signaling cascades such as the cell division cycle 42 (CDC42) pathway (Tada and Sheng, 2006). In this pathway, CDC42 is negatively regulated by a Rho GDP dissociation inhibitor alpha (ARHGDIA) (Gorvel et al., 1998) and acts through the CDC42-p21-activated serine/threonine kinase (PAK)-LIM domain kinase (LIMK) pathway to promote spine stability by phosphorylating and deactivating the neuronal cofilin, cofilin-1, an actin severing protein (Bernstein and Bamburg, 2010). In a separate pathway, negative regulation by CDC42 on CDC42 effector proteins 3 and 4 (CDC42EP3 and CDC42EP4) promotes spine outgrowth by opening the barrier at the base of dendritic spine necks formed by septin molecules such as Septin-7 (SEPT7) and allowing influx of molecules important in spine growth into the spine head (Joberty et al., 2001). RhoA is another GTPase which has a negative influence on dendritic spine maintenance, contributing to spine loss (Tashiro et al., 2000). In addition to the role that GTPases play in dendritic spine maintenance, other molecules contribute to the stability of spines. For example, neuronal cadherin (N-cadherin) has been shown to contribute significantly to nascent synapse stabilization and subsequent spine formation (Takeichi, 2007).

2. Methods

2.1. Animals and tissue preparation

Rhesus *(Macaca mulatta)* monkeys ($n = 20$) ranging in age from 3 days postnatal to 12 years old, ages which include the period between early neonatal to mid-life in this species, were used (Table 1). All monkeys were female except for one 3-day old male. The animals were divided into 5 age groups ($n = 4$ animals per group) based on previously identified inflection points in the developmental trajectory of spine density on layer 3 pyramidal cells in monkey DLPFC (Anderson et al., 1995): neonatal (3–8 days old), a period when the density of dendritic spines is rapidly increasing; infant (3 months), when spine density reaches a plateau; prepubertal (15–18 months), the end of the plateau period immediately prior to the onset of spine pruning during adolescence; postpubertal (43–48 months), following the end of spine pruning; and adult (8–12 years) when the density of dendritic spines is at stable, mature levels. The developmental trajectories of dendritic spines on layer 3 pyramidal cells are very similar in monkey (Anderson et al., 1995) and human (Petanjek et al., 2011) DLPFC. The inflection points in these trajectories were used to define age groups which were named based on the corresponding period in human development.

Monkeys younger than 6 months were housed with their mothers, juveniles 6–24 months were housed in groups, and those older than 24 months were housed either in pairs or in single cages in the same social setting. Seven animals were perfused transcardially with ice-cold artificial cerebrospinal fluid under deep anesthesia with ketamine and

Table 1
Rhesus monkeys used in study.

Age group	Subject	Age (months)	Sex	Perfusion Status	Biopsy	Weight (kg)	Storage time (months)
Neonatal	RH315	0.10	M	−	−	0.66	59
	RH311	0.23	F	−	−	0.66	64
	RH285	0.27	F	−	−	0.62	108
	RH331	0.27	F	−	−	0.55	14
Infant	RH310	3	F	−	−	1.14	65
	RH324	3	F	−	−	1.07	39
	RH241	3	F	+	+	1.02	148
	RH245	3	F	+	+	1.20	140
Prepubertal	RH264	15	F	+	+	2.50	126
	RH265	15	F	+	+	2.40	126
	RH317	16	F	−	−	2.70	59
	RH287	18	F	−	−	2.40	99
Postpubertal	RH239	43	F	+	+	5.50	148
	RH289	45	F	−	−	5.70	99
	RH258	47	F	+	+	6.30	128
	RH288	47	F	−	−	5.00	99
Adult	RH326	97	F	−	−	14.00	33
	RH259	104	F	+	+	6.4	127
	RH260	138	F	−	−	9.5	127
	RH354	155	F	−	−	7.7	5