Changes in expression of dendritic spine-enriched neuronal proteins in the prefrontal cortex during adolescence.

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ABSTRACT

Structural and functional alterations in the prefrontal cortex (PFC), including reduced spine density of PFC pyramidal cells, are thought to contribute to the cognitive symptoms of schizophrenia. These are not only debilitating, but do not respond to current treatments. It has been suggested that aberrant developmental synaptic elimination during adolescence contributes to the reduced spine density of PFC pyramidal cells (PCs) in schizophrenia. Recent data proposes that microglia are critically involved in synaptic elimination during early postnatal development. In an effort to better characterize the development of the PFC, which has a delayed and protracted maturation, we will examine the developmental expression pattern of neuronal proteins that are expressed at high levels in dendritic spines. We will use immunoblotting to quantify levels of PSD-95 and spinophilin at postnatal days 30 (peak spine density), 39 and 50 (decreasing spine density). We expect the changes in neuronal protein levels to mirror the developmental pattern of spine density, which in PFC PCs reach their peak density at P30, and thereafter decrease until a mature adult spine density is obtained. Along with data examining the developmental expression pattern of PFC glial proteins at the same postnatal days, we will obtain a better understanding of adolescent PFC development. These studies serve as a foundation for future work aimed at understanding the processes governing developmental PFC synaptic elimination, which is thought to be disrupted in schizophrenia, and which may contribute to cognitive symptoms. Future experiments will examine in the adult the structural and functional consequences of adolescent microglial ablation.

BACKGROUND

Dendritic spine number in the PFC is known to change throughout development, reaching a maximum number in early adolescence (~13 years of age in humans) and slowly decreasing afterwards until reaching a constant adult dendritic spine number (Fig 2). A similar trend is followed in rats, where a maximum number of spine density is reached around P35 (Fig 3). Changes in the PFC may be related to neurological diseases such as schizophrenia, where a dramatic decrease in dendritic spine density occurs early in adolescence. The underlying mechanism by which this occurs remains unknown, but it has been suggested that excessive pruning of dendritic spines by microglia contributes to the reduced spine density.

Figure 1. Top pyramidal cell. Bottom dendritic spine and synapse.

Synaptic density decreases early in adolescence in schizophrenia

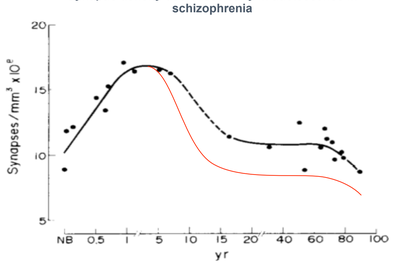


Figure 2. Normal synaptic density change throughout development in humans (black) in comparison to suggested synaptic density change in schizophrenia (red).

Spine density is at a maximum around P35 in rats

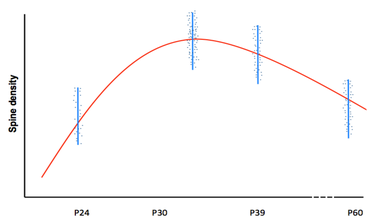


Figure 3. Dendritic spine density change through periadolescent development in rats.

Previous data from our lab suggests that microglial engulfment of spines increases at P39 compared to P30 (Fig 4), consistent with the decrease in dendritic spine density.

This project focused on studying the expression of neuronal proteins PSD-95 and spinophilin at different ages during the periadolescent period in rats.

Microglial engulfment of spines increases at P39, explaining decrease in dendritic spine number

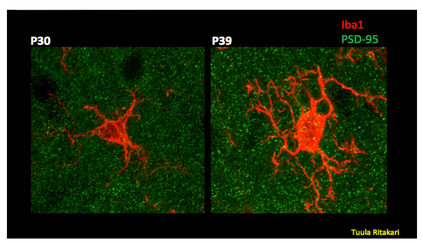


Figure 4. Previous data from our laboratory has shown an increase in microglial engulfment of spines at P39 relative to P30. This is consistent with the decrease in dendritic spine number already shown at this time point.

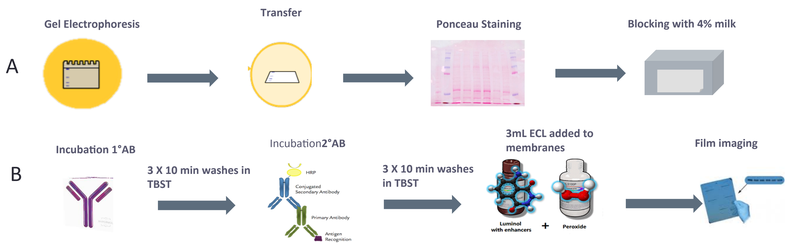
HYPOTHESIS

We hypothesize that the levels of the spine-enriched proteins PSD-95 and spinophilin will parallel the changes in spine density of PFC pyramidal cells across the different adolescent ages. Thus, we anticipate that both proteins will be at peak levels at P30 and thereafter decline.

METHODS

1. The PFC was dissected from 1.0 mm thick slices removed from rats at P30, P39, and P50. Tissue was homogenized in 2% SDS containing protease inhibitors. Protein concentration was determined using EZQ protein assay.

2. Immunoblotting was performed, probing for PSD-95 and spinophilin, two proteins enriched in spines: PSD-95 (1:3,000; EMD Millipore, Merck KGaA Darmstadt, Germany) and spinophilin (1:1,000; Upstate Biotechnology, NY, USA). Ponceau S Staining was used to visualize and calculate total protein transferred to membrane. After primary and secondary antibody incubation, membranes were developed using chemiluminescence.



3. Optical densities of PSD-95 and spinophilin bands were measured using ImageJ. Optical density was expressed as a ratio of total protein, and outliers were identified using Grubb's test. Data were analyzed by one-way ANOVA's.

RESULTS

PSD-95

No significant differences in PSD-95 levels at the three periadolescent ages examined.

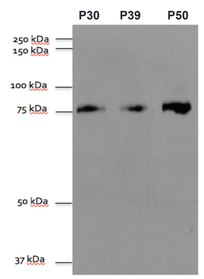


Figure 5. Representative western blot for PSD-95 at the three postnatal time points.

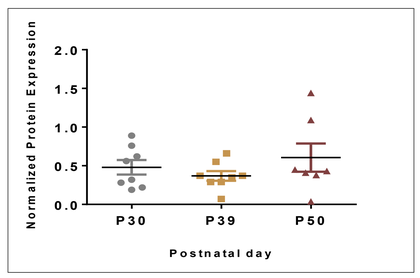


Figure 6. Scatter plot of normalized PSD-95 protein expression at the different postnatal time points (P30, P39, and P50). The one-way ANOVA did not uncover a significant overall difference [F (2, 20) = 0.99, NS]. Planned post-hoc comparisons found no significant differences between the different ages.

Spinophilin

Although no significant change was found in spinophilin expression across the different adolescent time points, a trend toward a decrease in spinophilin expression from P39 to P50 (p=.07) was noted.

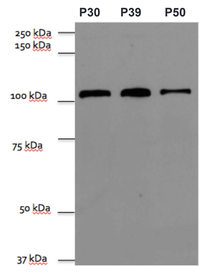


Figure 7. Representative western blot for spinophilin at the three postnatal time points.

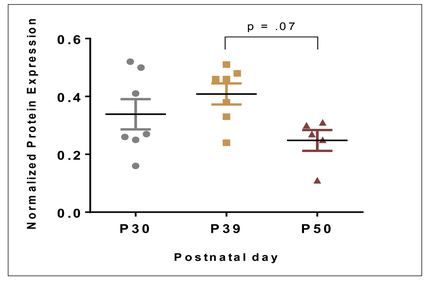


Figure 8. Scatter plot of normalized spinophilin values expression at P30, P39, and P50. One-way ANOVA revealed no significant overall difference, although a trend toward age-dependent differences in spinophilin levels was noted [F(2,16) = 3.05, p=.07]. Planned post-hoc comparisons showed that P50 spinophilin levels were significantly lower than at P39 (t(10)=3.0, p=.005), but not lower than P30.

DISCUSSION/CONCLUSIONS

PSD-95

- PSD-95 is located in the post-synaptic density of neurons. By virtue of its association with receptors such as the NMDA receptor, PSD-95 is critical for excitatory synaptic transmission. We observed no significant changes in PSD-95 expression in the PFC across adolescence. This may be due to too low a sample number. However, changes in PSD-95 levels may not correlate well with dendritic spine number: PSD-95 mainly reflects stronger and more stable synapses (Glantz et al. 2007) rather than spine number.

Spinophilin

- Spinophilin is mainly, but not exclusively, expressed in dendritic spines, where it interacts with actin and protein phosphatase–1.
- Although no significant changes were detected, a trend toward a decrease in spinophilin expression from P39 to P50 was noted. Interestingly, a planned post-hoc test revealed a significant decrease in spinophilin between P39 and P50, consistent with a decreased density of dendritic spines in late adolescence. However, spinophilin, while enriched in dendritic spines, is also present in other parts of the cell, as well as in interneurons, which lack spines. Therefore, the possible difference in spinophilin levels across adolescence may be due to spinophilin in extra-spinous sites.

Future directions and conclusions

- Both PSD-95 and spinophilin did not show any significant difference throughout the postnatal time points examined. In both cases, this may be due to a low number of samples, and therefore increasing the number of samples in future studies may help to detect significant changes. For spinophilin, subcellular fractionation may help to isolate specifically neuronal spinophilin. Immunogold localization would be useful in attempting to determine the precise intra-spinous localization of the two proteins.
- Future directions include studying other neuronal proteins that have been suggested to be dendritic spine markers, such as drebrin, and extending the analysis to include other postnatal time points.
- An overall understanding of how the PFC changes throughout development will be important when studying neuropsychiatric diseases such as schizophrenia that have abnormal developmental patterns.

ACKNOWLEDGEMENTS

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