Published in final edited form as:

Dev Psychobiol. 2017 July ; 59(5): 583–589. doi:10.1002/dev.21525.

Innervation of the Medial Prefrontal Cortex by Tyrosine Hydroxylase Immunoreactive Fibers during Adolescence in Male and Female Rats

Jari Willing¹, Laura R. Cortes¹, Joseph M. Brodsky², Taehyeon Kim¹, and Janice M Juraska^{1,2}

¹Department of Psychology, University of Illinois at Urbana-Champaign, University of Illinois at Urbana-Champaign, 603 E Daniels St, Champaign, IL 61820

²Neuroscience Program, University of Illinois at Urbana-Champaign, University of Illinois at Urbana-Champaign, 603 E Daniels St, Champaign, IL 61820

Abstract

Adolescence is associated with continued maturation of the cerebral cortex, particularly the medial prefrontal cortex (mPFC). We have previously documented pruning in the number of neurons, dendrites and synapses in the rat mPFC from preadolescence to adulthood, with the period of pubertal onset being particularly important. We hypothesized that dopaminergic innervation of this region, critical for executive functions, would also be influenced by pubertal onset. Here, we measured changes in the volume of tyrosine hydroxylase (TH) immunoreactive axons in all layers of the male and female mPFC from preadolescence to adulthood (postnatal day (P) 25, 35, 45, 60 and 90) as a marker of dopaminergic innervation. Assessing both total fiber volume and length, TH fibers were quantified by multiplying the mPFC volume by fiber density. While there were subtle layer-specific changes, TH fiber volume and length increased between P25 and P90 in both males and females. Contrary to our hypothesis, a role for pubertal onset in TH innervation of this region was not discernable. In summary, axons immunoreactive for TH increase with similar trajectories in the mPFC of male and female rats from pre-puberty to young adulthood.

Keywords

adolescent; development; dopamine; puberty; Long-Evans rat

1. Introduction

The mesocortical dopamine pathway, the projection from the ventral tegmental area (VTA) to the prefrontal cortex (PFC), plays a primary role in executive functions and behavioral inhibition, and has been implicated in a variety of clinical disorders. Particularly, development of this dopaminergic circuit is critical for the display of complex cognitive

Corresponding Author: Jari Willing, PhD, Department of Psychology, University of Illinois at Urbana-Champaign, 603 E Daniel St, Champaign, IL 61820, Phone: +1-518-253-0086, jwillin@illinois.edu.

Disclosure Statement

The authors declare no conflict of interest in regards to the content of this manuscript.

behaviors like strategy shifting and behavioral inhibition (Casey, Giedd, & Thomas, 2000; Seamans & Yang, 2004; Stefani & Moghaddam, 2006), and these behaviors improve significantly during adolescence. However, little is known regarding its potential development during the adolescent period.

Adolescence is a time of continuing development for the PFC. During adolescence, the volume of the PFC is known to decrease in both humans (Mills, Goddings, Herting, Meuwese, Blakemore, Crone, Dahl, Guroglu, Raznahan, Sowell, & Tamnes, 2016; Lenroot & Giedd, 2006) and rats (Markham, Morris, & Juraska, 2007), and there is a decrease in the number of neurons and axons projecting from the medial prefrontal cortex (mPFC) to the basal amygdala in male rats (Cressman, Balaban, Steinfeld, Shemyakin, Graham, Parisot, & Moore, 2010). Additionally, both neuronal and synaptic pruning have been documented in the adolescent rat mPFC during the pubertal period in mice and rats (Willing & Juraska, 2015; Drzewiecki, Willing, & Juraska, 2016; Pattwell, Liston, Jing, Ninan, Yang, Witztum, Murdock, Dincheva, Bath, Casey, Deissroth, & Lee, 2016). Coincidentally, the onset of puberty has also been associated with an increase in performance on a PFC-dependent cognitive task in both males and females (Willing, Drzewiecki, Cuenod, Cortes, & Juraska, 2016), suggesting that gonadal hormones may be involved in functional changes in the mPFC during adolescence.

Development of dopaminergic afferents to the PFC, as measured by tyrosine hydroxylase (TH), the rate-limiting enzyme for catecholamine synthesis (reviewed in Masserano & Weiner, 1983), begins with the rostral extension of axons from the VTA as early as embryonic day (E)13 (Smidt & Burbach, 2007; reviewed in Van den Heuvel & Pasterkamp, 2008). There is a substantial increase in TH immunoreactive (ir) fiber density during the neonatal period (Verney, Berger, Adrien, Vigny, & Gay, 1982; Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988). There is also a significant increase between the juvenile period and adulthood in male rats (Kalsbeek et al., 1988; Naneix, Marchand, Di Scala, Pape, & Coutureau, 2012). This is accompanied by changes in the firing patterns of dopaminergic neurons projecting to the PFC and an increase, followed by pruning in the number of PFC dopamine receptors between the juvenile period and young adulthood (Tseng & O'Donnell, 2007; O'Donnell, 2010; Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000).

There is some evidence that steroid hormones can affect the density of dopaminergic fibers of the cortex. Male rats gonadectomized during the perinatal period show a decrease in TH fiber density in the cingulate, sensory and motor cortices in adulthood (Kritzer, 1998). However, exogenous treatment with androgens during the perinatal period also decreased the density of TH fibers in the PFC of male spontaneously hypertensive rats (King, Barkley, Delville, & Ferris, 2000). Gonadectomy in adult male rats significantly increases TH axon density in the prelimbic mPFC and this increase is abolished with testosterone supplementation (Kritzer, Brewer, Montalmant, Davenport, & Robinson, 2007). In contrast, in adult female rhesus monkeys, ovariectomy decreases THir fiber density in the PFC and is raised to control levels after supplementation with both estrogen and progesterone (Kritzer & Kohama, 1998). Because gonadal hormones have been shown to impact the density of dopaminergic fibers in the PFC, it was hypothesized that the period of pubertal onset within adolescence would be associated with changes in TH fiber volume in male and female rats.

In the present study, we tracked changes in the volume of THir axons in the male and female rat mPFC at five time-points between the juvenile period and young adulthood (P25, P35, P45, P60, P90). Fiber density was multiplied by total mPFC volume to estimate the total volume and length of dopaminergic fibers across these ages. In addition, we examined both male and female rats while tracking their pubertal status.

2. Materials and Methods

2.1 Animals

Animals in this study were the offspring of Long-Evans hooded rats purchased from Harlan Laboratories (Indianapolis, IN) and bred in the vivarium of the Psychology Department at the University of Illinois. Animals were weaned on postnatal day (P) 24 and housed with same-sex littermates in pairs or triplets until the day of sacrifice. Rats were kept on a 12:12-h light-dark cycle with *ad libitum* access to food and water. Tissue from both male and female rats was collected at P25, P35, P45, P60 and P90 (n= 9–10 per group). P25 is within the juvenile period, P35 approximates the period of pubertal onset in females, P45 approximates puberty onset in males, and P60 and 90 represent the transition from adolescence to early adulthood. For animals not sacrificed prior to puberty, the day an animal reached puberty was recorded using vaginal opening as a marker for females and preputial separation for males, as these markers coincide with surges in pubertal hormones (Castellano, Bentsen, Sanchez-Garrido, Ruiz-Pino, Romero, Garcia-Galiano, Aguilar, Pinilla, Dieguez, Mikkelsen, & Tena-Sempere, 2011; Korenbrot, Huhtaniemi, & Weiner, 1977). Each age group was comprised of animals from a minimum of five litters, and at each age, no more than two animals of the same sex came from the same litter. All procedures were approved by the University of Illinois Institutional Care and Use Committee, and adhered to the National Institute of Health guidelines on the ethical use of animals.

2.2 Volume Estimation

Rats were given a lethal dose of sodium pentobarbital, and were perfused intracardially with 0.1M phosphate buffered saline (PBS) (pH 7.4) followed by 4% paraformaldehyde in PBS. Brains were removed and post-fixed for an additional 24 hours and were then cryoprotected in a PBS solution containing 30% sucrose for three days. After incubation in sucrose, brains were sliced coronally into 40µm sections with a freezing microtome. Parcellation and volume estimation were conducted on sections used for a previous study (Willing & Juraska, 2015). Every fifth section containing PFC was temporarily placed into 0.1M PBS and then mounted on gelatin-coated slides, while the other sections were stored in cryoprotectant. Once dried, sections were stained with Methylene Blue/Azure II. Staining and mPFC parcellation procedures were identical to those described in Markham et al. (2007). For each animal, the volume of the prelimbic and infralimbic mPFC was determined for layers 1, 2/3 and 5/6.

2.3 TH Immunohistochemistry

Sections from the mPFC (3 sections spaced at 120µm intervals per animal) were taken out of cryoprotectant and rinsed three times (five min each) in Tris-buffered saline (TBS), pH 7.6. They were then placed in blocking solution consisting of 1% hydrogen peroxide, 20%

normal goat serum (NGS), and 1% bovine serum albumin (BSA) in TBS for 30 min. They were then incubated in an anti-TH primary antibody (Mouse monoclonal, Millipore; MAB318) diluted at 1:1000 with 2% NGS and 0.3% triton-x-100 in TBS (TTG) for 48 hours. Sections were then rinsed in TTG three times for five minutes, followed by incubation in 5µg/ml biotinylated goat, anti-mouse secondary antibody (Vector; BA-9200) in TTG for 90 min. Sections were then rinsed twice in TTG and twice in TBS (5 min each) before being placed into avidin-biotin-complex diluted in TBS (Vector; PK-6100) for one hour at room temperature. Lastly, sections were rinsed three times in TBS, then reacted with Diaminobenzidin (DAB) (Sigma fast tabs; D4418). After excess DAB was rinsed, sections were mounted on gelatin-coated slides and cover-slipped with Permount for analysis.

2.4 Image Acquisition and THir Fiber Analysis

Images were acquired with Zeiss Axiovert 200M microscope from two sections containing the prelimbic (PL) and infralimbic (IL) mPFC. For each section, bilateral images were taken from layer 1, layers 2/3 and layers 5/6. Identification of these PFC subregions and cortical layers was made possible by their proximity to adjacent white matter under the frontal cortex and THir fiber orientation as was discussed in previous work from our laboratory (Chisholm, Kim, & Juraska, 2013). Briefly, layer 1 is comprised of a dense band of fibers with a dorsal/ventral orientation, fibers in layers 2/3 are arranged in a medial to lateral direction, while the fibers in 5/6 do not have a clear orientation (Figure 1). The images were taken throughout the entire thickness of the tissue at intervals of 0.275 µm resulting in approximately 100 images (240 x 160 microns) per Z-stack. Z-stacked images were compressed using Axiovision software. Since pictures were acquired bilaterally from two mPFC sections, this resulted in four sets of images per animal.

Image J software was used to analyze the compressed images. Before analysis, extraneous staining (blood vessels, cell bodies, diffuse background staining) was manually removed from the image by an experimenter blind to age and sex. Analysis was performed on both 'binary' and 'skeletonized' images (Chisholm et al., 2013). For binary images, the total number of saturated (ir) pixels was used in calculating TH fiber density, which includes both the length and thickness of fibers. When Image J converts compressed photos to skeletonized images, the fiber length is reduced to a uniform 1 pixel-wide thickness. This allows for the calculation of total fiber length, without fiber thickness. For each animal, THir density measurements were averaged across section and hemisphere, leaving two density calculations (binary and skeletonized) for each layer. The density of TH fibers was then multiplied by the volume of each layer to calculate the total volume and the length of TH fibers. These volumes and lengths were then added across layers for the total volume of THir in the mPFC.

2.5 Statistical Analysis

We hypothesized that puberty would affect TH fibers, and males and females reach puberty at non-overlapping ages. Therefore, a separate one-way ANOVA (age) was conducted for males and females for each mPFC layer, and for total THir (layers combined). This was done for binary and skeletonized images with litter run as a cofactor. For each of these measures, five *a priori* comparisons were examined for post hoc analysis (Fisher's LSD),

between consecutive ages: P25 vs. P35, P35 vs. P45, P45 vs. P60, P60 vs. P90 and between the extreme ages: P25 vs. P90. As with previous studies (Willing et al., 2015; Drzewiecki et al., 2016), for total THir volume, females in the P35 age group and males in the P45 age group were separated into those who had and had not gone through puberty. T-tests were used to compare THir volume between pre- and post-pubertal males and females at these ages.

3. Results

3.1 Binary

Analysis of total mPFC THir binary images revealed a significant main effect of age for males [$F(4,47)=2.76$, $p=0.039$] and females [$F(4,45)=2.87$, $p=0.036$]. Post hoc analysis revealed that in both sexes, there was a significant difference between P25 and P90 (males: $p=0.04$, females: $p=0.037$) (Figure 2). In females there was a non-significant trend ($p=0.09$) for an increase between P25 and P35. Layer specific findings are presented in Table 1. For all layers in both sexes, there were significant differences between P25 and P90 ($p<0.05$). For both sexes in layers 2/3, there was a non-significant trend for an increase in THir between P25 and P35 ($p<0.08$). In females only, there was a significant increase between P25 and P35 in layer 1 ($p=0.031$).

3.2 Skeletonized

Analysis of total THir for skeletonized images also revealed a significant main effect of age in males [$F(4,47)=3.44$, $p=0.016$] and females [$F(4,45)=3.59$, $p=0.013$]. Again, significant differences were found between P25 and P90 in males ($p=0.032$) and females ($p=0.036$) (Figure 3). There was a nonsignificant trend for an increase between P25 and P35 in females ($p=0.085$). Layer-specific effects are presented in Table 2. As with analysis of binary images, there was a significant difference between groups in males and females and in all layers between P25 and P90 ($p<0.05$). For males there was a significant difference between P25 and P35 for layers 2/3 ($p=0.011$). For females, this comparison was only a trend ($p=0.062$). Females did have a significant difference between P25 and P35 for layer 1 ($p=0.032$).

3.3 Pubertal Ages

For females, a comparison between animals that were prepubertal ($n=4$) and post-pubertal ($n=5$) at P35 revealed no significant difference in binary THir fiber volume [$t(7)=0.13$, $p=0.89$]. In males at P45, there were also no significant differences between pre- ($n=5$) and post-pubertal ($n=5$) animals [$t(8)=0.28$, $p=0.78$] (Figure 4).

4. Discussion

Both males and females gained TH fiber volume between the juvenile period and young adulthood, but the gains were gradual such that significant differences between the consecutive ages could not be detected. There were some indications, however, for a more rapid increase in TH fiber volume between the late juvenile/early adolescent periods. The similarity of the changes in the binary and skeletonized images implies that increases in total

fiber length, not fiber thickness, are the basis for the greater volume of fibers by adulthood. Given that dopamine cell number in the midbrain remains stable after the perinatal period (Lieb, Andersen, Lazarov, Zienecker, Urban, Reisert, & Pilgrim, 1996; Park, Kitahama, Geffard, & Maeda, 2000), this increase is not likely to reflect projections from new cells, but arborization of axons from existing neurons. In addition, the smaller projection from THir neurons of the locus coeruleus decrease in number between the juvenile and adolescent period (Benzin, Marcel, Debure, Ginovart, Rousset, & Pujol, 1994). While previous studies have examined the density of TH axons not the total amount, the present results are concordant with previous work examining THir density in the male mPFC showing an increase between early adolescence and adulthood (Kalsbeek et al., 1988; Naneix et al., 2012).

TH is the rate-limiting enzyme for catecholamine synthesis, raising the possibility that both noradrenergic and dopaminergic fibers were measured. However, this is not likely to have had a major impact on the conclusions of the present study because the density of noradrenergic fibers is sparse compared to dopaminergic fibers specifically within the mPFC (Devoto & Flore, 2006). In addition, experimental lesions of ascending noradrenergic fiber tracts had no effect on levels of TH in the neocortex (Lewis, 1997). Lastly, noradrenergic fibers, visualized with dopamine β -hydroxylase, the enzyme that converts dopamine to norepinephrine, remain stable in the PFC during adolescence (Naneix et al., 2012). This collectively suggests that the vast majority of immunoreactive TH fibers in the mPFC are in fact dopaminergic.

The similarity in the trajectory of THir fiber growth in males and females indicates that puberty, which is approximately 10 days apart in our rats, is not playing an important role. This is further supported by the lack of difference between pre- and post-pubertal rats at the same age and sex. Analysis of THir within distinct mPFC layers revealed trends towards a more rapid increase between P25 and P35, especially in the more superficial layers (layers 1–3). This age range coincides with the rise in pubertal hormones in female rats between juvenile and peri-pubertal, so that a minor role for ovarian hormones remains a modest possibility. However in general, a higher rate of dopaminergic innervation at early ages is consistent with studies showing heightened increases in THir through the perinatal/early juvenile period and a more gradual increase from adolescence to adulthood (Verney et al., 1982; Kalsbeek et al., 1988).

The increase in THir axons during adolescence corresponds with other facets of dopaminergic development within the PFC during this time. Coinciding with increased dopamine innervation in the PFC, dopamine availability as measured by HPLC increases between early adolescence and adulthood (Naneix et al., 2012). There are also changes during adolescence in the ability of dopamine to modulate the activity of GABAergic interneurons in the PFC via D1 and D2 receptors (Tseng & O'Donnell, 2007; reviewed in O'Donnell, 2010). In contrast to the ontogeny of dopaminergic innervation, the expression of multiple dopamine receptor subtypes in the PFC is known to change in male rats throughout the adolescent period, with an increase until ~P45, followed by a significant decrease thereafter (Andersen et al., 2000; Naneix et al., 2012), suggesting a different mechanism for regulation of PFC dopamine receptors. Both changes at the level of the

synapse and more broad structural reorganization are fine-tuned throughout the adolescent period, and these changes in connectivity and function of the mesocortical dopaminergic pathway likely play a role in enhanced performance on select cognitive tasks that are influenced by dopamine activity, including cognitive flexibility (Naneix et al., 2012; Stefani & Moghaddam, 2006; Willing & Wagner, 2016; Mizoguchi, Shoji, Tanaka, Maruyama, & Tabira, 2009; Chisholm et al., 2013).

Our previous studies have shown that the period of pubertal onset is associated with acute sex-specific changes in mPFC neuron number (Koss, Lloyd, Sadowski, Wise, & Juraska, 2015; Willing & Juraska, 2015) and synapse number (Drzewiecki et al., 2016). In contrast in the present study, we observed a similar developmental trajectory of mPFC dopaminergic innervation in males and females across adolescence. Contrary to our hypothesis, the analysis of females at P35 and males at P45 suggested that the pubertal onset was not accompanied by changes in THir fiber volume in either sex. This would seemingly conflict with previous studies documenting both long and short-term effects of gonadal hormones on TH expression in the PFC during early development (Kritzer, 1998; King et al., 2000; Willing & Wagner, 2016), young adulthood (Kritzer et al., 2007; Kritzer & Kohama, 1998) and in aged rats (Chisholm, Packard, Koss, & Juraska, 2012; Chisholm et al., 2013). Depending upon the presence of specific receptors, steroid hormone actions could have different or even contrasting effects during different periods of development. Sex differences in projection patterns from the VTA have been detected at different ages (reviewed in Gillies, Virdee, McCarthur, & Dalley, 2014), along with sex differences in the expression of estrogen and androgen receptors in forebrain targets of midbrain dopaminergic neurons (Kritzer & Creutz, 2008). Developmental changes in cellular function and receptor expression could allow time-specific openings for steroid hormones to affect this system.

While gonadal hormones have been shown to play a role in perinatal dopaminergic innervation of the mPFC (Kritzer, 1998; Willing & Wagner, 2016), results of the present study do not suggest a role for pubertal hormones in the increase in dopaminergic axon number after the juvenile period. However, they do not exclude a role for gonadal hormones in the maintenance of TH expression or activity within dopaminergic projections, which are acutely regulated by a complex feedback loop under the control of a variety of transcription factors (Tekin, Roskoski, Carkaci-Salli, & Vrana, 2014). Additionally, the analysis of THir fibers measures the volume and total length of dopaminergic axons but does not indicate the number of dopamine synapses, dopamine release or number of receptors, which may be differentially affected by pubertal hormones. Thus other measures of dopaminergic function that are not reflected in axonal THir may be altered at puberty.

The total volume of dopaminergic axons gradually increased in the male and female rat mPFC from the juvenile period into early adulthood, with a tendency towards a greater increase during in juvenile rats. Both sexes gained fibers at the same rate in spite of the sex difference in the day of pubertal onset. Though males and females seemed to have a similar developmental trajectory of dopaminergic innervation in the region, sex differences in other facets of dopaminergic functioning could play a role in the observed sex differences in the prevalence of mental disorders characterized by PFC dopaminergic dysfunction that manifest after puberty.

Acknowledgments

We thank the Microscopy Suite in the Beckman Institute for the use of the facility. This work was supported by NIH MH099625 to J.M. Juraska. JW is supported by T32 ES007326.

References

- Andersen SL, Thompson AT, Rutstein M, Hostetter JC, Teicher MH. Dopamine receptor pruning in prefrontal cortex during the periadolescent period in rats. *Synapse*. 2000; 37(2):167–9. [PubMed: 10881038]
- Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? *Neuroscience and Biobehavioral Reviews*. 2003; 27(1–2):3–18. [PubMed: 12732219]
- Bezin L, Marcel D, Debure LI, Ginovart N, Rousset C, Pujol JF, Weissmann D. Postnatal development of the tyrosine hydroxylase-containing cell population within the rat locus coeruleus: topological organization and phenotypic plasticity. *Journal of Neuroscience*. 1994; 14(12):7486–7501. [PubMed: 7996190]
- Casey BJ, Giedd JN, Thomas KM. Structural and functional brain development and its relation to cognitive development. *Biological Psychology*. 2000; 54(1–3):241–257. [PubMed: 11035225]
- Castellano JM, Bentsen AH, Sánchez-Garrido MA, Ruiz-Pino F, Romero M, Garcia-Galiano D, Aguilar E, Pinilla L, Diéguez C, Mikkelsen JD, Tena-Sempere M. Early metabolic programming of puberty onset: impact of changes in postnatal feeding and rearing conditions on the timing of puberty and development of the hypothalamic kisspeptin system. *Endocrinology*. 2011; 152(9):3396–408. [PubMed: 21712362]
- Chisholm NC, Packard AR, Koss WA, Juraska JM. The effects of long-term treatment with estradiol and medroxyprogesterone acetate on tyrosine hydroxylase fibers and neuron number in the medial prefrontal cortex of aged female rats. *Endocrinology*. 2012; 153(10):4874–82. [PubMed: 22903611]
- Chisholm NC, Kim T, Juraska JM. Males, but not females, lose tyrosine hydroxylase fibers in the medial prefrontal cortex and are impaired on a delayed alternation task during aging. *Behavioral Brain Research*. 2013; 243:239–46.
- Cressman VL, Balaban J, Steinfeld S, Shemyakin A, Graham P, Parisot N, Moore H. Prefrontal cortical inputs to the basal amygdala undergo pruning during late adolescence in the rat. *Journal of Comparative Neurology*. 2010; 518(14):2693–2709. [PubMed: 20506471]
- Devoto P, Flore G. On the origin of cortical dopamine: is it a co-transmitter in noradrenergic neurons? *Current Neuropharmacology*. 2006; 4(2):115–25. [PubMed: 18615131]
- Drzewiecki CM, Willing J, Juraska JM. Synaptic number changes in the medial prefrontal cortex across adolescence in male and female rats: A role for pubertal onset. *Synapse*. 2016; 70(9):361–8. [PubMed: 27103097]
- Gillies GE, Virdee K, McArthur S, Dalley JW. Sex-dependent diversity in ventral tegmental dopaminergic neurons and developmental programming: A molecular, cellular and behavioral analysis. *Neuroscience*. 2014; 282:69–85. [PubMed: 24943715]
- Kalsbeek A, Voorn P, Buijs RM, Pool CW, Uylings HB. Development of the dopaminergic innervation in the prefrontal cortex of the rat. *Journal of Comparative Neurology*. 1988; 269(1):58–72. [PubMed: 3361004]
- King JA, Barkley RA, Delville Y, Ferris CF. Early androgen treatment decreases cognitive function and catecholamine innervation in an animal model of ADHD. *Behavioral Brain Research*. 2000; 107(1–2):35–43.
- Korenbrod CC, Huhtaniemi IT, Weiner RI. Preputial separation as an external sign of pubertal development in the male rat. *Biology of Reproduction*. 1977; 17(2):298–303. [PubMed: 889997]
- Koss WA, Lloyd MM, Sadowski RN, Wise LM, Juraska JM. Gonadectomy before puberty increases the number of neurons and glia in the medial prefrontal cortex of female, but not male, rats. *Developmental Psychobiology*. 2015; 57(3):305–12. [PubMed: 25782706]
- Kritzer MF. Perinatal gonadectomy exerts regionally selective, lateralized effects on the density of axons immunoreactive for tyrosine hydroxylase in the cerebral cortex of adult male rats. *Journal of Neuroscience*. 1998; 18(24):10735–48. [PubMed: 9852608]

- Kritzer MF, Kohama SG. Ovarian hormones influence the morphology, distribution, and density of tyrosine hydroxylase immunoreactive axons in the dorsolateral prefrontal cortex of adult rhesus monkeys. *Journal of Comparative Neurology*. 1998; 395(1):1–17. [PubMed: 9590542]
- Kritzer MF, Brewer A, Montalmant F, Davenport M, Robinson JK. Effects of gonadectomy on performance in operant tasks measuring prefrontal cortical function in adult male rats. *Hormones and Behavior*. 2007; 51(2):183–94. [PubMed: 16942768]
- Kritzer MF, Creutz LM. Region and sex differences in constituent dopamine neurons and immunoreactivity for intracellular estrogen and androgen receptors in mesocortical projections in rats. *Journal of Neuroscience*. 2008; 28(38):9525–35. [PubMed: 18799684]
- Lenroot RK, Giedd JN. Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neuroscience and Biobehavioral Reviews*. 2006; 30(6):718–29. [PubMed: 16887188]
- Lewis DA. Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. *Neuropsychopharmacology*. 1997; 16(6):385–398. [PubMed: 9165494]
- Lieb K, Andersen C, Lazarov N, Zienecker R, Urban I, Reisert I, Pilgrim C. Pre- and postnatal development of dopaminergic neuron numbers in the male and female mouse midbrain. *Developmental Brain Research*. 1996; 94(1):37–43. [PubMed: 8816275]
- Markham JA, Morris JR, Juraska JM. Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood. *Neuroscience*. 2007; 144(3):961–968. [PubMed: 17137726]
- Masserano JM, Weiner N. Tyrosine hydroxylase regulation in the central nervous system. *Molecular and Cellular Biochemistry*. 1983; 53–54(1–2):129–152.
- Mills KL, Goddings AL, Herting MM, Meuwese R, Blakemore SJ, Crone EA, Dahl RE, Guroglu B, Raznahan A, Sowell ER, Tamnes CK. Structural brain development between childhood and adulthood: Convergence across four longitudinal samples. *NeuroImage*. 2016; 141:273–281. [PubMed: 27453157]
- Mizoguchi K, Shoji H, Tanaka Y, Maruyama W, Tabira T. Age-related working memory impairment is caused by prefrontal cortical dopaminergic dysfunction in rats. *Neuroscience*. 2009; 162(4):1192–1201. [PubMed: 19463906]
- Naneix F, Marchand AR, Di Scala G, Pape JR, Coutureau E. Parallel maturation of goal-directed behavior and dopaminergic systems during adolescence. *Journal of Neuroscience*. 2012; 32(46):16223–32. [PubMed: 23152606]
- O'Donnell P. Adolescent maturation of cortical dopamine. *Neurotoxicity Research*. 2010; 18(3–4):306–312. [PubMed: 20151241]
- Park M, Kitahama K, Geffard M, Maeda T. Postnatal development of the dopaminergic neurons in the rat mesencephalon. *Brain Development*. 2000; 22(Suppl 1):S38–44. [PubMed: 10984659]
- Pattwell SS, Liston C, Jing D, Ninan I, Yang RR, Witzum J, Murdock MH, Dincheva I, Bath KG, Casey BJ, Deisseroth K, Lee FS. Dynamic changes in neural circuitry during adolescence are associated with persistent attenuation of fear memories. *Nature Communications*. 2016; 7:11475.
- Seamans JK, Yang CR. The principal features and mechanisms of dopamine modulation in the prefrontal cortex. *Progress in Neurobiology*. 2004; 74(1):1–58. [PubMed: 15381316]
- Smidt MP, Burbach JP. How to make a mesodiencephalic dopaminergic neuron. *Nature Reviews Neuroscience*. 2007; 8(1):21–32. [PubMed: 17180160]
- Stefani MR, Moghaddam B. Rule learning and reward contingency are associated with dissociable patterns of dopamine activation in the rat prefrontal cortex, nucleus accumbens, and dorsal striatum. *Journal of Neuroscience*. 2006; 26(34):8810–8818. [PubMed: 16928870]
- Tekin I, Roskoski R Jr, Carkaci-Salli N, Vrana KE. Complex molecular regulation of tyrosine hydroxylase. *Journal of Neural Transmission*. 2014; 121(12):1451–1481. [PubMed: 24866693]
- Tseng KY, O'Donnell P. Dopamine modulation of prefrontal cortical interneurons changes during adolescence. *Cerebral Cortex*. 2007; 17(5):1235–1240. [PubMed: 16818475]
- Van den Heuvel DM, Pasterkamp RJ. Getting connected in the dopamine system. *Progress in Neurobiology*. 2008; 85(1):75–93. [PubMed: 18304718]

- Verney C, Berger B, Adrien J, Vigny A, Gay M. Development of the dopaminergic innervation of the rat cerebral cortex. A light microscopic immunocytochemical study using anti-tyrosine hydroxylase antibodies. *Brain Research*. 1982; 281(1):41–52. [PubMed: 6182953]
- Willing J, Juraska JM. The timing of neuronal loss across adolescence in the medial prefrontal cortex of male and female rats. *Neuroscience*. 2015; 301:268–275. [PubMed: 26047728]
- Willing J, Drzewiecki CM, Cuenod BA, Cortes LR, Juraska JM. A Role for Puberty in Water Maze Performance in Male and Female Rats. *Behavioral Neuroscience*. 2016; 130(4):422–427. [PubMed: 27054406]
- Willing J, Wagner CK. Progesterone Receptor Expression in the Developing Mesocortical Dopamine Pathway: Importance for Complex Cognitive Behavior in Adulthood. *Neuroendocrinology*. 2016; 103(3–4):207–22. [PubMed: 26065828]

Figure 1.
Tyrosine hydroxylase immunoreactivity in layers 1 (A), 2/3 (B) and 5/6 (C) in the medial prefrontal cortex (P90 male).

Figure 2.

Binary image analysis of tyrosine hydroxylase immunoreactive fiber volume in the male and female rat mPFC between P25 and P90. Males (A) gained THir fibers throughout adolescence, with a significant increase between P25 and P90. Females (B) also significantly showed increased fiber volume between P25 and P90 and a nonsignificant trend for an increase between P25 and P35. (* $p < 0.05$, # $p < 0.1$).

Figure 3.

Skeletonized image analysis of tyrosine hydroxylase immunoreactive fiber volume in the male and female rat mPFC between P25 and P90. Skeletonized images reduce fiber width to $1\mu\text{m}$. Males (A) gained THir fibers between P25 and P90. Females (B) significantly showed increased fiber volume between P25 and P90 and a nonsignificant trend for an increase between P25 and P35. (* $p < 0.05$, # $p < 0.09$).

Figure 4.

Acute effects of pubertal onset on TH immunoreactivity in males and females. Males at P45 and females at P35 were divided into pre- and post-pubertal groups. There was no significant effect of pubertal onset in either males (A) or females (B) on THir volume in the mPFC.

Table 1

<i>Males</i>	P25	P35	P45	P60	P90	<i>Females</i>	P25	P35	P45	P60	P90
Layer 1	2.0 +/- .19	2.5 +/- .36	3.1 +/- .25	3.0 +/- .26	*3.3 +/- .22	Layer 1	2.1 +/- .19	*3.0 +/- .35	3.1 +/- .30	3.2 +/- .38	*2.9 +/- .23
Layer 2/3	11 +/- 1.1	15 +/- 1.5	18 +/- 1.7	17 +/- 1.8	*18 +/- 2.5	Layer 2/3	13 +/- .99	16 +/- 1.6	17 +/- 1.8	20 +/- 2.2	*20 +/- 1.9
Layer 5/6	50 +/- 4.1	55 +/- 3.5	56 +/- 4.4	63 +/- 3.7	*67 +/- 3.7	Layer 5/6	46 +/- 3.3	56 +/- 4.03	60 +/- 5.9	65 +/- 4.7	*64 +/- 3.8

Layer specific changes in total volume of TH immunoreactive fibers (mean x 106 μm^3) in males and females,

* significantly different from P25.

Table 2

<i>Males</i>	P25	P35	P45	P60	P90	<i>Females</i>	P25	P35	P45	P60	P90
Layer 1	.52 +/- .05	.64 +/- .1	.79 +/- .06	.73 +/- .08	*.78 +/- .07	Layer 1	.53 +/- .05	*.77 +/- .09	.78 +/- .08	.78 +/- .1	*.76 +/- .07
Layer 2/3	2.8 +/- .33	*4.7 +/- .28	4.8 +/- .41	4.5 +/- .51	*4.6 +/- .66	Layer 2/3	3.1 +/- .28	4.2 +/- .46	4.5 +/- .44	5.3 +/- .67	*5.4 +/- .58
Layer 5/6	13 +/- 1.1	15 +/- 1.1)	15 +/- 1.3	17 +/- .95	*18 +/- .94	Layer 5/6	12 +/- .97	15 +/- 1.0	16 +/- 1.5	17 +/- 1.2	*16 +/- .70

Layer specific changes in total length of TH immunoreactive fibers (mean x 106 μm^3) in males and females,

* significantly different from P25.