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Adolescent testosterone influences BDNF and TrkB mRNA and neurotrophin–interneuron marker relationships in mammalian frontal cortex

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ABSTRACT

Late adolescence in males is a period of increased susceptibility for the onset of schizophrenia, coinciding with increased circulating testosterone. The cognitive deficits prevalent in schizophrenia may be related to unhealthy cortical interneurons, which are trophically dependent on brain derived neurotrophic factor. We investigated, under conditions of depleted (monkey and rat) and replaced (rat) testosterone over adolescence, changes in gene expression of cortical BDNF and TrkB transcripts and interneuron markers and the relationships between these mRNAs and circulating testosterone. Testosterone removal by gonadectomy reduced gene expression of some BDNF transcripts in monkey and rat frontal cortices and the BDNF mRNA reduction was prevented by testosterone replacement. In rat, testosterone replacement increased the potential for classical TrkB signalling by increasing the full length to truncated TrkB mRNA ratio, whereas in the monkey cortex, circulating testosterone was negatively correlated with the TrkB full length/truncated mRNA ratio. We did not identify changes in interneuron gene expression in monkey frontal cortex in response to gonadectomy, and in rat, we showed that only somatostatin mRNA was decreased by gonadectomy but not restored by testosterone replacement. We identified complex and possibly species-specific, relationships between BDNF/TrkB gene expression and interneuron marker gene expression that appear to be dependent on the presence of testosterone at adolescence in rat and monkey frontal cortices. Taken together, our findings suggest there are dynamic relationships between BDNF/TrkB and interneuron markers that are dependent on the presence of testosterone but that this may not be a straightforward increase in testosterone leading to changes in BDNF/TrkB that contributes to interneuron health.

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1. Introduction

Adolescence is a period of increasing circulating sex steroids as well as a period of brain development and maturation that contributes to changes in behaviour, cognition and social interactions (Spear, 2000; Sisk and Zehr, 2005; Richards et al., 2009; Schulz et al., 2009). Late adolescence in males is a period of greater susceptibility for the onset of schizophrenia compared to females — coinciding with increased circulating testosterone (Gaidano et al., 1980; Markham, 2012). In addition, males, as shown by two meta-analyses, have an overall ~40% greater chance of developing schizophrenia than females (Aleman et al., 2003; McGrath et al., 2004), have a worse course of the disease and are more treatment resistant (McGrath et al., 2008). This raises questions about how testosterone contributes to normal brain changes that may be relevant to the precipitation and progression of schizophrenia possibly in those individuals with an underlying (genetic) susceptibility to schizophrenia.

Cognitive deficits are a core feature of schizophrenia (Weickert et al., 2000) and are present throughout the adult life of most individuals with schizophrenia, including a typical drop in cognitive ability prior to the initial onset of psychosis, and thus, prior to antipsychotic treatment (Saykin et al., 1994; Davidson et al., 1999; Bora and Murray, 2014). Cognitive deficits include impairments in executive function, and working memory, abilities which are coordinated by cortical circuits, particularly in the prefrontal cortex (Levy and Goldman-Rakic, 2000). Cortical circuits are composed of two types of neurons, excitatory

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(pyramidal/projection neurons), which predominate, and inhibitory interneurons (Parnavelas, 2000; Lodato et al., 2014). Inhibitory interneurons use γ-aminobutyric acid (GABA) as a neurotransmitter and are crucial for the coordination and synchronization of excitatory neuron activity during working memory processes (Lewis and Hashimoto, 2007).

Interneuron health is compromised in schizophrenia as evidenced by reduced gene expression and protein levels of glutamic acid decarboxylase (GAD67), an enzyme that synthesizes GABA, in the dorsolateral prefrontal cortex (DLPFC) of individuals with schizophrenia (Akbarian et al., 1995; Woo et al., 1998; Guidotti et al., 2000; Lewis et al., 2004b; Thompson et al., 2009; Duncan et al., 2010). These cortical GABAergic inhibitory interneurons are a heterogeneous population of neurons that vary based on morphology, electrophysiological properties, laminar distribution, innervation of pyramidal neurons and expression of neuropeptides and calcium binding proteins (Markram et al., 2004; Petilla Interneuron Nomenclature GROUP, 2008). Three largely independent populations of interneurons expressing i) parvalbumin (PV), ii) somatostatin/calbindin (SST/CB) and iii) calretinin/vasoactive intestinal peptide (CR/VIP) have been identified (Somogyi et al., 1998). Expression of many biochemical markers of inhibitory interneurons is often reduced in postmortem cortex from schizophrenia patients including PV, SST, cholecystokinin (CCK), neuropeptide Y (NPY), and VIP (Hashimoto et al., 2003, 2008a,b; Morris et al., 2008; Fung et al., 2010, 2014; Joshi et al., in press) indicating functional deficits in cortical interneurons. On the other hand, CB expression may be increased in the DLPFC as found in three separate post mortem DLPFC cohorts in schizophrenia (Daviss and Lewis, 1995; Fung et al., 2010, 2014). Reports of decreased SST and PV gene expression appear to be the most robust and consistently reported in schizophrenia (Beasley and Reynolds, 1997; Lewis, 2000; Cotter et al., 2002; Hashimoto et al., 2008a,b; Morris et al., 2008; Fung et al., 2010, 2014). These two interneuron populations are known to be trophically dependent on BDNF/TrkB (Altar et al., 1997; Hashimoto et al., 2005; Glorioso et al., 2006; Woo and Lu, 2006).

Interneurons express tropomyosin related kinase (TrkB) and require pyramidal neuron synthesized brain derived neurotrophic factor (BDNF) for differentiation and maturation (Glorioso et al., 2006). Reductions in gene expression and protein level of both BDNF and its cognate tyrosine kinase receptor, TrkB, have been identified in the DLPFC of individuals with schizophrenia and these reductions have been proposed to contribute to the reduced health of interneurons in schizophrenia (Weickert et al., 2003, 2005; Hashimoto et al., 2005; Lewis et al., 2005; Wong et al., 2010; Ray et al., 2014). A recent meta-analysis has also shown that BDNF blood levels are reduced in both medicated and drug-naïve patients with schizophrenia (Green et al., 2011). Deficits in BDNF and TrkB have been linked to reduced GABAergic neuron density, reduced GAD67 gene expression, fewer GABAergic terminals and lower levels of somatostatin and parvalbumin in rodents (Huang et al., 1999; Villuendas et al., 2001; Glorioso et al., 2006; Arango-Gonzalez et al., 2009).

There is evidence to suggest that testosterone is able to modulate brain BDNF levels (Pluchino et al., 2013). Indeed, testosterone modulates BDNF and TrkB expression in the adult rodent cortex and songbird higher vocal centre (Rasika et al., 1999; Verhovshek et al., 2010; Hill et al., 2012). In the forebrain of male mice, increases in BDNF expression corresponded in time to the surge in testosterone at adolescence; and conversely, there was a significant decrease of TrkB protein with increased testosterone in the cortex (Hill et al., 2012). As such, the evidence that TrkB and BDNF signalling are reduced and interneuron health is compromised in schizophrenia, which raises the possibility of a role for testosterone in the pathophysiology of schizophrenia possibly *via* a reduction of cortical BDNF/TrkB expression levels ultimately impacting cortical interneurons. However, it is not known how testosterone and BDNF mRNAs are related to interneuron health in normal adolescent prefrontal cortex.

In normal male Sprague–Dawley rats and rhesus macaques, we investigated whether circulating testosterone during adolescence modulates gene expression of BDNF and TrkB, and we asked whether this was related to gene expression of interneuron specific genes — the expression of which is thought to be an index of the function of interneurons. The BDNF gene, in both rat and primate, can generate multiple transcripts by alternate splicing of 5′ exons (exons I–VIII) with the common 3′ exon IX (Aid et al., 2007; Pruunsild et al., 2007). All BDNF mRNA transcripts result in the same mature BDNF protein but the distinct alternate 5′ promoters enable distinct temporal and tissue specific expression and possibly different subcellular localization (Aid et al., 2007 a; Nanda and Mack, 1998; Wong et al., 2010). BDNF initiates intracellular signalling by binding to, and thereby activating, TrkB (Barbacid, 1994). However, truncated TrkB receptors that lack the tyrosine kinase domain have been identified in humans, non-human primates and rodents (Middlemas et al., 1991; Luberg et al., 2010; Wong et al., 2013) and are considered to be BDNF sinks whereby they bind BDNF preventing it from binding to and signalling *via* functional (full length) TrkB receptors (Eide et al., 1996). Although there is evidence that truncated TrkB proteins may activate alternate signalling pathways (Fenner, 2012).

In this study, we measured gene expression of the most highly expressed BDNF transcripts in the primate (monkey) (Aid et al., 2007; Pruunsild et al., 2007) and the rat cortex (Aid et al., 2007) under conditions of depleted (monkey and rat) and replaced testosterone (rat). We also measured gene expression of full length TrkB in both species (monkey: TrkB.TK+; rat: TrkB.FL), a single truncated TrkB isoform in monkey (TrkB.TK−) (Ohira and Hayashi, 2003; Ohira et al., 2005) and two truncated TrkB isoforms (TrkB.T1 and T2) in rats (Middlemas et al., 1991) to test if testosterone can influence cortical gene expression or splice variant levels of TrkB mRNA. Further, we asked whether changes in BDNF or TrkB transcripts are related to interneuron marker expression in either species. We measured PV, SST, CB and CR gene expression, which captures three largely independent populations of interneurons (Somogyi et al., 1998). Determining whether testosterone modulates neurotrophin mRNAs in relationship to interneuron markers that are involved in normal cortical maturation at adolescence may provide clues as to why adolescence is a period of risk for pathophysiology of cortical deficits that can be identified in those with chronic schizophrenia.

2. Methods

2.1. Experimental animals

2.1.1. Rhesus macaques

All procedures involving rhesus macaques were approved by the National Institute of Health (NIH) Animal Care and Use Ethics Committee. A total of twelve experimentally naive male rhesus macaque monkeys (*Macaca mulatta*) from the NIH Animal Centre's primate field station were used in the study, and were divided into two social groups. Subjects, housing conditions, surgery and endocrine measurements have been described in detail in Richards et al. (2009). 29 month old (~2.4 years) monkeys, showing no overt signs of puberty and testosterone levels less than 0.06 ng/ml were either gonadectomised (gdx, n = six) or sham-operated (intact, n = six) with a monkey from each group undergoing a yoked surgery (simultaneous anaesthesia, timing and recovery). Thus, sham animals underwent the same anaesthesia protocol but did not have any surgical manipulations performed. Blood samples were drawn every six–eight weeks beginning one month prior to surgery and continuing to the conclusion of the study. Samples were taken at 9:30 am and 11:00 pm, to track the diurnal variations in testosterone in addition to the seasonal variations. The average nighttime (pm) testosterone level (*i.e.*, 11 pm measurement) has been used in this study as daily circulating testosterone is highest at night. In intact monkeys, the average pm testosterone was 14.29 ± 1.53 ng/ml and this was reduced to 0.15 ± 0.01 ng/ml in the gonadectomised group. At 55 months of age (4.6 years) monkeys were saline perfused, brains were cut into 1 cm coronal sections and frozen. The fresh frozen tissue block containing frontal pole tissue was pulverized for homogenate studies and 14 μm

coronal sections were cut from the caudal-next frozen block of the frontal cortex containing the principle sulcus and tissue was thaw-mounted onto gelatinised glass slides for *in situ* hybridization studies (Brain Research Laboratories, Newton, MA).

2.1.2. Sprague–Dawley rats

All rat experiments were approved by the Animal Care and Ethics Committee of the University of New South Wales in accordance with the National Health and Medical Research Council of Australia's Code of Practice for the Care and Use of Animals for Experimental Purposes, which also conforms to standard international guidelines. Male Sprague–Dawley rats were used for all experiments (Animal Resource Centre, Perth, WA, Australia). Rats arrived at 32/33 days of age at our facility and were group housed (3–4/cage) in 12/12 h light/dark phases with constant humidity and temperature and free access to water and standard rat chow.

Adolescent male rats were gonadectomised at 45 days of age, prior to the adolescent testosterone increase (Saksena and Lau, 1979; Walker et al., 2012), and given continuous replacement testosterone (T) by subdermal silastic implant (Zirkin et al., 1989; Singh et al., 1995; Allan et al., 2010) for two weeks. By this age (60 days of age), intact animals have experienced the increase in testosterone associated with adolescence (Saksena and Lau, 1979; Walker et al., 2012). There were three groups (12–15 rats per group): intact (Intact), gonadectomy alone (Gdx) and gonadectomy plus testosterone (Gdx + T). Male rats (45 days old) were anaesthetized with an intraperitoneal (i.p.) injection of ketamine hydrochloride (60 mg/kg) and xylazine hydrochloride (10 mg/kg) (Provet, Castle Hill, Australia). The intact animals underwent sham abdominal surgery but gonads were left in place. Silastic implants were placed under the skin between the shoulder blades at time of gonadectomy. Gdx and Intact groups were given empty implants. Implants were 1 cm long, and have an internal diameter of 1.47 mm, and outer diameter of 1.95 mm (ends sealed with silastic adhesive). These implants have been characterized in previous studies and achieve supraphysiological, steady-state hormone levels and maintain seminal vesicle weights equal to that in untreated animals (Singh et al., 1995; Purves-Tyson et al., 2007). Seminal vesicles depend on androgen action for normal development and maintenance of structural and functional integrity (Mooradian et al., 1987). Weight of seminal vesicles at the end of experiment served as an index of the restoration of androgen action by the implants as previously reported (Purves-Tyson et al., 2007). Serum testosterone levels and seminal vesicle weights were used to confirm successful gonadectomy, and have been described in Purves-Tyson et al. (2012). Average circulating serum testosterone was 0.03 ± 0.001 ng/ml in the Gdx group ($n = 9$), 2.8 ± 0.6 ng/ml ($n = 12$) in the Intact group and 23.1 ± 12.0 ng/ml in the Gdx + T group ($n = 14$). Gonadectomy reduced seminal vesicles to 9.0% of the weight of the Intact group. Seminal vesicles were maintained at 94.2% of intact weights by replacement testosterone.

At 60 days of age rats were anaesthetized with 60 mg/kg sodium pentobarbital (Euthal, Delvet, Seven Hills, Australia). The brain was removed from the skull and a tissue block containing the frontal cortex was dissected following the Rat Brain Atlas as a guide (Paxinos and Watson, 2007). The frontal cortex block was trimmed such that cingulate, infralimbic and premotor cortices were collected for RNA extraction. Trunk blood was collected on day of euthanasia in 0.8 ml serum gel tubes (Z serum sep MiniCollect tube, Greiner Bio One, Wemmel, Belgium) and serum was collected by centrifugation after 30–60 min at room temperature.

2.2. RNA extraction and cDNA synthesis

Total RNA was extracted from frontal cortex samples in 800–1000 μl TRIzol reagent (Life Technologies Inc., Grand Island, NY, USA) as recommended by the manufacturer. RNA was quantified using a ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, USA). RNA integrity (RIN) was assessed with high resolution capillary electrophoresis (Agilent Bioanalyzer 2100, Agilent Technologies, Palo Alto, CA, USA). Rat samples: Two separate aliquots of 3 μg RNA from each sample were reverse transcribed to cDNA with SuperScript III First-Strand Synthesis Supermix and random hexamers, according to the manufacturer's protocol (Life Sciences) and pooled. Monkey samples: 3 μg RNA from each sample was reversed transcribed to cDNA with SuperScript II First-Strand Synthesis Supermix and random hexamers, according to the manufacturer's protocol (Life Technologies). In both rat and monkey samples, a parallel reaction was performed without reverse transcriptase as a genomic DNA amplification control.

2.3. Quantitative real-time PCR (qPCR)

Messenger RNA (mRNA) levels of genes of interest were measured by TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA) using an ABI Prism 7900HT Fast Real-Time PCR System and a 384-well format. Expression of four housekeeping genes, tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein (YWAZ), beta-glucoronidase (GusB), 18S ribosomal RNA (18S rRNA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), was used to calculate the normalizing control for rat gene expression (termed geometric mean), and were selected on the basis that they were unchanged by the treatment (Table 1).

The geometric mean of the four housekeeping genes used was calculated as described previously (Vandesompele et al., 2002). There were no group differences when the geomean of the three housekeepers was compared ($F = 1.67$, $df = 4$, $p = 0.17$). For monkey, SHDA, ACTB and TBP were used as the normalizing control for gene expression, and were selected on the basis that they were unchanged by gonadectomy [$t(10) = 1.79$, $p = 0.10$].

Genes of interest were targeted by TaqMan probes and listed in Table 1 (Applied Biosystems). Samples were run alongside a seven point standard curve using serial dilutions of pooled cDNA derived from PFC mRNA pooled from a subset of 25 rats (taken from all treatment groups) (Purves-Tyson et al., 2012) or pooled from all monkeys. No template controls were included which produced no signal for any mRNA examined. Measurements were performed in triplicate. PCR cycling conditions were: 50 °C for 2 min, 95 °C for 10 min, 50 cycles at 95 °C for 15 s and 60 °C for 1 min. PCR data were captured with Sequence Detector Software (SDS version 2.4, Applied Biosystems). SDS

Table 1
TaqMan gene expression assays.

Gene	Monkey	Rat
TrkB-TK+ TrkB.FL	Hs0193098_m1	Rn00820626_m1
TrkB-TK−	Hs0193110_m1	
TrkB.T1		AJSO7AX
TrkB.T2		AJRR84P
BDNF I	Hs00538277_m1	Rn01484924_m1
BDNF II	Hs00538278_m1	
BDNF IIA		Rn00560868_m1
BDNF IIB		Rn01484926_m1
BDNF IIC		Rn01484925_m1
BDNF IV		Rn01484927_m1
BDNF VI	Hs00601650_m1	Rn01484928_m1
PV	Hs00161045_m1	Rn00574541_m1
SST	Hs00356144_m1	Rn00561967_m1
CB	Hs01077191_m1	Rn00583140_m1
CR	Hs00242372_m1	Rn00588816_m1
SDHA	Hs01549169_m1	
TBP	Hs0042760_m1	
YWHAZ		Rn00755072_m1
GUSB		Rn00566655_m1
18S rRNA		Hs99999901
GAPDH		Rn01775763

software plotted real-time fluorescence intensity and set the threshold within the linear phase of the amplification profiles.

2.4. In situ hybridisation

Antisense riboprobes were generated for BDNF II and BDNF VI by amplifying transcript specific DNA fragments from cDNA using the following primer sets:

BDNF II forward 5′-TATCTCCAGGATCTAGCCACC-3′

BDNF VI forward 5′-ATCGGAACCACGATGTGACTCC-3′
BDNF common reverse 5′-ATCCAACAGCTCTTCTATCACG-3′.

Plasmids were constructed by annealing PCR products into a PCRII vector using the PCRII TA cloning kit (Invitrogen, CA, USA) as per manufacturer's instructions. Riboprobes were synthesized from linearised plasmid using SP6 and T7 polymerases respectively and an *in vitro* transcription kit as recommended by the manufacturer (Promega, WI, USA). Antisense and sense riboprobes were labelled to a specific activity of ~2×10^9 cpm/μg by addition of radiolabelled [^{35}S]rUTP (Perkin Elmer, Melbourne, Australia) and were purified by ethanol precipitation. 14 μm coronal sections containing the frontal cortex were used for *in situ* hybridisation histochemistry as previously described (Whitfield et al., 1990) using two slides per region per monkey. After hybridisation and washing steps, sections were exposed to autoradiographic film (Bio-Max, Kodak) for 2 months. All sections were assayed together to limit inter assay variability.

Calibrated densitometric analysis (ImageJ, NIH) was conducted blind to treatment group. Slides were first calibrated to radioactive standards (American Radiolabeled Chemicals Inc., MO, USA). In the dorsal bank of the principal sulcus on each section, three randomly placed lines were drawn perpendicular to the pial surface, traversing the entire cortical grey matter. The density of target mRNA as a function of cortical depth was computed from continuous optical density measurements recorded along the length of these lines. The percentage of full cortical width corresponding to the individual lamina was determined from published guidelines as follows: I (2–6%), II (14–18%), III (20–42%), IV (46–50%), V (54–68%) and VI (72–92%) (Kritzer and Goldman-Rakic, 1995; Rajkowska and Goldman-Rakic, 1995; Romanczyk et al., 2002).

2.5. Statistics

Statistical analyses were conducted using GraphPad (version 6) and Statistica (version 12) and $p \leq 0.05$ was considered statistically significant. Rat qPCR data are presented as percent change of mRNA levels relative to the Intact group ± SEM. Outlier detection of the triplicates obtained from the qPCR raw data was used to exclude measurement error (Weickert et al., 2010). qPCR raw data was normalized by the geomean of the housekeepers. Population outliers were removed by performing Grubb's test on the normalized data and data were analysed by one-way ANOVA followed by Fisher's LSD. Monkey qPCR data was analysed with two-tailed independent sample t-tests to determine the difference in mRNA expression between intact and gonadectomised monkeys, data are presented as percent change in mRNA levels relative to the intact group ± SEM.

In situ hybridization density measurements were analysed by repeated measures ANOVA with “treatment” (Intact *vs* Gdx) as the between subjects factor and “layer” (I–VI) as the within subjects factor. Significant interactions were followed up with Fisher's least significant differences *post hoc* analysis.

In the Intact monkey group, Pearson's correlations were performed to analyse the relationship between testosterone and neurotrophin transcripts, receptor transcripts and interneuron marker expression. Pearson's correlations were also used to determine changes in the relationship between interneuron markers and neurotrophin and receptor transcripts in Intact and testosterone replaced (rat only) and Gdx groups monkey and rat groups.

3. Results

3.1. Testosterone reverses gonadectomy-induced increases in selective BDNF transcripts in the rat and monkey frontal cortices

Investigation of BDNF II and BDNF VI mRNA transcript expression in the monkey prefrontal cortex by *in situ* hybridization revealed that testosterone removal during adolescence resulted in an increase in both transcripts. There was a main effect of group (Gdx *vs.* Intact) for both BDNF II and BDNF VI [BDNF II mRNA, $F(1,9) = 16.19$, $p = 0.003$ and BDNF VI mRNA, $F(1,10) = 22.90$, $p = 0.0007$] as well as a significant interaction between group and layer for both transcripts [BDNF II mRNA, $F(6,54) = 4.780$, $p = 0.0006$ and BDNF VI mRNA, $F(6,60) = 4.79$, $p = 0.0005$]. *Post hoc* comparisons revealed significant differences between gonadectomy and intact groups in all layers for both transcripts except for layer I (Fig. 1C and D).

To further investigate the effect of testosterone removal and replacement on BDNF gene expression, we investigated the effect on rodent cortex. In the rat, exon II gives rise to three distinct transcripts due to alternate donor splice sites (Aid et al., 2007), and transcripts IIa, IIb and IIc were measured in the rat. There was an effect of treatment group on BDNF IIa mRNA expression [$F(2,43) = 3.57$, $p = 0.04$] (Fig. 2A). Gonadectomy of male rats during adolescence revealed a significant 22.7% increase in BDNF-IIa mRNA expression in the gonadectomised group compared to the Intact group (Gdx *vs.* Intact, $p = 0.02$) and this increase was attenuated by testosterone replacement (Intact vs Gdx + T, p = ns; Gdx *vs.* Gdx + T, $p = 0.045$). In contrast, neither gonadectomy nor testosterone replacement resulted in a significant change in expression of BDNF IIb [$F(2,42) = 0.06$, $p = 0.90$] or BDNF IIc [$F(2,42) = 1.08$, $p = 0.35$] (data not shown). There was no significant effect of treatment group on gene expression of BDNF-VI [$F(2,42) = 1.96$, $p = 0.15$]. However, due to an *a priori* hypothesis based on findings in rhesus macaque, *post hoc* tests were carried out which revealed that testosterone replacement reduced BDNF-VI gene expression by 15% compared to gonadectomised rats (Gdx *vs.* Gdx + T, $p = 0.05$) (Fig. 2B). The expression levels of BDNF-I [$F(2,42) = 0.08$, $p = 0.9$] and BDNF-IV [$F(2,43) = 0.14$, $p = 0.87$] did not appear to be changed by gonadectomy and testosterone replacement (data not shown).

3.2. Testosterone modulates the relationship of full length to truncated TrkB mRNA in the rat and monkey frontal cortices

Analysis of gene expression in rodent frontal cortex, using qPCR, revealed no effect of treatment group when examining full length TrkB (TrkB.FL) [$F(2,44) = 2.38$, $p = 0.11$] mRNA or truncated TrkB transcript T1 [TrkB.T1, $F(2,42) = 0{,}95$, $p = 0.40$] expression (data not shown). However, there was a significant effect of treatment group on truncated TrkB transcript 2 [TrkB.T2, $F(2,43) = 3.32$, $p = 0.046$] (Fig. 3A). Testosterone removal increased TrkB.T2 by 28%, but this did not reach statistical significance (Intact vs Gdx, $p = 0.13$), however, testosterone replacement significantly reduced the level of TrkB.T2 mRNA by 46.2% compared to gonadectomised rats (Gdx vs Gdx + T, $p = 0.014$) (Fig. 3A left).

In the rat, there was no effect of treatment group on the TrkB.FL: TrkB.T1 (TrkB FL:T1) ratio [$F(2,43) = 0.68$, $p = 0.51$] (data not shown), whereas there was a significant effect of treatment group on TrkB.FL: TrkB.T2 (TrkB FL:T2) ratio [$F(2,43) = 3.97$, $p = 0.026$] (Fig. 3A right). Gonadectomy reduced the ratio of TrkB FL:T2 mRNA compared to intact animals by 37% (Intact *vs.* Gdx, $p = 0.027$) and the testosterone replaced group was not significantly different to the intact group but was increased 40% compared to the gonadectomised group (Intact *vs.* Gdx + T, p = ns; Gdx *vs.* Gdx + T, $p = 0.012$) (Fig. 3A left).

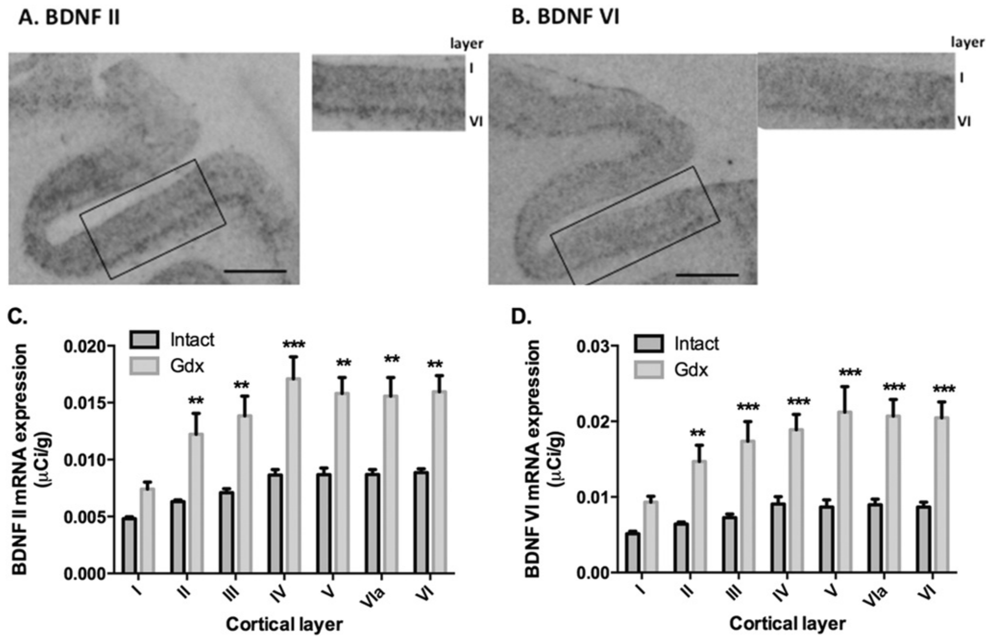

Fig. 1. Modulation of BDNF II and VI mRNA expression in rhesus macaque frontal cortex. The representative *in situ* hybridisation panels show BDNF II (A) and BDNF VI (B) mRNA expression in the frontal cortex of gonadectomised monkeys. Lower magnification panels show the principal sulcus of the frontal cortex (scale bar = 250 μm) and the higher magnification boxes show the dorsal bank of the principal sulcus. Three randomly placed lines were drawn perpendicular to the pial surface in the dorsal bank, traversing the whole grey matter and the density of target mRNA was measured (see Methods). There was a main effect of group (gdx *vs.* intact) for both BDNF II (C) and BDNF VI (D) [BDNF II mRNA, $F(1,9) = 16.19$, $p = 0.003$ and BDNF VI mRNA, $F(1,10) = 22.90$, $p = 0.0007$] as well as a significant interaction between group and layer for both transcripts [BDNF II mRNA, $F(6,54) = 4.780$, $p = 0.0006$ and BDNF VI mRNA, $F(6,60) = 4.79$, $p = 0.0005$]. Fisher's LSD *post hoc* revealed significant differences between intact and gonadectomised groups in layers II–VI but not layer I. $^{**}p < 0.01$, $^{***}p < 0.001$.

In the rhesus macaque, although qPCR did not reveal any changes in expression levels of TrkB full length mRNA (TrkB.TK+) or truncated TrkB (TrkB.TK−) (data not shown), Pearson's correlations between expression of TrkB transcripts and circulating testosterone levels in the intact group revealed a negative correlation between circulating testosterone and the TrkB TK+/TK− ratio ($r = -0.84$, $p = 0.036$) (Fig. 3C).

3.3. Gonadectomy and testosterone replacement changed expression levels of SST mRNA only in rat frontal cortex and did not change interneuron mRNAs in rhesus macaque frontal cortex

We detected a significant change in gene expression level in one out of four interneuron specific markers in the rodent prefrontal cortex following gonadectomy and testosterone replacement and detected no gene expression changes in any of these markers in monkey prefrontal cortex following gonadectomy.

In rat cortex, there was a significant change due to treatment group in SST mRNA expression [$F(2,43) = 3.17$, $p = 0.05$] (Fig. 4A). *Post hoc* tests revealed that gonadectomy reduced SST mRNA levels (Intact *vs.* Gdx, $p = 0.035$), however; this reduction was not prevented by testosterone replacement (Intact *vs.* Gdx + T, $p = 0.034$; Gdx *vs.* Gdx + T p = ns) (Fig. 4A). Pearson's correlations revealed in the testosterone replaced rat group (Gdx + T) that there was a significant positive relationship between testosterone and SST mRNA ($r = 0.65$, $p = 0.04$) (Fig. 4B, Table 2) and a significant negative relationship between testosterone and calretinin mRNA ($r = -0.74$; $p = 0.01$) (Table 2). There were no significant changes due to treatment group in PV [$F(2,42) = 2.19$, $p = 0.13$], CB [$F(2,43) = 0.88$, $p = 0.42$] or CR expression [$F(2,43) = 2.64$, $p = 0.08$] in the rat PFC (data not shown).

In the monkey PFC, there were no significant changes in PV [$t(10) = 0.075$, $p = 0.94$], SST [$t(10) = 0.14$, $p = 0.89$], CB [$t(9) = 0.69$, $p = 0.51$] or CR [$t(10) = 0.58$, $p = 0.57$] gene expression (data not shown). In the Intact monkey group, Pearson's correlations between circulating average pm testosterone and interneuron gene expression revealed no significant relationships (Table 2).

3.4. Circulating sex steroids alter the relationship between interneuron marker mRNA and BDNF/TrkB mRNA expression in the rat and monkey frontal cortices

In order to determine if the relationship between interneuron marker gene expression and BDNF/TrkB mRNA expression depended on the sex steroid environment, we separated monkeys into treatment groups

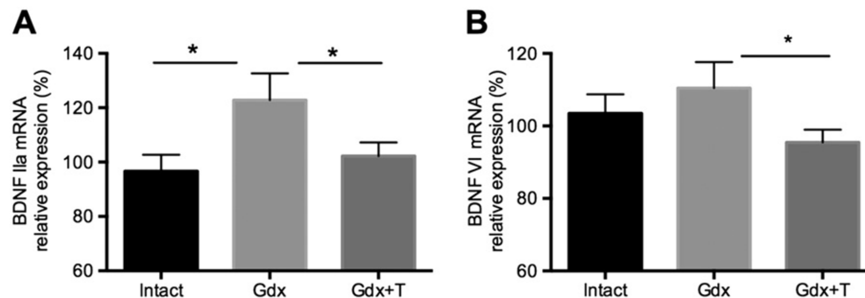

Fig. 2. In male rats testosterone replacement reversed the gonadectomy-induced increase in BDNF IIa mRNA (A) and reduced BDNF VI mRNA (B) relative to the gonadectomised group in the frontal cortex. Data are means ± SEM, expressed as a percentage of the intact group. * $p \leq 0.05$.

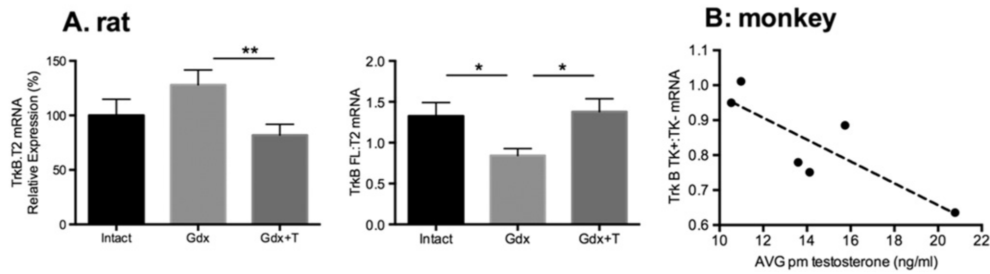

Fig. 3. Testosterone modulates TrkB transcripts in rat and monkey frontal cortices. A. In the rat cortex testosterone replacement reduced expression of TrkB.T2 mRNA. The TrkB FL:T2 mRNA ratio was reduced by gonadectomy and this was prevented by testosterone replacement. Data are means ± SEM, expressed as a percentage of the intact group. B. In the monkey cortex there was a negative association between the ratio of TrkB TK+:TK− mRNA and average nighttime circulating testosterone levels ($r = -0.84$, $p = 0.03$). * $p \leq 0.05$, ** $p < 0.01$.

(Intact and Gdx) and rats into those with testosterone (Intact plus Gdx + T) and those without (Gdx) testosterone and ran correlation analyses within each group (Table 3: monkey; Table 4: rat).

In the monkey prefrontal cortex, Pearson's correlations revealed many positive relationships between interneuron marker gene expression and BDNF and TrkB.TK+ transcript expression in the presence of testosterone that were not evident when testosterone was absent (Table 3). This pattern was most evident for correlations with BDNF/TrkB transcripts and SST gene expression, but was also found with CR and BDNF I, and with CB and TrkB TK+/TK−. Conversely, when testosterone was absent, a significant positive correlation between PV mRNA and TrkB.TK+ mRNA was revealed that was not present in the intact group.

In the rat cortex, Pearson's correlations revealed some negative correlations between interneuron markers and BDNF transcripts that depended on the presence of testosterone (Table 4). In particular, only in the presence of testosterone, SST mRNA was negatively correlated with BDNF IIc mRNA and PV mRNA was negatively correlated with BDNF IV and VI mRNA transcripts. When testosterone was removed, TrkB.FL mRNA was significantly positively correlated with CB mRNA and CR mRNA was negatively correlated with BDNF IIa and BDNF VI mRNAs.

4. Discussion

This study sought to determine if circulating testosterone during normal adolescence modulates cortical gene expression of BDNF and TrkB splice variants and whether this was related to gene expression of specific cortical interneuron markers. We show that, in both monkey and rat, testosterone removal over adolescence increased gene expression of specific BDNF transcripts, and testosterone replacement could prevent the increase in BDNF mRNA. The study also revealed similarities and differences in changes in TrkB mRNA levels by testosterone between species. The total level of TrkB full length mRNA was not changed by testosterone in rat PFC. The TrkB full length to truncated ratio increased in rat cortex when testosterone was present. If these changes were also reflected at the protein level, there may be more chance for classical TrkB signalling to be initiated by ligand binding when testosterone is present. We identified what appeared to be species-specific relationships between interneuron markers and BDNF/TrkB gene expression that were dependent on the presence of testosterone in the rat and monkey cortices, *e.g.*, specific BDNF transcripts were positively correlated with SST mRNA in the monkey but were negatively correlated with SST mRNA in the rat. Taken together, our findings suggest that there are dynamic relationships between BDNF/TrkB and interneuron markers that are dependent on the presence of testosterone and may contribute to interneuron health and cortical maturation during normal/healthy adolescence. The fact that BDNF mRNA is modulated by testosterone in the mammalian cortex during this developmental stage demonstrates that the sex steroid driven change of BDNF is possible, but does not predict the direction of change or alteration in schizophrenia.

A mature pattern of innervation by GABAergic synapses is not achieved until late childhood or adolescence in both rodents and primates (Morales et al., 2002; Chattopadhyaya et al., 2004; Lewis et al., 2004a; Di Cristo, 2007; Fung et al., 2010; Catts et al., 2013). As a main feature of adolescence is an increase in circulating sex hormones, it follows that sex hormones may contribute to the final maturation of cortical inhibitory interneurons. Given that GABAergic neurons require BDNF for maturation, and testosterone can induce BDNF in adults (Rasika et al., 1999), it was surprising that testosterone over adolescence decreased gene expression of some BDNF transcripts (BDNF II and VI) in the mammalian cortex, suggesting that BDNF may become more limited in supply during adolescence. This may have implications for GABAergic health and thus, cognition at adolescence as abnormally reduced BDNF action is associated with impaired spatial memory in male rats (Mu et al., 1999). Within the context of normal development, this decrease in specific BDNF transcripts due to testosterone may not have detrimental effects on cognition and indeed be part of the fine-tuning of cortical maturation, or part of a normal closing of developmental windows of increased plasticity. However, in circumstances where there is an underlying predisposition to cognitive dysfunction (such as genetic predisposition to schizophrenia) this testosterone-

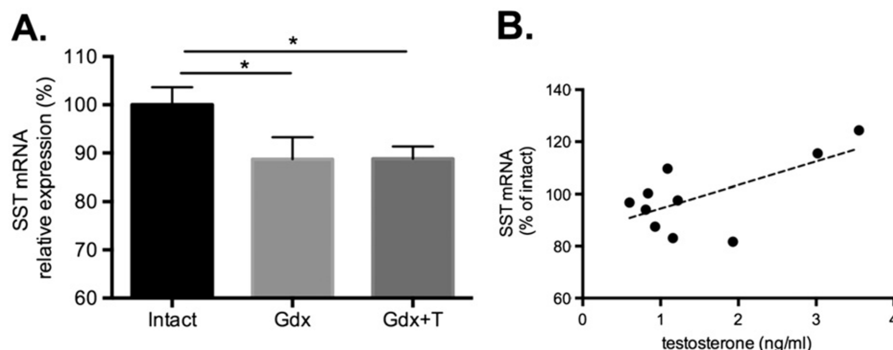

Fig. 4. A. In male rats gonadectomy reduced SST mRNA expression in the frontal cortex but this was not prevented by testosterone replacement. Data are means ± SEM, expressed as a percentage of the intact group. B. In the Gdx + T group there was a positive correlation between SST mRNA gene expression and testosterone level ($r = 0.65$, $p = 0.04$). * $p \leq 0.05$.

Table 2
Correlations between serum testosterone and interneuron marker gene expression in the frontal cortex in monkey and rat. Pearson's correlations between average nighttime testosterone in the intact monkeys revealed a trend for a negative correlation with CB gene expression only. In the testosterone-replaced rat group, Pearson's correlations revealed a significant positive correlation with SST and a significant negative correlation with CR with terminal circulating testosterone.

Testosterone average pm *vs.*	Intact monkey		Terminal testosterone *vs.*	Gdx + T	
	r value	p value		r value	p value
SST	−0.61	0.20	SST	**0.65**	**0.04***
CB	−0.85	0.07	CB	0.21	0.54
PV	−0.17	0.74	PV	−0.26	0.44
CR	−0.15	0.78	CR	**−0.74**	**0.01***

* $p \leq 0.05$.

related decrease in BDNF transcripts at adolescence may have negative consequences for interneurons and we speculate that this may contribute to the presentation and/or progression of cognitive deficits that typically emerge in schizophrenia during adolescence in males.

Although some transcripts were decreased by testosterone in both monkey and rat the relationships between BDNF/TrkB gene expression and interneuron marker gene expression diverged between the two species. In the monkey cortex, we found a positive relationship between the level of BDNF transcript expression and SST mRNA within intact animals. This aligns with full length TrkB mRNA and SST mRNA also being positively correlated in intact animals. The actual full length TrkB action would depend on the subcellular location of the TrkB isoforms and whether the truncated isoform was dimerising with TrkB.FL and therefore curtailing classic TrkB second messenger signalling pathways and/or acting as a BDNF sink and/or initiating other truncated TrkB independent functions (Fenner, 2012). A large proportion of SST+ interneurons also express CB (Di Cristo, 2007). The expression of two

Table 3
Correlations between interneuron marker gene expression and BDNF/TrkB transcript mRNA expression in the cortex within intact and gonadectomised monkey groups. The relationship between the expression of several BDNF/TrkB transcript mRNAs and the expression of interneuron marker mRNAs was altered by the presence or absence of adolescent testosterone.

	Intact		Gdx	
	r value	p value	r value	p value
SST *vs.* BDNF I	0.75	0.08	0.45	0.37
SST *vs.* BDNF II	0.75	0.08	0.49	0.32
SST *vs.* BDNF IV	**0.81**	**0.05***	0.11	0.8
SST *vs.* BDNF VI	0.80	0.06	0.8	0.06
SST *vs.* TrkB.TK+	**0.85**	**0.03***	−0.12	0.81
SST *vs.* TrkB.TK−	−0.34	0.52	−0.67	0.15
SST *vs.* TK+/TK−	−0.79	0.06	−0.64	0.17
CB *vs.* BDNF I	0.59	0.29	0.17	0.75
CB *vs.* BDNF II	0.37	0.53	0.20	0.70
CB *vs.* BDNF IV	0.84	0.07	0.23	0.66
CB *vs.* BDNF VI	0.83	0.08	0.10	0.86
CB *vs.* TrkB.TK+	0.65	0.24	0.24	0.65
CB *vs.* TrkB.TK−	**−0.99**	**0.001****	−0.004	0.99
CB *vs.* TK+/TK−	**0.91**	**0.03***	0.37	0.47
PV *vs.* BDNF I	0.76	0.08	−0.38	0.45
PV *vs.* BDNF II	0.50	0.30	0.25	0.63
PV *vs.* BDNF IV	0.37	0.47	0.05	0.93
PV *vs.* BDNF VI	0.30	0.56	−0.57	0.24
PV *vs.* TrkB.TK+	0.47	0.34	**0.88**	**0.02***
PV *vs.* TrkB.TK−	−0.07	0.89	0.79	0.06
PV *vs.* TK+/TK−	0.36	0.48	0.31	0.55
CR *vs.* BDNF I	**0.95**	**0.003****	0.27	0.60
CR *vs.* BDNF II	−0.05	0.9	−0.13	0.80
CR *vs.* BDNF IV	0.63	0.18	0.21	0.69
CR *vs.* BDNF VI	0.45	0.37	0.09	0.95
CR *vs.* TrkB.TK+	0.46	0.36	−0.10	0.86
CR *vs.* TrkB.TK−	−0.35	0.49	−0.70	0.75
CR *vs.* TK+/TK−	0.54	0.27	0.08	0.89

* $p \leq 0.05$.
** $p < 0.01$.

Table 4
Correlations between interneuron marker gene expression and BDNF/TrkB transcript mRNA expression, in the frontal cortex within intact and gonadectomised + testosterone (combined) and gonadectomised rat groups. The relationship between the expression of several BDNF/TrkB transcript mRNAs and the expression of interneuron marker mRNAs was altered by the presence or absence of adolescent testosterone.

	Intact and Gdx + T		Gdx	
	r value	p value	r value	p value
SST *vs.* BDNF I	0.10	0.60	0.35	0.21
SST *vs.* BDNF IIa	0.20	0.26	−0.02	0.95
SST *vs.* BDNF IIb	0.19	0.30	0.24	0.41
SST *vs.* BDNF IIc	**−0.45**	**0.01***	0.39	0.17
SST *vs.* BDNF IV	0.21	0.27	0.30	0.31
SST *vs.* BDNF VI	−0.35	0.07	0.18	0.53
SST *vs.* TrkB.FL	0.14	0.44	0.19	0.49
SST *vs.* TrkB.T1	0.05	0.76	−0.21	0.47
SST *vs.* TrkB.T2	0.29	0.11	0.10	0.72
SST *vs.* TrkB.FL/T1	0.05	0.78	0.31	0.27
SST *vs.* TrkB.FL/T2	0.18	0.33	−0.04	0.90
CB *vs.* BDNF I	−0.19	0.33	0.49	0.08
CB *vs.* BDNF IIa	0.12	0.51	0.26	0.36
CB *vs.* BDNF IIb	0.08	0.65	0.16	0.60
CB *vs.* BDNF IIc	0.32	0.08	0.17	0.56
CB *vs.* BDNF IV	−0.06	0.76	0.39	0.17
CB *vs.* BDNF VI	0.23	0.22	0.22	0.45
CB *vs.* TrkB.FL	0.25	0.16	**0.54**	**0.04***
CB *vs.* TrkB.T1	0.34	0.05	0.14	0.65
CB *vs.* TrkB.T2	0.28	0.12	0.33	0.26
CB *vs.* TrkB.FL/T1	−0.13	0.48	−0.07	0.80
CB *vs.* TrkB.FL/T2	−0.22	0.22	0.43	0.12
PV *vs.* BDNF I	−0.22	0.26	−0.30	0.32
PV *vs.* BDNF IIa	0.04	0.85	−0.01	0.96
PV *vs.* BDNF IIb	−0.07	0.85	−0.16	0.61
PV *vs.* BDNF IIc	−0.26	0.16	−0.09	0.78
PV *vs.* BDNF IV	**−0.50**	**0.01***	0.15	0.63
PV *vs.* BDNF VI	**−0.50**	**0.006****	0.02	0.9
PV *vs.* TrkB.FL	0.13	0.5	−0.3	0.29
PV *vs.* TrkB.T1	−0.14	0.45	−0.49	0.08
PV *vs.* TrkB.T2	−0.01	0.97	−0.20	0.49
PV *vs.* TrkB.FL/T1	0.32	0.08	0.05	0.87
PV *vs.* TrkB.FL/T2	0.08	0.67	0.24	0.42
CR *vs.* BDNF I	−0.16	0.41	−0.05	0.87
CR *vs.* BDNF IIa	−0.18	0.34	**−0.66**	**0.008****
CR *vs.* BDNF IIb	−0.33	0.07	−0.11	0.71
CR *vs.* BDNF IIc	−0.19	0.31	−0.05	0.86
CR *vs.* BDNF IV	−0.03	0.11	−0.41	0.15
CR *vs.* BDNF VI	−0.21	0.27	**−0.54**	**0.04***
CR *vs.* TrkB.FL	−0.19	0.31	−0.10	0.72
CR *vs.* TrkB.T1	−0.30	−0.10	−0.27	0.35
CR *vs.* TrkB.T2	−0.24	0.21	0.24	0.39
CR *vs.* TrkB.FL/T1	0.23	0.22	0.02	0.94
CR *vs.* TrkB.FL/T2	0.20	0.29	−0.05	0.88

* $p \leq 0.05$.
** $p < 0.01$.

BDNF transcripts (IV, VI) was positively correlated with CB in intact monkeys only and CB expression was positively correlated with the TrkB FL/TK− ratio. So in the SST+/CB+ interneurons there may be complex relationships between SST/CB gene expression and BDNF and TrkB gene expression which are only found in the presence of testosterone, as these correlations were not found in gonadectomised monkeys. This complex interaction may be a mechanism whereby the timing of the maturation of these neurons that synapse with pyramidal neuron dendrites is controlled over adolescence in the primate cortex.

In the rat cortex, there were fewer relationships between SST/CB and BDNF/TrkB than in the monkey cortex both in the presence or absence of testosterone. In contrast to the monkey, in the presence of testosterone BDNF IIc (and BDNF VI) mRNA in the rat cortex was negatively correlated with SST mRNA but, as in the monkey, positively correlated (BDNF IIc only) with CB. This may indicate a switch between predominantly expressing SST mRNA to predominantly expressing CB mRNA that is influenced by testosterone and may reflect the different developmental trajectories of the interneurons (Fung et al., 2010). No significant or distinct

relationships between SST and/or CB mRNA with TrkB receptor mRNA in the presence or absence of testosterone were identified in rats. This is partly in line with the model of Glorioso et al. (2006) who showed that SST expression was unchanged in male adult mouse cortex when TrkB was reduced, however their model also showed that SST was decreased when BDNF was decreased (Glorioso et al., 2006). However, their model is in adult mice rather than adolescence and there may be crucial differences in the relationships between BDNF and interneuron markers during adolescent cortical maturation and adulthood.

PV neurons are the second population of interneurons that are consistently reduced in schizophrenia. It has previously been reported that PV neurons are less dependent on BDNF for the maintenance of phenotype (Glorioso et al., 2006), although it has also been reported that approximately 80% of PV neurons express TrkB (Gorba and Wahle, 1999). Also, in mice, when BDNF IV transcription was specifically blocked there was a reduction in the number of PV neurons and impaired activity-dependent BDNF expression (Sakata et al., 2009). We found that PV mRNA appeared to be less broadly correlated than SST/CB mRNA with BDNF mRNAs in the monkey cortex and although there was more co-regulation of PV and BDNF mRNA in the rodent cortex in the presence of testosterone this was restricted to the mRNA derived from two BDNF promoters (BDNF IV and VI mRNAs). Calretinin expression in the cortex was the least correlated with BDNF and TrkB expression in the presence of testosterone. Indeed, studies in rodent have assessed that <20% of CR neurons express TrkB (Gorba and Wahle, 1999). No significant relationships with CR mRNA and BDNF/TrkB mRNAs were identified in the rat in the presence of testosterone, although in the monkey there was a strong positive correlation between BDNF I and CR mRNA that was dependent on testosterone. However, in the rat cortex, when testosterone was removed a negative relationship between CR mRNA and BDNF IIa and VI mRNAs was revealed. This may indicate that in the preadolescent, immature rodent cortex there is a suppression of CR expression by BDNF and that relationship may become uncoupled when testosterone increases at adolescence. Our study indicates that the relationship between testosterone and its regulation of BDNF and TrkB and thus, cortical neuron maturation is not a linear relationship but may rather be a complex interplay of differential regulation of BDNF/TrkB in distinct cortical interneuron populations.

Determining how testosterone contributes to cortical development in the healthy adolescent brain will assist in determining how, or indeed if, testosterone contributes to the derailment of normal development in individuals who may be susceptible to developing schizophrenia. This knowledge could aid the development of sex steroid based interventions to protect healthy cortical neuron development/maturation or to correct a derailed cortical system following diagnosis. Indeed, sex steroid based adjunctive treatment of schizophrenia patients with a selective oestrogen receptor modulator, raloxifene, has shown promising enhancement of cognitive function (Weickert et al., 2015). Future preclinical investigations will allow us to determine whether this is due to a modulation of the BDNF/TrkB/interneuron relationships identified here.

There are limitations in our study to be considered. We have identified differences in the relationships between BDNF/TrkB gene expression and interneuron markers between species and we have identified important similarities (*e.g.*, gonadectomy increased BDNF transcripts). The differences between rat and monkey may be due to species differences *e.g.*, species differences in the BDNF gene (Pruunsild et al., 2007). The differences between primates and rodents may also be due to differences in experimental design. Although the experimental interval of testosterone manipulation represented a similar period of time in terms of adolescent development, the time points we investigated in the two species may not be exactly equivalent with regard to timing of changes in the cortex (weeks in rats compared to months in monkeys). We must also acknowledge that circulating testosterone levels in the testosterone-replaced rats reached supra-physiological levels. It is also a limitation in our study that we have carried out multiple correlations without corrections and these studies require follow-up and repetition.

We report here that complex relationships exist between BDNF/TrkB gene expression and interneuron marker gene expression that appear to be dependent on the presence of testosterone at adolescence. Perturbations in these relationships, that may occur due to genetic or environmental factors that are associated with risk for schizophrenia, could well derail normal cortical brain development and contribute to cortical deficits apparent in schizophrenia. Indeed, BDNF genetic variants (Val66Met) may confer susceptibility to schizophrenia (Jonsson et al., 2006) that could be unmasked at adolescence. Cortical BDNF mRNA is lower (Weickert et al., 2003; Wong et al., 2010; Ray et al., 2014) and truncated TrkB may be increased (Wong et al., 2013) in schizophrenia. Suboptimal sex steroid signalling has been linked to schizophrenia in the form of a dominant negative variant of the oestrogen receptor (ER) (Perlman et al., 2005; Weickert et al., 2008). As testosterone in the male can act *via* androgen receptor and/or be aromatized to oestrogen and act *via* ERs, the presence of this ER variant confers risk to both genders. Reductions in cortical interneuron health have been implicated in schizophrenia (Lewis et al., 2005). We therefore propose that any of these factors may contribute to the disruption of the appropriate relationships between testosterone/BDNF/interneurons in schizophrenia. Given that we found that BDNF transcription can be changed normally in male adolescence this demonstrates that BDNF is a testosterone target in mammalian cortex; however, it does not allow us to determine the nature of a putative testosterone/BDNF/interneuron dysregulation in schizophrenia. Thus we speculate that we have demonstrated that it is possible that increased testosterone-induced changes in adolescence may precipitate or contribute to the progression of schizophrenia. As highlighted, adolescence is a risk period for schizophrenia, however it is also a risk period for other mental illnesses, including depression, anorexia nervosa and anxiety, some of which are more common in females. This emphasises that sex steroid signalling may play some role in the precipitation of many disorders. The fact that schizophrenia is more prevalent in males supports the suggestion that testosterone may play a specific role in the precipitation/progression of schizophrenia. Preclinical studies are required to determine how testosterone modulates BDNF/TrkB/interneuron relationships in schizophrenia-like models. As such, we conclude that the complex relationships between BDNF/TrkB and interneurons are modulated by testosterone during normal adolescence and may be critical for cortical interneuron maturation, particularly during the adolescent developmental window.

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Contributors

Tertia Purves-Tyson, contributed to rodent study design and conception, performed the rodent surgeries, the rodent qPCRs, statistical analyses and wrote the manuscript. Katherine Allen analysed the *in situ* radiographs, some monkey qPCRs and performed statistical analysis and contributed to drafting the manuscript. Samantha Fung performed the *in situ* hybridisation experiments and some monkey qPCRs. Deborah Rothmond and Pam Noble carried out the monkey experiments. David Handelsman analysed the rodent sex steroids and edited the manuscript. Cynthia Shannon Weickert conceived the monkey and rodent studies and edited the manuscript.

Conflict of interest

No author has a conflict of interest to declare.

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