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GREY MATTER VOLUME CORRELATES WITH VIRTUAL WATER MAZE TASK PERFORMANCE IN BOYS WITH ANDROGEN EXCESS

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Abstract—Major questions remain about the specific role of testosterone in human spatial navigation. We tested 10 boys (mean age 11.65 years) with an extremely rare disorder of androgen excess (Familial Male Precocious Puberty, FMPP) and 40 healthy boys (mean age 12.81 years) on a virtual version of the Morris Water Maze task. In addition, anatomical magnetic resonance images were collected for all patients and a subsample of the controls (*n*=21) after task completion. Behaviourally, no significant differences were found between both groups. However, in the MRI analyses, grey matter volume (GMV) was correlated with performance using voxel-based morphometry (VBM). Group differences in correlations of performance with GMV were apparent in medial regions of the prefrontal cortex as well as the middle occipital gyrus and the cuneus. By comparison, similar correlations for both groups were found in the inferior parietal lobule. These data provide novel insight into the relation between testosterone and brain development and suggest that morphological differences in a spatial navigation network covary with performance in spatial ability. Published by Elsevier Ltd on behalf of IBRO.

Key words: testosterone, spatial navigation, development, familial male precocious puberty, VBM, virtual water maze.

Testosterone plays a key role in brain development and sexual differentiation. These early effects are organizational, and permanently alter brain morphology (Romeo, 2003). Grey matter volume (GMV) of the brain has been shown to be influenced by sex and age (Neufang et al., 2009; Peper et al., 2009; Raznahan et al., 2010). Familial male precocious puberty (FMPP), an extremely rare, male-limited genetic disorder (up to nine in one million, Orphanet/NIH, Office of Rare Diseases), is characterised by isolated dysfunction of androgen secretion resulting in androgen excess. Signs of precocious puberty such as growth acceleration, skeletal advancement, pubic hair, acne and phallic enlargement emerge around 2–4 years of age (Leschek, 2004; Reiter and Norjavaara, 2005). Because of advanced growth acceleration and bone maturation, bone age rather than chronological age is commonly used in FMPP as an estimate of physiological maturation. Thus, FMPP represents an ideal and unique natural model to study the association between early androgen excess and brain development.

To date, very little information is known about the cognitive changes in FMPP. Prior studies in this patient group have shown functional perturbations in the neurocircuitry underlying emotional processing (Mueller et al., 2009a) as well as changes in brain morphometry (Mueller et al., 2011). One question immediately arising from these findings is how the structural neuroimaging findings relate to cognitive performance in this population.

Spatial navigation is an excellent candidate to address this question. First, spatial navigation depends on a wide cortical and subcortical network (Aguirre et al., 1996; Cornwell et al., 2008; Maguire et al., 1998; Grön et al., 2000; Iaria et al., 2003; Bohbot et al., 2004). Second, some studies suggest a developmental role of specific brain regions such as the temporo-parietal cortex in human spatial learning (Pine et al., 2002). Third, spatial abilities are among the most robust sexually dimorphic characteristics (Voyer et al., 1995; Grön et al., 2000), with differences in recruitment of brain areas including medial and lateral frontal and parietal regions. Fourth, studies in patients with hormonal perturbations (Hier and Crowley, 1982; Hines et al., 2003) have shown that spatial abilities covary with hormonal exposure. Drawing on this background, we examined spatial navigation abilities in girls suffering from congenital adrenal hyperplasia (CAH), a common disorder of prenatal virilisation (Merke and Bornstein, 2005). Girls with the highest exposure to androgens found the hidden platform as quickly as males and faster than girls with less severe forms of the disorder or unaffected girls on a virtual version of the Morris Water Maze (vMWM) (Mueller et al., 2008). Similarly, in healthy adults, women with high endogenous testosterone also completed the virtual maze faster than women with low testosterone levels (Burkitt et al., 2007).

The goal of this study is to extend our previous work with boys with FMPP. Based on (1) previous findings of sensitivity of frontal and parietal regions to sex differences in spatial navigation (Grön et al., 2000), (2) a crucial role of frontal and inferior parietal regions in spatial cognition during development (Pine et al., 2002), and (3) evidence of

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Abbreviations: FMPP, familial male precocious puberty; GMV, grey matter volume; IPL, inferior parietal lobule; VBM, Voxel-based morphometry; vMWM, virtual version of the Morris Water Maze.

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a relation between testosterone and spatial navigation (Roof and Havens, 1992), we hypothesised that behavioural performance on the vMWM would correlate with local grey matter volume (GMV) in these structures. Moreover, we expected that males with FMPP would perform better than healthy male volunteers on the virtual maze. To this aim, participants completed a version of the vMWM that has been used previously in our laboratory in adolescent populations (Mueller et al., 2008, 2009b).

EXPERIMENTAL PROCEDURES

Subjects

For the behavioural analyses, 10 boys with FMPP (mean age 11.65 years±SD 2.57, bone age 13.68 years±SD 2.75, range 9.12–15.50 years) and 40 healthy control males (mean age 12.81 years±SD 2.71, range 8.17–17.50 years) completed a vMWM task outside of the MRI environment. Given that FMPP is associated with advanced sexual maturation, patients were matched to controls on bone age, a biomarker of pubertal maturation and exposure to sex steroids, rather than chronological age. In healthy controls, bone age equals the chronological age. However, both groups did not differ on either chronological [$F(1,48)=1.51$, $P=.23$] or bone age [$F(1,48)=0.54$, $P=.47$]. Groups were also well-matched on IQ [FMPP: 109.7±SD 12.84; control: 110.30±SD 21.90, $F(1,48)=.007$, $P=.93$], but differed on pubertal status [FMPP: 4.1±SD 1.2; control: 2.94±SD 1.34, $F(1,40)=5.99$, $P<.05$], as assessed with the Tanner instrument (Marshall and Tanner, 1970).

For the MRI analyses, high-resolution structural MRI-scans were available for all FMPP patients and a subset of 21 male participants from the control group (mean age 13.52 years SD±2.85, range 8.42–17.50 years, IQ: 114.8±SD 16.2, pubertal status: 3.1±SD 1.5). Here, both groups did not differ on bone age [$F(1,29)=0.00$, $P=.99$] or IQ [$F(1,29)=0.76$, $P=.38$] but chronological age [$F(1,29)=3.10$, $P=.09$] and pubertal status [$F(1,26)=3.52$, $P=.07$] were trending toward significance. Subjects were matched on handedness as measured by the handedness questionnaire (Oldfield, 1971): of the FMPP, one subject was left-handed and one was ambidextrous. Of the controls, two subjects were left-handed. FMPP patients were recruited from an ongoing study of the Eunice Kennedy Shriver National Institute of Child Health and Human Development at the National Institutes of Health. Control subjects were recruited by advertisement in local newspapers. The Institutional Review Board of the NIMH approved the study. Parents signed consent forms and adolescents signed assent forms after being explained the study in detail.

All subjects completed a physical, neurological and psychiatric assessment. Psychiatric status was assessed via a standardized, structured psychiatric interview: the Kiddie Schedule for Affective Disorders and Schizophrenia – Present and Lifetime version (K-SADS-PL) (Kaufman et al., 1997). Three children with FMPP met criteria for current ADHD, two of whom were receiving medication (Strattera). All other children were free of psychiatric disorders. The Full-Scale IQ scores were prorated based on the Vocabulary and Block Design subtests of the Wechsler Intelligence Scales for Children (Wechsler, 1999).

Materials

A virtual version of the Morris Water Maze was used (NeuroInvestigations Inc., http://www.neuroinvestigations.com). It consisted of the display of a square room containing a circular pool of water. Four equally sized abstract rectangular paintings that were distinguishable by their shapes, colours and placements on the walls surrounding the pool, served as navigational cues to aid orientation. Each of these cues was placed on a different wall of the room and stretched from the ceiling to the pool wall. Participants navigated in the pool from a first-person perspective and moved around using the 'up', 'left' and 'right' arrow cursor keys of the keyboard. They were told they could not back up, and the 'back' arrow key was disabled. Instead, they were informed that if they wanted to turn around, they had to spin on their left or right axis for 180° using the left or right arrow keys. Following suggestions of previous authors (Skelton et al., 2000), the reason for this procedure was twofold: (1) to more adequately mirror the natural movement/swim pattern of rats in the Morris Water Maze and (2) to discourage participants from performing grid-like search patterns or focusing on a single cue. Participants completed the experiment on a laptop with a 17-inch monitor in a windowless room of the pediatric clinic of the Clinical Center at the NIH. The experiment was completed in one session without breaks and lasted about 15 min. The task consisted of 18 trials, including two initial practice trials and 16 experimental trials. On the first practice trial, participants had 30 s to explore the room and to learn to navigate comfortably in this environment. No platform was present during this first trial. The platform was introduced to the participants on the second practice trial. For this trial, participants were asked to simply 'swim' toward the visible platform. Over the next 16 experimental trials, the platform was always fixed in the same location but hidden. Participants were dropped randomly across trials at four locations on the side of the pool wall. For each trial, the task consisted of 'swimming' directly to the hidden platform. Once participants successfully reached the platform, a sound occurred. Participants remained on the platform for 2 s before the onset of the next trial. On each trial, participants were given 60 s to find the platform, after which the platform became visible and a written message appeared on the screen indicating the visibility of the platform and encouraging participants to move toward it.

Analysis

To assess behavioural performance we used two strategies: the first strategy assessed behavioural performance on standard measures in the water maze including (1) overall accuracy, that is, number of failed attempts, in which the latency exceeded 60 s; (2) heading error (in deg), that is, the angle between optimal heading direction and participant's heading direction sampled at the first instance when the subject's distance is greater than 25% of the pool diameter from the start position; (3) platform latency, that is, the time (s) spent to reach the platform; (4) path length, that is, the distance (relative to the pool diameter in arbitrary units) covered to reach the platform; (5) first-move latency (sec), an indicator of the length of time subjects remained at the wall edge at the beginning of a trial before they started moving toward the platform. Previous studies have implicated this variable in cognitive strategy to solve the task (Mueller et al., 2009b). For the analyses of these variables, the 16 experimental trials were binned into four blocks of four trials each, that is, block 1: trials 1–4, block 2: trials 5–8, block 3: trials 9–12 and block 4: trials 13–16. A repeated-measures ANOVA was then conducted for these performance parameters using a Block (1–4) by Group (FMPP vs. control) design. The second strategy served to obtain a more precise summary variable of learning abilities across the experiment and overall improvement in time to reach the platform (sec). We estimated learning abilities for each group by biasing the first and last blocks that reflect strongest and weakest periods of learning using the following formula: (2×mean block 1)+(1×mean block 2)+(−1×mean block 3)+(−2×mean block 4). A higher score on this variable would indicate a larger improvement in learning across the experiment. Given that learning patterns are presented as a single variable that summarises performance across blocks 1–4, potential group differences on this variable were calculated by using a multivariate ANOVA.

MRI data acquisition

A whole brain, high-resolution T1-weighted anatomical image was acquired on a 3 Tesla General Electric Signa Scanner (Waukesha, WI, USA). Head movement was restricted using foam padding. Due to reasons unrelated to this study, MRI acquisition sequences were switched during the study. A first set of subjects (n=21; 4 FMPP) were scanned using an MPRAGE sequence consisting of 124 1.2 mm axial slices (no-gap), FOV=220 mm, matrix=256×192, time to inversion (TI)=725 ms, flip angle 6 deg, and a voxel size of 0.86 mm×1.15 mm×1 mm. A second set of subjects (n=10; 6 FMPP) were scanned using an FSPGR imaging sequence consisting of 124 1.2 mm axial slices (no-gap), FOV=240 mm, matrix=256×256, flip angle 15 deg and a voxel size 0.94 mm×0.94 mm×1.2 mm. Finally, because of differences in MRI scan sequence acquisition (Fisher's exact test, P=.04), we evaluated the potential impact by close visual inspection of the scatterplots but could not find any systematic variation as a function of the MRI sequence, suggesting that extraction of voxelwise GMV was similar across both acquisitions. These data are not further discussed.

MRI processing

Voxel-based morphometry (VBM) was performed with SPM8 software (Wellcome Department of Imaging Neuroscience, University College of London, London, UK), Matlab 7 (The Mathworks Inc., Natick, MA, USA) and the DARTEL toolbox for VBM analysis as implemented in its standard version in SPM8. Several sequential processing steps were performed. (1) To enable preprocessing of the individual raw structural MRI data, it was first displayed and examined for scanner artefacts and gross anatomical abnormalities. Subsequently, the reference point for all images (image origin) was set at the anterior commissure (AC) for each subject. (2) Then, during the segmentation step, the data were partitioned into white matter, grey matter and CSF tissue classes. The resulting segmentation was assessed and confirmed by visual inspection. (3) A study-specific brain template was created from all individual grey and white matter images of each subject. This process included iterative alignments of the tissue-class images obtained from step (2) to the template. (4) Data were normalised to Montreal Neurologic Institute (MNI) space using an isotropic voxel size of 1.5 mm×1.5 mm×1.5 mm. (5) Subsequently, the data were modulated by using Jacobian-transformed tissue probability-maps and smoothed with a 10 mm isotropic Gaussian kernel (FWHM). The modulation step was utilized to obtain 'volume' differences rather than 'concentration' differences in grey matter (Ashburner and Friston, 2000). Although both groups did not differ significantly in total brain volume [$t(29)=-1.60$, ns], to avoid potential influence of this variable on the findings, total brain volume was modelled as a covariate in all MRI analyses. Although each performance variable measures a slightly different aspect of navigational abilities, these (latency to complete the trial, heading error, path length) were all highly correlated with one another (all $r^2(31)>.54$, $P\leq.002$). Therefore, to avoid intercorrelation effects of these variables in the design matrix of the MRI data, GMV correlations were computed for each performance parameter separately.

VBM analysis

Our analysis strategy was based on the idea that sex hormones and speeded development in FMPP would lead to differences in the correlations of spatial cognition with regional grey matter volume. A previous fMRI study of spatial navigation had reported sex differences in several frontal and parietal regions (BAs 6–9, 19, 30, 46) (Grön et al., 2000). In addition, developmental studies of virtual maze navigation have revealed parts of the temporal (BA 22) and parietal (BA 40) cortex to be sensitive to age differences (Pine et al., 2002). Based on these published works, we created a joint ROI mask of all of these regions (size=59,766 voxels) using the template available in the WFU Pickatlas and the Brodmann Area labelling/selection function (Tzourio-Mazoyer et al., 2002; Maldjian et al., 2003, 2004). A note of caution is required with regards to the use of Brodmann areas in structural imaging. Given the anatomical nature of our study, changes that are reported in BAs should be regarded as tentative. Yet, given widespread use of this classification system in previous relevant functional imaging work, this nomenclature seems appropriate to demonstrate a link between cognitive abilities and morphological changes in specific regions. With regards to statistical analyses, in order to balance statistical rigour with a small and valuable patient sample, we applied a Small Volume Correction (S.V.C.) for the complete ROI mask at $P_{uncorrected}<.001$ and report all significant coordinates that survived a Family Wise Error (FWE) correction for multiple comparisons of $P<.1$. Due to potential problems of non-stationarity in VBM analyses, significant P-values of individual peak voxels are reported rather than cluster-size P-values (Ashburner and Friston, 2000). Reported coordinates are world coordinates in mm in MNI space [x,y,z]. Only significant effects of 10 or more contiguous voxels are reported. To calculate the statistically significant differences in correlation coefficients, GMV for each significant voxel was extracted and standardised (z-scored) for each group separately. In addition, performance variables were also standardised by group. Then, both z-scored variables were subjected to an ANCOVA using SPSS with the standardized performance measure as the dependent variable, group as the between subject factor and the standardized individual GMV values as the covariate of interest that explicitly modelled the interaction between group and the covariate.

RESULTS

Behavioural findings

A Group by Block ANOVA was conducted on latency to find the platform, failure to complete trials, path length, heading error and first-move latency. Group had no significant effects on any of the performance variables, independently or in interaction with Block. However, significant learning effects (main effect of Block) in the pooled sample indicated progressively faster latencies to find the platform [$F(3,144)=4.31$, $P<.01$], which was also indicated by a significant linear effect [$F(1,48)=5.69$, $P=.02$]. This was further supported by significant speeding of the first-move latency (main effect of block: $F(3,144)=4.45$, $P<.01$, linear effect: $F(1,48)=4.7$, $P=.04$) and a marginally significant improvement of path length (block: $F(3,144)=2.61$, $P=.054$; linear effect: $F(1,48)=3.65$, $P=.06$) over the course of the task (Table 1). In addition, analyses of the individual learning effects did not differ significantly on any performance variable [all $F(1,48)<0.4$, $P>.55$]. Of note, the performance means between the full sample of controls (n=40) and the subsample (n=21) that was used for the MRI study did not differ substantially from each other and no differences emerged between the subgroup and the FMPP group (Table 1).

To examine the potential confounding effect of the comorbidity with ADHD in three boys with FMPP, we examined the directionality of behavioural performance means of these three patients relative to the rest of the patient group. Prior work in ADHD would suggest a worsening of performance in patients with this co-morbid diag-

Table 1. Means for behavioural performance variables for the FMPP group, the complete control group for the behavioural analysis (n=40) and the control group for the MRI analyses (n=21) split according to block (1–4, each block containing 4 trials each) and overall group means (SD). Learning across the experiment is summarised underneath. Lower score=better performance

	Block	Latency (s)	Path length (unit)	Heading error (deg)	Fail-to-complete	First-move (s)
FMPP (n=10)	1	20.9 (9.8)	1.4 (0.7)	23.7 (7.6)	0.1 (0.3)	4.1 (1.5)
	2	15.8 (9.7)	1.1 (0.7)	21.8 (20.3)	0.1 (0.3)	3.6 (1.3)
	3	16.5 (8.0)	1.2 (0.8)	19.2 (14.1)	0.1 (.03)	3.8 (2.0)
	4	14.5 (7.6)	1.0 (0.5)	15.7 (12.3)	0.0 (0.0)	3.1 (1.5)
	Learning	12.1 (18.8)	0.5 (1.9)	18.6 (27.4)	0.2 (0.8)	1.7 (3.1)
Control (n=40)	1	25.3 (12.5)	1.8 (1.1)	25.2 (15.8)	0.4 (0.8)	4.4 (2.1)
	2	18.5 (10.1)	1.3 (0.9)	22.7 (15.7)	0.1 (0.3)	3.2 (1.5)
	3	19.8 (14.1)	1.4 (1.0)	20.9 (14.5)	0.2 (0.5)	3.4 (1.8)
	4	18.0 (11.0)	1.2 (0.7)	20.0 (13.2)	0.1 (0.3)	3.6 (1.7)
	Learning	13.4 (35.9)	1.1 (2.7)	12.2 (39.9)	0.5 (1.9)	1.4 (4.7)
Control (n=21)	1	24.9 (14.3)	1.8 (1.4)	25.9 (19.3)	0.4 (1.0)	4.6 (2.5)
	2	20.0 (12.0)	1.3 (1.0)	21.9 (15.1)	0.2 (0.4)	3.3 (1.8)
	3	17.8 (10.4)	1.2 (0.8)	20.7 (14.7)	0.1 (0.3)	3.5 (2.0)
	4	17.8 (10.0)	1.2 (0.7)	20.1 (14.8)	0.1 (0.2)	3.5 (1.8)
	Learning	16.5 (37.4)	1.1 (3.3)	12.9 (40.5)	0.8 (2.1)	1.9 (3.9)
FMPP (n=10)	Mean	16.9 (7.5)	1.2 (0.5)	20.1 (11.2)	0.1 (0.2)	3.7 (1.4)
Control (n=40)	Mean	20.4 (9.2)	1.4 (0.7)	22.2 (11.3)	0.2 (0.3)	3.7 (1.5)
Control (n=21)	Mean	20.2 (8.9)	1.4 (0.7)	22.1 (13.2)	0.2 (0.3)	3.7 (1.9)

nosis relative to patients without. Although no statistical tests were conducted based on the small sample sizes (n=3 vs. n=7), mean latency to complete the trials was faster for FMPP+ADHD (mean 12.42 s SD 0.63) than for FMPP-ADHD (mean 18.85 s SD 8.36) or controls (mean 20.16 s SD 8.98), suggesting little impact of comorbid ADHD on the current findings.

VBM analysis

Significant group differences. Comparisons of regression coefficients between groups revealed significant differences in three regions. First, a significant difference in correlation coefficients was present between GMV and latency in the medial frontal cortex (BA 6) [4, 5, 52]

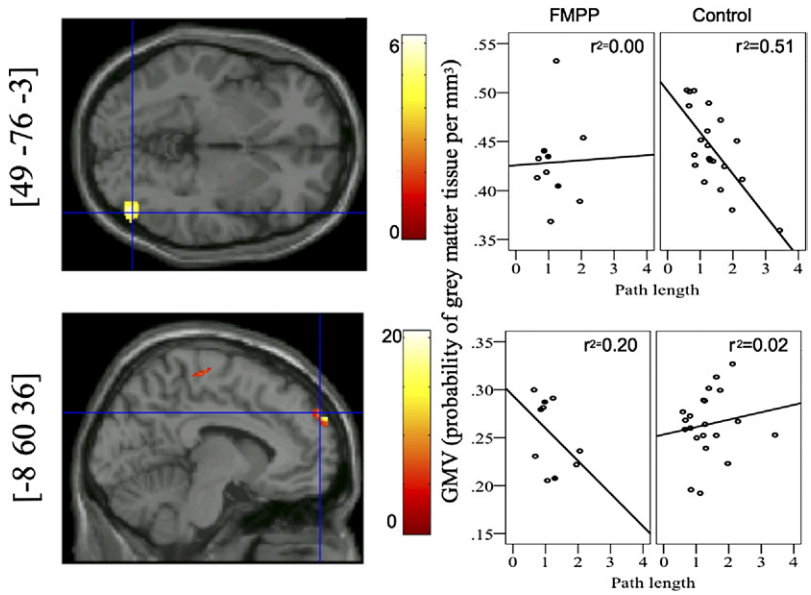

Fig. 1. Upper panel. Structural MRI image on the left illustrating the significant negative correlation of grey matter volume (GMV) in the middle occipital gyrus with path length for the control group. Corresponding scatter plots on the right for both groups with mean regression fit lines for GMV (y-axis) and performance variable (x-axis) for each group. Lower panel. Structural MRI image on the left illustrating the significant negative correlation in the superior frontal gyrus with path length for the FMPP group. Cross-hairs indicate coordinates provided in the axis-label. Corresponding correlation graphs on the right for both groups with mean regression fit lines for GMV (y-axis) and performance variable (x-axis). Images thresholded at $P<.001$, FWE<.10, left=left. Filled circles in the scatterplots of the FMPP group indicate the three participants with co-morbid ADHD. Colour bar indicates corresponding z-value of statistical test. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

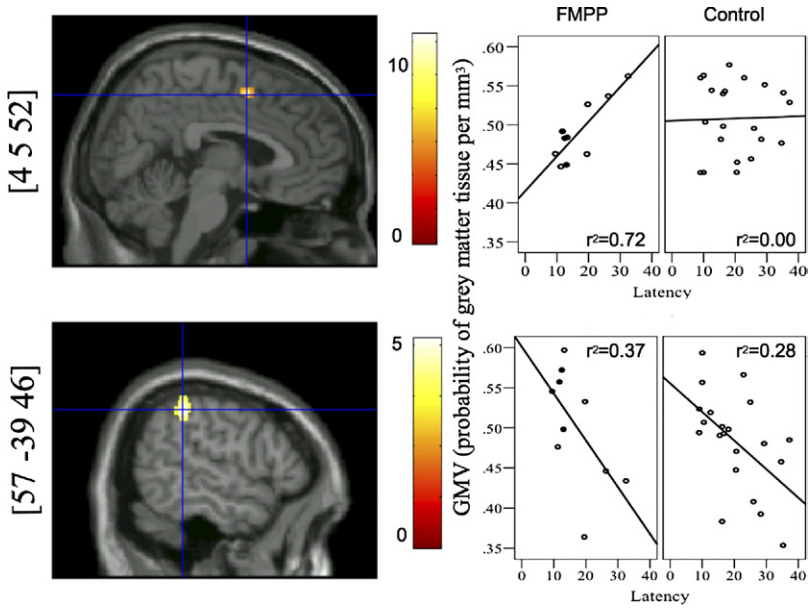

Fig. 2. Upper panel. Structural MRI image on the left illustrating the significant positive correlation of grey matter volume (GMV) in the medial frontal gyrus with latency to complete the trials for the FMPP group. Corresponding scatter plots on the right for both groups with mean regression fit lines for GMV (y-axis) and performance variable (x-axis) for each group. Lower panel. The significant negative correlation in the inferior parietal lobule with path length for FMPP. Cross-hairs indicate coordinates provided in the axis-label. Corresponding correlation graphs on the right for each group with mean regression fit lines for GMV (y-axis) and performance variable (x-axis). Images thresholded at $P<.001$, FWE$<.10$, left=left. Filled circles in the scatterplots of the FMPP group indicate the three participants with co-morbid ADHD. Colour bar indicates corresponding *z*-value of statistical test. For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.

[$F(1,26)=5.08$, $P<.05$] (Fig. 2). Here, GMV was positively associated with latency in FMPP, while no such correlation was apparent in controls. Second, a difference emerged between the right middle occipital gyrus and path length (BA 19) [49, −76, −3] [$F(1,26)=4.64$, $P<.05$] (Fig. 1). This difference showed that controls had a significant improvement in path length with increased GMV in this region, a correlation that did not exist for FMPP. Third, associations of heading error with GMV differed in the cuneus (BA19) [−14, −93, 19] [$F(1,26)=8.30$, $P<.01$]. These findings showed a positive association for FMPP and a negative correlation for controls (Table 2).

FMPP only. When FMPP patients were considered alone, path length and learning across the experiment correlated significantly (negatively) with grey matter volume in the superior frontal gyrus volume (BA 9) [−8, 60, 36] (Fig. 1) and the superior occipital gyrus (BA 19) [31, −93, 19], respectively. These associations indicated that greater GMV was associated with improved performance on these variables.

Table 2. Significant clusters of grey matter volume correlations with performance in the virtual water maze in the region-of-interest including the performance parameter, directionality [positive (+) or negative (−)], the cluster size, the specific region, the hemisphere (L=left, R=right), corresponding Brodmann area, t and z values and Montreal Neurological Institute peak voxel coordinates, *P*-value after Family Wise Error (FWE) correction ($P<.1$) with a spatial extent of at least 10 contiguous voxels. All peak voxel coordinates more than 4 mm apart are provided

	Cluster	Region	Side	BA	t	Z	x	y	z	FWE *P*
Group similarities (FMPP + controls)										
Latency	247	IPL	R	40	5.10	4.25	57	−39	46	.04
Path length	333	IPL	R	40	5.14	4.28	57	−39	46	.04
FMPP only										
Path length	68	SFG	L	9	11.00	4.39	−8	60	36	.06
Learning-	61	SOG	R	19	14.27	4.76	31	−93	19	.02
Control only										
Group differences (FMPP−controls)										
Latency	48	medFG	R	6	11.74	4.48	4	5	52	.04
Path length	26	MOG	R	19	5.91	4.35	49	−76	−3	.03
Heading error	29	Cuneus	L	19	10.65	4.34	−14	−93	19	.07

IPL, inferior parietal lobule; SFG, superior frontal gyrus; SOG, superior occipital gyrus; medFG, medial frontal gyrus; MOG, middle occipital gyrus.

Controls only. When controls were considered alone, no significant associations emerged.

Group similarities. When both groups were combined, greater GMV in the inferior parietal lobule (BA40) [57, −39, 46] (Fig. 2) was associated with improved performance on both latency to find the platform and path length.

Additional analyses. In order to confirm the robustness of our findings of between-group differences, we conducted additional analyses in which participants from either group were randomly assigned to one of two groups (group 1, $n=16$; group 2, $n=15$), which were then compared against one another for the selected ROIs. Patients were evenly divided among the new randomised groups ($n=5$ per group) and random assignment was repeated three times. No significant findings emerged for any of the three group comparisons for any performance variable (randomisation 1: *P*-values between .14–.42; randomisation 2: *P*-values between .12–.89, randomisation 3: *P*-values between .27–.91).

DISCUSSION

We hypothesised that adolescent youth exposed to excess androgen during childhood would show significant correlations between GMV of brain regions sensitive to age and sex differences and behavioural performance on a virtual maze. Moreover, we expected these correlations to differ from those of unaffected controls. As predicted, groups differed in correlations in regions of the prefrontal cortex and the occipital cortex. By contrast, similarities in associations were present in the parietal lobe. Finally, evidence for group differences in behavioural performance was not found.

Against expectations of improved performance in patients, FMPP boys did not differ significantly from controls on behavioural variables. A ceiling effect, that is, near optimal performance levels for both groups on this task, might have prevented the detection of significant group differences. Yet, these findings are consistent with reports of a stronger influence of testosterone levels on spatial navigation in girls than in boys, in both healthy volunteers (Burkitt et al., 2007) and in patients with CAH (Mueller et al., 2008). Alternatively, it is conceivable that no significant differences could be detected due to the relatively small sample size and subsequent reduction in statistical power.

In contrast to the behavioural results, significant group differences emerged for the regression slopes between regional GMV and performance. Significant differences between groups emerged in two areas of the occipital lobe (BA19), the cuneus and middle occipital gyrus, as well as the medial frontal gyrus (medFG, BA6). Middle occipital gyrus volume was negatively correlated with path length in controls but not patients, indicating a shorter path length to the platform with greater GMV. FMRI studies of spatial navigation frequently report involvement of BA19 in navigational processes in healthy participants (e.g. Grön et al., 2000; Moffat et al., 2006; Cornwell et al., 2008) corroborated by neuropsychological evidence, which indicates navigational deficits in patients with occipital lobe lesions (Barrash et al., 2000). Moffat et al. (2006) for instance showed that activity in this region correlated significantly with navigational accuracy in their version of the virtual maze and significantly activated BA19 more in younger (mean age 27 years) as compared with older (mean age 68 years) adults. Our findings suggest that early androgen excess moderates the role of extrastriate cortex in spatial navigation.

In comparison with the findings in the occipital lobe, associations between GMV and performance in the prefrontal cortex were stronger for patients than controls. Here, medial prefrontal cortical GMV was more strongly associated with performance in FMPP but not controls. However, this association differed between two regions of the PFC. In the supplementary motor area (SMA, BA6) greater GMV was paradoxically associated with worse latency, while greater volume in the medial portion of the superior frontal gyrus (BA9) was associated with shorter path length. Such data are consistent with prior work that suggests different roles of the parts of the PFC in spatial navigation. For example, the SMA and preSMA have been associated with learning of associations between visual cues and required responses (Nachev et al., 2008) and have been more active in simply following established paths rather than having to utilise spatial knowledge to create a new route (Hartley et al., 2003). Consistent with prior data that has suggested sensitivity of this region to age (Moffat et al., 2006) and sex (Grön et al., 2000), our findings suggest that excess androgen may negatively moderate the brain regions involved in learning movement sequences within a spatial context. By contrast, GMV of the medial portion of the superior frontal gyrus was associated with shorter path length to the platform in FMPP but not controls. This region has been reported to be more active during novel way-finding relative to route-following (Hartley et al., 2003) and during goal-directed navigation relative to sensorimotor control (Cornwell et al., 2008). These data would suggest an active role in the creation of novel routes. Taken together, our findings might suggest that excess androgen moderates specific associations of GMV with spatial skills including structures involved in novel way-finding, visual processing and learning of visual cue-response associations.

By comparison, when both groups were examined together, greater GMV in the inferior parietal lobule (IPL) was associated with significantly improved latency and path length. The right inferior parietal cortex is involved in the coding of spatial relationships (Jeannerod and Jacob, 2005) supported by neuropsychological evidence that has reported impairments on virtual mazes in patients with parietal cortex lesions (Weniger et al., 2009). Developmental studies have documented a relationship between age and allocentric memory in this region (Pine et al., 2002) consistent with increased activations in right IPL in younger relative to older adults during virtual maze navigation (Moffat et al., 2006). Recently, the IPL has been implicated in the calculation of heading vectors in a visually

sparse water maze task (Rodriguez, 2010). These data suggest that larger IPL volume may facilitate computations for successful navigation during development. The data also indicate that computations achieved in the IPL may be insensitive to fluctuations in androgen levels.

Three patients of the present sample suffered from co-morbid ADHD, and a potential impact of this comorbidity on the findings deserves discussion. Indeed, a behavioural study has reported impairments in visuo-spatial abilities in adolescent ADHD (Gitten et al., 2006). Although FMPP patients exhibit an estimated 44% comorbidity with ADHD (Mueller et al., 2010), it is unlikely that it impacted the current results. First, despite an absence of behavioural differences between patients and controls, latencies to find the platform were faster for FMPP with co-morbid ADHD than for patients without co-morbid ADHD, making an impairment in spatial abilities unlikely. Second, scatterplots of associations between GMV and performance did not reveal any systematic distribution as a function of ADHD comorbidity, suggesting little or no impact of co-morbid psychopathology on the present results.

Although tentative, these data offer several compelling hypotheses that could be addressed in experimental animals to help validate the effects of androgen excess on the neurocircuitry subserving spatial cognition. First, while some studies have shown that chronic administration of testosterone in healthy animals leads to a decrement in spatial learning (Goudsmit et al., 1990), it remains to be seen whether this effect is accompanied by greater grey matter volume in the regions we observed. Second, animal models could help delineating the precise developmental contribution of these structures to spatial navigation. Third, only animal models could permit to examine the degree to which these developmentally specific regions are vulnerable to hormonal manipulation during sensitive periods (e.g. Romeo, 2003; Schulz et al., 2009) and distinct cognitive processes such as spatial navigation (Galea et al., 1994). Addressing these issues in future studies would aid in the understanding of the role of sex steroids in spatial navigation and brain development.

Finally, some strengths and limitations of the current study need to be addressed. Most importantly, the small sample size of the FMPP group limited the statistical power of this study. Accordingly, any negative findings must be viewed with caution. Prior work on extremely rare conditions suggests the importance of using so-called “experiments of nature” to generate novel insights in areas where research using other methods are not ethically justified. In-line with this prior work, our sample of FMPP provides a similar unique opportunity to examine a selective disturbance in sex steroid hormone metabolism arising early in development. Although limited, this sample represents the recruitment of patients among 1.5 million adolescents based on the FMPP estimated prevalence-rate of $<$9/1 million (Orphanet). To our knowledge, this is the largest study to examine cognitive abilities in FMPP compared with the previous case report studies. Unfortunately, this study cannot discriminate between the prenatal effects of testosterone and the ongoing impact during postnatal development. However, the present study sought to provide evidence suggesting a potential role of androgens on the anatomical network subserving spatial navigation. Another limitation that deserves mention is the lack of a probe trial in the current study. Previous studies in our laboratory have suggested that a substantial number of human subjects quickly form the belief on probe trials that the hidden platform has indeed been removed (or moved elsewhere). In such a situation, it is no longer the case that performance metrics are valid indices of spatial memory. Accordingly, we provided a more streamlined procedure to participants. However, given that no differences were found on the other trials, a probe trial may have been useful. A major strength of this study is the ability to examine within-sex factors contributing to spatial abilities in an all-male sample, reducing interpretational limitations of between-sex comparisons.

In sum, this is the first study, to our knowledge, to examine spatial navigation in male youth exposed to androgen excess during development. Striking changes in correlations between performance on a virtual water maze task and GMV were found in several brain regions, most notably in the prefrontal and occipital cortex. This study extends previous functional MRI work by providing information on morphological changes associated with androgen perturbation related to spatial cognition. These novel data spur hypotheses that can be tested in future research in animals.

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