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Testosterone levels correspond with increased ventral striatum activation in response to monetary rewards in adolescents

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ABSTRACT

Risk taking is an integral part of learning and development, particularly during adolescence the prevalence of risky behaviors peak. It is hypothesized that the tendency to take risks is related to pubertal maturation, where there is interplay between gonadal hormones, the neural mechanisms that underlie affective (e.g., reward) processing, and risky behavior. To test this hypothesis, fifty healthy adolescents (aged 10–16 years; 33 girls, 17 boys) at different stages of puberty performed a gambling task while lying in the MRI scanner, and provided saliva samples for hormone assessment. Gonadal hormone levels were correlated with the neural response to receiving a monetary reward. Results showed that testosterone level correlated positively with activation in the striatum for both boys and girls, suggesting that individual differences in hormones at puberty are related to the way adolescents respond to reward, which can ultimately affect risk-taking behavior.

1. Introduction

The onset of adolescence heralds a period of vulnerability—a time in development when natural tendencies to explore and take risks leads to a sharp increase in risky behaviors with a myriad of negative health consequences (Institute of Medicine & National Research Council, 2011). Yet, it is equally important to recognize that most youth navigate this developmental period quite well, and that a great deal of the exploration and risk-taking that occurs in adolescence is normative and can contribute to learning, discovery and positive development.

For these reasons there is growing interest in understanding at a deeper, more mechanistic level, normative developmental processes that underpin some of these maturational changes and may provide insights into the risks and vulnerabilities during adolescence. There has been particular interest in sensation seeking which appears to increase in association with pubertal maturation (Steinberg, 2008; Forbes and Dahl, 2010). Sensation seeking is regarded as a personality trait that is related to risk-taking behavior (Llewellyn, 2008). Sensation seeking not only peaks in adolescence, but also girls reach their peak at a younger age than boys (Romer and Hennessy, 2007), possibly due to sex differences in pubertal maturation. One study that replicated this developmental peak in risky behavior in an experimental setting showed that the preference for risk taking peaks at around age 14 (Burnett et al., 2010).

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1.1. Developmental peak in reward sensitivity

The focus of a second line of research is on developmental changes in reward processing in adolescence, often assessed using risk-taking paradigms, and thought to be associated with risk-taking behavior (e.g., Van Leijenhorst et al., 2008, 2010a). The adult literature using such paradigms has shown that the striatum is sensitive to (monetary) rewards (Breiter et al., 2001; Delgado, 2007; McClure et al., 2004). Developmental studies have shown that in response to rewards, adolescents recruit similar brain regions (including the striatum) as children and adults. However, the extent to which these brain regions are recruited differs across age groups (Geier and Luna, 2009). Based on contradicting results in the field of developmental neuroimaging, two opposing models have been proposed to describe the nature of reward processing in typically developing adolescents; one model proposes that adolescents recruit reward-related brain regions, such as the striatum, to a lesser extent than children and adults (Bjork et al., 2004), the other model proposes that adolescents recruit these brain regions to a greater extent (Ernst et al., 2005; Galvan et al., 2006; Geier et al., 2010; Van Leijenhorst et al., 2010a,b). However, the convergence of evidence appears to support the model that specifically at the moment of receiving a reward, the striatum response is stronger in adolescents compared to children and adults (Galvan, 2010), suggesting that the adolescent inclination to take risks might be associated with increased sensitivity to rewards, as indicated by an adolescent-specific peak in activation of the striatum.

1.2. Pubertal maturation, gonadal hormones, and reward processing

According to Nelson et al. (2005) changes in affective processing during adolescence (e.g., reward processing and reorientation to peer social stimuli) may be associated with the increase of gonadal hormones at puberty that influence neural processing in the limbic brain regions, such as the striatum (see SIPN model: Nelson et al., 2005). This model suggests that changes in gonadal hormone levels (or different levels of puberty) are associated with changes in the magnitude and/or extent of the response to reward, specifically in the striatum. Thus, heightened sensitivity to rewards in adolescents could be related to structural and neurochemical changes that are unique to the adolescent brain. However, the exact nature of these changes, the relation with gonadal hormones, and how they affect motivational behavior in adolescents is not yet well understood (Doremus-Fitzwater et al., 2010). Therefore, our goal was to directly test the relationship between gonadal hormone concentrations and activity in the striatum in response to reward outcomes in adolescents across different stages of puberty.

Previous studies have shown in adults that the exogenous administration of testosterone increases the likelihood of disadvantageous or risky decision-making. More specifically, when performing the Iowa gambling task, higher testosterone levels lead participants to choose more often from card decks that resulted in large (as opposed to moderate) monetary rewards, despite a net monetary loss. This was interpreted as testosterone contributing to a shift to less punishment sensitivity and relatively greater reward sensitivity (Van Honk et al., 2004). Another study in adults that administered testosterone and focused on neural processing during reward anticipation showed that higher testosterone levels resulted in increased striatal activity (Hermans et al., 2010). Similar results were found in adolescents at different pubertal stages; the natural occurrence of higher testosterone levels corresponded with increased striatal activity during reward anticipation, but with decreased striatal activity during reward outcome processing (Forbes et al., 2010), suggesting that the relation between testosterone and striatal activity differs depending on the phase of risky decision making. Few studies have investigated the relation between estradiol, a pubertal hormone that is indicative of pubertal development in girls, and reward processing. However, it has been found that reward processing changes with menstrual cycle phase (Dreher et al., 2007).

1.3. Present study

In this fMRI study, we investigated the relation between reward processing and gonadal hormones in adolescent boys and girls. We used the Jackpot gambling task, in which participants could actively choose whether to take a (low or high) risk or not (i.e., skip the trial), and when they chose to take the risk participants received feedback indicating whether they had won or lost (10 Eurocents). This task design has several advantages above passive gambling paradigms, as reward-related activity in the striatum is modulated by perceived control (Rao et al., 2008; Zink et al., 2004) and willingness of the participant to take a risk (Tricomi et al., 2004). Based on the previous findings showing that striatum activation peaks in mid-adolescence (e.g., Van Leijenhorst et al., 2010a), and that testosterone is associated with striatal activity during reward processing (Hermans et al., 2010; Forbes et al., 2010), we hypothesized that individual differences in gonadal hormone levels at different stages of puberty correlate with individual differences in reward-related activity in the striatum.

2. Material and methods

2.1. Participants

In this study, 50 healthy, right-handed adolescents participated. All participants were aged between 10 and 16 years, 17 boys (M age = 13.5, SD = 2.3) and 33 girls (M age = 12.9, SD = 1.8). The sample of girls was doubled relative to the boys, because less variation in testosterone levels was expected. Prior to enrollment, participants were screened for psychiatric or neurological conditions, history of head trauma, and history of attention or learning disorders. Parents of the children filled out the Child Behavior Checklist (CBCL; Achenbach, 1991) to screen for psychiatric symptoms. All participants scored below clinical levels on all subscales of the CBCL.

All participants and their parents gave written informed consent, and participants were instructed and prepared for

scanning in a quiet room with a mock scanner, which was used to explain the scanning procedure. The study was approved by the local Medical Ethical Committee.

2.2. Pubertal assessment

Participants were asked to complete two self-report measures of pubertal maturation, as well as to provide saliva samples to test for gonadal hormone levels. The self-report scales were (1) the picture-based interview about puberty (PBIP; Shirtcliff et al., 2009), and (2) the Pubertal Development Scale (PDS; Petersen et al., 1988). The PBIP consists of an interview with a research assistant about changes that happen when you grow up, with the assistance of a script and photographs. After this conversation, the research assistant leaves the room while participants report their assessment of their pubertal stage based on the presented photographs. Scores could range from 1 to 5, where "1" corresponds with no physical signs of puberty, and "5" corresponds with (seemingly) completed physical development. The PDS consists of five questions about physical development, where scores range from 1 (no physical changes) to 4 (development seems complete). Prior research has shown that the reliability of the PDS was high ($\alpha = .77$ for boys, $\alpha = .81$ for girls), and has demonstrated that the self-report data provide similar or even better indices of pubertal maturation than when the assessment was done by a nurse practitioner in the form of a physical examination, possibly because self-assessments are based on more continuous judgments as opposed to a one-visit decision (Shirtcliff et al., 2009).

Saliva was obtained by passive drool (Shirtcliff et al., 2001); each participant was requested to collect six saliva samples across two consecutive days, at fixed times in the evening (at 8, 8:30, and 9 pm). These samples were collected at home, and stored in a fridge or freezer until participants brought them in on the day of the MRI scan. Collected samples were immediately stored in a freezer at the university to prevent deterioration, and after collection was completed all samples were transported to an external institute where they were analyzed. For each participant, saliva was essayed for testosterone, estradiol, and dehydroepiandrosterone (DHEA), a precursor to the gonadal hormones. The mean hormone levels across the three samples that were collected each day correlated highly between the two days for both testosterone ($r = .93$, $p < .001$), estradiol ($r = .86$, $p < .001$), and DHEA ($r = .83$, $p < .001$), indicating that hormone levels were relatively stable across days, and hence a reliable indicator of the participant's basal hormone level. In the current study, the main focus was on testosterone, as this measure is most valid in both boys and girls (Shirtcliff et al., 2000). The self-report measures of pubertal status were used to validate the hormone measures (see also Shirtcliff et al., 2009).

2.3. Experimental task

While lying in the scanner, participants performed the Jackpot Gambling Task, an active gambling task in which participants could choose to take a (small or large) risk (i.e., to play) or not take a risk at all (i.e., to skip or reset the trial). On each trial, a slot machine was presented with two out of three slots showing two similar fruit types (e.g., 2 plums). In a yellow frame presented above the slot machine, three possible outcomes for the third slot were shown. In the low-risk condition, participants had a 66.6% (2/3) chance that the third slot would show a similar fruit type; in the high-risk condition, the chance was 33.3% (1/3). Based on this information, participants could choose to play (i.e., spin) or to skip the trial (i.e., reset). Upon selecting "spin", the outcome could be positive (i.e., monetary reward) or negative (i.e., monetary loss); upon "reset", the outcome was neutral (i.e., no monetary reward/loss; Fig. 1).

Participants were given 2 Euros to play; if participants won, 10 Eurocents were added, and if participants lost, 10 Eurocents were deducted. If participants chose to reset, no money was won or lost. Participants were told that they would be paid according to the final outcome at the end of the experiment.

Each trial started with a fixation cross, which was presented in the middle of the screen. Fixation was followed by the stimulus presentation (3000 ms), during which the participant had to select a choice (spin or reset). After a choice was made (i.e., by button press), feedback was given (reward, loss, or reset) for 2000 ms, before the next trial started (Fig. 1). If no response was given within the specified timeframe, the text "too slow!" was presented. Periods of fixation lasted between 1 and 6 s, jittered in increments of 500 and 1000 ms. In each condition, the choice to spin resulted in positive feedback in 50% of the trials, or negative

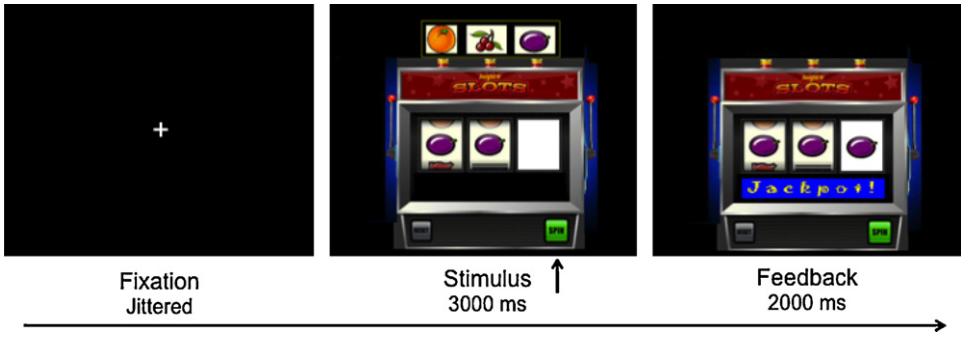

Fig. 1. The Jackpot gambling task. Example of a high-risk trial in which the participant chooses to spin (by a right button press) and wins (i.e., receives a monetary reward).

feedback in 50% of the trials (independent of the presented risk). This was done to have a similar number of observations for reward and loss trials.

2.4. MRI data acquisition

Fifty trials (20 low-risk; 30 high-risk) were presented in total, over the course of one event-related scan that lasted approximately 5 min (1 run). The visual stimuli were projected onto a screen that participants could see via a mirror attached to the head coil. Scanning was performed using a standard whole-head coil on a 3 Tesla Philips scanner. Functional data were acquired using a T2*-weighted gradient-echo echo-planar pulse sequence (38 contiguous 2.75 mm oblique axial slices, using interleaved acquisition, TR = 2.2 s, TE = 30 ms, 2.75 × 2.75 mm in-plane resolution, 140 volumes per run). The first two volumes of each scan were discarded to allow for T1-equilibration effects. High-resolution T2* weighed images and high resolution T1 anatomical images were collected at the end of the scan session. Head motion was restricted due to foam inserts that surrounded the head. Average head movement was .09 mm (SD = .05) for boys and .09 mm (SD = .05) for girls, and there were no significant sex differences in head motion ($p > .05$).

2.5. fMRI preprocessing and statistical analysis

Data preprocessing and analysis was conducted using SPM5 (Wellcome Department of Cognitive Neurology, London). Images were corrected for differences in timing of slice acquisition, followed by rigid body motion correction. Functional volumes were spatially normalized to echo planar imaging templates, respectively. The normalization algorithm used a 12-parameter affine transformation together with a nonlinear transformation involving cosine basis functions. During normalization the data was resampled to 3-mm cubic voxels. Templates were based on the MNI305 stereotaxic space (Cocosco et al., 1997). Functional volumes were smoothed with an 8-mm full-width at half maximum isotropic Gaussian kernel.

Statistical analyses were performed on individual subjects' data using the GLM in SPM5. In the whole-brain analysis, reward and loss outcomes were modeled as single events with zero duration at the onset of the presentation of the outcome. High risk and low risk outcomes were modeled separately, and collapsed in the analysis. Reset trials and trials on which the participant did not respond within the 3-s time frame were modeled separately and were not included in the contrasts because on these trials participants did not receive feedback; they did not win or lose money after they had selected "reset" (i.e., chose *not* to play, or not to take a [low or high] risk), as opposed to when they chose to play, and selected "spin". Only in the latter case did participants receive feedback indicating either monetary gain or loss.

Whole-brain analyses tested the contrast reward > loss which was computed across all participants, and for boys and girls separately. A two-sample *t*-test was performed to examine whether there were sex differences in neural activation to reward > loss.

Because the time-course, physiology, hormones, and component physical changes of pubertal maturation differ markedly for boys and girls (Dorn et al., 2006), all analyses were performed separately for each sex, so that testosterone, estradiol, and DHEA levels were added as regressors to the reward > loss contrast for boys and girls separately. Task-related responses were considered significant if they consisted of at least 10 contiguous voxels that exceeded an uncorrected threshold of $p < .001$, unless otherwise specified.

To further describe patterns of activation, we used the MarsBaR toolbox for use with SPM5 to perform region of interest (ROI) analyses.

3. Results

3.1. Task performance

Performance (i.e., risk taking) was measured as the percentage of spinning trials, and compared across task conditions. As predicted, participants chose to play more often on low-risk trials (mean = 90.4%) than on high-risk trials (mean = 37.3%; $F(1, 49) = 162.95$, $p < .001$). No significant sex differences in choice selection were found; both boys and girls selected "spin" more often in the low-risk (LR) condition compared to the high-risk (HR) condition, and did so to the same extent (boy vs. girl for LR: 91.5% vs. 89.8%, for HR: 31.0% vs. 40.6%), $F(1, 48) = .99$, $p > .05$ (Fig. 2). Three boys and 3 girls never selected "spin" or selected "spin" only once or twice, after which they received only positive (reward) or negative (loss) feedback in the high-risk condition. For these participants the contrast reward > loss could not be calculated for the high-risk condition, and they were thus excluded from the analysis. This resulted in a sample of 14 boys (*M* age = 13.4, SD = .56) and 30 girls (*M* age = 12.9, SD = .38). There was no significant age difference between these groups, $F(1, 42) = .38$, $p > .05$.

3.2. Hormone results

Table 1 shows the average PDS and PBIP puberty scores, and overall mean levels of testosterone, estradiol, and DHEA for boys and girls separately.

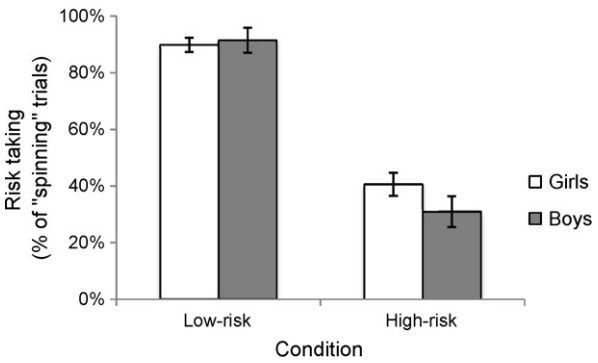

Fig. 2. Risk-taking behavior. Percentage of "spinning" trials in both low-risk (LR) and high-risk (HR) conditions, plotted for boys and girls separately.

Table 1
Puberty measures for boys and girls separately. Upper: Means (sd) for self-report and hormone measures. Lower: Bivariate correlations.

	Boys ($n = 14$)	Girls ($n = 30$)
Puberty measures:		
PDS	2.00 (.92)	2.46 (.81)
PBIP	2.96 (1.47)	3.10 (.97)
Testosterone*	26.11 (27.39)	14.48 (16.10)
Estradiol	4.79 (3.94)	5.30 (3.96)
DHEA	114.65 (69.35)	144.30 (96.93)
Bivariate correlations (Pearson r):		
PDS-PBIP	.886**	.718**
PDS-Testosterone	.786**	.385*
PDS-Estradiol	.801**	.508**
PDS-DHEA	.902**	.426*

* Significant at $p < .05$.
** Significant at $p < .01$.

Average PDS score did not differ significantly between boys (mean = 2.0) and girls (mean = 2.5, $p > .05$), and similarly average PBIP score demonstrated no significant differences between boys (mean = 3.0) and girls (mean = 3.1, $p > .05$; Table 1). Furthermore, because both measures (PDS and PBIP) correlated highly with each other for both boys ($r = .89$, $p < .01$) and girls ($r = .72$, $p < .01$; Table 1), only one measure (i.e., average PDS score) was selected and used for further analyses.

Next, we tested for sex differences in gonadal hormone levels. As predicted, testosterone levels were significantly higher in boys than in girls, $t(42) = 1.77$, $p = .04$. Estradiol levels and DHEA levels did not differ significantly between boys and girls (both p's > .05).

Correlations were computed between PDS scores and salivary hormone levels for boys and girls separately. These correlations were significant for testosterone, estradiol and DHEA (Table 1), indicating that the hormone levels assessed by saliva samples provided a sensitive index of puberty level. Given that testosterone level is the most reliable measure for both boys and girls, and previous studies had shown an impact of testosterone on neural systems of reward anticipation, analyses mainly focused on testosterone for testing for neural correlations in both groups. In addition, because testosterone level did not correlate significantly with PDS scores in girls, estradiol level was used to test for neural correlations in girls only (this measure has previously been found to be non-reliable for boys; Shirtcliff et al., 2009). Together, these relations set the stage for examining neural activation patterns in the Jackpot task, and how this activation is related to gonadal hormone levels.

3.3. Reward processing: main effects

First, we conducted a GLM analysis on the functional data modeled at the onset of the feedback presentation, and computed the voxelwise contrast of reward > loss averaged across high-risk and low-risk trials. The analysis was first performed across all participants, and then for boys and girls separately. The whole-brain analysis including all participants resulted in several areas of activation, particularly in reward-related brain regions including the dorsal and ventral striatum, and the medial orbitofrontal cortex (Fig. 3A). Whole-brain results for boys and girls separately resulted in bilateral activation in the striatum in both groups (Fig. 3B). A two-sample t-test did not result in different levels of activation in boys versus in girls. An overview of significant clusters and corresponding MNI coordinates are reported in supplementary Table S1.

3.4. Hormone level as predictor

A whole-brain regression analysis with testosterone level as predictor on the contrast reward > loss in boys ($n = 14$) showed that boys with higher testosterone levels had more activation in the bilateral ventral striatum (Fig. 4A, left panel). An overview of significant clusters and corresponding MNI coordinates is reported in supplementary Table S2.

A similar whole-brain regression analysis with testosterone level as predictor on the reward > loss contrast was performed for girls ($n = 30$). This analysis did not result in activation at the threshold $p < .001$, but when the threshold was lowered to $p < .005$, activation was observed in the left ventral striatum at a similar location as in boys (Fig. 4A, right panel). An overview of significant clusters and corresponding MNI coordinates are reported in supplementary Table S2. Additionally, results of a whole-brain regression analysis with testosterone level as predictor on the reward > loss contrast including all participants ($n = 44$; 14 boys, 30 girls) also resulted in robust activation in the ventral striatum. These results are reported in supplementary Figure S1.

To further visualize patterns of activation sphere ROIs with a radius of 6 mm were created for boys and girls separately, based on the peak voxel of activation within the striatum that correlated positively with testosterone level in the specific groups (coordinates: $x = -24$, $y = 9$, $z = -9$ [boys]; $x = -12$, $y = 12$, $z = -12$ [girls]), and for both groups together, based on the point of overlap at $p < .005$ (coordinates: $x = -9$, $y = 9$, $z = -9$). As can be seen in Fig. 5, testosterone level predicted the extent of activation in these several areas of the ventral striatum, such that higher levels of testosterone corresponded with increased reward-related activation in both boys and girls.

Next, we chose to select an ROI in the left nucleus accumbens (coordinates, $x = -9, y = 6, z = 12$) that was based on a prior study by Van Leijenhorst et al. (2010a), with a radius of 6 mm. This region was chosen because this prior study also concerned a developmental study on risk taking using the same scanner and processing software, and it provides an ROI based on an independent sample. Similarly to our previous results testosterone level predicted the extent of activation in this area of the ventral striatum. Follow-up tests confirmed the whole-brain analyses and resulted in positive correlations for both groups (boys, $r = .75$, girls, $r = .34$, both p's < .05). To test whether testosterone level, and not age, significantly explained individual differences in reward-related activation in the ventral striatum, we conducted a hierarchical regression analysis predicting striatal activation (i.e., parameter estimates from the independent ROI) based on age and testosterone level. Results of this analysis showed that age as a single predictor did not account for a significant proportion

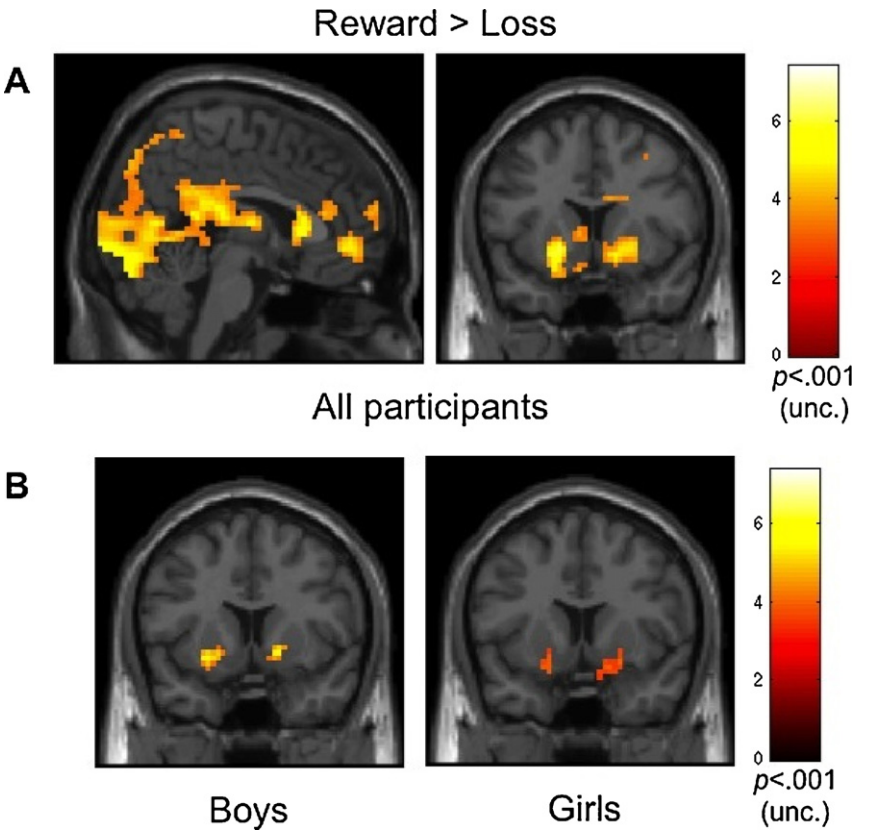

Fig. 3. Whole-brain results for the contrast reward > loss. (A) Regions of activation for all participants included the dorsal and ventral striatum, and the medial orbitofrontal cortex. (B) Regions of activation for boys and girls separately included the bilateral striatum in both groups.

of the variance in activation of the ventral striatum in boys, $R^2 = .26$, $F(1, 12) = 4.18$, $p = .06$, nor in girls, $R^2 = .00$, $F(1, 28) = .00$, $p = .99$. When testosterone level was added to the regression, a significant contribution was made to explaining the variance in reward-related activation for boys, $\Delta R^2 = .31$, $p = .017$, and girls, $\Delta R^2 = .14$, $p = .044$. The model in which striatal activation was predicted by age and testosterone was significant in boys, $F(2, 11) = 5.71$, $p = .01$, and tests of the individual regression coefficients showed that only testosterone level explained a significant proportion of the variance in activation in the ventral striatum, $b = .031$, $t(11) = 2.8$, $p = .017$. In girls, the model including both predictors was not significant, $F(2, 27) = 2.24$, $p = .126$, however, there was a positive relation between testosterone and striatal activation, $b = .044$, $t(27) = 2.12$, $p = .044$. Despite a significant correlation between testosterone and age in both boys, $r = .58$, $p = .015$, and girls, $r = .41$, $p = .012$, there was no multicollinearity, as indicated by the variance inflation factor (i.e., $\sqrt{\text{VIF}} < 2.0$). These results suggest that individual differences in reward-related activation in the ventral striatum can be better explained by testosterone level as opposed to age.

Finally, a whole-brain regression analysis with estradiol level as predictor on the contrast reward > loss was performed in girls ($n = 30$). This analysis did not result in activation at the threshold $p < .001$, but when the threshold was lowered to $p < .005$, activation was found in the dorsal striatum, DLPFC, and medial PFC (Fig. 4B). An overview of significant clusters and corresponding MNI coordinates is reported in supplementary Table S3.

4. Discussion

The goal of this study was to investigate the relation between gonadal hormone levels and reward processing in adolescents. To test this, participants provided saliva samples and performed a simple gambling task while in the MRI scanner. During this task participants chose on each trial whether to take a (low or high) risk, or not (i.e., to skip the trial). When they had chosen to take the risk, participants either received or lost a monetary reward.

4.1. Girls and boys exhibit similar risk taking behavior

As predicted, participants showed increased risk taking on low-risk trials compared to high-risk trials, and this pattern of behavior was similar for boys and girls. In a prior behavioral study in which participants had to select between a response option with low probability of a high reward and high probability of a small reward (i.e., a forced gamble), boys were found to take more risks than girls. In this study, like the current study, the participants also played for small amounts of money (i.e., 10 Eurocents; Van Leijenhorst et al., 2008). Thus, it is unlikely that the absence of sex differences is related to small

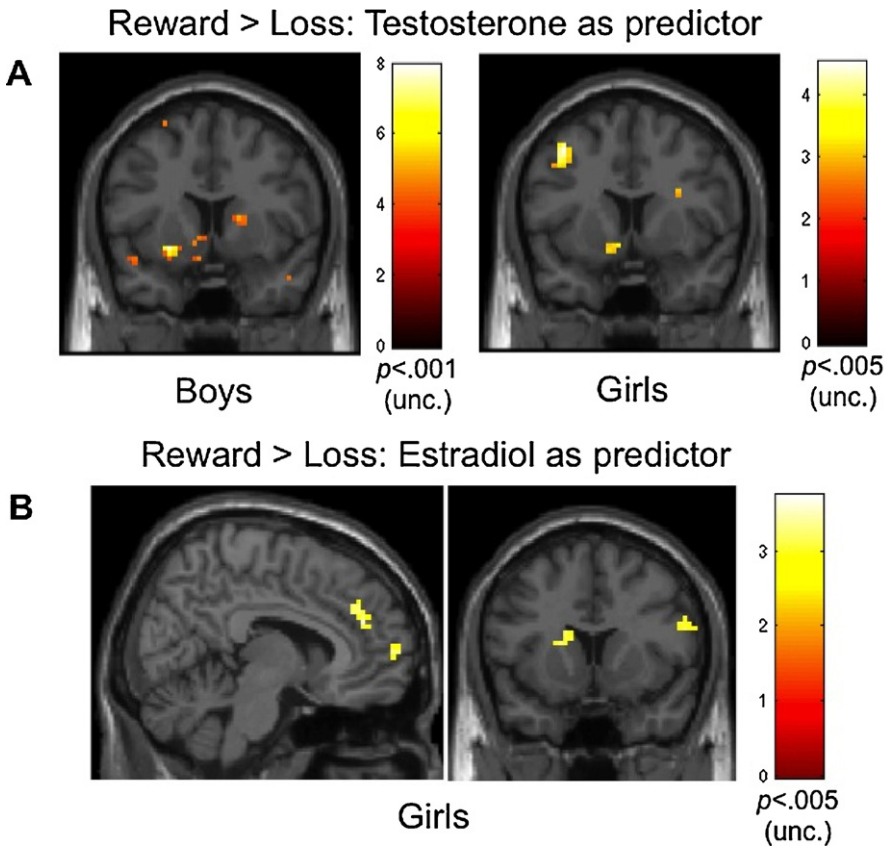

Fig. 4. Results for the regression analyses with gonadal hormones. (A) Regions of activation for reward > loss with testosterone as predictor included the bilateral ventral striatum in boys (left), and left ventral striatum in girls (right), at a threshold of $p < .005$. (B) Regions of activation for reward > loss with estradiol as predictor included dorsal striatum, DLPFC, and medial PFC in girls only, at a threshold of $p < .005$.

rewards per se, but rather, it is likely that the absence of a forced gamble results in different patterns of risk taking.

4.2. Girls and boys recruit similar brain areas in response to monetary reward

When participants chose to take a risk and won (i.e., received a monetary reward), they recruited brain areas including the dorsal and ventral striatum, and the medial orbitofrontal cortex (OFC). These brain areas play a key role in reward processing (Haber and Knutson, 2010). Whereas the ventral striatum has been associated with coding for subjective value of reward (Peters and Büchel, 2010), previous studies have shown that in the context of uncertainty (e.g., gambling task) also the dorsal striatum responds to valence (reward or loss) and magnitude of outcomes, showing strongest activation to large monetary rewards, and weakest activation to large monetary losses (Delgado et al., 2003). The medial OFC specifically responds to abstract rewards, such as monetary gain (Kringelbach and Rolls, 2004).

Adolescents showed no sex differences in reward processing; boys and girls displayed similar bilateral activation of the striatum. The absence of sex differences could be because divergence of the sexes in reward processing arises later in development, possibly influenced by puberty-related changes (Sisk and Zehr, 2005; Schulz et al., 2009). However, in this study we did not have enough power (i.e., observations per age group) to test this age by sex interaction. Most importantly, the task elicited strong and robust activation in the ventral striatum in both boys and girls, which sets the stage for the examination of hormone effects.

4.3. Gonadal hormone levels correspond with stronger reward-related activation

Results showed that testosterone levels were positively associated with activation in the ventral striatum in response to a monetary reward. Specifically, in the nucleus accumbens it was found that both in boys and girls higher testosterone levels predicted more reward-related activation. In girls this relation was only found at a less stringent threshold but was statistically confirmed using an independent sphere ROI analysis. These findings are in line with Nelson's social information processing network (SIPN) model, which predicts that affective changes (e.g., changes in reward processing) are associated with changes in limbic brain regions, such as the nucleus accumbens, that are specifically influenced by gonadal hormones (Nelson et al., 2005). Furthermore, neuroanatomical studies have shown

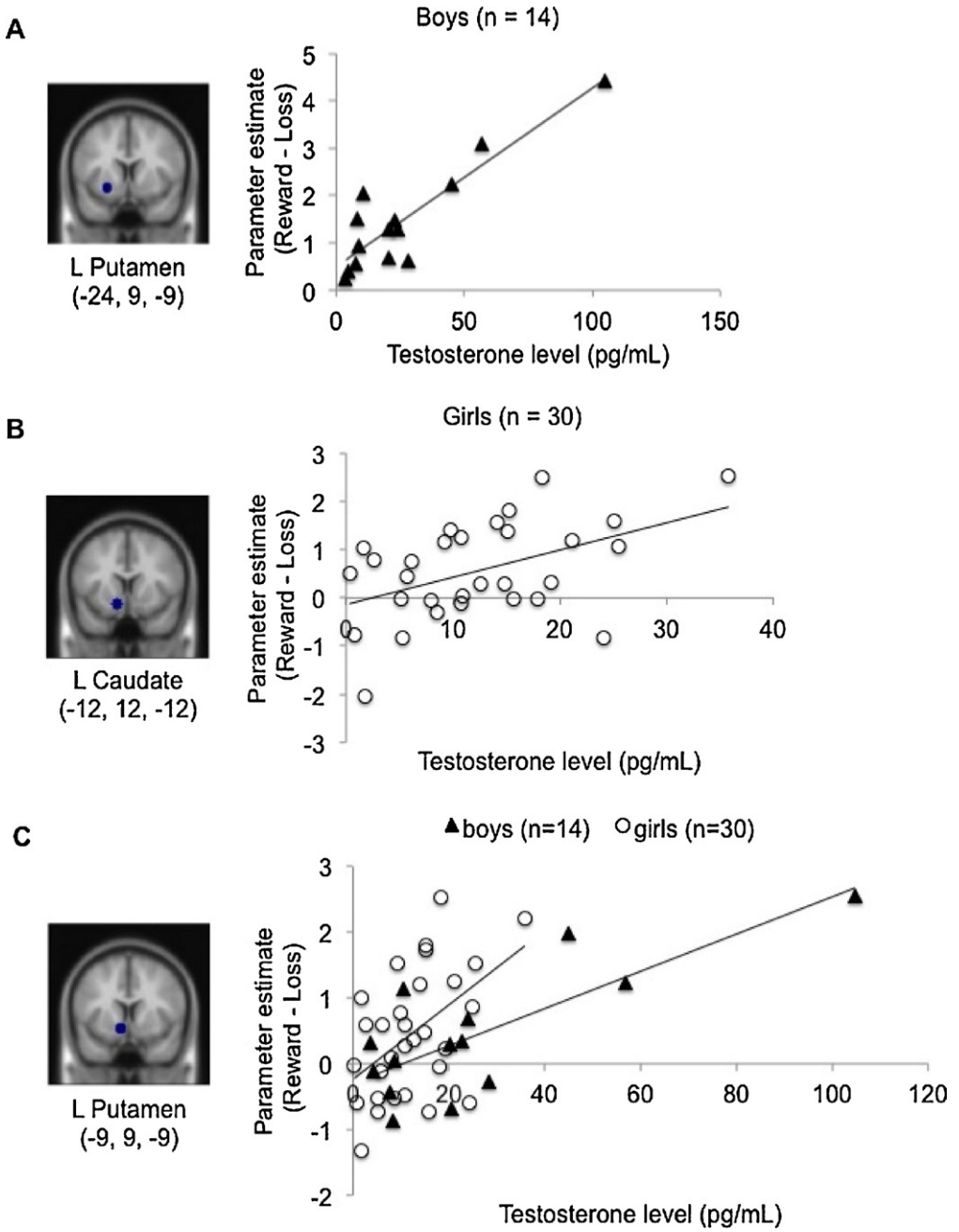

Fig. 5. Results of sphere ROIs (radius 6 mm) based on the peak voxel of reward-related activation that correlates positively with testosterone level for boys in (A) left putamen, for girls in (B) left caudate, and for boys and girls (i.e., overlap in activation) in (C) left putamen.

that gonadal hormones at puberty are associated with changes in both gray and white matter, with testosterone and estradiol showing differential effects in adolescent boys and girls (Peper et al., 2011). These findings suggest that both functional and structural changes in the brain are associated with individual differences in gonadal hormone levels at puberty.

These findings are also in line with previous literature showing that competition is associated with increased testosterone levels, and more importantly, winning as opposed to losing a monetary reward during a hypothetical competition is associated with a higher increase of testosterone levels (Archer, 2006). Furthermore, high basal levels of testosterone are associated with neurochemical and behavioral changes in response to winning as opposed to losing, whereas low basal levels of testosterone are not (Mehta et al., 2008), strengthening our conclusion that individual differences in testosterone levels at puberty may explain individual differences in the neural response to reward versus loss.

A previous study that also examined the relation between gonadal hormones and activation in reward-related brain regions, such as the striatum, resulted in opposite findings; not only did they find that striatal activity decreased with pubertal maturation, but also that testosterone level was negatively correlated with the neural response to reward (Forbes et al., 2010). A possible explanation for this discrepancy is the difference in experimental paradigms; in Forbes et al.'s study a card-guessing game was used in which participants guessed whether the next playing card would be lower or higher than the stimulus card presented. After participants selected a response, and were shown whether the trial was a possible gain or loss trial (anticipation phase), the next card was shown, followed by feedback that indicated whether they had won ($1), lost ($0.50), or nothing happened ($0; outcome phase). The neural response to reward was measured during the outcome phase, and was time-locked to feedback presentation, indicating gain, loss, or nothing. This occurred separate from, and after the outcome was presented (i.e., the next card). In the Jackpot task outcome (i.e., appearance of fruit in the third slot) and feedback (i.e., appearance of blue or red bar indicating gain or loss respectively) were presented simultaneously, and the neural response to reward was time-locked to this "combined" presentation. Thus, the neural response to reward may have represented different phases of reward processing in these two paradigms, possibly explaining the discrepancy in results. For future research it is important to disentangle the different phases of reward processing, as they also involve activation of different brain regions (Rademacher et al., 2010).

The relation between testosterone and reward-related activation was more robust in boys than in girls, possibly due to lower variability in testosterone level in girls than in boys. Results for estradiol, a more reliable measure of pubertal development in girls (Shirtcliff et al., 2009), also showed a positive relation with reward-related activation in the dorsal striatum, DLPFC, and medial PFC, although again at a less stringent threshold. Interestingly, the relation between reward-related activation with estradiol was in a different set of brain regions, namely those associated with cognitive control (Nelson et al., 2005). Indeed previous studies have shown that cognitive performance (e.g., working memory) changes across the menstrual cycle (Jacobs and D'Esposito, 2011), suggesting that fluctuations in levels of estrogen (or estradiol) contribute to changes in prefrontal functioning. Also, estrogen-replacement therapy in postmenopausal women protects against cognitive decline across different domains of cognitive functioning, including attention, memory, and reasoning (Sherwin, 2002). These findings support the likelihood that individual differences in estradiol levels are associated with functional differences in brain regions that are involved in cognitive control. However, it is unclear which aspect of cognitive control is influenced by estrogen, and future research is needed to determine which brain regions are involved, and whether these overlap with the regions reported in this study.

Furthermore, these results should be interpreted with caution, because the analyses did not survive strict corrections for multiple comparisons, but provide interesting hypotheses for future research. A possible explanation for the absence of a robust relation between gonadal hormones and reward-related activation in girls, despite showing similar neural responses to reward compared to boys, might be that girls have less stable hormone levels due to the menstrual cycle, or possible measurement errors which are summarized below.

4.4. Limitations

One limitation of this study calls for cautious interpretation of the findings, namely that age was correlated with puberty score and testosterone level, possibly confounding the relation between testosterone and striatum activation. Reassuringly, hierarchical regression analyses showed that testosterone, not age, was the best predictor for neural activity in boys and girls. However, future studies should disentangle age and pubertal development by using a more narrow age range, matching girls and boys on age and comparing them across different levels of puberty (see also Forbes et al., 2010).

4.5. Future directions

The finding of neural differences in the context of risk taking in adolescents compared to children and adults, or across different stages of puberty is a first step towards understanding how neurodevelopment relates to changes in risk-taking behavior during adolescence. To fully comprehend the association between neural and behavioral changes (i.e., to know *when* neural differences become explicit behaviorally) it is important to note that adolescents make more risky choices for themselves than for others (Crone et al., 2008), that they are especially sensitive to social rewards (Doremus-Fitzwater et al., 2010), and (social) changes in the context of reward. For example, the presence of peers increases risk taking behavior, and the response of the striatum to reward (Chein et al., 2010). For future research, adding social context as factor in the risk-taking paradigm may provide insight into the relation between risk-taking behavior and neural processes.

5. Conclusions

Results of the present study showed that individual differences in gonadal hormone levels at different stages of puberty are positively associated with individual differences in the neural response to monetary reward, suggesting that the drastic rise of gonadal hormone levels at puberty may contribute to increased reward sensitivity (i.e., enhanced striatum response to reward) that is observed in adolescents. Despite that this finding was more robust in boys (for testosterone) than in girls (for testosterone and estradiol), these results provide insight into the underlying mechanism of reward processing, and further our understanding about the role of gonadal hormones in individual neural differences.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.dcn.2011.06.003.

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