SHORT COMMUNICATION

Hair testosterone and visuospatial memory in middle-aged men and women with and without depressive symptoms

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Summary

Background: Testosterone binds to androgen receptors, which can be found abundantly in the hippocampus. Associations between testosterone levels and visuospatial memory have been reported, albeit with inconsistent results. Previous studies have used point sampling of testosterone levels (blood, saliva) rather than long-term secretion measures. Hair analysis for steroids allows for retrospective ascertainment of cumulative steroid measures over several months. We examined hair testosterone and its association with verbal and visuospatial memory in middle-aged men and women with and without major depression.

Methods: We examined a total of 73 middle-aged individuals (35 depressed patients, and 38 age-, sex- and education-matched healthy subjects). We tested verbal (Auditory Verbal Learning Task) and visuospatial (Rey figure) memory and measured testosterone in the hair by liquid chromatography tandem mass spectrometry.

Results: Hair testosterone levels did not differ between patients and controls (mean 1.35 pg/mg vs. 1.40 pg/mg, SD 0.61 and 0.80, respectively). In men (n = 24) but not women (n = 49), hair testosterone was associated with visuospatial memory in a multiple regression analysis after controlling for age, education, body mass index, and depression (adjusted $R^2 = 0.56$).

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1. Introduction

Testosterone is an anabolic steroid hormone and belongs to the androgen group. It is secreted by the testicles in men and ovaries in women, and, in some part, by the adrenal glands. Testosterone exerts its effects in many parts of the body including muscles, bones, hematopoietic system, reproductive and sexual organs, and adipose tissue. In the brain, testosterone binds to androgen receptors, which can be found abundantly in the hippocampus (Beyenburg et al., 2000), a region of the brain closely involved in verbal and visuospatial memory.

Interestingly, studies using cell cultures and in vivo animal models showed that testosterone promotes neuroprotection including dendritic sprouting and increased expression of nerve growth factor in the hippocampus (see Hogervorst et al., 2010 for review). Consequently, studies have investigated associations between testosterone levels and hippocampus-dependent verbal and visuospatial memory, albeit with inconsistent results. Most rodent studies showed that gonadectomy leads to impaired memory performance in hippocampus-dependent tasks with restoration of memory performance through testosterone replacement in male rats (Kritzer et al., 2001; Edinger and Frye, 2004).

However, in humans results are equivocal. In line with rodent models, Moffat and colleagues demonstrated in large prospective studies with healthy elderly males that low baseline plasma testosterone levels were associated with worse performance on measures of visuospatial memory during follow-up (Moffat et al., 2002, 2004). Consistent with these results, Ackermann et al. (2012) demonstrated a role for testosterone in enhancing memory through increased brain activation in the amygdala during encoding. They found that higher salivary testosterone levels at encoding were associated with a greater number of freely recalled neutral pictures in men but not in women. Martin et al’s cross-sectional study results, however, show poorer memory performance with higher testosterone levels in men over fifty years of age (Martin et al., 2007, 2008). In sum, the relationship between testosterone levels and memory function remains somewhat unclear.

There are a number of reasons why human studies on testosterone and its effect on memory may present with controversial results. First, the type of memory process that is being investigated may play an important role (e.g., hippocampus-dependent vs. other brain area-dependent memory functions). Second, the age range of the participants may be important because most studies investigated elderly men (e.g., Moffat et al., 2002, 2004; Martin et al., 2007, 2008). Third, health factors may be important to control for, especially in elderly samples (e.g. body mass index). Finally, so far, all studies have used blood or saliva sampling for ascertainment of testosterone levels. Blood and saliva samples represent point samples reflecting momentary testosterone levels, which undergo a circadian rhythm. Some criticize that salivary testosterone measurements are highly unreliable as cotton collection devices, storage time and temperature may bias testosterone levels (Wirth et al., 2012). Hence, for associations with compromised memory, a cumulative measure of testosterone may be of much greater importance.

Recently, hair analysis for steroids has been introduced to the field of biopsychology. Hair analysis allows for the retrospective ascertainment of cumulative steroid levels over several months (Dettenborn et al., 2012; Stalder and Kirschbaum, 2012; Russell et al., 2012). Here, we present for the first time, hair testosterone levels and their association to cognitive functioning in middle-aged men and women with and without depressive symptoms.

2. Methods

2.1. Subjects

As part of a larger study specifically designed to study hair steroids in depressed and non-depressed individuals, we recruited 35 in- and outpatients (24 women and 11 men, mean age 42.3, SD 11.6) from specialized depression clinics at the Department of Psychiatry and Psychotherapy and the Department of Psychosomatic Medicine, University Medical Center Hamburg (Germany) with a diagnosis of major depressive disorder, single or recurrent according to DSM-IV criteria and a minimum baseline score of 18 points on the Hamilton Rating Scale for Depression, 17-item version (HAM-D-17). Patients were moderately depressed with a mean HAM-D-17 score of 22.0 (SD 4.1) and a mean Beck Depression Inventory (BDI) score of 32.1 (SD 9.2). Whereas 17 patients were free of psychotropic medication, 18 patients were treated with SSRIs (n = 7), SNRI (n = 1), SSNRI (n = 4), Mirtazapin (n = 2), Agomelatine (n = 1), St. Johns Worth (n = 2), and Opipramol (n = 1). Criteria for exclusion were dementia, schizophrenia spectrum disorder, bipolar disorder, substance dependence < 6 months, serious medical conditions, pregnancy, and nursing.

A control group of 38 healthy subjects (25 women, 13 men, mean age 41.5 years, SD 10.5) matched for age, sex, and years of education were enrolled in the study. Subjects were free of former and present DSM-IV axis I disorders according to the MINI-interview, had no physical illness, and had been free of any medication at least 3 months prior to study entry.

The study was approved by the local ethics committee. After complete description of the study to the subjects, written informed consent was obtained.

2.1.1. Hormonal assessment

Hair strands of a total thickness of approximately 3 mm (diameter) were taken from the scalp — near posterior vertex position. Testosterone concentrations were determined from
the first 3-cm hair segments most proximal to the scalp. Based on an average hair growth rate of 1 cm/month, hair segments were assumed to represent the hair grown over the 3-month period prior to hair sampling. Wash and steroid extraction procedures followed the protocol described in (Stalder et al., 2012), involving 10 mg of whole, nonpulverized hair being washed with 2.5 mL isopropanol. Two changes were made to the protocol to allow analysis by liquid chromatography tandem mass spectrometry (LC–MS/MS): methanol incubation was carried out in the presence of 50 μL testosterone-d5 as internal standard and reconstitution following methanol evaporation with 250 mL double-distilled water. Afterwards 200 μL of the medium were injected into a Shimadzu HPLC-MS/MS system (Shimadzu, Canby, OR, USA) coupled to an AB Sciex API 5000 Turbo-ion-spray(R) triple quadrupole tandem mass spectrometer (AB Sciex, Foster City, CA, USA). The system was controlled by AB Sciex Analyst(R) software (version 1.5.1).

2.1.2. Neuropsychological assessment
Auditory verbal learning test (AVLT) (Lezak, 1995): The AVLT is a measure of short-term and long-term verbal memory. The experimenter reads a list of 15 words (list A), which the participant is requested to repeat in loose order. After list A has been presented five times, requesting subsequent immediate reproduction, the subject is asked to reproduce words from a newly presented list (list B). Following this, the subject is instructed to recall the words from list A without renewed presentation. After 30 min, the subject is again asked to repeat the words from list A without renewed presentation.

Rey–Osterrieth complex figure test (RCFT) (Osterrieth, 1944): This test measures visuospatial memory. The participant is first required to copy a complex figure. Immediately thereafter and 20 min later the figure has to be re-drawn from memory without renewed presentation.

2.1.3. Statistical analyses
Demographic characteristics between patient groups and healthy controls (HC) were compared using ANOVA for continuous variables and chi-square tests for dichotomous variables.

Mixed analyses of variance ANOVA were conducted to investigate differences in testosterone values, AVLT and Rey scores between patients and HC and between women and men. Partial correlation analyses and linear regression models for testosterone and cognitive tests were applied. In all analyses, two-sided tests were used and as nominal level of significance, α = 0.05 was accepted.

3. Results
There were no significant differences between groups on demographic variables except BMI (patients mean 27.3 (SD 5.9) vs. HC mean 23.3 (SD 3.4) (p = 0.01)) and BDI (patients 32.1 (SD 9.2) vs. HC 2.0 (SD 2.1) ratings. Only one woman in the patient group and none of the HC used oral contraceptives, which was, therefore, not controlled for. Patients had been depressed for a mean of 231 days (SD 357), 22 patients (63%) had recurrent depression with a mean number of episodes of 3.8 (SD 4.7).

3.1. Hormone concentrations
Univariate ANOVA with testosterone as dependent variable revealed a significant effect of sex (F(1;72) = 11.86, p = 0.001) indicating higher mean testosterone concentrations in men (mean 1.8 pg/mg SD 1.01) compared to women (mean 1.2 pg/mg, SD 0.38), while the main effect of group (patients vs. HC) and the group by sex interaction was not significant. In patients, antidepressant treatment was not associated with testosterone concentrations.

Legend. Pat w AD depressed patients with antidepressant treatment, Pat w/o AD depressed patients without antidepressant treatment, HC healthy control subjects

Figure 1 Association of hair testosterone and memory function in (a) men and (b) women. Pat w AD depressed patients with antidepressant treatment, Pat w/o AD depressed patients without antidepressant treatment, HC healthy control subjects.

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3.2. Memory function

Visuospatial memory function: Age was associated with visuospatial memory function (main effect of age, $F(1;68) = 17.3$, $p < 0.01$) and, therefore, controlled for in consequent analyses. Repeated-measures ANCOVA with Rey copy, and immediate and delayed recall as within-subject factors, and sex and group as between-subject factors revealed no significant differences between patients and HC nor between men and women (all $p > 0.1$).

Verbal memory function: Age was not associated with verbal memory and, therefore, omitted from analysis. Univariate ANOVA revealed an effect of sex at trend level ($F(1;70) = 3.5$, $p = 0.065$) indicating better verbal memory in women than in men. The effect of group was only a trend ($F(1;70) = 2.9$; $p = 0.09$) indicating better verbal memory in HC than in patients. No significant interaction emerged.

3.3. Association of testosterone and memory function

Partial correlation analyses (adjusted for age and depressive symptoms) revealed a significant association between testosterone and visuospatial memory function in men (Rey immediate recall, $r = 0.45$, $p = 0.04$; delayed recall, $r = 0.34$, $p = 0.04$) (see Fig. 1), but not in women. Stepwise linear regression analysis for men and women separately, with age, school years, BMI (step 1), BDI (step 2), and testosterone (step 3) confirmed the association between steroid hormones and visuospatial memory in men, but not in women. No associations between verbal memory and testosterone emerged. The same regression analysis with depression status as group variable (depressed vs. healthy controls) instead of continuous variable produced the same results (see Table 1).

4. Discussion

In our study, long-term testosterone secretion as measured by hair analysis was significantly associated with visuospatial memory in men after controlling for age, education, BMI, and depression. We did not find differences in hair testosterone levels (representing testosterone secretion over the past three months) between depressed patients and HC.

Our results are in line with recent results from Ackermann et al. (2012) who report a role for testosterone in improving hippocampus-dependent memory function in men but not women. While Ackerman and colleagues used saliva sampling immediately before cognitive testing, we were specifically interested in long-term testosterone secretion and memory function. Interestingly, Wirth et al. (2012) commented on Ackerman’s work by questioning the reliability of salivary sampling due to the effect of collection devices, storage time, and temperature and asked for a replication of results using more reliable testosterone measurement techniques. Our study applying hair analysis for testosterone confirms the association between testosterone and visuospatial memory in men. Currently, it remains unknown why this association appears to be limited to men. This may simply be due to the overall lower testosterone values and less variance among women prohibiting to see an association with memory function. Or else, as testosterone can be converted to estradiol by aromatase (Roselli et al., 2001), there may be a sex-specific activity of this conversion, thus leading to differential receptor binding (androgen vs. estrogen receptor), which are located in different brain areas in men and women.

Importantly, hair analysis ascertains steroid concentrations over several months in a retrospective fashion. This relatively new method has produced a number of new research results with regards to the steroid cortisol (Stalder and Kirschbaum, 2012; Dettenborn et al., 2012; Russell et al., 2012, for review), and is now expanding to other steroids including testosterone. It is tempting to speculate that a cumulative measure of testosterone secretion may provide a better understanding of its association with verbal and visuospatial memory because neuroprotection through testosterone may be more likely through long-term testosterone stimulation rather than sporadic testosterone peaks. Moffat et al.’s (2002) large prospective study indicating that lower testosterone levels earlier in life are associated with worse performance on measures of memory performance later in life may support this notion.

In addition to the source of testosterone (plasma, saliva, hair), other methodological factors may influence the results regarding testosterone effects on memory such as the type of memory tested, age range of the participants, and health factors. In contrast to most studies, we investigated middle-aged participants rather than the elderly who are characterized by declining testosterone levels. Our results are in line with Ackermann et al. (2012) who investigated an even younger sample (age range 18–32 years), but contrast to Martin et al.’s (2007, 2008) work who investigated an elderly sample (over fifty years of age). Whether our and Ackermann’s findings regarding the association between testosterone and memory are limited to young men who present with overall higher testosterone levels compared to elderly men needs to be tested in larger studies.

Table 1 Regression analysis in men only.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Predictor variable</th>
<th>Model</th>
<th>$R^2$</th>
<th>Beta</th>
<th>T</th>
</tr>
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<tbody>
<tr>
<td>Rey delayed recall</td>
<td>Age</td>
<td>1</td>
<td>0.34†</td>
<td>−0.55†</td>
<td>−2.25</td>
</tr>
<tr>
<td></td>
<td>Education</td>
<td></td>
<td></td>
<td>0.36</td>
<td>2.02</td>
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<tr>
<td></td>
<td>BMI</td>
<td></td>
<td></td>
<td>0.29</td>
<td>1.20</td>
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<tr>
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<td>Depression</td>
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<td>−0.82</td>
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</tr>
<tr>
<td></td>
<td>Testosterone</td>
<td>3</td>
<td>0.56†</td>
<td>0.56†</td>
<td>2.92</td>
</tr>
</tbody>
</table>

* Indicates $p < 0.05$, BMI: body mass index.
The current results emerge from a larger project designed to investigate steroid hormones in depression. To test memory function, we applied the AVLT and the RCFT, both regarded as gold standard instruments with multiple studies asserting their validity (e.g., Lezak, 1995). However, like all neuropsychological tests, the AVLT and RCFT capture several cognitive and perceptual domains beyond memory, thus making it difficult to test isolated domains. For example, visuospatial abilities are a prerequisite for nonverbal memory and sufficient command of the test language is a prerequisite for verbal memory.

While the current results emerge from a rather small sample, the effect size for testosterone and its association with visuospatial memory is noteworthy. Larger studies are needed to confirm or disprove our findings which will also allow a better evaluation of the effect of testosterone on memory function in women. Overall, hair steroids may provide a new approach to understanding steroid-memory associations overcoming reliability issues inherent to saliva measures.

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Conflict of interest statement

Dr. Otte has received honoraria fees for lectures from Astra Zeneca, Berlin-Chemie, Lundbeck, and Servier.

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