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Steroid abnormalities and the developing brain: Declarative memory for emotionally arousing and neutral material in children with congenital adrenal hyperplasia

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Summary

Steroid hormones modulate memory in animals and human adults. Little is known on the developmental effect of these hormones on the neural networks underlying memory. Using Congenital Adrenal Hyperplasia (CAH) as a naturalistic model of early steroid abnormalities, this study examines the consequences of CAH on memory and its neural correlates for emotionally arousing and neutral material in children. Seventeen patients with CAH and 17 age- and sex-matched healthy children (ages 12 to 14 years) completed the study. Subjects were presented positive, negative and neutral pictures. Memory recall occurred about 30 minutes after viewing the pictures. Children with CAH showed memory deficits for negative pictures compared to healthy children ($p < 0.01$). There were no group differences on memory performance for either positive or neutral pictures ($p > 0.1$). In patients, 24h urinary-free cortisol levels (reflecting glucocorticoid replacement therapy) and testosterone levels were not associated with memory performance. These findings suggest that early steroid imbalances affect memory for negative material in children with CAH. Such memory impairments may result from abnormal brain organization and function following hormonal dysfunction during critical periods of development.

Keywords

Development; glucocorticoids; declarative memory; congenital adrenal hyperplasia; steroid hormones

Introduction

Previous findings report that steroid hormones may influence cognitive function (McEwen, 1994; Cherrier, 2005; Lupien et al., 2007). Acute elevations in glucocorticoids (cortisol in humans) and androgens were shown to modulate declarative memory for emotional and neutral

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material (e.g., Vazquez-Pereyra et al., 1995; Naghdi et al., 2001; Cherrier, 2005; de Kloet et al., 2005; Lupien et al., 2007). Declarative memory refers to the conscious or voluntary recollection of previously learned information (Milner et al., 1998). Glucocorticoids and androgens may influence declarative memory for emotional and neutral material through their interactions with a large number of their receptors located in the frontal cortex, hippocampus and amygdala (Handa et al., 1994; Beyenburg et al., 2000; Lupien and Lepage, 2001; Roozendaal, 2003; de Kloet et al., 2005; Wilson and Davies, 2007), three brain structures implicated in declarative memory and the processing of emotional information (Milner et al., 1998; Davidson, 2002; Phelps, 2006).

Steroids can have two types of effects, organizational and activational effects. The organizational effects refer to permanent changes in brain structure, organization or function *in utero* or during critical periods of development. The activational effects refer to the acute effects of circulating steroids during adulthood. Although most research on the mnemonic effects of steroids focused on the activational mechanisms of these hormones (e.g., Vazquez-Pereyra et al., 1995; Naghdi et al., 2001; Cherrier, 2005; see de Kloet et al., 2005 and Lupien et al., 2007), organizational influences of steroids on declarative memory are now widely accepted (Welberg and Seckl, 2001; Owen et al., 2005).

Indeed, animal models provide important evidence that dysfunction of glucocorticoids prenatally or early in life can induce organizational changes in structure and function of the hippocampus, a central neural substrate of declarative memory, and amygdala, a key to emotional coding. Altered hippocampal architecture, such as neuronal degeneration of hippocampal pyramidal neurons or reduced hippocampal glucocorticoid gene expression, was observed following pre-natal and early life chronic exposure to elevated glucocorticoid levels in young rat offspring (Francis et al., 1999; Matthews, 2001; Welberg and Seckl, 2001). This animal model was also associated with increased glucocorticoid gene expression in the amygdala of juvenile offspring (Welberg and Seckl, 2001). Behaviorally, pre-natal and early life chronic glucocorticoid excess was found to be associated with increased anxiety behaviors in threatening situations and deficits in discrimination learning and spatial memory tasks in young rodents (Owen et al., 2005; Welberg and Seckl, 2001).

Though a few studies report on the organizational effects of glucocorticoid insufficiency on brain structure and function in juvenile mammals, such findings remain scarce. Interestingly however, data reported so far show that removal of glucocorticoids by adrenalectomy (ADX) leads to similar consequences as those observed in young offspring exposed to elevated glucocorticoid levels. Hence, reduced number and branching of dendrites in hippocampal cells were reported following neonatal ADX in juvenile rodents (Hashimoto et al., 1989). Moreover, degeneration in granule cells throughout the dentate gyrus of the hippocampus was reported in rat pups submitted to ADX during the early post-natal period (Gould et al., 1991; Sloviter et al., 1993). No studies measured the effects of early post-natal ADX on the amygdala in young offspring. Behaviorally, glucocorticoid depletion in early post-natal life was associated with impaired fear expression to threat in rat pups tested a few days after ADX (Takahashi, 1994; Moriceau et al., 2004).

Androgen levels in pre-natal and early life were also found to influence structure and function of both hippocampus and amygdala in animals. More specifically, these organizational effects were shown to modulate synaptic plasticity in hippocampal CA1 cells in juvenile rats (Hebbard et al., 2003). Moreover, sex differences in the shape and synaptic organization of the medial amygdala are influenced by pre-natal and early life androgen levels in young rodent offspring (Cooke et al., 1998; Cooke and Wolley, 2005; Wilson and Davies, 2007). Behaviorally, elevated levels of pre-natal and early life androgens were shown to reduce social memory performance (Hebbard et al., 2003), and to sexually differentiate the development of spatial

memory abilities (Roof, 1993) and visual discrimination learning (Hagger and Bachevalier, 1991) in young and late puberty juvenile mammal offspring.

Despite animal findings documenting organizational effects of glucocorticoids and androgens on the hippocampus or amygdala, and on learning performance, no studies examined such consequences in humans. An initial step could be the study of declarative memory in children with congenital steroid dysfunction such as CAH.

Classic CAH is an autosomal recessive disorder with a prevalence rate estimated at one in 15,000 live births (Merke and Bornstein, 2005). Classic CAH due to 21-hydroxylase (OH) deficiency is characterized by a deficiency in cortisol biosynthesis and severe androgen excess. In addition, a rodent model of CAH reports that the lack of cortisol negative feedback results in overproduction of corticotropin-releasing hormone (CRH)mRNA in the hypothalamus, as well as overproduction of proopiomelanocortin (POMC)mRNA (precursor of adrenocorticotrophic hormone (ACTH) in the pituitary; Tajima et al., 1999). Such overproduction of ACTH stimulates the adrenals and leads to excess adrenal androgen production (Merke and Bornstein, 2005).

Pre-natal dysfunction of glucocorticoids and androgens in CAH patients has led to the notion that brain organization, behavior and cognition may be affected in this population. With respect to brain organization, we recently reported irregularities in the structure and function of the amygdala in children with CAH. Specifically, we documented reduced volume in both male and female patients (Merke et al., 2003), and enhanced response to negative facial emotions in female patients (Ernst et al., 2007). Behaviorally, some studies have suggested that prenatal androgen excess masculinizes the brain of females with CAH, as these young girls exhibit male-like behaviors in their play interests, report themselves as being more aggressive and have enhanced spatial abilities compared to their healthy counterparts (Berenbaum, 2001; Hines et al., 2003; Cohen-Bendahan et al., 2005). Cognitively, research in CAH patients has reported inconsistent results. Johannsen and collaborators (2006) found that adult patients with the most severe form of classic CAH, and especially those who experienced salt-wasting adrenal crisis as neonates, showed lower IQ and were at risk for cognitive deficits. On the other hand, a number of studies failed to detect any cognitive impairments on IQ, declarative memory for neutral material, executive function or spatial abilities (Malouf et al., 2006), and even reported cognitive enhancement in the form of IQ advantage (Nass and Baker, 1991; see Berenbaum, 2001) in adult CAH patients. Such discrepancies in results are possibly attributable to genetic or socioeconomic factors (Nass and Baker, 1991; see Berenbaum, 2001).

In the present study, we extend this work further by investigating declarative memory for emotionally arousing and neutral material in children with CAH. To this aim, memory for positive, negative and neutral pictures was measured in pediatric patients with classic CAH and healthy control children. We predicted that children with CAH would show memory impairments for both emotional and neutral information compared to healthy children, and memory for emotional pictures would be more affected than memory for neutral pictures in the patient group based on amygdala irregularities associated with this disorder. Furthermore, we examined the influence of sex on memory performance to evaluate possible androgen-specific effects which would affect CAH females more than CAH males.

Methods

Participants

Thirty-four children participated to this study. Seventeen children with classic CAH due to 21-OH deficiency (10 boys; mean $\pm$ S.D.: age 13.6 ± 3.0 , bone age 14.0 ± 2.5) were compared to 17 healthy control children (10 boys; mean $\pm$ S.D.: age 14.1 ± 2.2). Patients with CAH and

healthy control children were age- and sex-matched. Socioeconomic status (SES) was determined by the four-factor Hollingshead scale (Hollingshead, 1973) in both groups. Patients with CAH were being followed at the NIH Clinical Center and enrolled in the study on a voluntary basis. Healthy control subjects were recruited in the community. The study was approved by the institutional review boards of the National Institute of Mental Health and National Institute of Child Health and Human Development. Prior to participation in the study, parents and children gave written consent and assent, respectively.

All subjects underwent physical and psychiatric examinations. Exclusion criteria included chronic medical condition (except for CAH) and pharmacological treatment unrelated to CAH. All participants were free from any present or lifetime history of psychiatric disorders, as assessed by DSM-IV criteria using the Schedule for Affective Disorders and Schizophrenia for School-Age Children- Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997) with parents and children separately. All participants scored within the average/above average on the Wechsler Abbreviated Scale of intelligence for children (Wechsler, 1999).

According to the clinical phenotype, all patients with CAH had the classic or severe form of CAH. Eight of the 17 patients were salt wasters by genotype, and a ninth patient was a salt waster by phenotype only. Seven of the nine SW CAH children suffered prior salt-wasting crises. Of these seven, five had no more than two salt-wasting crises, and two SW CAH children had more than two crises (seven and nine crises, respectively). All females were diagnosed at birth, except for one patient who was diagnosed at two years of age. The mean age of diagnosis for males was 1.7 years of age (S.D.: 1.93; range *in utero* to 4.5 yr). All CAH patients were receiving glucocorticoid replacement therapy at the time of testing: fifteen were treated with hydrocortisone administered thrice daily at 0800h, 1500h and 2200h (mean $\pm$ S.D.: 12.2 ± 4.96 mg/m²-day; hydrocortisone doses ranged from 6.14 mg/m²-day to 24 mg/m²-day, with a median of 11.8 mg/m²-day) and two with dexamethasone administered once daily at 2200h (0.3 and 0.5 mg/m²-day, respectively). Dexamethasone was converted to hydrocortisone dose equivalent using a relative potency ratio of 70 to 1. One CAH boy was treated from the 5th to the 8th week of gestation with dexamethasone, while all other patients were treated post-natally (from birth (n=5) or within the first 4.5 years of life (n=11)). Patients with CAH receiving hydrocortisone (n= 15) had 24-hour urinary free cortisol levels within the normal range (see Table 1). No cortisol levels are reported for patients treated with dexamethasone (n= 2) as this drug is not easily measured in urine. All CAH patients were also receiving fludrocortisone (aldosterone agonist). Finally, testosterone levels were within the normal range for age, sex and pubertal status for all patients (Table 1).

Declarative memory task

Subjects performed an incidental declarative memory task (subjects were not aware of the later memory test; see Baddeley, 1997). The memory test consisted of two phases, encoding in an fMRI environment and recognition outside the scanner. Because only a subset of the brain imaging data was sufficiently free of artifacts to be analyzed. Therefore, we present here only the behavioral data, which are both important and timely as no other study has measured memory for emotional and neutral material in children suffering from steroid dysfunction.

During the encoding phase, participants viewed a series of 32 pictures, 11 negative, 11 positive and 10 neutral images, selected from a pool of 96 (32 negative, 32 positive, 32 neutral) pictures chosen from the International Affective Picture System (IAPS) database (Lang et al., 1999). Pictures from the IAPS database have previously been used successfully in adults in paradigms measuring memory for emotional and neutral stimuli (e.g., Abercrombie et al., 2003). Each subject viewed a different set of 32 pictures that were randomly selected from the pool of images.

The 128-trials memory task was composed of four 32-trial blocks. Each block was divided into eight segments, with four pictures presented in random order per segment (8 segments $\times$ 4 pictures $\times$ 4 blocks = 128 trials). Therefore, all 32 pictures were presented once in each block. Four sets of instruction were used per block, and each set of instruction was presented twice in a row. Pairs of instructions were randomly distributed across subjects. The instruction sets were presented at the beginning of each segment. Participants were asked to either (1) passively view the pictures, (2) rate the valence (how happy or unhappy does the picture make you feel?), (3) rate the arousal (how excited or calm does the picture make you feel?) or (4) rate the greenness level (how green is the picture?) of the images on a scale of 1 (not very) to 5 (extremely) using a five-key response box. Hence, each picture was viewed four times, i.e., once in each of the four blocks, under each of the four instruction sets: passive viewing, valence, arousal and greenness ratings. As described earlier, the use of these different attention manipulations facilitates encoding success by reducing boredom associated with repeated viewing of the same pictures (Nelson et al., 2003; Pine et al., 2004; Roberson-Nay et al., 2006; Maheu et al., in press).

The 18 min. task was programmed in the e-prime software package and the order of presentation for blocks and segments was randomized across subjects. Pictures and instruction sets were presented for 3000 ms. Picture presentation was followed by a fixation point that appeared for 3000 ms and during which subjects were to execute their ratings. Stimuli were presented in the MRI environment using the Avotec Silent Vision Glasses (Stuart, FL) and responses were recorded using a five-key button box developed by MRI Devices (Waukesha, WI). Before testing, subjects were familiarized with the task in an MRI simulator using a practice set of IAPS pictures to ensure understanding of picture rating and acclimation to the MRI environment. The pictures presented during the practice session were different than the ones used during the actual memory task. Because of the incidental nature of the declarative memory task, memory recall was only performed following the actual memory task.

Therefore, the surprise free recall of the pictures occurred immediately after the scanning session. During recall, subjects were encouraged to remember as many pictures as they could for 90s while the experimenter was writing down the participants' answers. Participants were credited with the recall of a picture (one point per picture) if they remembered elements that were unique to this picture and not present in any other picture. Because the number of pictures presented for each category of emotions varied (11 positive, 11 negative, 10 neutral pictures), the memory score for each category of emotions was reported as a percentage of correct responses. The experimental session lasted an hour, and all participants were evaluated between 5 and 7 pm.

Hormonal Measures and Assays

Twenty-four hour urine collection was performed in CAH patients to measure urinary free cortisol levels (see Table 1). Urine samples were stored at -80°C until assayed. Cortisol levels were measured using an extraction-based competitive chemiluminescence immunoassay kit at the Mayo Medical Laboratories (Rochester, MN). Cortisol levels were adjusted by body surface area. Plasma testosterone levels in CAH patients were collected in serum separator tubes (SST) containing barrier gels and stored at -80°C until assayed. Testosterone levels were measured with high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) at the Mayo Medical Laboratories (Rochester, MN). Urine and blood samples were taken within a day or two of memory testing. Both urinary free cortisol levels and plasma testosterone levels were not measured in healthy control children. Norms for cortisol and testosterone levels are presented in Table 1 for comparison.

Statistical Analysis

Demographic and clinical characteristics were compared between groups using two-sample two-sided *Student's t*-tests for continuous measures or chi-squares tests for nominal measures. Memory scores were evaluated for assumptions of normality and sphericity. Data was distributed normally, and Greenhouse-Geiser (1959) corrections were applied when the sphericity criterion was not met.

A mixed three-way analysis of variance (ANOVA) with diagnosis (CAH patients *vs.* healthy control children) and sex (males *vs.* females) as the between-subjects factors and picture emotion (positive *vs.* negative *vs.* neutral) as the within-subjects factor was performed to measure the influence of diagnosis, sex and emotion type on mean percent recall of emotionally arousing and neutral pictures.

In CAH patients, an ANOVA with disease severity (salt-wasters (n=9) *vs.* non salt-wasters (n=8)) as the between-subjects factor and picture emotion (positive *vs.* negative *vs.* neutral) as the within-subject factor was performed as a follow-up analysis to assess the influence of disease severity on memory performance.

Interaction effects, simple effects and Tukey's honestly significant difference analyses were conducted on significant findings. Correlations were performed to assess the contribution of cortisol and testosterone levels measured within two days of testing to memory recall for positive, negative and neutral pictures in CAH patients. Correlations were also performed to assess the contribution of clinical variables to memory performance in CAH children. The clinical variables investigated were: severity of disease, hydrocortisone dose (analysis performed both including and excluding the two patients receiving dexamethasone), and age at which hydrocortisone treatment was started. Bonferroni corrections were applied to account for multiple correlational analyses.

Results

Demographic and clinical characteristics

Groups were compared on age, IQ, Tanner stage and SES. Because of data lost, IQ measures were available for only 11 CAH patients, and Tanner stage was available for only 15 healthy children. Children with CAH (mean $\pm$ SD: age 13.6 ± 3.0 ; IQ 108.1 ± 12.7 ; Tanner stage: 2.9 ± 1.7 ; SES: 61.1 ± 19.3) and healthy children (mean $\pm$ SD: age 14.1 ± 2.2 ; IQ 109.9 ± 11.0 ; Tanner stage: 3.0 ± 1.1 ; SES: 46.4 ± 19.4) did not differ significantly with respect to age ($t(32) = 0.57$, $p = 0.6$), IQ ($t(26) = 0.40$, $p = 0.7$), Tanner stage ($X^2(4) = 8.77$, $p = 0.07$) or SES ($X^2(19) = 26.13$, $p = .13$).

Cognitive Findings

The ANOVA measuring the influence of diagnosis and sex on memory recall for positive, negative and neutral pictures showed a significant two-way interaction between diagnosis and picture emotion ($F(2, 60) = 4.81$, $p < 0.01$). Post-hoc comparisons between groups showed that memory for negative pictures was significantly impaired in children with CAH compared to healthy control children ($p < 0.01$). Recall for positive and neutral pictures was not significantly different among groups (p 's > 0.1 ; Fig. 1). There was no main effect of sex, and no interaction effects of sex with diagnosis or picture emotion on memory recall (p 's > 0.1).

To determine if prior salt-wasting crises could influence results, we removed the memory performance data for the two CAH children with more than two salt-wasting crises (see Methods) from the statistical analysis. Findings were not modified by doing so: the ANOVA measuring the influence of diagnosis and sex on memory for positive, negative and neutral

pictures showed a significant two-way interaction between diagnosis and picture emotion ($F(2, 56) = 3.79, p = 0.028$). Post-hoc comparisons between groups showed that memory for negative pictures was significantly impaired in children with CAH compared to healthy control children ($p = 0.017$). Recall for positive and neutral pictures was not significantly different among groups (p 's > 0.1). These findings therefore suggest that the results were not driven by these two subjects with history of more than two salt-wasting crises. Moreover, in CAH children, severity of disease (salt-wasters ($n=9$) vs. non salt-wasters ($n=8$)) had no impact on memory performance ($F(2, 30) = 1.39, p > 0.1$). Therefore, a history of prior salt-wasting episodes did not seem to have influenced the memory performance observed in the CAH group.

Finally, memory recall for positive, negative and neutral material was not correlated with any clinical or hormonal variables in the patient group (p 's > 0.1). The absence of correlations between hormonal measures and memory performance is not surprising because steroid levels were maintained therapeutically. The iatrogenic origin of these hormones prevented the potential fluctuation of their levels at the presentation of the pictures.

Because prenatal exposure to dexamethasone has been associated with behavioral problems and working memory deficits (Trautman et al., 1995; Welberg and Seckl, 2001; Hirvikoski et al., 2007), memory performance was also analyzed after removal of the one CAH boy treated *in utero* with dexamethasone. This patient showed memory scores in the same range as those observed in the CAH group and none of the findings were altered by the removal of his data.

Discussion

This study examined the influence of early abnormal steroid function on declarative memory for emotionally arousing and neutral material in pediatric patients with CAH relative to normal healthy children. In line with predictions, children with CAH were found to present significantly poorer recall for negative pictures, when compared to healthy children. However, contrary to our hypothesis, no differences in recall for positive or neutral images were observed in CAH patients compared to healthy control children. In the patient group, memory performance was not associated with 24-hrs urinary free cortisol levels and testosterone levels, or with clinical variables (severity of disease, glucocorticoid treatment dose, age at the start of treatment). Overall, this study shows, for the first time, that declarative memory for negative material may be altered in CAH pediatric patients. Impaired memory for negative information may be deleterious as memorizing threat stimuli helps avoid dangerous situations (Hamann, 2001).

We recently reported amygdala irregularities in CAH children. Reduced amygdala volume was observed in both male and female CAH patients compared to healthy children (Merke et al., 2003), and hyperactive amygdala function to negative facial expression was detected in CAH girls compared to healthy females (Ernst et al., 2007). The amygdala is particularly implicated in the processing of emotional stimuli and modulates memory for these stimuli via interactions with the hippocampus (Richardson et al., 2004; see Cahill, 2000; Roozendaal, 2003). The present findings support amygdala dysfunction since the selective memory deficit for negatively valenced pictures is consistent with the predominant role of the amygdala in the processing of aversive stimuli (LeDoux, 2003; Zald, 2003; Burgdorf and Panksepp, 2005).

Various factors could explain our observations. The organizational effects of androgens on the development of the amygdala could be one factor explaining the memory deficits seen in the CAH group. Our recent functional neuroimaging study reported an abnormally high amygdala response to negative facial emotions that was confined to the girls and was not present in the boys with CAH, suggesting organizational effects of the *in utero* androgen excess (Ernst et al., 2007). Indeed, the amygdala possesses androgen receptors (Handa et al., 1994; Wilson and

Davies, 2007) and acute elevations in androgen levels were shown to modulate responses to negatively valenced stimuli in rodents (Vazquez-Pereyra et al., 1995; Naghdi and Asadollahi, 2004). In this study, however, no group differences were observed in memory performance when comparing CAH boys and girls with their respective counterparts. This suggests that other mechanisms than organizational effects of androgens may be at play, as detailed below. Nevertheless, as a cautionary note, the failure to detect sex differences may also reflect insufficient statistical power, and a larger sample size might have been able to uncover such effects. Therefore, we cannot rule out that organizational effects of androgens may have contributed to the memory deficits of the CAH patients.

A second explanation to our findings may reside in the organizational effects of *in utero* decreased glucocorticoid production and circulating levels. The amygdala possesses many glucocorticoid receptors (Roozendaal, 2003; de Kloet et al., 2005) and, as reported in the introduction, organizational effects of glucocorticoid dysfunctions have been associated with alterations in amygdala function, and impairments in emotional behavior. Thus, organizational effects of decreased glucocorticoid synthesis may have influenced amygdala development and memory for negative material in our group of CAH children.

Alterations in pre-natal and early life corticotropin-releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) could also account for our findings. Depletion in glucocorticoid production and circulating levels leads to lack of negative feedback on the hypothalamic-pituitary-adrenal (HPA) axis. This absence of negative feedback, as observed in 21-OH deficient mice (an animal model paralleling CAH; Tajima et al., 1999) and in humans with CAH (Merke and Bornstein, 2005), results in hypersecretion of CRH and ACTH. Animal models show that acute excess in CRH and cerebral ACTH levels may impair emotional learning and memory, most probably through their receptors in the amygdala (McGaugh, 1983; Croiset et al., 2000; Roozendaal et al., 2002; Gulpinar and Yegen, 2004; Chaki and Okuyama, 2005). Indeed, many CRH receptors are found in the amygdala (Aguilera et al., 2004), and ACTH receptors were also recently observed in this brain region (Gantz and Fong, 2003; Chaki and Okuyama, 2005). Though melanocortin-2 receptors (MC2R) are the main ACTH receptors, they are not observed in the brain. Melanocortin-4 receptors (MC4R) are actually the ACTH receptors abundantly expressed in the amygdala and recently found to modulate anxiety and depression-like behaviors in rodents (Chaki et al., 2003; Yamano et al., 2004; Chaki and Okuyama, 2005). Thus, it is possible that pre-natal and early life excess in CRH and cerebral ACTH may have influenced amygdala development and functioning, resulting in impaired memory for negative material in our sample of CAH children.

Finally, our findings could be explained by the intermittent periods of over- or under-treatment that may have occurred in the CAH patients. Such disruptions in glucocorticoid substitution therapy could have affected amygdala development and function, leading to the memory deficits observed in the CAH children.

A number of caveats should be mentioned. First, the absence of sex differences in memory performance should be considered carefully. As already eluded to, the sample size may have been too small to detect a sex effect. Similarly, the sample size may have limited our ability to detect correlations of memory performance with clinical characteristics. Another caveat concerns the period of development investigated in this study. Memory performance was measured at the beginning of the pubertal period, when acute changes in steroid function take place. This period may thus be characterized by more interindividual variability since pubertal stage varies across individuals. However, CAH patients and healthy controls were similar regarding age and pubertal status, and our ability to detect significant differences between the two groups suggests that the findings were robust and unlikely to be related to activational effects. Nevertheless, this will need to be tested either longitudinally or in younger populations.

Finally, although similar on all demographic characteristics to the CAH children, our healthy group did not permit us to control for the effects of a chronic illness on brain development. A comparison group with a chronic illness that does not directly affect the brain would be important to add to this type of study.

Despite these limitations, we report, for the first time, that memory for negative material may be impaired in children with CAH. Memory for negative material is critical for avoiding dangerous situation, and such deficits can be deleterious to adaptive behaviors. Findings observed in the CAH patients support the proposal of a dysfunctional amygdala, possibly reflecting organizational effects of androgens, glucocorticoid depletion, HPA axis-related hormones (CRH, ACTH) and/or iatrogenic glucocorticoid levels on this brain structure. Future cognitive and neuroimaging studies in pediatric patients with androgens and HPA axis imbalances could provide new insights on the specific influence of these hormonal dysfunctions on brain development and memory function. Including pre-pubertal as well as post-pubertal individuals needs to be considered.

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Figure 1.

Mean ($\pm$ SEM) percentage correct recall in the Congenital Adrenal Hyperplasia (CAH) patients and healthy control children. ** CAH children showed significantly poorer recall for negative pictures compared to healthy control children ($p < 0.01$).

Table 1

Hormonal measures in patients with congenital adrenal hyperplasia (CAH) and normal hormonal ranges.

	CAH patients 10B/7G	Normal range ² for healthy children
24 hrs urinary free cortisol ($\mu\text{g}/24\text{ h}$)	71.2 ± 8.4 ($n=14$) ¹	8–77
24 hrs urinary free cortisol corrected for body surface area ($\mu\text{g}/\text{m}^2\ 24\text{ h}$)	46.9 ± 4.9 ($n=14$) ¹	<68
Testosterone (ng/dL)	B: 299.6 ± 111.2 G: 71.9 ± 19.4	B: 26–800 G: 17–75

All values are mean $\pm$ SEM. B: boys; G: girls.

¹ Reduced n because 2 patients received dexamethasone therapy, which is not measured in urine; data lost for one patient.

Hormonal measures were taken within 1–2 days of memory testing.

² Normal range for comparable pubertal stage.