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# Neuroactive Steroids are Altered in Schizophrenia and Bipolar Disorder: Relevance to Pathophysiology and Therapeutics

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Evidence suggests that neuroactive steroids may be candidate modulators of schizophrenia pathophysiology and therapeutics. We therefore investigated neuroactive steroid levels in post-mortem brain tissue from subjects with schizophrenia, bipolar disorder, nonpsychotic depression, and control subjects to determine if neuroactive steroids are altered in these disorders. Posterior cingulate and parietal cortex tissue from the Stanley Foundation Neuropathology Consortium collection was analyzed for neuroactive steroids by negative ion chemical ionization gas chromatography/mass spectrometry preceded by high-performance liquid chromatography. Subjects with schizophrenia, bipolar disorder, nonpsychotic depression, and control subjects were group matched for age, sex, ethnicity, brain pH, and post-mortem interval ($n = 14$–15 per group, 59–60 subjects total). Statistical analyses were performed by ANOVA with *post-hoc* Dunnett tests on log transformed neuroactive steroid levels. Pregnenolone and allopregnanolone were present in human post-mortem brain tissue at considerably higher concentrations than typically observed in serum or plasma. Pregnenolone and dehydroepiandrosterone levels were higher in subjects with schizophrenia and bipolar disorder compared to control subjects in both posterior cingulate and parietal cortex. Allopregnanolone levels tended to be decreased in parietal cortex in subjects with schizophrenia compared to control subjects. Neuroactive steroids are present in human post-mortem brain tissue at physiologically relevant concentrations and altered in subjects with schizophrenia and bipolar disorder. A number of neuroactive steroids act at inhibitory $GABA_A$ and excitatory NMDA receptors and demonstrate neuroprotective and neurotrophic effects. Neuroactive steroids may therefore be candidate modulators of the pathophysiology of schizophrenia and bipolar disorder, and relevant to the treatment of these disorders.
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## BACKGROUND

Neuroactive steroids can be synthesized *de novo* in the brain from cholesterol (neurosteroids) or in the periphery by the adrenals and gonads (Paul and Purdy, 1992). Neuroactive steroids rapidly alter neuronal excitability by acting at inhibitory $GABA_A$ and/or excitatory NMDA receptors, among others (Rupprecht and Holsboer, 1999, review). For example, the neuroactive steroid 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) potentiates $GABA_A$ receptor response with greater potency than benzodiazepines or barbiturates (Majewska *et al*, 1986; Morrow *et al*, 1987, 1990). Certain neuroactive steroids also demonstrate activity at nicotinic acetylcholine (Mayo *et al*, 2003; Darnaudery *et al*, 1998, 2002; Pallares *et al*, 1998; Rhodes *et al*, 1996, 1997) and $\sigma_1$ ($sigma_1$) receptors (Maurice *et al*, 2001). Pregnenolone sulfate and dehydroepiandrosterone (DHEA) are positive modulators of NMDA receptors (Irwin *et al*, 1994; Wu *et al*, 1991; Compagnone and Mellon, 1998; Debonnel *et al*, 1996; Bergeron *et al*, 1996) and negative modulators of $GABA_A$ receptors (Majewska *et al*, 1988, 1990; Imamura and Prasad, 1998; Park-Chung *et al*, 1999). Many of these endogenous molecules are present at physiologically relevant concentrations in both male and female rodent brain, but little is currently known regarding

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their regulation in the human central nervous system. Available data suggest that neuroactive steroids are also present in human brain, but few investigations have been conducted to date. Several small post-mortem studies have used radioimmunoassay approaches (Lacroix *et al*, 1987; Lanthier and Patwardhan, 1986; Bixo *et al*, 1997; Brown *et al*, 2003). Two studies to our knowledge have utilized mass spectrometry-based techniques to determine neuroactive steroid levels in human post-mortem brain tissue from subjects with Alzheimer's disease (Weill-Engerer *et al*, 2002, 2003), the most highly sensitive and specific current methodology (Purdy *et al*, in press). It is currently unknown if neuroactive steroid levels are altered in post-mortem brain tissue from subjects with schizophrenia, bipolar disorder, or depression. Since converging evidence suggests that neuroactive steroids may be candidate modulators of the pathophysiology and therapeutics of these disorders, we determined levels of these endogenous molecules in post-mortem brain tissue from the Stanley Neuropathology Consortium.

Neuroactive steroids are lipophilic and cross the blood–brain barrier readily in their unsulfated forms. In addition to neuroactive steroids formed in the brain from cholesterol or other precursors, it is likely that peripheral neuroactive steroids enter the brain and contribute to central nervous system neuroactive steroid levels, as has been demonstrated in rodents (Marx *et al*, 2003; Wang *et al*, 1997; Karavolas *et al*, 1979; Raisinghani *et al*, 1968). The precise dynamics of neuroactive steroid compartmentalization have been very challenging to determine (Baulieu *et al*, 2001). Rodent evidence suggests that brain neuroactive steroid levels are generally higher than plasma or serum levels, achieving central nervous system concentrations known to modulate inhibitory $GABA_A$ (Morrow *et al*, 1987, 1990) and excitatory NMDA (Compagnone and Mellon, 1998; Akwa *et al*, 2001) receptor actions, and to influence GABA release (Mtchedlishvili and Kapur, 2003) and glutamate release (Meyer *et al*, 2002). Nanomolar concentrations of allopregnanolone modulate $GABA_A$ receptors (Morrow *et al*, 1987, 1990; Gee *et al*, 1988; Puia *et al*, 1990; Shu *et al*, 2004) and clearly demonstrate behavioral effects at physiologically relevant concentrations. Micromolar concentrations of pregnenolone sulfate, DHEA, and DHEAS modulate $GABA_A$ (Majewska *et al*, 1988, 1990; Imamura and Prasad, 1998; Park-Chung *et al*, 1999) and NMDA (Irwin *et al*, 1994; Wu *et al*, 1991; Bowlby, 1993) receptors. In addition, nanomolar levels of pregnenolone sulfate, consistent with levels found in rodent hippocampus (Kimoto *et al*, 2001), decrease GABA release from hippocampal neurons (Mtchedlishvili and Kapur, 2003), and DHEAS (100 nM) may increase glutamate release (Meyer *et al*, 2002). A number of neuroactive steroids therefore exhibit the capacity to modulate the major excitatory and inhibitory neurotransmitter systems in the mammalian brain. It is thus important to characterize neuroactive steroid levels in human brain, since alterations in glutamatergic and GABAergic neurotransmitter systems have been implicated in a number of psychiatric disorders including schizophrenia and bipolar disorder.

An expanding literature in animal models documents additional neuroactive steroid properties that may be relevant to the pathophysiology and therapeutics of psychiatric disorders. For example, both clozapine (Marx *et al*, 2003; Barbaccia *et al*, 2001) and olanzapine (Marx *et al*, 2000a, 2003) dose dependently elevate the neuroactive steroid allopregnanolone in rodent brain, and we have hypothesized that neuroactive steroid induction may contribute the therapeutic efficacy of these compounds (Marx *et al*, 2003, 2005). Furthermore, recent data support the possibility that allopregnanolone potentiates olanzapine actions on dopamine-mediated behaviors in rodents (Ugale *et al*, 2004). In addition to certain antipsychotics, fluoxetine also elevates allopregnanolone levels in animal models (Uzunov *et al*, 1996; Pinna *et al*, 2003, 2004), and these increases have been linked to its antidepressant-like effects in rodent behavioral models (Khisti and Chopde, 2000a; Khisti *et al*, 2000b) and to the mitigation of aggression in socially isolated rats (Pinna *et al*, 2003). We have also determined that olanzapine and fluoxetine elevate pregnenolone levels in rodent hippocampus (Trost *et al*, 2005). Since pregnenolone (Flood *et al*, 1992) and pregnenolone sulfate (Flood *et al*, 1992, 1995; Vallee *et al*, 1997, 2003; Akwa *et al*, 2001) enhance learning and memory in rodents, and since low pregnenolone levels have been associated with depressive symptoms in humans (George *et al*, 1994), pregnenolone elevations may be relevant to cognitive deficits in schizophrenia, and could potentially contribute to olanzapine and fluoxetine effects on depressive symptoms, including decreased concentration. Although data in humans are limited, it has also been demonstrated that fluoxetine- and fluvoxamine-induced elevations in cerebrospinal fluid allopregnanolone levels are correlated with symptom improvement in patients with depression (Uzunova *et al*, 1998). Initial evidence therefore suggests that neuroactive steroid alterations may contribute to the therapeutic effects of antipsychotics and selective serotonin reuptake inhibitors.

In addition to alterations in neuroactive steroid levels following certain antipsychotic and antidepressant agents, an extensive literature documents the neuroprotective and neurotrophic properties of a number of neuroactive steroids, and these actions may be relevant to the pathophysiology of schizophrenia and other psychiatric disorders. For example, DHEA and DHEAS decrease neuronal death following anoxia (Marx *et al*, 2000b), and obstetrical complications resulting in hypoxia are associated with increased schizophrenia risk (Dalman *et al*, 2001; Cannon *et al*, 1999, 2000; Geddes *et al*, 1999). Allopregnanolone demonstrates anticonvulsant actions in a number of seizure paradigms (Kokate *et al*, 1994, 1996; Devaud *et al*, 1995; Belelli *et al*, 1989) and exhibits pronounced protective effects against neurodegeneration in a mouse model of Niemann–Pick type C disease (Griffin *et al*, 2004). DHEA and DHEAS increase axonal and dendritic outgrowth, respectively (Compagnone and Mellon, 1998), and DHEA increases neurogenesis in rodent hippocampal dentate gyrus (Karishma and Herbert, 2002) and human neural stem cells (Suzuki *et al*, 2004). Pregnenolone sulfate may also increase neurogenesis in rodent hippocampus (Mayo *et al*, 2005), although results are conflicting (Wang *et al*, 2005). Allopregnanolone increases proliferation in rodent and human neural progenitor cells (Wang *et al*, 2005). Neuroactive steroids therefore appear to impact neuroplasticity, a possibility that

has critical ramifications for neuronal function. In addition, neuroactive steroids appear to be regulated differently in males and females (Bjornerem *et al*, 2004; Laughlin and Barrett-Connor, 2000; Genazzani *et al*, 1998; Pearson Murphy and Allison, 2000), and may therefore modulate the neurobiology of sex differences in a number of psychiatric disorders. Finally, augmentation with the neuroactive steroid DHEA decreases negative symptoms, anxiety, and depressive symptoms in patients with schizophrenia (Strous *et al*, 2003), suggesting a potential role for neuroactive steroids in schizophrenia therapeutics.

Since converging evidence suggests that neuroactive steroids may be candidate modulators of schizophrenia pathophysiology and treatment strategies, we investigated these molecules in subjects with schizophrenia, bipolar disorder, nonpsychotic depression, and control subjects in two brain regions, posterior cingulate and parietal cortex.

## METHODS

Posterior cingulate and parietal cortex post-mortem brain tissue was generously provided by the Stanley Neuropathology Consortium. A number of brain regions including posterior cingulate (Haznedar *et al*, 2004) and parietal cortex (Danckert *et al*, 2004) have been linked to schizophrenia pathophysiology; however, tissue availability within the Stanley Neuropathology Consortium ultimately dictated the two brain regions to be tested. Characteristics of this post-mortem tissue collection have been published previously (Torrey *et al*, 2000, 2005; Knable *et al*, 2004; Jarskog *et al*, 2000). Briefly, subjects were group matched for age, sex, ethnicity, brain pH, and post-mortem interval. For each brain region, frozen tissue from 15 subjects each with schizophrenia and bipolar disorder, 14–15 subjects with nonpsychotic depression, and 15 nonpsychiatric control subjects was analyzed for neuroactive steroids. The posterior cingulate collection was missing tissue from one subject with depression, and therefore 14 specimens from this group were analyzed. Neuroactive steroid analyses for both posterior cingulate and parietal cortex were performed in a manner blind to group condition.

Neuroactive steroid analyses were performed by highly sensitive and specific gas chromatography/mass spectrometry (GC/MS) preceded by high-performance liquid chromatography (HPLC) purification as previously described (Uzunova *et al*, 1998; Dong *et al*, 2001), with several modifications. All glasssware was silanized. Brain tissue was homogenized in five volumes of distilled water containing a trace amount of tritiated neuroactive steroid (4000 dpm/injection, New England Nuclear) to detect the HPLC fraction of interest, as well as a constant amount of deuterated allopregnanolone (D4-allopregnanolone) and deuterated pregnenolone (D4-pregnenolone) as the internal standards. Supernatants were extracted three times with three volumes of ethyl acetate and dried under nitrogen prior to HPLC. HPLC purification was performed with 900 μl injections per sample on an 1100 Series Agilent HPLC equipped with a Packard 500TR Flow Scintillation Analyzer for radiopeak detection. Each steroid was collected into a separate fraction based upon the retention time of its radioactive analogue, utilizing hexane, tetrahydrofuran, and ethanol in the mobile phase and a Phenomenex LiChrosorb DIOL (5 μm particle size) 250 mm × 4.6 mm column; flow rate 1.0 ml/min.

Samples were then transferred to 1 ml Reacti-Vials, evaporated to dryness, and derivatized utilizing heptafluorobutyric acid anhydride (HFBA) (50 μl HFBA added to 450 μl ethyl acetate at room temperature for 2 h). Derivatized samples were transferred to autosampler vials equipped with deactivated glass inserts. Standards and samples were injected onto an Agilent 5973 Mass Spectrometer (MS) coupled to an Agilent 6890N Gas Chromatograph (GC) equipped with an Agilent HP-5MS 30 meter × 0.250 mm × 0.25 μm capillary column, and analyzed in the negative ion chemical ionization mode (NICI) utilizing methane as the reaction gas and helium as the carrier gas. Each sample was injected in duplicate. The derivatized steroids of interest subjected to NICI yield negative ions in the mass range between *m/z* 100 and *m/z* 700. In addition to the GC retention time characteristic of each steroid, the structural identification of each neuroactive steroid assayed was provided by its unique mass fragmentation pattern. Mass spectrometer single ion monitoring mode was utilized to focus on the most abundant ion fragment for each HFBA steroid derivative (pregnenolone 492.3, 472.4; DHEA 464.4, 444.4; allopregnanolone 474.4, 494.3).

For neuroactive steroid quantification, the standard curve for the steroid of interest was prepared by combining varying known quantities of steroids (Steraloids) with a constant amount of deuterated internal standard. Identical to the experimental samples, each standard curve sample was extracted three times in ethyl acetate prior to HPLC purification and GC/MS injection; standard curve $r^2 = 0.99$ for each neuroactive steroid. The area under the peak of a known quantity of each steroid was divided by the area under the peak of the internal standard. This ratio was then plotted on the *y*-axis against known quantities of each steroid to generate the standard curve. Only peaks with a signal to noise ratio greater or equal to 5 : 1 were integrated. The limit of neuroactive steroid detection with this method was 2 pg for DHEA and allopregnanolone, and 10 pg for pregnenolone. The intra-assay coefficients of variation were 3.9% for pregnenolone, 2.1% for DHEA, and 6.6% for allopregnanolone.

### Statistical Analyses Methods

*Primary analyses.* Neuroactive steroid levels were log transformed due to the skewness of the distributions and the relationship of the mean and SD. We calculated means and standard deviations for all transformed neuroactive steroid levels. For each neuroactive steroid level (pregnenolone, DHEA, allopregnanolone), we performed a one-way, four-group analysis of variance (ANOVA) with post-hoc Dunnett tests comparing each of the diagnostic group means with the mean of the control group (no psychiatric diagnosis) in each brain region: (1) posterior cingulate and (2) parietal cortex.

*Secondary analyses.* Three secondary analyses were performed subsequent to the initial primary analyses above: (1) We performed separate one-way, four-group ANOVAs on

log-transformed neuroactive steroid levels with *post hoc* Dunnett tests for males and females as exploratory secondary analyses. Since the existing literature suggests that neuroactive steroids may be regulated differently in males and females, separate analyses stratified by sex were judged to be the most appropriate statistical approach to begin to address the question of possible sex differences in neuroactive steroid levels in human brain. Since we recognize that separating the sample into male and female groups may greatly reduce statistical power, we regard these secondary analyses as preliminary investigations for future hypothesis testing in a larger cohort of subjects. (2) Given rodent evidence suggesting that clozapine and olanzapine elevate the neuroactive steroid allopregnanolone in rodent brain but that haloperidol and risperidone do not demonstrate this effect (Marx *et al*, 2003; Barbaccia *et al*, 2001), we performed a Mann–Whitney *U* statistic comparing neuroactive steroid levels in patients with schizophrenia or bipolar disorder receiving clozapine at the time of death ($n=7$) with neuroactive steroid levels in patients receiving a typical antipsychotic, risperidone, or no antipsychotic at this time point ($n=23$). No patients were receiving olanzapine at the time of death. (3) Finally, we investigated if neuroactive steroids were associated with lifetime antipsychotic exposure (in fluphenazine equivalents) by determining the Pearson's correlation coefficient for each neuroactive steroid and this variable.

## RESULTS

### Overview: Selected Neuroactive Steroid Biosynthetic Pathways

Both DHEA and allopregnanolone are downstream metabolites of the precursor steroid pregnenolone (Figure 1). In addition to metabolism to DHEA and allopregnanolone, pregnenolone can be metabolized to a number of other neuroactive steroids. Figure 1 represents a partial listing of steroid metabolites and the enzymes involved in each biosynthetic step.

### Primary Analyses

*Neuroactive steroids in posterior cingulate.* Pregnenolone, DHEA, and allopregnanolone are present in posterior cingulate at physiologically relevant nanomolar concentrations, Table 1. Median pregnenolone and allopregnanolone levels in posterior cingulate exceed typical serum or plasma concentrations observed in males, follicular-phase females, and postmenopausal females by approximately 10-fold or greater in all subject groups. Median DHEA levels in posterior cingulate are comparable to typical serum and plasma levels, Table 1. Pregnenolone levels and DHEA levels (log transformed) are significantly elevated in posterior cingulate tissue from subjects with schizophrenia and bipolar disorder compared to control subjects; allopregnanolone levels (log transformed) are not significantly altered in posterior cingulate tissue from subjects with a psychiatric diagnosis compared to control subjects, Figure 2.

*Neuroactive steroids in parietal cortex.* Similar to posterior cingulate, pregnenolone and allopregnanolone levels in parietal cortex exceed typical serum or plasma levels observed in males, follicular-phase females, and postmenopausal females by approximately 10-fold or greater in each subject group; median DHEA levels in parietal cortex are comparable to typical serum and plasma levels, Table 2. Also similar to posterior cingulate, pregnenolone and DHEA levels (log transformed) are significantly elevated in parietal cortex tissue from subjects with bipolar disorder compared to control subjects; pregnenolone and DHEA levels (log transformed) also tended to be higher in the

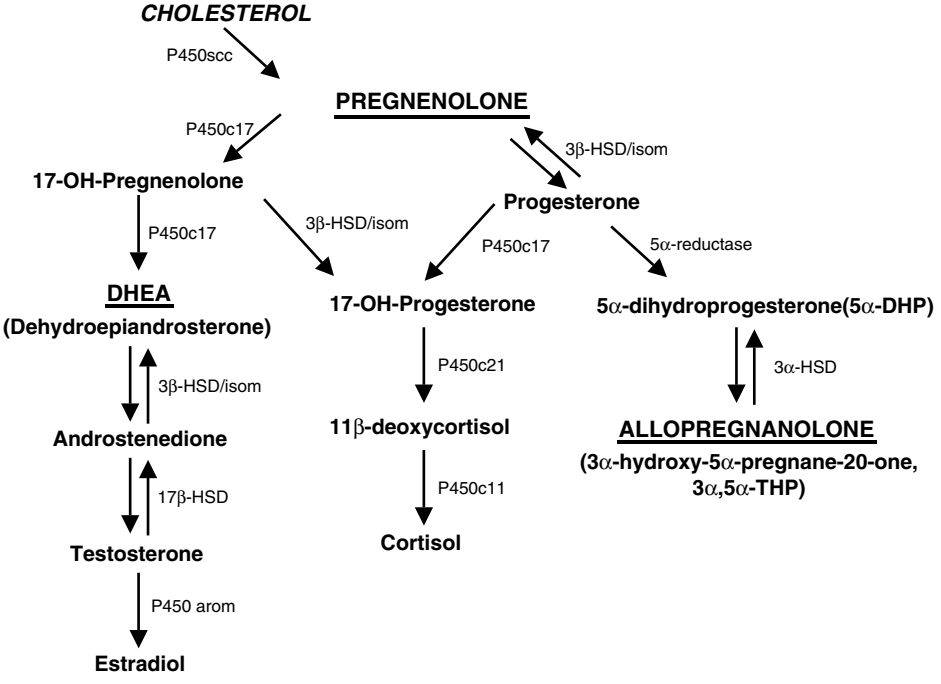


**Figure 1** Partial listing of selected biosynthetic pathways.

**Table 1** Neuroactive Steroid Levels (ng/g) in Posterior Cingulate

| | *N* | Min | Q1 | Med | Q3 | Max |
|---|---|---|---|---|---|---|
| *Pregnenolone* | | | | | | |
| Control | 15 | 3.14 | 6.05 | 9.32 | 13.50 | 46.06 |
| Schizophrenia | 15 | 2.89 | 14.31 | 22.27 | 34.69 | 107.87 |
| Bipolar | 15 | 8.50 | 12.21 | 31.00 | 50.65 | 163.62 |
| Depression | 14 | 6.34 | 8.75 | 14.77 | 19.35 | 186.22 |
| *DHEA* | | | | | | |
| Control | 15 | 1.24 | 1.63 | 5.68 | 10.07 | 24.58 |
| Schizophrenia | 15 | 2.21 | 7.54 | 13.38 | 18.80 | 44.22 |
| Bipolar | 15 | 3.32 | 8.15 | 16.35 | 26.50 | 41.71 |
| Depression | 14 | 1.82 | 3.22 | 7.64 | 13.68 | 27.28 |
| *Allopregnanolone* | | | | | | |
| Control | 15 | 1.68 | 5.20 | 6.77 | 10.32 | 54.98 |
| Schizophrenia | 15 | 1.90 | 4.64 | 6.56 | 9.56 | 30.58 |
| Bipolar | 15 | 2.82 | 5.27 | 8.12 | 21.77 | 64.23 |
| Depression | 14 | 1.89 | 4.02 | 12.42 | 15.50 | 70.33 |

*N* = number of subjects; Min = minimum value in group; Q1 = lower quartile; Med = median; Q3 = upper quartile; Max = maximum value in group.

**Table 2** Neuroactive Steroid Levels (ng/g) in Parietal Cortex

| | *N* | Min | Q1 | Med | Q3 | Max |
|---|---|---|---|---|---|---|
| *Pregnenolone* | | | | | | |
| Control | 15 | 4.70 | 10.94 | 14.96 | 25.69 | 101.49 |
| Schizophrenia | 15 | 5.87 | 21.70 | 40.42 | 52.94 | 170.21 |
| Bipolar | 15 | 9.29 | 17.76 | 41.14 | 139.84 | 331.86 |
| Depression | 15 | 3.70 | 8.23 | 14.12 | 34.04 | 138.33 |
| *DHEA* | | | | | | |
| Control | 15 | 0.82 | 3.67 | 6.04 | 10.59 | 33.21 |
| Schizophrenia | 15 | 3.39 | 7.22 | 9.31 | 24.88 | 42.26 |
| Bipolar | 15 | 4.63 | 6.38 | 18.33 | 30.80 | 41.92 |
| Depression | 15 | 2.39 | 2.83 | 8.93 | 11.53 | 34.17 |
| *Allopregnanolone* | | | | | | |
| Control | 15 | 5.20 | 7.44 | 11.42 | 14.22 | 42.92 |
| Schizophrenia | 15 | 4.13 | 5.40 | 7.16 | 10.09 | 11.44 |
| Bipolar | 15 | 3.01 | 6.37 | 7.42 | 12.91 | 17.87 |
| Depression | 15 | 3.89 | 4.86 | 9.02 | 10.68 | 16.82 |

*N* = number of subjects; Min = minimum value in group; Q1 = lower quartile; Med = median; Q3 = upper quartile; Max = maximum value in group.

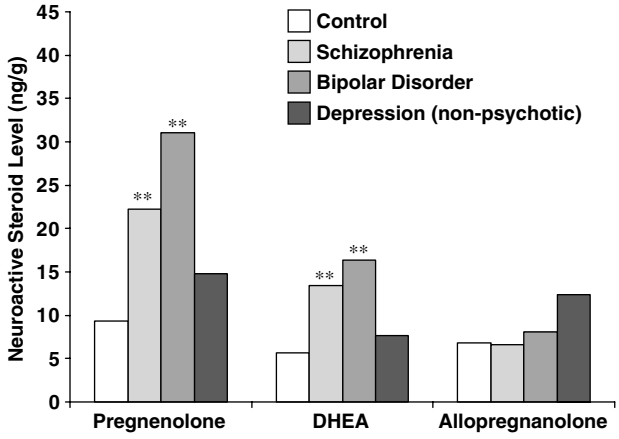


**Figure 2** Median neuroactive steroid levels in posterior cingulate in control subjects without a psychiatric diagnosis and in patients with schizophrenia, bipolar disorder, and depression (nonpsychotic). Pregnenolone levels (log transformed) are significantly higher in posterior cingulate tissue from subjects with schizophrenia and bipolar disorder compared to control subjects (ANOVA $p = 0.0017$; df 3,55; $F = 5.73$; *post hoc* Dunnett $^{**}p < 0.01$ for both schizophrenia and bipolar disorder groups, $n = 15$ per group). DHEA levels (log transformed) are significantly higher in posterior cingulate tissue from subjects with schizophrenia and bipolar disorder compared to control subjects (ANOVA $p = 0.0015$; df 3,55; $F = 5.84$; *post hoc* Dunnett $^{**}p < 0.01$ for both schizophrenia and bipolar disorder groups, $n = 15$ per group).

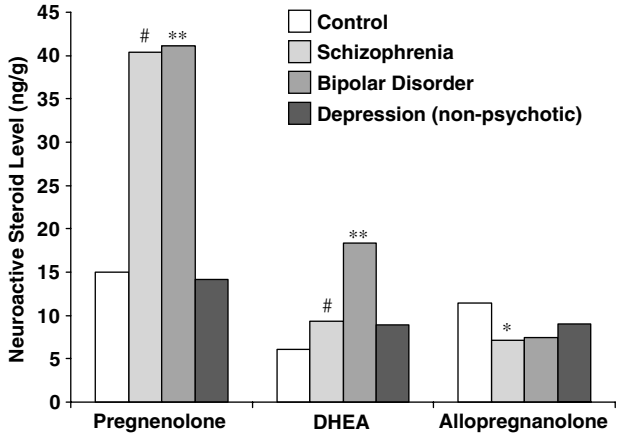


**Figure 3** Median neuroactive steroid levels in parietal cortex in control subjects without a psychiatric diagnosis and in patients with schizophrenia, bipolar disorder, and depression (nonpsychotic). Pregnenolone levels (log transformed) were significantly higher in parietal cortex tissue from subjects with bipolar disorder compared to control subjects (ANOVA $p = 0.0046$; df 3,56; $F = 4.844$; *post-hoc* Dunnett $^{**}p < 0.01$ for the bipolar disorder group, $n = 15$). Pregnenolone levels also tended to be higher in the schizophrenia group, but this finding was reduced to a trend in this brain region (*post hoc* Dunnett $^{\#}p = 0.06$, $n = 15$). DHEA levels (log transformed) were significantly higher in parietal cortex tissue in subjects with bipolar disorder compared to control subjects (ANOVA $p = 0.0087$; df 3,56; $F = 4.272$; *post hoc* Dunnett $^{**}p < 0.01$ for the bipolar disorder group, $n = 15$). DHEA levels also tended to be higher in the schizophrenia group, but this finding was reduced to a trend in this brain region (*post hoc* Dunnett $^{\#}p = 0.06$, $n = 15$). Allopregnanolone levels (log transformed) tended to be lower in parietal cortex tissue from subjects with schizophrenia compared to control subjects (ANOVA $p = 0.0911$; df 3,56; $F = 2.263$; *post hoc* Dunnett $^{*}p = 0.04$ for the schizophrenia group, $n = 15$).

schizophrenia group, Figure 3. Allopregnanolone levels (log transformed) tended to be lower in parietal cortex tissue from subjects with schizophrenia compared to control subjects, Figure 3.

The neuroactive steroids pregnenolone and DHEA are therefore similarly altered in both brain regions tested (posterior cingulate and parietal cortex) in patients with schizophrenia and bipolar disorder. It is thus possible that pregnenolone may be preferentially metabolized to DHEA

rather than allopregnanolone in these subjects, potentially leading to a shift toward DHEA formation and away from allopregnanolone synthesis (Figure 1). A preferential shift toward DHEA formation could potentially result in a net increase in neuronal excitation, since DHEA (which is increased in patients with schizophrenia and bipolar disorder) is a positive modulator of excitatory NMDA receptors and allopregnanolone (which tends to be decreased in patients with schizophrenia in parietal cortex) potentiates inhibitory $GABA_A$ receptor response.

## Secondary Analyses

*Preliminary findings in posterior cingulate and parietal cortex: neuroactive steroids analyzed separately by sex.* Since a number of neuroactive steroids appear to be regulated differently in males and females, separate statistical analyses were conducted for posterior cingulate and parietal cortex neuroactive steroid levels in male and female subjects. These analyses should be viewed as preliminary and exploratory, given small sample sizes for male subjects ($n = 8$–9) and female subjects ($n = 6$) in each group. Briefly, significant findings persist in male subjects with schizophrenia and bipolar disorder (and may even be strengthened), but do not appear to persist in female subjects with these disorders in either posterior cingulate or parietal cortex, Table 3. These findings may simply reflect decreased power in the analyses of the female subjects, however, since there are 50% fewer subjects in this group compared to male subjects (hence introducing the possibility of Type II error). Findings will clearly require replication in a larger cohort.

**Table 3** Median Neuroactive Steroid Levels (ng/g) in Posterior Cingulate and Parietal Cortex in Males and Females

| | Posterior cingulate | | Parietal cortex | |
|---|---|---|---|---|
| | **Male** | **Female** | **Male** | **Female** |
| *Pregnenolone* | | | | |
| ANOVA *P*-value | 0.0014 | 0.128 | 0.0019 | 0.190 |
| Control | 7.49 | 12.60 | 14.44 | 26.44 |
| Schizophrenia | 31.45** | 13.55 | 44.87** | 27.66 |
| Bipolar | 21.45** | 37.63 | 35.30* | 63.41 |
| Depression | 11.44 | 18.62 | 12.50 | 33.85 |
| *DHEA* | | | | |
| ANOVA *P*-value | <0.0001 | 0.542 | 0.0004 | 0.652 |
| Control | 5.17 | 6.51 | 4.87 | 6.49 |
| Schizophrenia | 17.39*** | 8.00 | 16.15* | 6.86 |
| Bipolar | 19.81*** | 11.68 | 29.55** | 12.17 |
| Depression | 4.87 | 11.08 | 4.14 | 9.12 |
| *Allopregnanolone* | | | | |
| ANOVA *P*-value | 0.164 | 0.810 | 0.478 | 0.283 |
| Control | 6.42 | 8.53 | 8.80 | 12.40 |
| Schizophrenia | 6.55 | 7.51 | 7.16 | 7.38 |
| Bipolar | 8.12 | 7.98 | 7.00 | 10.43 |
| Depression | 13.75 | 3.03 | 8.63 | 9.84 |

ANOVAs performed on log-transformed neuroactive steroid levels.
*Post hoc* Dunnett's test *$p<0.05$, **$p<0.01$, ***$p<0.001$.

*Neuroactive steroids and clozapine.* Median levels of the neuroactive steroid allopregnanolone were higher in posterior cingulate (but not parietal cortex) in patients with schizophrenia or bipolar disorder who were receiving clozapine at the time of death (12.45 ng/g, $n = 7$) compared to those who were not receiving clozapine at this time point (7.44 ng/g, $n = 23$); however, these differences did not achieve statistical significance (Mann–Whitney *U*-test statistic $p > 0.05$). Median DHEA levels were lower in patients receiving clozapine (7.70 ng/g, $n = 7$) compared to subjects who were not receiving clozapine (18.33 ng/g, $n = 23$), but these differences were also not statistically significant (Mann–Whitney *U*-test statistic $p > 0.05$). Similarly, analyses within the schizophrenia and bipolar groups comparing subjects receiving clozapine to those who were not receiving clozapine at the time of death were not statistically significant. It is possible, however, that the small number of subjects in the clozapine-treated group(s) resulted in reduced statistical power and the introduction of Type II error in this cohort.

*Neuroactive steroids and lifetime antipsychotic exposure.* In posterior cingulate (but not parietal cortex), allopregnanolone levels tended to be positively correlated with lifetime antipsychotic exposure (in fluphenazine equivalents) in schizophrenia subjects (Pearson's $r = 0.45$, $p = 0.094$, $n = 15$) and bipolar disorder subjects (Pearson's $r = 0.47$, $p = 0.077$, $n = 15$). The absence of significant correlations may not be entirely unexpected, since only $\sim$33% of patients with schizophrenia ($n = 5$) and $\sim$13% of patients with bipolar disorder ($n = 2$) in this study were receiving clozapine at the time of death (none were receiving olanzapine), and the conventional antipsychotic haloperidol and the second generation antipsychotic risperidone do not appear to alter neuroactive steroids in rodent models (Marx *et al*, 2003; Barbaccia *et al*, 2001; Trost *et al*, 2005).

## DISCUSSION

A primary finding in this investigation is that a number of neuroactive steroids (pregnenolone, DHEA, allopregnanolone) are present in human post-mortem brain tissue at physiologically relevant concentrations in the nanomolar range. For example, pregnenolone and DHEA (Flood *et al*, 1992) and pregnenolone sulfate (Flood *et al*, 1992, 1995; Akwa *et al*, 2001) enhance learning and memory in rodents at low nanomolar concentrations. These DHEA, pregnenolone, and pregnenolone sulfate actions may therefore be particularly relevant to cognitive deficits observed in subjects with schizophrenia. DHEA and DHEAS also have trophic effects on axonal and dendritic outgrowth, respectively, at similar concentrations (Compagnone and Mellon, 1998), and allopregnanolone positively modulates $GABA_A$ receptors at low nanomolar concentrations (Morrow *et al*, 1987, 1990). Since decreased neuropil (Glantz and Lewis, 2000; Harrison and Weinberger, 2005, review) and changes

in the GABA neurotransmitter system (Benes and Berretta, 2001; Wassef *et al*, 2003; Costa *et al*, 2004; Lewis *et al*, 2004; Coyle, 2004) are consistent findings in the schizophrenia literature, neuroactive steroids may be candidate modulators of these alterations and merit investigation. In addition, evidence suggests that patients with bipolar disorder may also demonstrate neuropil reductions (Gray *et al*, 2003, review). Pregnenolone, DHEA, and allopregnanolone may therefore have a functional impact on a number of different neurophysiological processes that are relevant to schizophrenia and bipolar disorder. To our knowledge, this is the first investigation to report neuroactive steroid levels in postmortem brain tissue from subjects with these disorders.

Our primary analyses determined that pregnenolone and DHEA levels were higher in subjects with schizophrenia and bipolar disorder compared to control subjects in both posterior cingulate and parietal cortex. The precise significance of these neuroactive steroid elevations in two brain regions in patients with schizophrenia and bipolar disorder is currently unclear, and a number of questions remain. For example, could higher levels be the result of enhanced biosynthesis and/or decreased metabolism to downstream neuroactive steroids, reflecting changes in the regulation of biosynthetic pathways? Could these changes potentially alter the regulation of excitatory and/or inhibitory neurotransmission? Do these increases reflect compensatory upregulation, pathologic dysregulation, or epiphenomena? We provide a brief framework for these and other inquiries below.

In our secondary analyses, neuroactive steroid findings appear to persist in male subjects with schizophrenia and bipolar disorder when neuroactive steroid levels are analyzed separately by sex, but this may not be the case for female subjects with these disorders (although the possibility of Type II error cannot be excluded, given smaller sample sizes in the female groups). Our secondary analyses addressing antipsychotic exposure did not result in significant findings, but possible trends emerged and are discussed below.

## Potential Changes in the Regulation of Neuroactive Steroid Biosynthetic Pathways in Schizophrenia and Bipolar Disorder

The regulation of neuroactive steroids in the brain is likely exceedingly complex, as these molecules are components of dynamic biosynthetic pathways that include both reversible and irreversible reactions and encompass a hundred or more steroids. In the current investigation, pregnenolone and DHEA are elevated in subjects with schizophrenia and bipolar disorder in both posterior cingulate and parietal cortex. Since pregnenolone is a potential precursor for many steroids, it is important to consider downstream pregnenolone metabolite effects that include a large number of possibilities and a myriad of combinations. For example, pregnenolone can be metabolized to neuroactive steroids that have opposite effects on $GABA_A$, NMDA, and other membrane-bound ligand-gated ion channel receptors, potentially resulting in a highly versatile mechanism impacting the regulation of net inhibitory or excitatory neurotransmission. This investigation suggests that pregnenolone may be preferentially metabolized to DHEA rather than allopregnanolone in subjects with schizophrenia and bipolar disorder. Since both pregnenolone sulfate and DHEA are positive modulators of excitatory NMDA receptors and allopregnanolone is a potent positive modulator of inhibitory $GABA_A$ receptors, the neuroactive steroid milieu in subjects with schizophrenia and bipolar disorder may be one of a net increase in neuronal excitation. Neuroactive steroid alterations may therefore represent a possible mechanism for the complex orchestration of major brain neurotransmitter systems in patients with schizophrenia and bipolar disorder.

Also potentially relevant to the regulation of neuroactive steroid biosynthetic pathways, genes involved in cholesterol biosynthesis appear to be downregulated in subjects with schizophrenia (Prabakaran *et al*, 2004). Since cholesterol is a precursor for all steroids, alterations in cholesterol synthesis may also impact downstream neuroactive steroid metabolites. Conversely, upregulated pathways in patients with schizophrenia appear to include a metabolism pathway encompassing transcripts of the P450scc (CYP11A1) gene encoding the enzyme catalyzing the conversion of cholesterol to pregnenolone, and the START domain in the outer mitochondrial membrane, among others (Prabakaran *et al*, 2004). Although no significant changes were observed in transcripts for these individual genes utilizing stringent criteria addressing multiple comparisons, significant upregulation in the overall hormone metabolism pathway that was targeted suggests that neuroactive steroid regulation may be altered in schizophrenia (Prabakaran *et al*, 2004). These steroid metabolism pathway changes may therefore represent a potential mechanism for higher levels of pregnenolone and DHEA (a pregnenolone metabolite) in subjects with schizophrenia.

## Neuroactive Steroid Alterations and Possible Ramifications for Glutamatergic and GABAergic Neurotransmission

Pregnenolone and DHEA are elevated in two brain regions in subjects with schizophrenia and bipolar disorder in these investigations. Since both pregnenolone sulfate and DHEA demonstrate positive modulatory actions at NMDA receptors, the glutamatergic neurotransmitter system may be impacted by these alterations in posterior cingulate and parietal cortex. Relevant to this possibility, the glutamatergic neurotransmitter system may be altered in subjects with schizophrenia and is theorized to be a logical target for the development of new therapeutic strategies (Javitt, 2004, review). It has been demonstrated that NMDA antagonists such as ketamine are psychotomimetic, exacerbating psychotic symptoms in subjects with schizophrenia and inducing psychotic symptoms in healthy control subjects (Krystal *et al*, 1994; Malhotra *et al*, 1997; Lahti *et al*, 1995, 2001). These findings have informed the hypothesis that NMDA receptor hypofunction represents a key component of schizophrenia pathophysiology. Since pregnenolone sulfate and DHEA have positive modulatory actions at NMDA receptors (Irwin *et al*, 1994; Wu *et al*, 1991; Compagnone and Mellon, 1998; Debonnel *et al*, 1996; Bergeron *et al*, 1996; Sliwinski *et al*, 2004) and increase learning and memory in rodent models (Flood *et al*, 1992, 1995; Akwa *et al*, 2001; Vallee *et al*, 1997, 2003), elevated

levels of these compounds could have therapeutic value. The NMDA receptor antagonist D-2-amino-5-phosphonovalerate (D-AP5) prevents the memory-enhancing effect of pregnenolone sulfate, suggesting this action may involve NMDA receptors (Akwa *et al*, 2001). Pregnenolone sulfate administration also prevents cognitive deficits following the NMDA receptor antagonists dizoclipine maleate (MK-801) and D-AP5 (Romeo *et al*, 1994; Mathis *et al*, 1996), supporting the possibility that NMDA receptor modulation contributes to the positive actions of this neuroactive steroid on learning and memory. Furthermore, pregnenolone sulfate appears to increase long-term potentiation in rat hippocampus by an NMDA receptor-mediated mechanism (Sliwinski *et al*, 2004). Elevations in pregnenolone and DHEA in posterior cingulate and parietal cortex may thus be relevant to hypothesized NMDA receptor hypofunction and cognitive symptoms in patients with schizophrenia.

### Neuroactive Steroid Alterations in Schizophrenia and Bipolar Disorder: Compensatory Upregulation *vs* Pathological Dysregulation, and Potential Impact on Brain Function

*Neuroactive steroids and neuroprotection.* DHEA levels are elevated in subjects with schizophrenia and bipolar disorder in posterior cingulate and parietal cortex, and these changes may impact brain function. For example, DHEA is neuroprotective against a number of insults resulting in oxidative stress, and these protective actions may be relevant to schizophrenia pathophysiology. DHEA and its sulfated derivative DHEAS protect neurons against NMDA-induced excitotoxicity (Kimonides *et al*, 1998; Kurata *et al*, 2004) and anoxia (Marx *et al*, 2000b) in rodent models. DHEA also protects neurons against glutamate and amyloid $\beta$-protein toxicity (Cardounel *et al*, 1999), $CuSO_4$-induced oxidative damage (Boccuzzi *et al*, 1997), $H_2O_2/FeSO_4$-stimulated lipid oxidation (Bastianetto *et al*, 1999), and hyperglycemia-induced toxicity (Aragno *et al*, 1997). In addition, DHEA appears to have direct actions on mitochondrial function, dose dependently protecting mitochondrial membranes from anoxia-reoxygenation oxidative stress (Morin *et al*, 2002). Since DHEA levels are elevated in two brain regions in subjects with schizophrenia and bipolar disorder in the current investigation, it is possible these alterations may result in neuroprotection against oxidative stressors and represent an adaptive change. Initial evidence suggests that administering agents known to induce oxidative stress, such as ferrous sulfate and $\beta$-amyloid peptide, result in increased DHEA formation (Brown *et al*, 2003). Oxidative stressors my therefore precede and possibly precipitate elevated DHEA levels observed in these investigations.

*Neuroactive steroids and myelination.* Pregnenolone and DHEA elevations in subjects with schizophrenia and bipolar disorder may be relevant to the regulation of myelination in these disorders. Changes in cholesterol biosynthetic pathways in patients with schizophrenia (Prabakaran *et al*, 2004) likely also impact myelination processes, since cholesterol is a critical myelin component and genes involved in cholesterol synthesis and myelin formation are coregulated (Nagarajan *et al*, 2002). Converging evidence from microarray (Hakak *et al*, 2001; Tkachev *et al*, 2003) and neuroimaging (Szeszko *et al*, 2005) investigations suggest a dysregulation in myelination in schizophrenia. Efforts demonstrating alterations in myelin-producing oligodendrocytes in patients with schizophrenia also support this possibility (Uranova *et al*, 2001; Hof *et al*, 2003). Since an expanding literature suggests a role for neuroactive steroids in the regulation of myelination, it is possible that neuroactive steroid alterations observed in this investigation may impact this component of schizophrenia pathophysiology. For example, pregnenolone increases myelin sheath thickness following sciatic nerve cryolesion (Koenig *et al*, 1995). Also implicating a modulatory role for neuroactive steroids in myelination, chronic pregnenolone treatment improved locomotor behavior in myelin mutant mice (Bloom *et al*, 2002). DHEA treatment increases the number of myelinated fibers in rat sciatic nerve and enhances functional recovery following crush injury (Gudemez *et al*, 2002), and increases axonal outgrowth *in vitro* (Compagnone and Mellon, 1998). Allopregnanolone increases myelin basic protein expression *in vitro* (Ghoumari *et al*, 2003), and deficits in this neuroactive steroid have been linked to neurodegenerative disorders in which myelination is dysregulated, including Neimann–Pick type C disease (Mellon *et al*, 2004) and Alzheimer's disease (Trost *et al*, 2004). Together these investigations suggest a positive modulatory role for neuroactive steroids in myelination, and it is therefore possible that pregnenolone and DHEA elevations in subjects with schizophrenia represent a compensatory upregulation of these neuroactive steroids.

*Neuroactive steroids as candidate modulators of stress regulation.* DHEA levels were higher in both posterior cingulate and parietal cortex in subjects with schizophrenia and bipolar disorder compared to control subjects, yet the administration of exogenous DHEA as an augmentation strategy appears to have therapeutic effects on negative, depressive, and anxiety symptoms in patients with schizophrenia (Strous *et al*, 2003). Since DHEA has positive modulatory actions on NMDA receptors (Debonnel *et al*, 1996; Bergeron *et al*, 1996) and also enhances learning and memory in rodent models (Flood *et al*, 1992; Vallee *et al*, 2001), it is tempting to speculate that DHEA elevations in brain may reflect a compensatory process. It is also possible that subjects with schizophrenia and bipolar disorder may be physiologically resistant to DHEA actions in some manner (potentially resulting in the increased formation of this neuroactive steroid), or that there is dysregulation in a feedback system involving the hypothalamic–pituitary–adrenal (HPA) axis. Specifically, DHEA increases following corticotrophin-releasing hormone (CRH) (Genazzani *et al*, 1998; Bernardi *et al*, 2000) and ACTH administration (Rasmusson *et al*, 2004) in humans, and therefore persistent DHEA elevations may reflect a prolonged upregulation of the HPA axis. DHEA also demonstrates antiglucocorticoid actions (Kimonides *et al*, 1999; Karishma and Herbert, 2002; Hu *et al*, 2000; Kalimi *et al*, 1994), supporting a role for DHEA in HPA axis regulation. DHEA administration to human subjects appears to result in significantly higher serum allopregnanolone and lower cortisol levels (Genazzani *et al*, 2003), and these findings may be consistent with allopregnanolone-induced decreases in CRH, ACTH, and

corticosterone release observed in rodents (Patchev *et al*, 1994, 1996; Guo *et al*, 1995) and DHEA antiglucocorticoid effects. Increased DHEA levels in subjects with schizophrenia and bipolar disorder and a trend for decreased allopregnanolone levels in parietal cortex in subjects with schizophrenia may therefore be relevant to HPA axis regulation and stress modulation.

## Neuroactive Steroids and Potential Implications for Sex Differences in Schizophrenia and Bipolar Disorder

An extensive literature documents sex differences in the epidemiology and course of patients with schizophrenia. Female patients with schizophrenia demonstrate a later age of onset, better premorbid functioning, and fewer negative symptoms than male subjects with the disorder (reviews Seeman, 2004; Tamminga, 1997; Hafner, 2003; Aleman *et al*, 2003). Imaging investigations suggest that female patients with schizophrenia may demonstrate fewer structural brain alterations compared to male subjects with the disorder (Andreasen *et al*, 1990; Nopoulos *et al*, 1997; Gur *et al*, 2000; Goldstein *et al*, 2002). In addition, there appear to be sex differences in functional connectivity (Slewa-Younan *et al*, 2004) and neuropsychological correlates to hippocampal volumes (Szeszko *et al*, 2002) in patients with schizophrenia. It has been hypothesized that the hormonal environment is neuroprotective in some manner in female patients with schizophrenia (Halbreich and Kahn, 2003; Hafner, 2003; Seeman, 2004), but the precise neurobiology of these sex differences has remained elusive. Similarly to schizophrenia, there also appear to be sex differences in the age of onset of mania and bipolar disorder, with males demonstrating earlier onset after controlling for premorbid factors (Kennedy *et al*, 2005). Female subjects with bipolar disorder may be more likely to demonstrate depressive episodes and rapid-cycling (Arnold, 2003, review), and there appear to be sex differences in vesicular monoamine transporter binding in bipolar patients (Zubieta *et al*, 2000). Sex differences therefore appear relevant to the pathophysiologies of both schizophrenia and bipolar disorder.

Our analyses demonstrate that significant pregnenolone and DHEA elevations persist (and may possibly be strengthened) in male patients with schizophrenia and bipolar disorder in both posterior cingulate and parietal cortex when data are analyzed separately by sex. When female subjects are analyzed separately, however, these pregnenolone and DHEA findings do not appear to persist in either brain region. It is therefore possible that pregnenolone and DHEA elevations in patients with schizophrenia and bipolar disorder may be specific to males, potentially yielding clues to the neurobiology of sex differences in these disorders. These secondary analyses are exploratory, however, and the possibility of sex differences in brain neuroactive steroid levels must be tempered with acknowledgement that sample sizes in female subjects are very limited ($n = 6$ per group), and that these female groups may thus be too small to detect differences. Nonetheless, separate analyses by sex were very similar in both posterior cingulate and parietal cortex, potentially providing initial evidence that neuroactive steroids may represent candidate modulators of sex differences in these disorders. Replication of these findings in a larger cohort of subjects will be required to test this possibility, and to inform future hypotheses regarding the relevance of neuroactive steroids to the clinical manifestations of sex differences in schizophrenia and bipolar disorder.

## Antipsychotic Drug Exposure and Neuroactive Steroids

Based on prior rodent findings (Marx *et al*, 2000a, 2003; Barbaccia *et al*, 2001; Trost *et al*, 2005), we hypothesized that patients with schizophrenia or bipolar disorder receiving clozapine at the time of death would demonstrate elevated allopregnanolone levels compared to subjects with these disorders receiving conventional antipsychotics, no antipsychotic, or risperidone at this time point. Although median allopregnanolone levels were 67% higher in patients receiving clozapine compared to those who were not receiving clozapine, this difference did not achieve statistical significance. The analysis was limited by small sample size, however, since only seven patients were receiving clozapine at the time of death. The concurrent use of other psychiatric medications is also potentially confounding. For example, the selective serotonin reuptake inhibitor (SSRI) fluoxetine increases brain allopregnanolone levels in rodent models (Uzunov *et al*, 1996) and appears to increase cerebrospinal fluid allopregnanolone levels in patients with depression (Uzunova *et al*, 1998). Carbamazepine also appears to increase allopregnanolone levels in rodents (Serra *et al*, 2000). A total of four subjects with schizophrenia or bipolar disorder were receiving SSRIs at the time of death (two in each group) and three subjects were receiving carbamazepine (one and two in each group, respectively), but neither SSRI nor carbamazepine status appeared related to neuroactive steroid levels in patients with these disorders (data not shown). Analyses addressing medication status at the time of death with regard to agents hypothesized to alter neuroactive steroid levels are clearly challenging. Limitations include small sample sizes, substantial numbers of patients receiving more than one psychiatric medication at the time of death (10/15 and 12/15 patients with schizophrenia and bipolar disorder, respectively), and the presence of a number of agents known to alter neuroactive steroid levels in rodents.

Allopregnanolone levels tended to be positively correlated with lifetime antipsychotic exposure in both schizophrenia and bipolar disorder in posterior cingulate, but these results did not achieve statistical significance and trends were observed in only one brain region. Lifetime antipsychotic exposure is very difficult to assess, however, and it is possible that these estimates add variability to the sample. Although clozapine and olanzapine dose dependently increase allopregnanolone in rodents, antipsychotic-induced neuroactive steroid changes following haloperidol or risperidone were not detected in animal models (Marx *et al*, 2003; Barbaccia *et al*, 2001; Trost *et al*, 2005). Since neuroactive steroid changes appear specific to the administration of certain second generation antipsychotics in rodents, it might be expected that correlations would be less strong in a mixed cohort of subjects exposed to both conventional and second generation agents. Analyzing a variable that converts two classes of antipsychotics to a single common denominator (ie fluphenazaine equivalents) may thus be somewhat limited.

### Study Limitations and Potential Confounding Factors

A major study limitation is small sample size ($n = 15$ in each group), a challenge confronting many human post-mortem tissue investigations. Given the scarcity of well-characterized samples from patients with psychiatric disorders, however, this limitation is not unexpected. Data regarding neuroactive steroid levels in human brain tissue are extremely limited, as previously discussed. To address potential confounding elements, we must therefore extrapolate from rodent investigations and human studies in the periphery. Possible confounders in this investigation include uncontrolled medication status at the time of death, as discussed above. In addition, both acute (Morrow *et al*, 1995; Girdler *et al*, 2001; Purdy *et al*, 1991; Barbaccia *et al*, 1998; Vallee *et al*, 2000) and chronic (Dong *et al*, 2001; Pinna *et al*, 2003; Wolkowitz *et al*, 2001) stressors impact neuroactive steroid levels in rodent brain and human plasma, and it is possible that a number of stressors may also alter neuroactive steroid levels in human brain. Smoking also increases DHEA levels (Field *et al*, 1994; Feldman *et al*, 1998; Mendelson *et al*, 2005), and we have recently determined that serum levels of allopregnanolone are correlated with salivary levels of cotinine (a nicotine metabolite) in subjects with nicotine dependence (Marx *et al*, in press). Nicotine also elevates neuroactive steroid levels in rodent models (Porcu *et al*, 2003). Smoking status at the time of death is hence an important potential confounder, given high rates of nicotine dependence in patients with schizophrenia and bipolar disorder. Unfortunately, tobacco history is not currently available for this cohort of subjects. Alcohol use may also alter neuroactive steroid levels (VanDoren *et al*, 2000; Pierucci-Lagha *et al*, in press). In addition, it is possible that menstrual cycle phase in female premenopausal subjects (and hormone replacement status in postmenopausal subjects) may affect neuroactive steroid levels in human brain, since rodent evidence suggests that central allopregnanolone levels fluctuate across the estrous cycle (Purdy *et al*, 1990; Corpechot *et al*, 1997; Frye and Bayon, 1998). Peripheral plasma allopregnanolone levels also demonstrate variability across the menstrual cycle in humans (Genazzani *et al*, 1998, 2002; Paul and Purdy, 1992; Pearson Murphy and Allison, 2000). Menstrual cycle phase data and hormone replacement status are unfortunately not available for this tissue collection. Since it was not feasible to control for these variables in female subjects, it is quite possible that they may represent additional confounding elements in our analyses of female patients, potentially contributing to the loss of a statistically significant signal. Finally, it is unknown if any patients were receiving cholesterol-lowering medications at the time of death. Since it is theoretically possible that decreasing cholesterol precursor could influence downstream steroid formation, this could also constitute a potential confounder.

### Conclusions

Neuroactive steroids represent candidate modulators of schizophrenia and bipolar disorder pathophysiology and therapeutics. The neuroactive steroids pregnenolone and DHEA are elevated in subjects with schizophrenia and bipolar disorder in both posterior cingulate and parietal cortex. Since the number of subjects in this investigation is small, results will require replication in a larger cohort. Nonetheless, very similar neuroactive steroid profiles were observed in two brain regions utilizing a highly sensitive and specific mass spectrometry-based method. Furthermore, pregnenolone, DHEA, and allopregnanolone levels determined in human post-mortem brain in these investigations are known to be physiologically relevant, suggesting that neuroactive steroid alterations in schizophrenia and bipolar disorder may have a functional impact on the pathophysiology of these disorders and do not merely represent epiphenomena. In addition, neuroactive steroid induction represents a potential mechanism contributing to the efficacy of several antipsychotic agents, and therefore these molecules merit further investigation as targets for pharmacological intervention. Future efforts will be required to characterize the precise regulation of neuroactive steroids in human brain, but initial findings that these molecules are significantly altered in post-mortem brain tissue from subjects with schizophrenia and bipolar disorder suggest a role for neuroactive steroids in these disorders.

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