Changes in the Cholinergic System between Bipolar Depression and Euthymia as Measured with [123I]5IA Single Photon Emission Computed Tomography

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Abstract

Background—The cholinergic system is substantially altered in individuals with major depression and is partially restored when depression remits. We quantified the availability of α_2-subunit-containing nicotinic acetylcholine receptors (α_2*-nAChR) in subjects with bipolar disorder.

Methods—Twenty-five subjects with bipolar disorder (15 depressed, 10 euthymic) and 25 sex- and age-matched control subjects had a [123I]5IA-85380 single photon emission computed tomography scan to quantify α_2*-nAChR V_T/f_P (total volume of distribution, corrected for individual differences in metabolism and protein binding of the radiotracer). Average V_T/f_P was compared between groups and correlated with clinical characteristics. Postmortem analysis of α_2*-nAChRs was conducted using equilibrium binding with [125I]5IA in subjects with bipolar disorder and matched control subjects.

Results—We showed significantly lower α_2*-nAChR availability (20%–38%) in subjects with bipolar depression compared with euthymic and control subjects across all brain regions assessed (frontal, parietal, temporal, and anterior cingulate cortex, hippocampus, amygdala, thalamus, striatum). The postmortem binding study in which endogenous acetylcholine was washed out did not show a statistically significant difference in α_2*-nAChR number in temporal cortex of the bipolar depressed and control groups (15% difference; $p = .2$).

Conclusions—We show that the alteration in the cholinergic system observed during a depressive episode appears to resolve during euthymia. We suggest that lower V_T/f_P observed in vivo may be due to a combination of higher endogenous acetylcholine levels during depression, which could compete with radiotracer binding to the receptor in vivo, and lower receptor number in bipolar depression. Identification of differences in cholinergic signaling in subjects with bipolar depression may improve our understanding of its etiology and reveal new treatment targets.

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Keywords

Acetylcholine; α_2*nAChR; bipolar disorder; depression; SPECT; tobacco smoking

Bipolar disorder is a chronic, debilitating illness with a 12-month prevalence of 2.6% and a lifetime prevalence of 3.9% (www.nimh.nih.gov/statistics). It is a leading cause of worldwide disability (1), mostly because of the depressive episodes (2), and is associated with increased risk of suicide, which is highest during depressive episodes. Although many mood stabilizers and antipsychotics are efficacious in the acute treatment and long-term prevention of manic episodes (3), fewer options are available for the treatment of the depressive phase of the illness (4,5). Antidepressant medications, which are efficacious in unipolar depression, are less effective for the acute treatment of bipolar depression (6). Therefore, there is a substantial need to develop medications that are more efficacious for the treatment of bipolar depression.

Several lines of evidence implicate dysfunction of the cholinergic system in mood disorders (7,8). Animal studies and human challenge studies suggest that decreased cholinergic activity is associated with mania, while increased acetylcholine (ACh) activity is associated with depression (9,10). ACh, a major neurotransmitter in the peripheral and central nervous systems, is released throughout the brain and binds to either muscarinic or nicotinic acetylcholine receptors (nAChRs), and brain ACh levels appear to be higher in individuals with major depressive disorder (MDD) (11). Rats bred for increased sensitivity to cholinergic agents show behaviors that resemble symptoms of depression, such as lethargy, reductions in self-stimulation, and increased behavioral despair (12). Furthermore, challenge with physostigmine, a cholinesterase inhibitor that increases ACh levels in the brain, induces significant depression-like symptoms both in human subjects with affective disorders and individuals with no history of psychiatric illness (13,14). The cholinergic system plays a major role in the sleep–wake cycle (15), learning, memory, attention (16,17), motivation, and reward (18,19); thus, dysfunction in this system may result in circadian abnormalities, cognitive deficits, and impaired reward function in individuals with bipolar depression.

Our understanding of the involvement of different neurotransmitters in bipolar depression has been hampered, in part due to lack of neuroreceptor imaging studies in this population. The substantial evidence implicating ACh and nAChRs in mood regulation and our previous findings of altered cholinergic activity in MDD that persists into remission (11) led us to determine whether there is any change in availability of α_2-subunit-containing (α_2*)-nAChRs in individuals with bipolar depression. We performed a $[^{123}I]$-5-iodo-3-[2(S)-2-azetidinylmethoxy]pyridine ($[^{123}I]5IA$) single photon emission computed tomography (SPECT) study to evaluate availability of α_2*-nAChR in subjects with active bipolar depression and in subjects who are currently euthymic and comparison control subjects. $[^{123}I]5IA$ has high affinity for the α_2*-nAChR and has been used previously to conduct studies of receptor availability (11,20,21) and occupancy (22,23). Our primary hypothesis was that subjects with bipolar disorder (currently depressed or euthymic) would have lower α_2*-nAChR availability compared with control subjects based on our previous studies of individuals with unipolar depression (11). We followed with a postmortem binding study as described previously (11) to evaluate whether there are differences in α_2*-nAChR number between individuals with and without bipolar disorder under conditions in which endogenous ACh is washed out. We hypothesized, based on previous findings in MDD subjects, that there would not be a difference in α_2*-nAChR number between bipolar and control groups in vitro.

Methods and Materials

Study Design

Each subject participated in one magnetic resonance imaging and one [123I]5IA SPECT scan within 3 weeks of screening. In subjects with bipolar disorder, mood symptoms were reassessed at the time of the SPECT scan to confirm that each subject was still in a depressive or euthymic episode.

Regulatory Approvals

The study was approved by the Institutional Review Boards for Yale University School of Medicine and Veteran Affairs Connecticut Healthcare System. The use of [123I]5IA was allowed by the U.S. Food and Drug Administration. All subjects signed informed consent before full study participation.

Screening and Eligibility

As described previously (11), each prospective subject had an interview with an experienced psychiatrist who elicited a complete psychiatric and medical history. Subjects underwent the Structured Clinical Interview for DSM-IV Disorders (SCID-I) (24) to confirm the diagnosis of bipolar depression, current episode (depression or euthymia), or absence of any psychiatric disease. The Montgomery-Åsberg Depression Rating Scale and the Beck Depression Inventory were used to determine the severity of the current depressive episode, and the Young Mania Scale was used to rule out a current manic or mixed episode. The study participants were 25 subjects with bipolar disorder and 25 age- and gender-matched healthy comparison subjects. Fifteen of the subjects with bipolar disorder were acutely depressed (9 medicated nonsmokers, 6 unmedicated nonsmokers), and 10 were euthymic for at least 2 months (all unmedicated, 5 smokers and 5 nonsmokers). Subjects with any other significant Axis I diagnosis were excluded from the study (except nicotine dependence for smoking subjects), and only those with a primary diagnosis of bipolar disorder were included. The unmedicated subjects had been medication-free for at least 3 months, and the acutely ill subjects scored above 16 on the Hamilton Rating Scale for Depression. Comparison nonsmoking subjects were included in the study only if they had no lifetime history of any Axis I as judged with the SCID-I (except for nicotine dependence for smoking comparison controls). One control subject was recruited for each bipolar subject and matched based on age (within 3 years), smoking status, and sex. Nonsmokers defined as smoking less than 40 cigarettes in their lifetime and none in the past year; smokers were defined as smoking more than 10 cigarettes per day for at least 1 year.

Pregnancy in women was ruled out by a serum beta-human chorionic gonadotropin (hCG) test at screening and a urine beta-hCG test on the day of the SPECT scan. Nonsmoking status was confirmed by plasma cotinine levels of less than 15 ng/mL at screening and on SPECT scan day, urine cotinine levels of less than 100 ng/mL (at screening and on the day of the scan), and exhaled carbon monoxide levels of less than 8 ppm at screening and on the day of the scan. Smoking status was confirmed by plasma cotinine levels greater than 50 ng/mL, urine cotinine levels greater than 200 ng/mL, and exhaled carbon monoxide levels greater than 11 ppm at screening.

Smoking Abstinence

Nicotine has high affinity for $_2$*-nAChRs in the brain and blocks or displaces the radioligand from binding to these receptors (22,23). Previous studies revealed that 1 week of smoking cessation is required for all nicotine to leave the brain to accurately quantify $_2$*-nAChR availability (25). All smoking subjects were asked to abstain from tobacco smoking and any nicotine products for about 1 week before SPECT scan. Smoking abstinence was

achieved through daily contingency management meetings and twice-daily urine cotinine and carbon monoxide collections. Subjects were paid for these appointments as described previously (26). On SPECT scan day, smoking abstinence was confirmed by plasma cotinine levels of less than 15 ng/mL, urine cotinine levels of less than 100 ng/mL, and exhaled carbon monoxide levels of less than 8 ppm.

Magnetic Resonance Imaging

Magnetic resonance imaging for coregistration was performed on a 3-Tesla Siemens Scanner (Erlangen, Germany; Magnetom Trio A Tim System; Software: Numaris/4; Version: syngo MR B17) to guide placement of regions of interest for SPECT scans (Series 1: 3 plane localizer; Series 2: Sag 3d Turbo Flash; 250 field of view; 1-mm slice thickness; 176 slices total; echo time 3.53; repetition time 2500; inversion time 1100; flip angle 7; 256 × 256 2 averages).

SPECT Imaging

SPECT emission scans were obtained on the Phillips PRISM 3000 XP (Cleveland, Ohio), a three-headed SPECT camera equipped with a low-energy, ultra-high-resolution fan beam collimator (photopeak window, 159 keV ± 10%; matrix 128 × 128) with a uniform sensitivity across the field of view. A 57Co-distributed source was measured with each experiment to control for day-to-day variation in camera sensitivity. The axial resolution (full width at half maximum) is 12.2 mm, measured with a ^{123}I line source in water in a cylindrical phantom. [^{123}I]5IA was synthesized as previously described (27) and administered through a venous catheter in the upper extremity, using a bolus-plus-constant-infusion paradigm with a ratio of 7.00 ± .03 hours for the bipolar depressed group, 7.00 ± .04 hours for the bipolar euthymic group, and 7.00 ± .04 hours for the control group, and a total injected dose (accounting for decay) of 341 ± 43.8 MBq for the bipolar depressed group, 347 ± 27.9 MBq for the bipolar euthymic group, and 337 ± 48.2 MBq for the control group. Blood samples for metabolite analyses were collected from another venous catheter. Approximately 6 hours after injection of [^{123}I]5IA, a simultaneous transmission emission protocol scan and three 30-minute equilibrium emission scans were obtained. Blood samples were collected at the midpoint of the scans to quantify total parent and free fraction (fP), to correct for individual differences in metabolism and protein binding of [^{123}I]5IA.

SPECT Image Analysis

As previously described (27), SPECT emission images were reconstructed using a filtered back projection algorithm with a ramp filter on a 128 × 128 matrix to obtain 50 slices with a pixel size of 2.06 × 2.06 × 3.56 mm in the x, y, and z axes. A three-dimensional (3D) Butterworth filter (order 10, cutoff frequency .24 cycle/pixel) was applied post hoc. A coregistered magnetic resonance image was used to guide the placement of standard two-dimensional region of interest templates using MEDx software (Medical Numerics, Germantown Maryland) as previously described (22,28). A 3D volume of interest was generated for each region and transferred to the coregistered SPECT image to determine regional radioactive densities. The chosen regions were known to contain $_2$*-nAChRs and included the frontal, anterior cingulate, and temporal cortices, thalamus, striatum (an average of caudate and putamen), hippocampus, and amygdala (29,30). We previously showed significant sex differences in metabolism and protein binding of [^{123}I]5IA; thus, regional [^{123}I] 5IA uptake was determined by V_T/f_P (total volume of distribution corrected for metabolism and protein binding of radiotracer), which corrects for this variability.

In Vitro Binding Assays

Samples of temporal cortex from eight depressed individuals with bipolar disorder (three nonsmoking, aged 55.7 ± 18.2 years; five smoking, aged 31.7 ± 19.2 years) who had committed suicide, and from eight sex-, smoking-, and age-matched control subjects (three nonsmoking, aged 56.7 ± 18.2 years; five smoking, aged 36.6 ± 18.6 years) were obtained from the Canadian Brain Bank. The tissue prisms were split on an ice-cold surface in an orientation that provided approximately equal amounts of gray and white matter. Tissue was homogenized in a chilled glass tissue grinder in 1 mL .1 × physiological buffer (PB) (14.4 mmol/L NaCl, .22 mmol/L KCl, .2 mmol/L CaCl2, .1 mmol/L MgSO4, 25 mmol/L (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) Hemi-Na) plus protease inhibitors (10 μg/mL aprotinin, leupeptin, pepstatin A). The resulting homogenate was centrifuged at 20,000 g for 5 minutes, and the supernatant was collected and saved. The pellet was resuspended in 1 m: of .1 × PB. For membrane binding with [^{125}I]5IA (Perkin Elmer, Waltham, Massachusetts), a 100-μL aliquot of each homogenate was diluted 1:10 in .1 × PB, centrifuged at 20,000 relative centrifugal force for 5 minutes, supernatant discarded, and the pellet was resuspended in 1 mL .1 × PB (procedure was repeated three times). Protein content was determined using the Lowry method, aliquots of 10 μL were used for 5IA binding, taken in triplicate (duplicate total binding, one for nonspecific), and 10 μL of 3 × PB was added to each well. [^{125}I]5IA (2200 Ci/mmol) was diluted in water, and 10 μL was added to each well at room temp for 4 hours. Nonspecific binding was measured in the presence of 100 μmol/L cytisine. Samples were collected by vacuum filtration (Packard Cell Harvester, Waltham, Massachusetts) on Pall (Washington, New York) glass fiber filters (type A/C and type A/D, sandwiched), soaked in .5% polyethylenimine, rinsed three times with approximately 1 mL per well of ice-cold PB. Radioactivity was quantified by liquid scintillation (Beckmann LS6000LL; Brea, California). Saturation of [^{125}I]5IA was calculated using a two-parameter hyperbolic equation of the form B = B$_{max}$[L]/Kd + [L] where B is measured binding at concentration of ligand [L] with apparent maximal binding (B$_{max}$) and equilibrium constant (K$_d$). To ensure the calculated parameters conformed to simple Michaelis-Menton kinetics, a double-reciprocal plot of each saturation curve was taken, and goodness of fit was assessed to a single polynomial equation of the form y = mx + b, where y = 1/measured binding, b = 1/B$_{max}$, and m = 1/K$_d$.

Statistical Analysis

Statistical analysis was performed using IBM SPSS v19.0 (Armonk, New York). Statistical significance was set at $p < .05$, two-tailed. Multivariate analysis of variance (MANOVA), which controls for Type II error, was used to measure differences in V_T/f_P between control, depressed and euthymic bipolar groups. Post hoc analyses (Bonferroni) were used to specify between group differences. Secondary analyses using MANOVA were conducted to examine the effect of medication on $_2$*-nAChRs in depressed bipolar subjects and the effect of smoking in euthymic bipolar subjects. The Spearman correlation coefficient was used to measure associations between receptor availability and clinical variables. Two-way analysis of variance was used to analyze the in vitro [^{125}I]5IA binding data.

Results

Demographic and Clinical Characteristics

Clinical and demographic characteristics are shown in Table 1 for bipolar depressed group and matched control subjects; Table 2 for euthymic bipolar group and matched control subjects; and Table 3 for all euthymic bipolar, depressed bipolar, and controls. The medication list for bipolar depressed subjects was as follows: Subject 1—aripiprazole, escitalopram, and bupropion; Subject 2—lamotrigine and oxcarbazepine; Subject 3—lithium and phenelzine; Subject 4—quetiapine, aripiprazole, and hydroxyzine; Subject 5—valproic

acid; Subject 6—lithium, fluoxetine, and topiramate; Subject 7—oxcarbazepine, dextroamphetamine, and citalopram; Subject 8—lamotrigine; and Subject 9—lithium, risperidone, alprazolam, and methylphenidate.

V_T/f_P in the Overall Sample

Results of the omnibus model including three groups revealed a significant effect of group (Hotelling's trace $F = 2.5$, $p = .004$). Post hoc tests showed that this difference was due to statistically significantly lower V_T/f_P in bipolar depressed compared with bipolar euthymic and control subjects (Table 1 and Table 3; Figure 1). Because the euthymic bipolar group and associated controls included smoking subjects, we removed the smoking subgroups and repeated the analyses. Omnibus model including three groups (bipolar depressed, bipolar euthymic nonsmokers, and control non-smokers) revealed a trend toward a significant effect of group (Hotelling's trace $F = 1.5$, $p = .1$). Post hoc tests showed that this difference was due to statistically significantly lower V_T/f_P in bipolar depressed compared with bipolar euthymic and control subjects.

Differences in V_T/f_P and Clinical Variables Due to Medication Status in Currently Depressed Subjects

We did not detect significant differences in clinical variables between medicated and unmedicated bipolar depression groups (all ps > .1). Results of the omnibus MANOVA modeling did not detect significant differences in V_T/f_P between medicated and unmedicated subjects with bipolar depression (Hotelling's trace $p = .85$). However, when we examined groups on the basis of medication status, in the unmedicated sample, we observed a significant negative correlation between self-reported depression score on the Beck Depression Inventory and V_T/f_P in the frontal cortex ($r_5 = -.83$, $p = .04$).

V_T/f_P in Euthymic Subjects as a Function of Smoking Status

To determine whether there is upregulation of $_2$*-nAChRs bipolar disorder, we performed a MANOVA within the euthymic bipolar group and detected that in vivo, smokers with bipolar disorder do not have higher V_T/f_P compared with age- and sex-matched nonsmokers with bipolar disorder (Hotelling's trace $F = 5.6$, $p = .16$; Figure 2). We confirmed previous findings in control smokers: significantly higher V_T/f_P compared with nonsmokers (Hotelling's trace $F = 10.0$, $p = .04$), and this was significant in the anterior cingulate, frontal, and parietal cortices ($F_{55} = 6.0$, $p = .04$; $F_{5,5} = 10.7$, $p = .01$; and $F_{5,5} = 8.8$, $p = .02$, respectively, with a trend in the temporal cortex $F_{5,5} = 4.0$, $p = .08$). No significant associations between V_T/f_P and clinical variables were observed.

In Vitro [^{125}I]5IA Binding

There were no significant differences in calculated [^{125}I]5IA K_d values for ^{125}I-A85380 binding in control versus bipolar smokers ($p > .9$) or control versus bipolar nonsmokers ($p > .8$; Student t test). The K_d values by group are as follows (mean ± SEM): control nonsmoker, 2.25E-8 ± 2.1E-9 mmol/L; bipolar nonsmoker, 2.6E-8 ± 4.5E-9 mmol/L; control smoker: 3.94E-8 ± 3.7E-9 mmol/L; bipolar smoker: 3.45E-9 ± 5.6E-9 mmol/L. Calculated B_{max} values are as follows: control nonsmoker, 47.5 ± 13.5 fmol/mg; bipolar nonsmoker, 37 ± 8.2 fmol/mg, control smoker, 127 ± 8.6 fmol/mg; bipolar smoker, 110 ± 11.4 fmol/ mg. Two-way analysis of variance showed a main effect of smoking status on K_d ($F_{1,15} = 7$, $p = .02$), and post hoc two-sided t tests showed $p = .017$ for the comparison of control smokers versus control nonsmokers (the others were all ps > .05). A small shift in affinity in the control smoker group may reflect differential posttranslational modification that could be worthy of further consideration. Measurement of high-affinity (200 pM) [^{125}I]5IA binding in tissue samples from human temporal cortex revealed a highly

significant effect of smoking status ($F_{1,14} = 104.4$, $p < .0009$) and a trend toward a bipolar diagnosis effect ($F_{1,14} = 2.13$, $p = .17$). The effect of smoking on 200 pM [^{125}I]5IA binding was further examined with saturation analysis of radioligand binding and detected a significant increase in the calculated B_{max} of [^{125}I]5IA ($F_{1,15} = 24.74$, $p < .0009$).

Discussion

We showed that compromise in the cholinergic system associated with depression in subjects with bipolar disorder appears to resolve during euthymia. Consistent with our previous study in unmedicated subjects with MDD (11), we found that currently depressed subjects with bipolar depression had significantly lower V_T/f_P of cortical (25%–38%) and subcortical (20%–24%) $_2$*-nAChRs compared with age- and sex-matched bipolar euthymic and control subjects. Contrary to the findings of the MDD study, we did not detect significant differences in V_T/f_P between bipolar euthymic and control subjects. In vitro $_2$*-nAChR quantification in the temporal cortex did not detect significant differences in receptor number in bipolar depressed subjects compared with control subjects, although there was a trend toward significance likely due to the 15% lower B_{max}/K_d in the bipolar depressed sample.

There are several possible explanations for the lower V_T/f_P in the bipolar depressed sample. Lower $_2$*-nAChR V_T/f_P in vivo might be due to fewer receptor complexes available on the cell surface or intracellularly in bipolar depression, lower nondisplace-able binding in the bipolar depressed group, or could result from more ACh competing with the radiotracer. In studies using intact cells, nicotinic ligands bind with equal efficiency to intracellular and extracellular nAChRs (31). Given the 29% difference in temporal cortex V_T/f_P and 15% difference in B_{max}/K_d, it is plausible that the observed lower binding in vivo is in part due to lower intracellular or extracellular pool in bipolar depression. Use of V_T/f_P as an outcome measure (sum of specific and nondisplaceable bindings) assumes uniform nondisplaceable binding across experimental groups. Although we previously calculated nondisplaceable binding in control smokers, this has not been done in individuals with mental illness. It is possible that individuals with bipolar disorder have lower nondisplaceable binding, which would reflect lower V_T/f_P and is a limitation to using V_T/f_P as outcome measure. Competition for radioligand binding by higher endogenous levels of ACh would be consistent with Janowsky's adrenergic-cholinergic balance hypothesis of depression, which posits that a depressive state may be due to overactivity of brain ACh (7,14). Preclinical evidence (32) and current clinical evidence (33) show that increasing endogenous ACh levels by blocking acetylcholinesterase (AChE) effectively competes with the radioligand for the binding to $_2$*-nAChRs. Thus, we suggest that the observed lower V_T/f_P in the current study may be due to higher extracellular ACh levels that could be associated with a depressed state, in combination with lower intra- and extracellular complexes.

The lack of observed differences in $_2$*-nAChR V_T/f_P between control and bipolar euthymic subjects in the in vivo sample was surprising. We had previously reported that the apparent lower V_T/f_P in vivo (i.e., higher endogenous ACh) during depression in unmedicated subjects with unipolar depression is persistent even during euthymia (11). Our finding of no difference, which may be interpreted as a resolution in the cholinergic compromise during euthymia, may be critical in understanding the role of cholinergic system in the mood states in individuals with bipolar disorder and may also contribute to the growing literature regarding neurochemical differences between unipolar and bipolar depression.

The reduced V_T/f_P in bipolar depression contributes to a growing literature showing that ACh can play a role in regulation of mood and motivation. The role of ACh in mood regulation may derive from the fact that ACh output to the cortex is driven in large part by

the limbic system (34), which is largely responsible for mood regulation. In turn, alterations in cholinergic tone affect signaling through nAChRs, and there are several lines of evidence suggesting that nAChR signaling may contribute to depression (7). Furthermore, some studies suggest that medications that act at nAChRs may have antidepressant properties (35,36). For example, up to 60% of individuals with bipolar depression smoke, likely because of the antidepressant effects of nicotine, which has high affinity for α_2*-nAChRs and desensitizes these receptors (for review see Mineur and Picciotto) (7). In addition, partial agonists (37) and antagonists (38) of α_2*-nAChR can enhance the anti-depressant effects of serotonin or norepinephrine reuptake inhibition, and a large number of studies suggest that decreasing the activity of ACh at nAChRs using antagonists or partial agonists results in antidepressant effects (39). Such data suggest that nAChRs, specifically those containing the α_2 subunit, are involved in mood regulation in humans.

Medication status did not appear to affect α_2*-nAChR V_T/f_P during a depressive episode in bipolar depressed subjects, although affirmation is required in a larger sample of medicated and unmedicated subjects. However, this finding is consistent with a previous study showing that treatment with selective serotonin reuptake inhibitors (SSRIs) did not have an effect on [^{123}I]5IA binding (40). Although SSRIs have been shown to block nAChRs, these effects appear to be non-competitive through actions on the channel pore (41). Interestingly, although there were no significant differences in self-reported symptoms of depression between the medicated and unmedicated groups in the current sample, in the unmedicated group, lower α_2*-nAChR V_T/f_P was significantly associated with greater numbers of reported depressive symptoms. This is an important observation that requires further investigation in a larger sample of medicated and unmedicated subjects with bipolar depression.

Our in vivo data show an apparent lack of upregulation in α_2*-nAChR V_T/f_P in smokers versus nonsmokers with bipolar disorder, whereas postmortem findings show a significant upregulation in the temporal cortex, although the upregulation appears to be blunted in tissue from bipolar subjects. It is likely that smokers with bipolar disorder do upregulate, and, because of the small sample size, we did not observe a detectable upregulation in vivo. Similar findings exist in subjects with schizophrenia (42), where postmortem quantification shows blunted upregulation of α_2*-nAChRs in smokers with versus without schizophrenia. Further examination is required to confirm whether α_2*-nAChRs are regulated in a different manner in individuals with mood disorders compared with control smokers.

There are several limitations to this study. First, we included both medicated and unmedicated bipolar depressed subjects The effect of medications on α_2*-nAChR V_T/f_P cannot be ruled out. For example, bupropion is a noncompetitive high-affinity nAChR antagonist (43) and may alter α_2*-nAChR V_T/f_P. Some reports also suggest SSRIs may weakly bind to α_2*-nAChR; however, a [^{123}I]5IA SPECT study showed that SSRI treatment did not affect radio-ligand binding (i.e., receptor availability) (40). Because we did not include a homogenous sample of medications, evaluations and conclusions of the effect of medications on α_2*-nAChR are limited. Also, subject compliance to medication routine was not evaluated (i.e., we did not ensure they adhered to their daily medication routine, and we did not check blood for therapeutic dose levels). Second, the postmortem sample is small (only three nonsmokers and five smokers) and limited to the temporal cortex; however, it is in line with previous findings in MDD sample. Furthermore, the quantitative evaluation of the static receptor number requires fewer subjects to obtain statistically relevant findings, and the greater effect of smoking on nAChR number in the postmortem study is in line with the published effects of smoking on nAChR upregulation in postmortem brain (30,42). Third, scanning smokers after smoking abstinence limits understanding of brain ACh levels

during active smoking. However, given that nicotine directly competes with radioligand for binding to the $_2$*-nAChRs, it is required that smokers abstain from smoking.

In conclusion, this is a unique examination of the cholinergic system across mood states in individuals with bipolar depression. We showed that during depression, there is lower $_2$*-nAChR V_T/f_P in vivo regardless of medication status, which is likely due to a combined effect of lower actual V_T/f_P and in vivo competition between radioligand and ACh. We also showed that there appears to be a restoration of cholinergic functioning during euthymia. Additional studies are needed to clarify the molecular underpinnings of the observed lower V_T/f_P during depressive state and to determine whether the cholinergic system may be a novel target for drugs aimed at treating bipolar depression or to help smokers with bipolar disorder during smoking abstinence to achieve smoking cessation without relapsing into a depressive episode.

Acknowledgments

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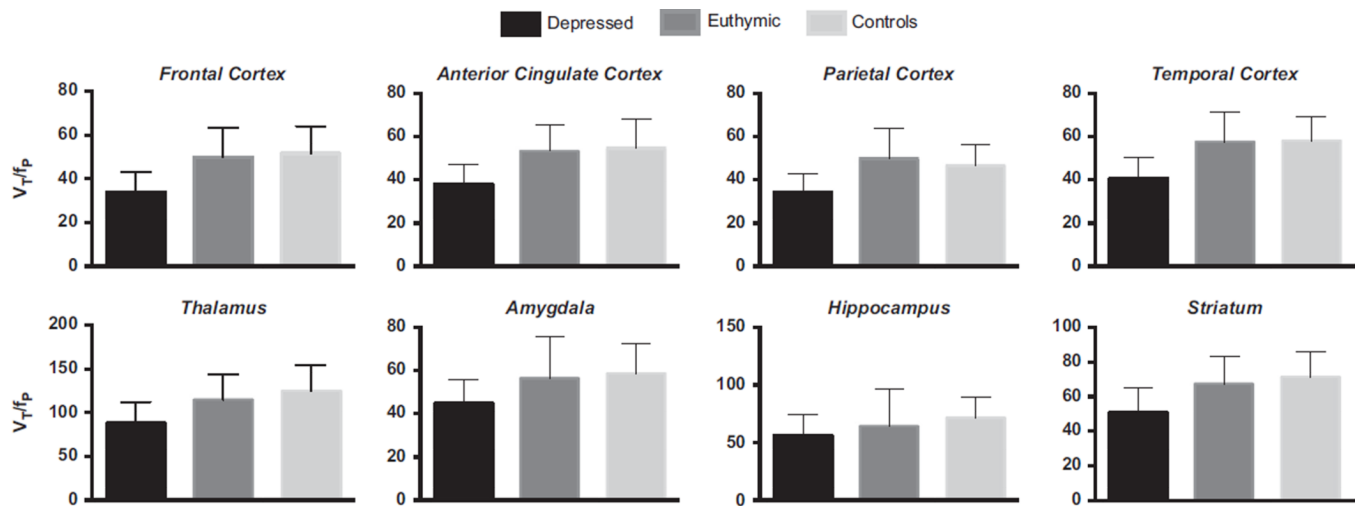
Figure 1.
Bars illustrating in vivo V_T/f_P in bipolar depressed ($n = 15$), bipolar euthymic ($n = 10$), and control ($n = 25$) subjects. Lower V_T/f_P was observed across all brain regions in bipolar depressed compared with bipolar euthymic and control subjects (Hotelling's trace $F = 2.5$, $p = .004$). V_T/f_P, receptor availability.
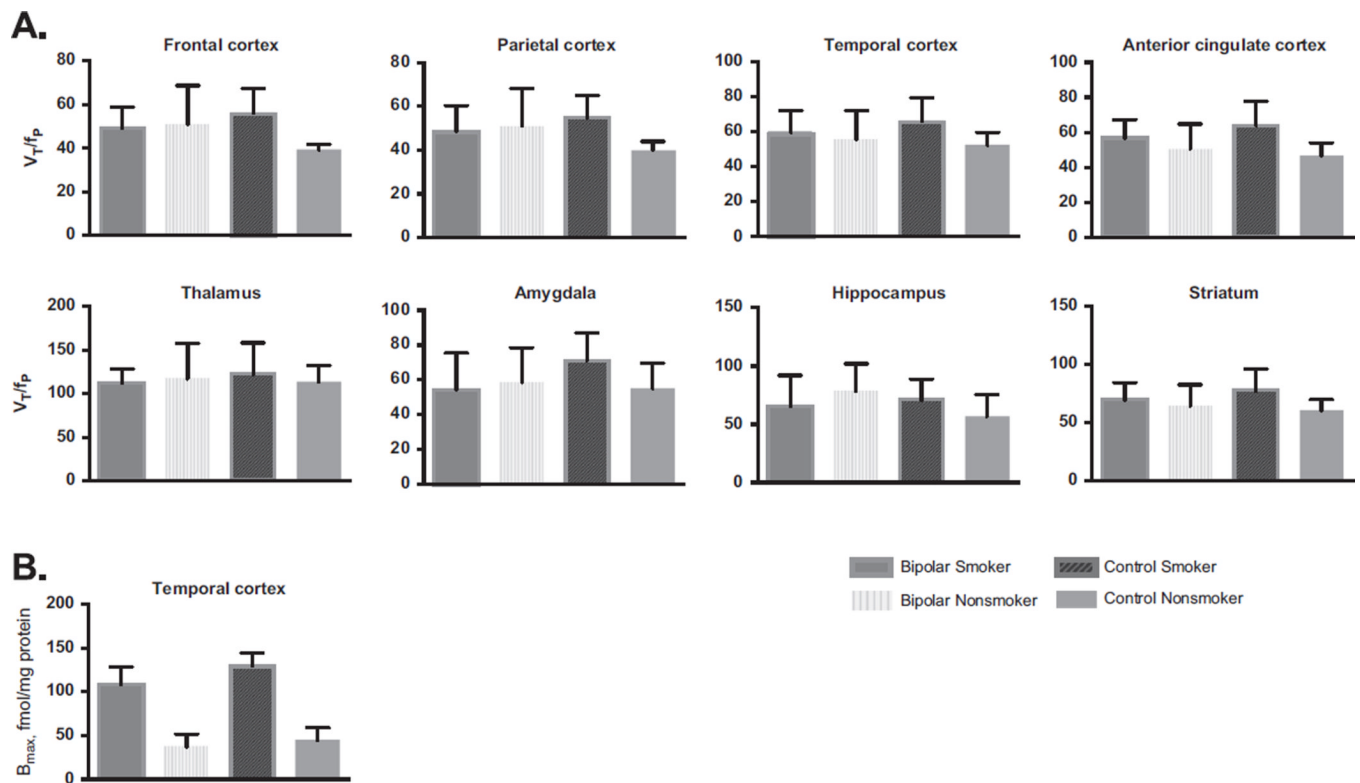
Figure 2.
(A) Illustration of in vivo V_T/f_P in euthymic smokers with ($n = 5$) and without ($n = 5$) bipolar disorder and nonsmokers with ($n = 5$) and without ($n = 5$) bipolar disorder. No significant upregulation was observed in V_T/f_P in bipolar euthymic sample (Hotelling's trace $F = 5.6$, $p = .16$). As we have shown previously, there was higher V_T/f_P in control smokers compared to nonsmokers (Hotelling's trace $F = 10.0$, $p = .04$), and this was significant in the anterior cingulate, frontal, and parietal cortices ($F_{5,5} = 6.0, p = .04$; $F_{5,5} = 10.7, p = .01$; and $F_{5,5} = 8.8$, $p = .02$, respectively, with a trend in the temporal cortex $F_{55} = 4.0$, $p = .08$). (B) Calculated B_{max} (fmoles/mg protein) for [^{125}I]5IA binding in homogenates of temporal cortex samples in control nonsmokers ($n = 3$), control smokers ($n = 5$), nonsmokers with bipolar disorder ($n = 3$) and smokers with bipolar disorder ($n = 5$). No significant differences were detected as an effect of diagnosis ($F_{1,14} = 2.13$, $p = .17$), but nicotinic acetylcholine receptors upregulation was greater in control smokers than in smokers with bipolar disorder ($F_{1,14} = 104.4$, $p < .0009$). V_T/f_P, receptor availability.

Table 1

Demographics and Clinical Characteristics and V_T/f_P Data in Depressed Subjects with Bipolar Disorder and Control Subjects

	Bipolar Unmedicated (n = 6)	Bipolar Medicated (n = 9)	Control Subjects (n =15)
Age (yrs), Mean (SD)	42.3 ± 12.6	40.7 ± 14.5	40.8 ± 13.2
Sex	4 men, 2 women	6 men, 3 women	10 men, 5 women
Years Diagnosed with BD	10.2 ± 8.8	11.7 ± 13.3	NA
Duration of Current Episode (wks)	27.2 ± 15.7	106.0 ± 176.3	NA
Lifetime No. of Depressive Episodes	30.2 ± 40.4	30 ± 40	NA

Assessments	Bipolar Unmedicated (n = 6)				Bipolar Medicated (n = 9)				Control Subjects (n =15)			
	Screening		Scan Day		Screening		Scan Day		Screening		Scan Day	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CES-D	36.8	8.3	38.3	11.7	32	6.9	29.0	10.7	4.6	6.8	NA	
BDI	27.5	10.6	26.7	6.4	21	5.0	17.3	6.6	.8	1.6	NA	
STAI-S	48	10	45.2	11.3	46	8.4	44.3	12.9	NA			
STAI-T	50	8.3	54.0	11.2	54	5.9	51	5.9	NA			
HAM-D	21	8.3	20	6.8	18	4.6	15	6.9	NA			
Young	2.8	2.7	4.0	4.5	2.9	1.6	1.6	1.8	NA			
MADRS	31	8.0	30	4.1	26	7.1	19	9.9	NA			

V_T/f_P

ROI	Bipolar Med (n =9)		Bipolar Unmed (n=6)		All Bipolar (n =15)		Control (n=15)		% Diff	F1,27	p^a
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
Frontal	32	7.2	36	12.5	33.8	9.4	54.4	12.6	−37.70	24.7	<.0001
Anterior Cingulate	35	7.7	42.2	10.8	37.8	9.4	53.5	9.8	−29.40	21.1	<.0001
Temporal	38	7.7	45.2	11.4	40.8	9.7	57.2	10.9	−28.70	19.7	<.0001
Parietal	33	6.4	55.6	36.5	34.4	8.3	48.2	11.6	−28.60	14.1	.001
Hippocampus	58.3	10.8	52.3	28.8	56.2	18.3	76.6	15.5	−26.70	10.6	.003
Amygdala	45	8.0	43.7	16.4	44.8	11.1	55.7	11.7	−19.50	6.6	.016
Striatum	46.7	12.1	56.6	16.9	50.6	14.5	72.4	13.7	−33.70	18.1	<.0001
Thalamus	80.7	15.8	97.7	32.9	87.5	24.6	129.8	30.9	−32.60	17.5	<.0001

BD, bipolar disorder; BDI, Beck Depression Inventory; CES-D, Center for Epidemiologic Studies Depression scale; Diff., difference; HAM-D, Hamilton Rating Scale for Depression; MADRS, Montgomery-Åsberg Depression Rating Scale; Med, medicated; NA, not applicable; ROI, region of interest; STAI-S and STAI-T, State-Trait Anxiety Inventory State and Trait subscales; Unmed, unmedicated; Young, Young Mania Scale; V_T/f_P, receptor availability.

[a] $p < .05$ statistically significant.

Table 2

Demographic Characteristics, Scan-Day Assessments, and V_T/f_P Data in Euthymic Bipolar Disorder Subjects and Matched Control Subjects

	CNS (n = 5)		CS (n = 5)		BDNS (n = 5)		BDS (n = 5)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (yrs)	33.6	10.6	36.2	12.3	35.0	13.3	37.6	13.9
Years Diagnosed	NA		NA		9.4	10.1	14	9.6
Months Euthymic	NA		NA		8.1	9.0	9.8	11
Cigarettes/Day	NA		18.0	6.7	NA		15.6	7.2
Years Smoking	NA		16.4	11.7	NA		13.0	13.2
Scan-Day Mood Assessments								
BDI	2.0	4.5	6.5	6.7	5.6	2.7	7.8	6.0
CES-D	5.2	6.8	10	9.2	15	6.9	14.4	11.3
HAM-D	NA		NA		3.6	2.7	3.8	1.1
MADRS	NA		NA		2.4	2.1	4.2	4.1
STAI-S	28	7.8	35.6	14.4	36	7.5	43	7.2
Young	NA		NA		1.4	2.6	3.2	2.8
Scan-Day Smoking Assessments								
Desire	NA		7.8	4.5	NA		6.5	5.7
Relief	NA		8.8	6.3	NA		7.3	4.7
Withdrawal	NA		7.4	8.4	NA		9.4	8.0

V_T/f_P

	CNS		CS		BDNS		BDS		% Diff.[a]	% Diff.[b]	% Diff.[c]	% Diff.[d]
ROI	Mean	SD	Mean	SD	Mean	SD	Mean	SD				
Frontal	39	2.8	54.9	11.3	50.6	17.9	49.1	9.8	4.8	3.2	−10.6	29.7
Anterior Cingulate	47	7.3	63	14	50.4	14.2	56.5	10.6	−3.2	−11	−11	8.0
Temporal	52	7.5	65.6	13.6	55.4	16.7	59.6	12.6	−2.6	−7.1	−9.1	6.1
Parietal	44	5.3	63.8	12.4	53.6	17.7	57.9	13.4	2.5	−7.4	−9.3	21.5
Hippocampus	56.6	19.3	71.4	17.8	67.2	32.8	75.8	13.2	−2.3	4.2	−8.6	3.3
Amygdala	54.9	14.7	68	16	53	22	60.7	17.9	−2.9	−6.8	−8.8	6.5
Striatum	61	9.4	76	19	64.6	18.4	69.3	15.3	12	−11	6.1	19

	CNS (*n* = 5)		CS (*n* = 5)		BDNS (*n* = 5)		BDS (*n* = 5)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Thalamus	114	18.5	123	36.9	117	40.4	112	16.2	−11	−14	−10	−4.3

% Diff., percent difference; BDI, Beck Depression Inventory; BDS, smokers with bipolar disorder, BDNS, nonsmokers with bipolar disorder; CES-D, Center for Epidemiologic Studies Depression scale; CS, control smokers, CNS, control nonsmokers; HAM-D, Hamilton Rating Scale for Depression; NA, not applicable; MADRS, Montgomery-Åsberg Depression Rating Scale; STAI-S, State–Trait Anxiety Inventory State subscale; Young, Young Mania Scale; V_T/f_P, receptor availability.

[a] All bipolar disorder vs. all control subjects.

[b] BDNS *vs.* BDS.

[c] BDS *vs.* CS.

[d] BDNS *vs.* CNS.

Table 3

Demographics and Clinical Characteristics and V_T/f_P Data in All Bipolar Disorder and Control Subjects

	Euthymic Bipolar (n =0)				Depressed Bipolar (n =5)				Control Subjects (n =25)			
Age (yrs) Mean (SD)	36.3 ± 12.9				41.6 ± 13.1				38.4 ± 12.4			
Sex	5 men, 5 women				10 men, 5 women				15 men, 10 women			
Duration Current Episode (wks)	NA				77.9 ± 144				NA			
	Screening		Scan Day		Screening		Scan Day		Screening		Scan Day	
Assessments	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CES-D	16	9.0	14	9.4	33	6.6	29.3	10.4	7.6	8.0	NA	
BDI	8.8	7.4	6.7	4.8	21	5.0	19	6.2	3.5	5.5	NA	
STAI-S	39	9.7	35	8.8	47	8.4	43.6	11.7	NA			
STAI-T	50	8.3	42	8.6	53	6.8	50	6.4				
HAM-D	5.7	5.0	3.9	2.0	18	4.6	15	6.1				
Young	2.4	2.6	2.4	3.1	2.6	2.2	2.6	2.7				
MADRS	4.7	4.6	3.1	3.4	25	6.1	19.3	9.8				

V_T/f_P

ROI	Eu BD (n =10) Mean	SD	CS for Eu (n =10) Mean	SD	Diff. Eu vs. CS	Dep BP (n =15) Mean	SD	CS for Dep BD (n =15) Mean	SD	Diff. Dep vs. CS	Diff. Dep vs. EU BD	F	p[a]
Frontal	49.9	11.9	47.6	11.9	4.8%	34	9.4	54.4	12.6	−37.9%	−32.3%	10.8	<.0001
Anterior Cingulate	53.4	13.9	55.2	13.9	−3.3%	38	9.4	54	9.8	−29.3%	−29.2%	11.7	<.0001
Temporal	57.5	12.6	59.1	12.6	−2.7%	41	9.7	57.2	10.9	−28.7%	−29.0%	11.8	<.0001
Parietal	49.7	14.1	47.3	10.7	5.1%	34	8.3	48.2	11.6	−28.6%	−30.8%	8.6	.001
Hippocampus	71.0	19.0	63.7	19.0	11.5%	56.2	18.3	76.6	15.5	−26.6%	−20.8%	2.3	.12
Amygdala	56.2	16.8	62.9	16.8	−10.7%	44.8	11.1	55.7	11.7	−19.6%	−20.3%	3.4	.04
Striatum	67.0	16.7	69.0	16.7	−2.9%	50.6	14.5	72.4	13.7	−30.1%	−24.5%	9.1	<.0001
Thalamus	115	27.8	118	27.8	−2.3%	87.5	24.6	130	30.9	−32.6%	−23.8%	8.5	.001

BDI, Beck Depression Inventory; BD, bipolar disorder; CES-D, Center for Epidemiologic Studies Depression scale; CS, control subject; Dep, depressed; Diff., difference; Eu, euthymic; HAM-D, Hamilton Rating Scale for Depression; MADRS, Montgomery-Åsberg Depression Rating Scale; NA, not applicable; ROI, region of interest; STAI-S and -T, State–Trait Anxiety Inventory State and Trait subscales; Young, Young Mania Scale; V_T/f_P, receptor availability.

[a] *p* value is for bipolar depressed subjects compared with bipolar euthymic and CS (multivariate analysis of variance).