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Decreased Muscarinic Receptor Binding in the Frontal Cortex of Bipolar Disorder and Major Depressive Disorder Subjects

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Abstract

Background—Dysfunction of the cholinergic muscarinic receptors has been implicated in the pathology of bipolar disorder and major depressive disorder. However, there is conflicting evidence regarding the association between individual muscarinic receptors and the two disorders.

Methods—We used the muscarinic receptor selective radioligands [^{3}H]pirenzepine, [^{3}H] AFDX-384 and [^{3}H]4-DAMP to measure the levels of muscarinic$_1$ (CHRM1) and muscarinic$_4$ (CHRM4) receptors, muscarinic$_2$ (CHRM2) and muscarinic$_4$ (CHRM4) receptors and muscarinic$_3$ (CHRM3) receptor, respectively. Radioligand binding was measured in Brodmann's area (BA) 10 of the rostral prefrontal cortex, BA 46 of the dorsolateral prefrontal cortex and BA 40 of the parietal cortex in the post-mortem CNS from subjects with bipolar disorder or major depressive disorder and control subjects.

Results—[^{3}H]AFDX-384 binding was decreased in BA 46 in both bipolar disorder ($p<0.01$) and major depressive disorder ($p<0.05$). [^{3}H]4-DAMP binding was decreased in BA 10 in bipolar disorder ($p<0.05$) but not major depressive disorder ($p>0.05$). [^{3}H]AFDX-384 and [^{3}H]4-DAMP binding were unaltered in any other cortical region examined for either disorder ($p>0.05$). [^{3}H] pirenzepine binding was not significantly altered in either disorder in any cortical region examined ($p>0.05$).

Limitations—9 bipolar disorder, 9 major depressive disorder and 19 control subjects were used in the study.

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Conclusion—Our data is consistent with previously published data implicating a role for CHRM2 receptors in the pathology of bipolar and major depressive disorder. The demonstration of a novel association between decreased CHRM3 receptor expression and bipolar disorder suggests bipolar and major depressive disorder differ in the underlying nature of their cholinergic dysfunction.

Keywords

Bipolar Disorder; Depression; Muscarinic Receptors; Post-Mortem; Frontal Cortex

1. Introduction

The pathologies of bipolar disorder and major depressive disorder have long been thought to have a cholinergic component (Janowsky et al 1972; Janowsky et al 1994). Early studies found that insecticidal cholinesterase inhibitors (Roundtree et al 1950) induced depression in individuals with bipolar disorder. Further evidence from clinical studies into the effects of drugs that act on the cholinergic and adrenergic neurotransmitter systems lead to a cholinergic-adrenergic hypothesis of mania and depression (Janowsky et al 1972). Thus, a hypocholinergic-hyperadrenergic state could induce mania while a hypercholinergic-hypoadrenergic state would produce depression. Supporting this hypothesis, clinical studies have shown have shown that scopolamine, a non-selective cholinergic muscarinic receptor antagonist, has antidepressant effects in both bipolar disorder and major depressive disorder (Furey and Drevets 2006). In addition, it has been shown that the mood stabiliser, lithium, upregulates hippocampal cholinergic muscarinic receptors (Marinho et al 1998). These observations suggest that muscarinic receptors may have a role in the pathology of bipolar disorder and major depressive disorder.

The potential role of muscarinic receptors in bipolar disorder and major depressive disorder has underpinned genetic studies to determine if these receptors are associated with an increased risk of developing the disorders. Two association studies have identified several non-coding single nucleotide polymorphisms (SNP's) within the cholinergic muscarinic$_2$ receptor (*CHRM2*) gene associated with the incidence of major depressive disorder (Comings et al 2002; Wang et al 2004). The reported association between SNPs within *CHRM2* and a combined cohort of subjects with bipolar disorder, major depressive disorder and seasonal affective disorder suggests a potentially broader role for *CHRM2* in the pathology of affective disorders (Luo et al 2005). Conversely, a recent analysis of 19 cholinergic genes, including the five members of the muscarinic receptor family, failed to detect an association between SNP's in *CHRM2* incidence of bipolar disorder (Shi et al 2007). Amongst the other muscarinic receptors, this same study also failed to detect an association between SNP's in any other muscarinic receptor gene and the incidence of bipolar disorder. The lack of association between other muscarinic receptors and the pathology of affective disorders is further supported by linkage studies show, which no association between bipolar disorder and the long arm of chromosome 11, the chromosomal region containing the cholinergic muscarinic$_1$ receptor (*CHRM1*) gene (Ewald et al 1995).

Neuroimaging studies have also implicated the cholinergic muscarinic receptors in the pathology of affective disorder. PET studies have reported a decrease in the CHRM2 selective agonist [^{18}F]FP-TZTP in the anterior cingulate cortex of individuals with bipolar disorder but not major depressive disorder (Cannon et al 2006). By contrast, post-mortem tissue has failed to show any change in binding of the CHRM2/CHRM4 selective antagonist [^{3}H]AFDX-384 or the CHRM1/CHRM4 selective antagonist [^{3}H]pirenzepine in the anterior cingulate cortex from subjects with bipolar disorder or major depressive disorder (Zavitsanou et al 2004; Zavitsanou et al 2005). [^{18}F]FP-TZTP selectively binds to the high affinity binding state of CHRM2, compared to the high and low affinity binding of the radioligands employed in the

postmortem studies. Thus the decrease in functional pool of CHRM2 observed in neuroimaging studies are suggestive of an increase in intrasynaptic acetylcholine levels and/or a reduction in the proportion of receptors in their high affinity state associated with bipolar disorder rather than a decrease in receptor expression.

The muscarinic receptor antagonists [^{3}H]pirenzepine, [^{3}H]AFDX-384 and [^{3}H]4-DAMP have been shown to be effective in selectively measuring muscarinic receptor protein expression, whereby [^{3}H]pirenzipine binds with high affinity to CHRM1 and CHRM4, [^{3}H]AFDX-384 binds with high affinity to CHRM2 and CHRM4 and [^{3}H]4-DAMP displays high affinity binding to CHRM3 (Hammer et al 1980, Michel et al 1989, Miller et al 1991). We used the radioligands [^{3}H]pirenzepine, [^{3}H]AFDX-384 and [^{3}H]4-DAMP to examine muscarinic receptors in post-mortem tissue from subjects with bipolar disorder or major depressive disorder. We focused our studies on the frontal cortex, as this region has been shown to be affected by both mania and depression (Blumberg et al 1999; Drevets et al 1997; Soares and Mann 1997). We also measured muscarinic receptors in the parietal cortex as this area has not been shown to be markedly affected by the pathology of bipolar disorder or major depressive disorder; thus we hypothesised that muscarinic receptor expression would be unchanged in this region.

2. Methods

2.1. Tissue collection

All tissue was obtained from the Victorian Brain Bank Network, Mental Health Research Institute of Victoria, Parkville, Australia. Approval for the study was obtained from both the Ethics Committee of the Victorian Institute of Forensic Medicine and the Mental Health Research and Ethics Committee of Melbourne Health. Tissue from Brodmann's Area 10 (BA 10), Brodmann's Area 46 (BA 46) and Brodmann's Area 40 (BA 40) of the left hemisphere was obtained post-mortem from 9 subjects diagnosed with bipolar I disorder, 9 subjects diagnosed with major depressive disorder and 19 subjects with no history of psychiatric illness (controls). BA 10 was taken as the most rostral portions of the superior frontal gyrus and middle frontal gyrus, bounded ventrally by the superior rostral sulcus. BA 46 was taken as the lateral surface of the frontal lobe and includes approximately the middle third of the middle frontal gyrus and the most rostral portion of the inferior frontal gyrus. BA 40 was taken as the lateral surface of the parietal lobe including primarily the supramarginal gyrus surrounding the posterior segment of the lateral fissure.

For each subject, clinical case histories were used to enable a senior psychologist and psychiatrist to reach a diagnostic consensus using the Diagnostic Instrument for Brain Studies (DIBS) (Keks et al 1999). The DIBS is a semi-structured protocol for post-mortem assessment allowing psychiatric diagnosis according to DSM-IV criteria (American Psychiatric Association, 1994). Demographic factors and tissue quality markers, including gender (control= 13 male: 15 female; BD= 4 male: 5 female; MDD= 3 male: 6 female), age (mean ±SD; range: control= 48.2 ±16.4 years; 21–77 years; BD= 54.4 ±12.5 years; 29–66 years; MDD= 51.7 ±19.9 years; 19–79 years), duration of illness (mean ±SD; range: BD= 17.0 ±12.9 years; 3–40 years; MDD= 14.2 ±9.2 years; 1–25 years), incidence of suicide (control= 0/19; BD= 2/9; MDD= 7/9), post-mortem interval (mean ±SD; range: control= 33.7 ±13.7 hr; 15.5–60 hr; BD= 33.8 ±15.9 hr; 15.0–58.0 hr; MDD= 47.9 ±14.8 hr; 27.0–67.0 hr) and CNS pH (mean ±SD; range: control= 6.28 ±0.21; 5.86–6.59; BD= 6.26 ±0.29; 5.68–6.46; MDD= 6.45 ±0.15; 6.17–6.64) were used to assess the impact of subject variability on the experiment. The medication histories of the bipolar disorder and major depressive disorder subjects included in this study are summarised in Table 1. These demographic factors and tissue quality markers were used to assess the impact of subject variability on the experiment. Cadavers were refrigerated within 5 hr and frozen to −70°C within 30 min of autopsy. Where death was

witnessed, the time between death and autopsy was taken as the post-mortem interval. Where death was not witnessed, tissue was only collected from subjects who had been seen alive up to 5 hr prior to being found dead. In such cases, post-mortem interval was measured from the midpoint between the subject being found and being last seen alive. The pH of the CNS was measured as described previously (Kingsbury et al 1995). RIN values were not obtained for this study as they have no additional value in tissue quality assessment for studies focused on proteins (Stan et al 2006).

2.2. *In situ* radioligand binding with autoradiography

20μm frozen sections were cut from BA 10, BA 40 and BA 46 tissue with a cryostat and mounted on to gelatinised slides. The sections were used for radioligand binding with [^{3}H] AFDX-384, [^{3}H]4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP) and [^{3}H] pirenzepine. For each radioligand, 5 sections were used per subject.

Prior to [^{3}H]AFDX-384 binding, sections were incubated in 10mM KH_2PO_4, 10 mM Na_2HPO_4 (pH 7.4) (assay buffer 1) for 30 min at room temperature. The sections were then rinsed in water and dried. [^{3}H]AFDX-384 binding was performed by incubating 3 sections/subject in 7nM [^{3}H]AFDX-384 (PerkinElmer, Waltham, MA, USA) in assay buffer 1. 2 sections/subject were used to measure non-specific binding by displacing 7nM [^{3}H]AFDX-384 with 1μM tropicamide (Tocris Bioscience, Bristol, UK) in assay buffer 1 (Crook et al 1999). The sections where incubated for 1 hr at room temperature. Following radioligand binding, the slides were washed twice for 2 min in ice cold assay buffer 1, rinsed in ice cold water and dried in a stream of cool air before being partially fixed overnight in paraformaldehyde buffer.

Prior to [^{3}H]4-DAMP binding, sections were incubated in 50mM Tris-HCl (pH 7.4) (assay buffer 2) for 15 min at room temperature, then rinsed in water and dried. [^{3}H]4-DAMP binding was performed by incubating 3 sections/subject in 3nM [^{3}H]4-DAMP (PerkinElmer, Waltham, MA, USA) in assay buffer 2. 2 sections/subject were used to measure non-specific binding by displacing 3nM [^{3}H]4-DAMP with 10μM 4-diphenylacetoxy-N-methylpiperidine methiodide mustard hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) in assay buffer 2 (Araujo et al 1991). The sections where incubated for 1 hr at room temperature. Following radioligand binding, the slides were washed twice for 5 minutes with ice cold assay buffer 2, rinsed in ice cold water, dried and partially fixed overnight in paraformaldehyde vapour.

To measure [^{3}H]pirenzepine binding, 3 sections/subject were incubated in 15nM [^{3}H] pirenzepine (PerkinElmer, Waltham, MA, USA) in assay buffer 1 for 30 min at room temperature. 2 sections/subject were used to measure non-specific binding by displacing 15nM [^{3}H]pirenzepine with 1μM quinuclidinyl xanthene-9-carboxylate hemioxilate (Sigma-Aldrich, St. Louis, MO, USA) in assay buffer 1 (Dean et al 1996). Following radioligand binding the slides were wash twice for 2 min with ice cold assay buffer 1, rinsed in ice cold water, dried and partially fixed overnight in paraformaldehyde vapour.

The fixed slides were apposed to a BAS-TR2025 plate (Fujifilm, Tokyo, Japan) with autoradiographic [^{3}H]microscales™ (Amersham Biosciences, Little Chalfort, UK). [^{3}H] pirenzepine and [^{3}H]4-DAMP labelled slides were apposed for 3 days. AFDX-384 labelled slides were apposed for 7 days. The plates were scanned in a BAS 5000 high resolution phosphoimager (Fujifilm, Tokyo, Japan) and the resulting images analysed using AIS imaging software (Imaging Research, St. Catharines, ON, Canada). Radioligand binding was measured as an integrated measurement of signal intensity across the entire region of binding. Signal intensities were calibrated against the microscales and expressed as the average amount of total bound radioligand/estimated tissue equivalent subtracted from the average non-specific binding for each subject.

2.3. Statistics

The data was first analysed by the D'Agostino & Pearson omnibus normality test to determine whether they followed a Gaussian distribution. The data was then analysed using the general linear model. Separate analyses were performed across diagnoses for each individual region examined and for each radioligand used. Post test analysis was performed using Bonferroni's multiple comparison test, correcting for three comparisons. P values resulting from post test analysis are expressed as corrected values. Statistical significance was accepted at $p<0.05$. The relationship between demographic and tissue condition data were assessed using Pearson product moment correlation. Analyses were conducted using using Prism 5.01 (Graphpad Software, La Jolla, CA, USA) and Minitab 13.01 (Minitab, State College, PA, USA) software.

3. Results

3.1 Distribution of radioligand binding within the grey matter

Within all cortical regions examined, binding of [^{3}H]AFDX-384, [^{3}H]4-DAMP and [^{3}H] pirenzepine appeared homogeneous across the cortical laminae (Figure 1). Thus, an integrated measurement of radioligand binding was taken across the cortical laminae for each section. Using these measures, the radioligand binding data set showed non-Gaussian distribution of the binding data and thus non-parametric analyses were completed.

All binding experiments were performed using radioligand concentrations 3-fold greater than their Kd value. Thus, differences in radioligand binding between groups are unlikely to reflect a change in the binding affinity of the receptors. Thus observed differences in binding levels were, therefore, taken to be reflective of a difference in the level of protein expression of the radioligand's target receptors.

3.2. Muscarinic receptor selective-radioligand binding

Analysis of the binding data for the CHRM2 and CHRM4 selective radioligand [^{3}H]AFDX-384 across diagnoses showed significant variation in [^{3}H]AFDX-384 binding levels with diagnosis in BA 46 of the dorsolateral prefrontal cortex ($F_{2,36}=8.80$; $p<0.001$) but not in BA 10 of the rostral prefrontal cortex ($F_{2,36}=1.57$; $p=0.223$) nor in BA 40 of the parietal cortex ($F_{2,36}=1.55$; $p=0.226$). Post test analysis of [^{3}H]AFDX-384 binding within BA 46 showed a 37.1% $\pm 15.1\%_{SE}$ ($F_{2,36}=8.80$; $p_{corrected}=0.001$) decrease in binding levels within the bipolar disorder group and a 28.0% $\pm 14.7\%_{SE}$ ($F_{2,36}=8.80$; $p_{corrected}=0.014$) decrease in binding levels within the major depressive disorder group compared to the controls (Figure 2A). This data suggests a decrease in CHRM2 and/or CHRM4 protein expression associated with both bipolar disorder and major depressive disorder. While significant changes in receptor expression appear regionally localised within the cortex, there appeared to be consistent trend towards lower [^{3}H]AFDX-384 binding levels in bipolar disorder compared to major depressive disorder in all cortical regions examined.

There was no significant variation in [^{3}H]pirenzepine binding with diagnosis in BA 10 ($F_{2,36}=1.33$; $p=0.277$), BA 46 ($F_{2,36}=0.07$; $p=0.928$) or BA 40 ($F_{2,36}=1.97$; $p=0.155$) (Figure 2B) in either the bipolar disorder or the major depressive disorder groups. As [^{3}H]pirenzipine selectively binds to CHRM1 and CHRM4, the lack of altered [^{3}H]pirenzipine binding across diagnoses implies the observed decrease in [^{3}H]AFDX-384 binding levels reflects a decrease in CHRM2 in BA 46 rather than a decrease in CHRM4.

Analysis of the [^{3}H]4-DAMP binding data across diagnoses showed significant variation with diagnosis in BA 10 ($F_{2,36}=3.69$; $p=0.036$) but not BA 46 ($F_{2,36}=2.67$; $p=0.084$) or BA 40 ($F_{2,36}=1.45$; $p=0.248$). Within BA 10, an 18.7% $\pm 10.6\%_{SE}$ ($F_{2,36}=3.69$; $p_{corrected}=0.022$) decrease in [^{3}H]4-DAMP binding levels was seen in tissue from the bipolar disorder group

whilst no significant difference in [^{3}H]4-DAMP binding was observed in the major depressive disorder group ($F_{2,36}$=3.69; $p_{corrected}$=0.505) compared to controls (Figure 2C). The high affinity of [^{3}H]4-DAMP for CHRM3 is suggestive of a decrease in CHRM3 expression, specific to bipolar disorder. As with the [^{3}H]AFDX-384 binding data, while a significant decrease in binding was only observed in BA 10, there appeared to be a trend towards consistently lower levels of [^{3}H]4-DAMP binding across the other cortical regions examined.

3.3. Effects of confounding variables

The effect of potential confounding factors, such as age, tissue pH, postmortem interval, duration of illness and antipsychotic and mood stabilising medication, on radioligand binding were assessed using Pearson correlations: a value of r^2>0.25 indicating the potential for a significant relationship (Gliner et al 2002). No significant correlations were observed between any of these variables (r^2 from <0.001 to 0.179).

4. Discussion

Our data suggests that decreased muscarinic receptor expression in the rostral and dorsolateral and prefrontal cortices is associated with both bipolar disorder and major depressive disorder. There are both similarities and differences in the nature of the cholinergic dysfunctions in these disorders. Binding of the CHRM2/CHRM4 selective radioligand [^{3}H]AFDX-384 was decreased by 37% in subjects with bipolar disorder and by 28% in subjects with major depressive disorder compared to controls within BA 46, suggesting a reduction in expression of CHRM2 and/or CHRM4 within the dorsolateral prefrontal cortex is associated with both disorders. This data is supported by *CHRM2* SNP association studies, which have suggested an involvement of *CHRM2* in major depressive disorder (Comings et al 2002; Wang et al 2004). Binding of [^{3}H]pirenzepine was not significantly altered in either bipolar disorder or major depressive disorder. As [^{3}H]pirenzepine selectively binds to both CHRM1 and CHRM4, this would suggest that CHRM1 and CHRM4 expression are not altered in either bipolar disorder or major depressive disorder. Furthermore, the decrease in [^{3}H]AFDX-384 binding in bipolar disorder and major depressive disorder would appear to reflect a decrease in CHRM2 expression associated with these two affective disorders rather than altered CHRM4 expression. Supporting the lack of involvement of either CHRM1 or CHRM4 in affective disorders, previous post-mortem studies have also reported no change in [^{3}H]pirenzipine binding levels in the anterior cingulate cortex of individuals with bipolar disorder or major depressive disorder (Zavitsanou et al 2004). Linkage analysis has failed to show any association between the long arm of chromosome 11, which contains the *CHRM1* gene and the incidence of bipolar disorder (Ewald et al 1995).

In contrast to our data, post-mortem studies have shown no change in [^{3}H]AFDX-384 binding in the anterior cingulate cortex of individuals with either bipolar disorder or major depressive disorder compared to controls (Zavitsanou et al 2005). It is possible that such differences reflect differences in the genotypes of the subjects involved in these studies. However, these differences might also reflect different regionally specific changes in CHRM2 expression associated with bipolar disorder and major depressive disorder. PET studies have reported decreased binding of [^{18}F]FP-TZTP, a CHRM2 selective agonist, within the anterior cingulate cortex of individuals with bipolar disorder, however, no change in [^{18}F]FP-TZTP binding was found in individuals with major depressive disorder (Cannon et al 2006). As [^{18}F]FP-TZTP binds predominantly to the high affinity binding state of CHRM2 (Jagoda et al 2003), the apparent decrease in the functional receptors binding sites may represent elevated intrasynaptic acetylcholine concentrations and/or a shift of the CHRM2 pool into the low affinity binding state, possibly in response to elevated acetylcholine levels. Thus, the decreased expression of CHRM2 in bipolar and major depressive disorder and CHRM3 in bipolar disorder within

discrete regions of the frontal cortex suggested by our data may reflect a regionally specific down regulation of CHRM2 and CHRM3 in response to elevated acetylcholine associated with affective disorders.

Contrasting the [^{3}H]AFDX-384 binding data, there was a 19% decrease in binding of the CHRM3 selective radioligand [^{3}H]4-DAMP in subjects with bipolar disorder compared to controls within in BA 10 of the rostral prefrontal cortex but no significant change in [^{3}H]4-DAMP binding in subjects with major depressive disorder. Decreased expression of the food intake associated protein, pro-melanin concentrating hormone (PMCH), has been reported in the hippocampus of *CHRM3* knockout mice (Yamada et al 2001). While the role of PMCH is poorly defined in affective disorders, bipolar disorder has been linked to an increased incidence of obesity (McElroy et al 2004) and PMCH is a potent regulator of appetite (Qu et al 1996). Importantly, association studies have implicated the PMCH receptor, melanin concentrating hormone receptor$_1$, (MCHR1) in the pathology of bipolar disorder (Severinsen et al 2006). Thus, a decrease in [^{3}H]4-DAMP binding may be associated with bipolar disorder specific alterations in the expression of PMCH or the signalling pathway of MCHR1.

Our finding of decreased muscarinic receptors within BA46 and BA10 is of importance given the strong evidence from neuroimaging studies implicating both the dorsolateral and rostral prefrontal cortices in the pathology of affective disorders (Blumberg et al 1999; Haldane and Frangou 2006). The dorsolateral prefrontal cortex is known to play an important role in executive functioning and working memory (Collette et al 1999; Levy and Goldman-Rakic 1999; Manoach et al 1997), while the rostral prefrontal cortex has been proposed to act as an attentional gateway, mediating stimulus dependant and stimulus independent cognition and, thus, facilitating the processing of more complex cognitive tasks (Burgess et al 2007). Accordingly, cognitive deficits have been characterised within the symptomatology of both bipolar disorder and major depressive disorder (Burdick et al 2005; Gualtieri and Morgan 2008). Thus, reduced grey matter volume in BA 46 of individuals with major depressive disorder has been reported to correlate with a greater number of perseverative errors in cognitive tests (Vasic et al 2008). PET studies have also reported decreased activation of BA 10 in bipolar disorder subjects in a manic state during word generation tasks (Blumberg et al 1999). Furthermore, individuals with bipolar disorder in a euthymic state display reduced reward learning (Pizzagalli et al 2008), a function that appears to be mediated by BA 10 (Rogers et al 1999). The potential involvement of the dorsolateral prefrontal cortex in the cognitive deficits of affective disorders is pertinent in light of the cognitive deficits reported in *CHRM2*−/− mice (Seeger et al 2004).

However, the cognitive role of CHRM3 within the rostral prefrontal cortex is unclear. While the *CHRM3*−/− mouse does not appear to have cognitive deficits (Yamada et al 2001), CHRM3 is expressed in cortical and subcortical areas of the brain that are involved with cognitive function (Levey et al 1994). Thus, it's possible that the reduced CHRM3 expression in BA 10 of subjects with bipolar disorder has little impact on the cognitive deficits associated with this region. Alternatively, the cognitive effects of this receptor may not be perceptible in mouse behavioural tests.

The dorsolateral and rostral prefrontal cortices also appear to be important in the cognitive mediation of emotional responses. Individuals with major depressive disorder display a suppression of the neural activity within BA 46 that is normally associated with ignoring a fear response (Fales et al 2008). Neuroimaging studies have also reported increased neural activity in BA 10 associated with both positive and negative emotional responses in hypomanic individuals with bipolar disorder (Malhi et al 2004). Importantly, CHRM2 and CHRM3 antagonists are able to suppress cataplexy, an abrupt episode of muscle atonia commonly induced by strong emotional responses, in the canine narcoleptic model, suggesting both

receptors have roles in regulating mood (Reid et al 1998). Therefore, dysfunction of muscarinic receptor-mediated cholinergic neurotransmission within the dorsolateral and rostral prefrontal cortices, evidenced by our data, may underlie the cognitive and emotional symptoms of bipolar disorder and major depressive disorder.

Common molecular causes have been proposed for affective disorders and schizophrenia (Citrome et al 2005; Craddock et al 2006; Van Den Bogaert et al 2006). Our data suggests bipolar disorder and major depressive disorder are associated with abnormal muscarinic receptor expression, as has been reported in schizophrenia (Dean et al 2003). However, in contrast to the widespread decreases in [^{3}H]pirenzepine binding reported in the frontal cortex (Crook et al 1999; Crook et al 2001; Dean et al 2002; Zavitsanou et al 2004; Zavitsanou et al 2005), no change in [^{3}H]pirenzepine binding was observed within this bipolar disorder or major depressive disorder cohort. This data is supported by other post-mortem studies that have also found no change in [^{3}H]pirenzepine binding in the anterior cingulate cortex of bipolar disorder and major depressive disorder subjects (Zavitsanou et al 2004). Thus, not only do the cholinergic abnormalities within these disorders appear to have different molecular bases, bipolar disorder and major depressive disorder appear to be associated with more regionally discrete alterations in the cholinergic system within the brain.

The cholinergic hypothesis of affective disorders proposes that depression is driven by a hypercholinergic state, whereas mania results from a hypocholinergic state (Janowski *et al* 1972). While it is difficult to make assumptions about the affective state of our subjects at the time of death, the lack of association between the incidence of suicide and the level of radioligand binding would suggest reduction in CHRM2 and CHRM3 protein expression the frontal cortex is sustained during both manic and depressive states. The elevated acetylcholine levels in bipolar disorder suggested by published observations of decreased CHRM2 high affinity binding sites within the anterior cingulate cortex (Cannon et al 2006) is consistent with the hypercholinergic nature of depression. Thus, the regionally discrete reduction in CHRM2 density in major depressive disorder and CHRM2 and CHRM3 in bipolar disorder may reflect an attempt to maintain normal cholinergic function within the prefrontal cortex during a hypercholinergic state. The ability of cholinomimetic drugs to induce depression in manic individuals suggests mania is associated with a reduction in acetylcholine levels (Davis et al 1978). Maintaining reduced CHRM2 and CHRM3 expression in the prefrontal cortex of individuals with bipolar disorder could exacerbate a shift towards reduced cholinergic neurotransmission as acetylcholine levels decrease following recovery from a depressive state, thus driving the brain into a manic state. The decreased CHRM3 expression and greater reduction in CHRM2 expression associated with bipolar disorder compared to major depressive disorder potentially creates a greater susceptibility of individuals with bipolar disorder to enter into a hypocholinergic state in response to decreasing acetylcholine. This potential for the exacerbation of a manic state in response to the normalisation of acetylcholine levels is supported by the greater trend towards reduced [^{3}H]AFDX-384 and [^{3}H]4-DAMP binding in all cortical areas examined in bipolar disorder compared to major depressive disorder, apparent from our data. Importantly, the rostral prefrontal cortex, where we have observed a bipolar disorder specific decrease in CHRM3 expression, has been implicated in the pathology of mania by PET studies (Blumberg et al 1999). Thus, the hypocholinergic state of mania proposed by the cholinergic hypothesis of affective disorders may reflect a reduction in muscarinic receptor mediated cholinergic neurotransmission resulting from an aberrant regulatory response to a hypercholinergic state.

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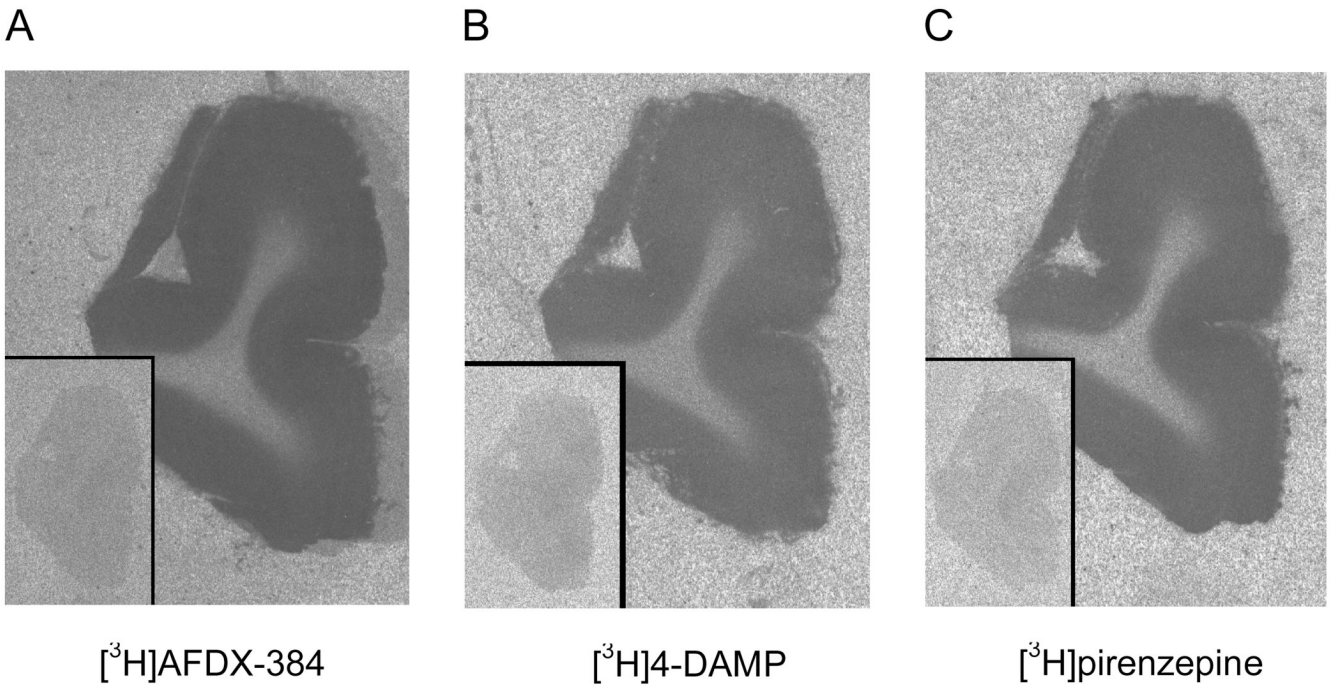

Figure 1.

Representative autoradiographs showing (A) [^{3}H]AFDX-384, (B) [^{3}H]4-DAMP and (C) [^{3}H] pirenzipine binding in Brodmanns area (BA) 10 from the same subject. The signal intensity of the bound radioligand was corrected non-specific background signal by subtracting the binding levels of the radioligand in the presence of a displacing agent (non-specific binding) from the total binding level of the radioligand. The displacing agents used to correct for [^{3}H]AFDX-384, (B) [^{3}H]4-DAMP and (C) [^{3}H]pirenzipine background signal were quinuclidinyl xanthene-9-carboxylate hemioxilate, tropicamide and 4-diphenylacetoxy-N-methylpiperidine methiodide mustard hydrochloride, respectively. Non-specific binding of the radioligands in the presence of displacing agents are shown in the inset images.

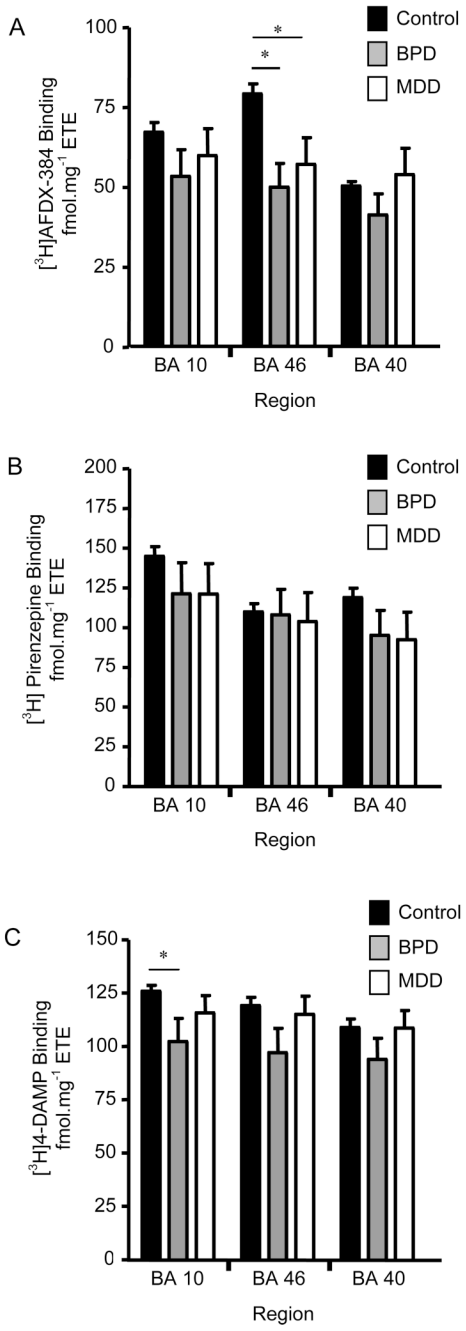

Figure 2.

(A) [^{3}H]AFDX-384, (B) [^{3}H]Pirenzepine and (C) [^{3}H]4-DAMP binding in the post-mortem cortex of bipolar disorder (BPD) (n=9), major depressive disorder (MDD) (n=9) and control (n=19) subjects. A significant decrease in [^{3}H]AFDX-384 binding was seen in BA 46 of subjects with bipolar disorder ($F_{2,36}$=8.80; $p_{corrected}$=0.001) and major depressive disorder ($F_{2,36}$=8.80; $p_{corrected}$=0.014). A significant decrease in [^{3}H]4-DAMP binding was seen in BA 10 of subjects with bipolar disorder ($F_{2,36}$=3.69; $p_{corrected}$=0.022). While changes in [^{3}H] AFDX-384, [^{3}H]Pirenzepine and [^{3}H]4-DAMP binding did not reach significance in any other region, there appeared to be a trend towards a greater decrease in [^{3}H]AFDX-384 and [^{3}H]4-DAMP binding in bipolar disorder compared to major depressive disorder in all cortical regions

examined. Values are expressed as fmol of bound radioligand per mg of estimated tissue equivalent. * =$p<0.05$; ** = $p<0.01$

Table 1

A summary of the drug history of the bipolar 1 disorder and major depressive disorder subjects used in this study. For each drug, the final recorded daily dose (FRDD), as recorded in the subject's clinical history, is provided in mg/day. Drugs and drug metabolites present in the subject's blood, or where otherwise stated, at the time of death are recorded in brackets along with the concentration in mg/L, or as otherwise stated. The FRDD of the antipsychotic drugs are recorded as chlorpromazine equivalents. Antidepressants and mood stabilisers are recorded as the actual dosage. N/A = data not available.

Bipolar 1 Disorder

Case #	Antipsychotics	FRDD	Antidepressants	FRDD	Mood Stabilisers	FRDD
1	Fluphenazine	100	Dothiepin	150	(Na Valproate)	(10.0)
				(4.0)	[Urine]	
			(Amitriptyline)	(4.9)		
			(Nortriptyline)	(3.1)		
2			(Moclobemide)	(8.0)		
3	Chlorpromazine	300	Paroxetine	20	Lithium	750
4	Modecate	166				
5	Chlorpromazine	300			Lithium	1500
		(0.3)			[Serum]	(0.8 mmol/L)
6	Stelazine	N/A			Lithium	1000
7	Flupenthixol	N/A			Na Valproate	2500
8					Lithium	500
9			Venlafaxine	300	Epilum	2500

Major Depressive Disorder

	Antipsychotic s	FRDD	Antidepressants	FRDD	Mood Stabilisers	FRDD
10	(Thioridazine)	(0.35)	(Desipramine)	(2.8)		
	(Mesoridazine)	(0.08)				
11	(Thioridazine)	(0.79)	(Doxepin)	(0.02)		
	(Mesoridazine)	(0.49)	(Nordoxepin)	(0.03)		
	(Sulforidazine)	(0.10)				
12			(Sertraline)	(2.1)		
13	Olanzapine	N/A				
		(0.2)				

Major Depressive Disorder						
	Antipsychotic s	**FRDD**	**Antidepressants**	**FRDD**	**Mood Stabilisers**	**FRDD**
14			(Sertraline)	(1.0)		
15						
16						
17						
18			Luvox	N/A	Lithium	N/A
			Mirtazapine	N/A		
			Fluoxetine	N/A		