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Effects of Mental Illness and Aging in Two Thalamic Nuclei

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Abstract

We previously reported a schizophrenia associated reduction of neuronal and oligodendrocyte number in the anterior principal thalamic nucleus (APN) in a cohort of severely impaired elderly subjects with schizophrenia (SZ) relative to age matched nonpsychiatric controls (NCs). The present study was undertaken to determine 1) if those findings could be replicated in an independent sample of less chronically impaired subjects with SZ and NCs stratified across a broader age range; 2) if the findings are specific to SZ or are also seen in unipolar major depressive (MDD) or bipolar disorder (BPD); and 3) if the findings are specific to the APN or also seen in another thalamic nucleus.

Computer assisted stereological methods were employed to determine the number of neurons and oligodendrocytes in the APN and centromedian nucleus (CMN) of the Nissl-stained thalamic sections maintained by the Stanley Foundation Brain Bank. This collection includes specimens from NCs and age matched subjects with diagnoses of SZ, MDD, or BPD who died between the ages of 25 and 68. Data were analyzed by mixed-effects linear regressions adjusting for demographic variables and known history of exposure to psychotropic medications.

Oligodendrocyte number was decreased in both nuclei relative to NCs in subjects with SZ and in that subset of subjects with BPD who had experienced psychotic episodes. Compared to NCs both of these patient groups also exhibited an attenuation of an age-related increase in the number of oligodendrocytes. Contrary to our previous report, we did not detect a SZ-associated deficit in neuronal number in the APN. A history of exposure to neuroleptics, however, was associated with a decrease in neuronal number in both nuclei, but this decrease did not vary in relation to cumulative lifetime neuroleptic exposure in fluphenazine equivalents. Among subjects with psychiatric diagnoses, exposure to lithium was associated with an increase in the number of oligodendrocytes. No effects were detected for exposure to anticonvulsants or for abuse of alcohol or other substances.

1. Introduction

The thalamus is a major hub of communication exchange not only between cortical and subcortical structures but also between different cortical regions. As such, its component nuclei provide links in the multiple functional circuits implicated in mental illness. Because each thalamic nucleus has a unique pattern of efferent and afferent projections, each carries different implications regarding mental illness. The anterior principal nucleus (APN) projects reciprocally to the cingulate and paracingulate gyri, and also sends efferents to the hippocampus. The hippocampus then projects to the mammillary bodies which, in turn, project

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back to the APN (van Veen and Carter 2002; van Groen et al. 1999; Saunders et al. 2005; Vogt et al. 1987; Swanson and Cowan 1977). The APN, therefore, provides a link for intralimbic circuits that have been implicated in SZ and affective disorders (Benes et al. 1991; Benes et al. 2001; Bouras et al. 2001; Carter et al. 1994; Dracheva et al. 2006; Stark et al. 2004; Young et al. 2004) and which mediate complex functions including memory, emotion, motivation, conflict monitoring and attention (van Veen and Carter 2002).

We previously reported a SZ-associated reduction in the number of neurons and oligodendrocytes in the APN in a cohort of chronically and severely impaired elderly subjects with SZ and age matched NCs (Byne et al. 2006). The present study was undertaken to determine if those findings could be replicated in an independent collection of specimens maintained by the Stanley Foundation (Torrey et al. 2000). Compared to the specimens examined in our initial study, those from the Stanley Foundation collection are from a cohort of less severely impaired subjects with SZ who had a much younger mean age at the time of death (well stratified between the ages of 25 and 68). The Stanley Foundation collection also has equal numbers of specimens from NCs and from subjects with unipolar major depressive disorder (MDD) and bipolar disorder (BPD). The collection, therefore, enabled us to examine the specificity of our previous findings to SZ as opposed to these other chronic psychiatric disorders. We also examined the specificity of our findings to the APN by examining the thalamic centromedian nucleus (CMN). A previous MRI study found a reduced CMN volume in SZ (Kemether et al. 2003); however, to date no study has reported either neuronal or oligodendrocyte counts for the CMN in relation to mental illness. The CMN has widespread cortical projections and is also a major regulator of the striatum (Sadikot et al. 1992; Jones 1985).

2. Experimental Design and Methods

2.1. Specimens

All data were obtained from the preexisting Nissl-stained thalamic sections from the Stanley Foundation Brain Collection. The demographics and histological processing of these specimens has been described fully elsewhere (Torrey et al. 2000). Briefly, the collection comprises every 16th 60 micron thick section through the thalamus of 60 subjects divided equally (15 per group) among those with diagnoses of SZ, MDD, or BPD, and NCs. Approximately half of the specimens in each group are from the left hemisphere, half from the right; half from males and half from females. History of psychotic episodes, medication history (exposure antipsychotics, antidepressants, anticonvulsants, lithium) and cause of death, including suicide are documented for each subject as is information regarding alcohol and other substance abuse. Anatomical data are not complete for all specimens due to missing or incomplete sections or inadequate staining. The demographics for all subjects from whom any data were gathered in the present study are summarized in Table 1. The exact numbers of subjects in each diagnostic category whose data were employed in each comparison are indicated in Table 2 and relevant figures.

2.2. Parcellation and Morphometrics

APN and CMN are depicted in Figures 1 and 2 and their delineations described in the accompanying legends. Every 16th serial section was available for study giving a minimum of 9 sections through each nucleus (range 9–16). Each slide containing at least one region of interest (ROI) was covered with a sheet of clear acetate on which one of the investigators (WB) drew its borders with an ultra fine point marking pen while viewing the slide under a binocular dissecting microscope. After registering the profiles of each ROI with the image analysis system, the acetate overlay was removed to allow microscopic examination. Counting frames (75 microns per side) positioned at the intersections of a randomly placed grid (800 microns/

side) within each ROI were then examined (40 ×, NA 1.25 oil objective) for cell counts which were made through the full thickness of the sections. Neurons, defined by the presence of distinct cytoplasm, nucleus and nucleolus (Figure 3), were counted in every available section if the nucleolus did not touch an exclusion line of the counting frame. Small, round, densely and homogeneously staining profiles (Figure 3) that did not touch exclusion lines were counted as nuclei of oligodendrocytes. In a previous study, close agreement was found between such profiles and immunocytochemically identified oligodendrocytes (Hof et al. 2003). Prior to initiating this study both cell counters had been trained to identify neurons and oligodendrocytes and had correctly identified video images of 100 thalamic cell profiles as neurons, oligodendrocytes or “other” with 100% agreement for neurons and 96% agreement for oligodendrocytes. One investigator (GY) counted neurons in all specimens, and another investigator (AT) counted oligodendrocytes in all specimens.

Nuclear volumes, cell numbers and cell densities were calculated using Bioquant software as described previously (Byne et al. 2007). Split-cell corrections using the method of Abercrombie (Abercrombie and Johnson 1946) were applied to the cell densities upon which cell number estimates were based. The corrected density of objects (Den) is based on the observed density (den), section thickness (t), and the diameter (d) of the objects counted (nuclei in the case of oligodendrocytes; nucleoli in the case of neurons): $Den = den(t/(t + d))$. These corrected densities were then multiplied by the volume of each ROI to give cell number estimates corrected for split cells. A separate correction factor was calculated for each cell type in each specimen based on the average section thickness for that specimen after histological processing and the average z-axis diameter of 25 oligodendrocyte nuclei and 25 neuronal nucleoli for each specimen.

Coefficients of error (CE) were calculated by the Bioquant software according to the formula of West (1991). The number of cells sampled per specimen (mean ± SD) for the APN was 181 ± 60 for neurons and 422 ± 329 for oligodendrocytes, giving CE's (mean ± SD) of $.08 \pm .01$ and $.03 \pm .02$ for neurons and oligodendrocytes, respectively. For the CMN the number of neurons sampled per specimen was 107 ± 33 (CE $.10 \pm .02$) and the number of oligodendrocytes sampled per specimen was 220 ± 154 (CE $.08 \pm .02$). Investigators were blind to diagnosis until counts were completed. Diagnostic and demographic information were made available to the investigators only after all data had been gathered and electronically relayed to the Stanley Medical Research Institute.

2.3. Statistical Analyses

Mixed-effects models are appropriate for the analysis of biological measures from more than one brain region per subject. Without such adjustment, statistical inferences may be incorrect (Gibbons 2000). We employed Supermix software to perform mixed-effects linear regressions (Hedeker and Gibbons 1996) with the following dependent variables for each nucleus: nuclear volume, number and density of neurons, and number and density of oligodendrocytes. Each dependent variable was adjusted for the two thalamic nuclei (APN and CMN) using a random effect for brain region.

Three sets of regressions were performed. The first set of regressions looked for an association between each of the diagnostic groups (NC, SZ, MDD, BPD) and the dependent variables. Each dependent variable was regressed upon region, 3 dummy variables for diagnosis (MDD, BPD, or SZ versus NC), sex, and hemisphere. Age, brain weight, PMI, storage time and interaction terms were included when significant.

A second set of regressions looked for an association between psychosis and the dependent variables using the same covariate adjustment described above. This was done once for the entire sample and again comparing only the BPDs with a history of psychosis to the NCs. A

third set of regressions looked at the association between the dependent variables and medication exposure, known history of alcohol and other substance abuse and death by suicide. The parameters of medication history examined were cumulative neuroleptic exposure in fluphenazine equivalents and history vs no history of exposure to neuroleptics, antidepressants, lithium, or anticonvulsants. The variability among individual medication regimens precluded a meaningful analysis of exposure to particular types of neuroleptics. All available details of medication history as well as alcohol and substance use are given for each subject in Supplementary Table 1.

3. Results

3.1. Effects of SZ and Age

For subjects with SZ compared to NCs, oligodendrocyte number was reduced by 45% in the APN and 40% in the CMN. These differences were statistically significant as evidenced by a main effect of diagnosis ($p=.013$) in the absence of a nucleus by diagnosis interaction (Figure 4, Table 2). These differences were primarily due to a SZ-associated reduction in the density of oligodendrocytes as the volumes of these nuclei did not differ between NCs and SZs. Mean oligodendrocyte density in subjects with SZ was reduced by 39% in the APN and 40% in the CMN, giving rise to a significant main effect of diagnosis ($p = .004$) (Figure 4, Table 2). There were no significant main effects for the other diagnoses, no diagnosis by nucleus interactions and no significant effects for nuclear volumes or neuronal number (Table 2). Compared to other diagnostic groups, the MDD group had the greatest number of neurons; however, we did not find that increase to be statistically significant relative to NCs as reported by others (Young et al. 2004). When the diagnosis-by-age interaction was included it was significant in the comparison of NCs with SZs for number of oligodendrocytes ($p = .02$) and approached significance for their density ($p = .05$). This interaction was due to a greater increase in oligodendrocyte number as a function of age in NCs compared to subjects with SZ (Figure 5).

3.2. Effects of Psychosis

When all subjects were categorized on the basis of history versus no history of psychosis, neuronal number was decreased in those with a history of psychosis by 19% in the APN and 18% in the CMN, giving rise to a significant main effect of psychosis ($p=.01$) (Table 2). Neuronal density, however, did not differ between groups as a function of psychotic history. Compared to subjects with no history of psychosis, those with such a history also exhibited reductions of both oligodendrocyte number (43% in the APN, 22% in the CMN) and oligodendrocyte density (31% in the APN and 19% in the CMN). These differences gave rise to significant main effects of psychosis ($p=.03$ for number, $p = 0.01$ for density) in the absence of significant nucleus by psychosis interactions. The psychosis by age interaction approached significance for number of oligodendrocytes ($p = 0.05$) with the age response in nonpsychotics being similar to that of the NCs (increasing with age) and the response of the psychotics similar to that of the SZs (attenuated age response relative to nonpsychotics).

An additional analysis was done in which the psychotic BPDs were only compared to the NCs. In this analysis, the main effect of psychosis remained significant for the number of oligodendrocytes which was decreased relative to NCs by 49% in the APN and 42% in the CMN ($p=.02$). The main effect of psychosis approached significance for number of neurons ($p = .07$); however, psychosis by age interactions did not reach statistical significance for either oligodendrocyte or neuronal numbers, and there were no significant main effects or interactions for density data.

3.3. Other Effects

A third set of regressions limited to subjects with a psychiatric diagnosis looked at the association between the dependent variables and medication history, alcohol and substance abuse, and suicide. In these analysis, neuronal number was lower in both nuclei (24% in APN; 19% in CMN) in those who had ever received neuroleptics compared to those with no neuroleptic exposure giving rise to a significant main effect of antipsychotic exposure ($p=.018$) (Figure 6). The main effect of exposure to antipsychotics approached significance ($p=.069$) when restricted to subjects with BPD. In these subjects neuroleptic exposure was associated with a mean reduction of 40% in the APN and 27% in the CMN. Among subjects with a history of antipsychotic exposure, however, neuronal number was not correlated with cumulative life time dosage in fluphenazine equivalents. No other effects of antipsychotics were seen.

A history of exposure to lithium, was associated with an increased number and density of oligodendrocytes in both nuclei when the analysis included all subjects with a psychiatric diagnosis ($p<.001$ for each nucleus) (Figure 7). Among subjects with a psychiatric diagnosis, those who died from suicide ($N=21$ for APN, 20 for CMN) on average had fewer neurons in both nuclei (14% for APN, 18% for CMN) compared to those who died of other causes ($N=19$ for both nuclei). This resulted in a trend level main effect of suicide ($z = -1.91$, $p = .05$) in the absence of an interaction with diagnosis or region. No significant effects were seen for history (positive vs negative) of exposure to antidepressants or anticonvulsants, or known history of alcohol or other substance abuse. No statistically significant effects of diagnosis were seen for the volume of either nucleus, and no main or interactive effects of sex or hemisphere were seen for any dependent variable.

4. Discussion

The present study replicates our previous finding of a SZ-associated deficit in the number of oligodendrocytes in the APN and suggests a comparable deficit in the CMN. Additionally, both nuclei exhibited an age-related increase in oligodendrocyte number in NCs that was attenuated in subjects with SZ. These findings are reminiscent of those of an MRI study of subjects between the ages of 20 and 65 (similar to the 25–68 year range of the present study) that detected evidence for age-related myelin changes in NCs but not SZs (Jones et al. 2006). We did not replicate our previous report of a SZ-associated neuronal deficit in the APN; however, relative to NCs, neuronal number was decreased in both nuclei in that subset of subjects with BPD who had experienced psychotic episodes. Neuronal number was also decreased in the group comprising all subjects (including those with SZ) who had experienced psychotic episodes and the group comprising all subjects who had been exposed to neuroleptics. These latter two groups are essentially the same since neuroleptics are generally given only in the context of psychosis. It is, therefore, not possible to determine whether this effect was due to psychosis or neuroleptic exposure. Similarly, the observation that the differences in number of oligodendrocytes in NCs and subjects with SZ diverged as a function of age raises the possibility that that difference also may be related to neuroleptic exposure instead of, or in addition to, SZ. The same holds true for the current observations of oligodendrocytes in that subset of subjects with BPD with a history of psychotic episodes, most of whom had also received neuroleptics.

While there is evidence from animal studies that neuroleptics may decrease oligodendrocyte number (Konopaske et al. 2008) and the expression of some myelin genes (Narayan et al. 2007), several human studies have not detected an influence of neuroleptic exposure in humans on oligodendrocyte number, morphology or gene expression (Uranova et al. 2001; Uranova et al. 2004; Dracheva et al. 2006; Katsel et al. 2005). Moreover, none of the cellular parameters examined in the present study varied as a function of cumulative antipsychotic dose. The similarities observed here between subjects with SZ and those with BPD and psychotic

episodes may, therefore, reflect psychosis as a common denominator rather than neuroleptic exposure. The attenuation of the age-related increase in oligodendrocyte number in these subjects may reflect an intrinsic abnormality of oligodendrocytes, their progenitors, or factors provided by other cells that influence the normal age-related proliferation of oligodendrocytes.

Although the present study did not examine myelination, it is possible that variations in oligodendrocyte number reflect variations in the myelination of axons originating in or passing through the nuclei investigated. Such differences, whether in the context of mental illness, aging or medication exposure could impact conduction velocities and thus the temporal pattern of convergence and summation of inputs to neurons within the circuits linked by the affected nuclei. Given the diffuse cortical projections of the CMN (Jones 2002) and the medial prefrontal and limbic projections of the APN (van Veen and Carter 2002; van Groen et al. 1999; Saunders et al. 2005; Vogt et al. 1987; Swanson and Cowan 1977), anomalous temporal summation within these nuclei would be expected to disrupt a multiplicity of functions including cognition, arousal, and affect.

4.1. Changes Myelin Structure and Oligodendrocyte Number with Age

It has long been appreciated that myelination in particular regions of the human brain may increase into the third decade (Benes 2004; Bartzokis 2004); however, more recent evidence suggests that in at least some brain regions myelination may increase during the fifth and sixth decades (Benes et al. 1994). Studies of aging animals have also shown changes in myelin structure and function and have linked these late occurring changes to the cognitive deficits of aging (Hinman et al. 2006). Electron microscopic studies show that the length of myelinated internodes (i.e., individual segments of myelin sheath) along the course of axons in visual cortex decreases progressively as monkeys progress beyond middle age so that more myelinated internodes are needed to cover a given length of axon (Peters 2002). That observation led the investigators to ask whether each oligodendrocyte produces additional myelin segments with advancing age, or whether additional oligodendrocytes are produced to provide the myelin for the increasing number of internodes. They found that the number of oligodendrocytes in gray matter increases with age as internodal length decreases and suggested that oligodendrogenesis accompanies the age-related remodeling of internodes (Peters and Sethares 2004). Their interpretation is consistent with the recent observation that oligodendrocytes may possess the potential to make new internodes only during a restricted period of their development (Watkins et al. 2007). Thus, remyelination, even in the context of normal age related myelin turnover, may require the generation of new myelinating oligodendrocytes. An age-related increase in oligodendrocytes accompanied by a decrease in internodal length has also been described in the mouse (Lasiene et al. 2007) and the expression of genes involved in myelin turnover increases with aging in rats (Blalock et al. 2003).

4.2. Oligodendrocyte/Myelin Deficits in Mental Illness

Numerous studies have described deficits of oligodendrocytes or other glial cells in various brain regions of subjects with SZ or affective disorders (Hof et al. 2003; Byne et al. 2006; Hamidi et al. 2004; Pakkenberg 1990; Stark et al. 2004; Pakkenberg and Gundersen 1988; Uranova et al. 2004; Rajkowska 2000; Rajkowska et al. 1999; Rajkowska and Miguel-Hidalgo 2007) although there have been some negative studies, e.g., (Benes et al. 1991). In these same patient populations neuroimaging studies have detected evidence of decreased myelin integrity (Jones et al. 2006; Kanaan et al. 2005; Kubicki et al. 2005; Haznedar et al. 2005; Auer et al. 2001), and molecular biological studies have also described deficits in the expression of genes associated with the oligodendrocyte lineage including some of those encoding proteins of mature myelin (Hakak et al. 2001; Dracheva et al. 2006; Katsel et al. 2005; Tkachev et al. 2003; Aston et al. 2004; Aston et al. 2005). The present data together with the animal research reviewed above suggest that the age by diagnosis (SZ vs NC) interaction for oligodendrocyte number may

reflect an inability of oligodendrocyte progenitors in subjects with SZ (or perhaps with exposure to neuroleptics) to proliferate and/or mature appropriately as required for the normal turnover and remodeling of internodes that occurs with aging.

4.3. Limitations of the Present Study and Questions for Future Research

The present study relied on cytoarchitectonic criteria to identify oligodendrocytes in a preexisting collection of Nissl-stained material. Although Nissl staining is not specific, in a previous study we found close agreement between profiles identified by these criteria and those stained immunocytochemically for using antibodies specific to oligodendrocytes (Hof et al. 2003). Additionally, oligodendrocytes identified by such criteria are likely to be functionally heterogeneous. For example, it has been proposed that the oligodendrocytes that occur as satellites to neurons do not form myelin but possess the potential to transform into oligodendrocyte precursor cells under appropriate conditions (Ludwin 1984; Ludwin 1979; Butt et al. 2005), whereas those that occur in pairs or clusters may represent recently generated nonmyelinating oligodendrocytes (Peters and Sethares 2004). While the specificity afforded by immunocytochemical labeling would be desirable, immunocytochemical staining often gives variable results in postmortem human material due to variations in agonal state, postmortem interval, fixation and storage. Moreover, the extent to which the decreased expression of myelin/oligodendrocyte genes in SZ and BPD reflects down regulation of their expression as opposed to a numerical deficit of oligodendrocytes is not certain (Tkachev et al. 2003; Davis and Haroutunian 2003). The gene expression deficits are not likely to reflect simple cell deficit since not all oligodendrocyte/myelin genes appear to be affected. To the extent that down regulation of genes might preclude immunocytochemically detectable staining for their protein products, immunocytochemical studies could lead to incorrect inferences regarding oligodendrocyte number.

Another limitation of the present study is that it did not assess either myelin turnover or integrity. In the absence of such data, strong inferences regarding the functional significance of variations in the number of putative oligodendrocytes cannot be made. Finally, as discussed above, it is, not possible to conclude that the observed disorder-associated effects reported here are independent of neuroleptic exposure. An age-related increase of oligodendrocyte number has been demonstrated in animal models (Lasiene et al. 2007; Peters and Sethares 2004) in which the potential effects of neuroleptic exposure suggested by the present data could be tested.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Delineation of APN

Coronal 60 micron thick Nissl stained sections through the APN arranged rostrocaudally from panels **a**) to **c**). The APN comprises both the anteroventral (AV) and anteromedial (AM) nuclei. Because the boundary between these two subdivisions is indistinct, they were not assessed separately in this study. At most levels, the APN is clearly delineated by the ventricular space medially, the internal medullary lamina medially, laterally and superiorly (arrowheads), and clusters of deeply staining neurons (arrows) of the internal medullary lamina ventrally and ventrolaterally. **a**) At this most anterior level both the AV and AM are present and a wide lamina separates them medially from the ventral anterior nucleus (VA). **b**) More caudally, AM has disappeared and only AV remains. Here AV is well circumscribed by the lamina. Ventrally, CL, a prominent member of the intralaminar nuclei, intervenes between AV and the mediodorsal nucleus (not shown in this panel). **c**) In its most caudal extent, AV is separated from the ventricle by LD whose neurons stain less intensely than those of AV. The boundary between AV and LD is indicated by the dotted line. At higher power, or with dark field optics (not shown), fascicles can be seen separating these two nuclei. The white arrow indicates the superior border of CL which intervenes between AV and the MDN. The scale bar corresponds to 5 mm.

Abbreviations: AM anterior medial nucleus, APN anterior principal nucleus, AV anteroventral nucleus, CL central lateral nucleus, LD lateral dorsal nucleus, VA ventral anterior nucleus, VLp ventral lateral posterior nucleus.

Figure 2. Delineation of CMN

Coronal 60 micron thick Nissl stained sections through the CMN. **a)** In the coronal plane, CMN emerges anteriorly just as LD replaces the APN more dorsally. The CMN is bounded at this level by the MDN medially, Pf ventromedially and VPM laterally. At most levels the CMN is clearly circumscribed by the internal medullary lamina which can be seen in the higher power views of panels **b)** and **c)**. **b)** Higher power view of same section depicted in **a)**: Here the internal medullary lamina (arrowheads) can be seen surrounding the CMN medially and laterally. Ventromedially, the border with Pf is less clear. At times fascicles of the lamina (arrowheads) may be seen along this border and in other places deeply staining clusters (arrows) of neurons associated with the lamina may be seen. Care was taken to minimize the extent to which these clusters were included within the perimeter drawn for the CMN. **c)** In the posterior extent of CMN, PA replaces VPM medially. CMN is well circumscribed by the lamina (black arrowheads) and clusters of intralaminar neurons (arrows). A prominent grouping of neurons, sometimes designated as the magnocellular division of the CMN (Dewulf 1971) is conspicuous at this level (white arrow). No attempt was made to subdivide the CMN in the present study. The scale bar corresponds to 5 mm. **Abbreviations:** CMN centromedian nucleus, LD lateral dorsal nucleus, MDN mediodorsal nucleus, PA anterior pulvinar nucleus, Pf parafascicular nucleus, VLp ventral lateral posterior nucleus, VPL ventral posterior lateral nucleus, VPM ventral posterior medial nucleus.

Figure 3. Characteristics Cells Counted

Neurons were only counted if in addition to cytoplasm, a clear nucleus containing a nucleolus (white arrows) was visible. Small, nuclei which were almost perfectly round, uniformly darkly stained, and lacking visible cytoplasm were counted as presumed oligodendrocytes (arrowheads). **a)** A single neuron with conspicuous nucleolus (white arrow) is seen. Several oligodendrocytes are seen, one in close association with the neuron (probably a satellite), one with no apparent association with neurons or other oligodendrocytes, and three closely spaced and aligned as if associated with a single axon. **b)** Although 2 neurons are seen, only one has a distinct nucleolus (white arrow) in focus here. A satellite oligodendrocyte (arrowhead) is seen closely opposed to one of the neurons. The other darkly stained profiles are not in focus and, therefore, not classified here. **c)** A single neuron with nucleolus (white arrow) is seen and 2 nuclei meeting our criteria for presumptive oligodendrocytes. **d)** Only two nuclei meeting criteria for oligodendrocytes are seen (black arrowheads). Scale bar corresponds to 10 microns.

Figure 4. Oligodendrocyte Number and Density Across Diagnostic Categories

The main effect of SZ was significant for both number ($z = -2.47$, $p = .01$) and density ($z = -2.89$; $p = .004$) of oligodendrocytes. Main effects for other diagnoses were nonsignificant. There were no statistically significant nucleus by diagnostic group interactions. In the absence of such interactions, post hoc tests are not warranted and it cannot be concluded that any diagnosis differentially affected either nucleus. Means and SEs for each group are given in Table 2. The numbers in parentheses refer to the numbers of subjects in each group.

Figure 5. Age by Diagnosis Interaction for NC vs SZ

The diagnosis by age interaction was significant in the comparison of NCs with SZs for number of oligodendrocytes ($z = -2.40$, $p = .02$). As shown here, the regression of number on age has a greater positive slope in NCs than subjects with SZ. This interaction approached significance for density of oligodendrocytes ($z = -1.99$, $p = .05$) (data not shown). When all subjects were stratified by history vs no history of psychotic episodes, the psychosis by age interaction approached significance for number of oligodendrocytes ($z = -2.00$, $p = .05$) with the age response in nonpsychotics being similar to that of the NCs and the response of the psychotics similar to that of the SZs (data not shown).

Figure 6. Neuronal Number in Patients With and Without History of Neuroleptic Exposure

Mean neuronal number was lower in both nuclei in patients with (+) compared to those without (-) a history of neuroleptic exposure giving rise to a significant main effect of exposure ($z = -2.35, p = .018$). The numbers in parentheses refer to the numbers of subjects in each group for which the comparisons were made.

Figure 7. Oligodendrocyte Number in Patients With and Without History of Lithium Exposure
Mean oligodendrocyte number was higher in both nuclei in patients with (+) compared to those without (-) a history of lithium exposure giving rise to a significant main effect of exposure ($z=3.57$, $p=.0004$). The main effect was also significant for oligodendrocyte density ($z=4.41$, $p=.00001$) (Data not shown). The numbers in parentheses refer to the numbers of subjects in each group.

Table 1

Characteristics of Sample

Dx	N	Age (Years)	Sex (F/M)	Side (R/L)	Psy (+/-)	Suicide (+/-)	AP(+/-)	AD(+/-)	AC (+/-)	Lithium (+/-)	Br wt (gms)	PMI (hrs)	Storage (months)
NC	14	48.6 ± 10.9	6/8	6/8	0/14	0/14	0/14	0/14	0/14	0/14	1515 ± 161	24.6 ± 9.7	6.6 ± 5.0
SZ	14	43.3 ± 12.6	5/9	6/8	14/0	3/10	12/1	4/9	1/12	2/11	1486 ± 96	34.2 ± 15.0	8.8 ± 5.9
MDD	13	46.8 ±	6/7	6/7	0/13	7/6	0/13	10/3	0/13	2/11	1440 ± 129	26.2 ± 10.6	7.2 ± 4.2
BPD	15	42.3 ± 11.7	6/9	8/7	11/4	9/6	12/3	8/7	7/8	4/11	1441 ± 171	32.5 ± 16.1	12.0 ± 8.5

Numbers and characteristics of specimens for which any data were gathered in present study. Abbreviations: AC anticonvulsant exposure, AD antidepressant exposure, AP antipsychotic exposure, Dx diagnosis, F female, L left hemisphere, M male, N number, R right hemisphere, PMI postmortem interval Psy history of psychosis. More complete demographic information for each subject is available in table S1.

Table 2

Summary of Results by Diagnosis

	NC	MDD	BPD (all)	BPD (psychotic)	All With Psychosis	SZ	All Without Nonpsychosis
Volume (cc)	APN	0.15 ± 0.01 (13)	0.13 ± 0.01 (15)	0.12 ± 0.01 (11)	0.13 ± 0.01 (25)	0.13 ± 0.01 (14)	0.16 ± 0.01 (31)
	CMN	0.13 ± 0.01 (14)	0.13 ± 0.01 (14)	0.12 ± 0.01 (10)	0.13 ± 0.01 (22)	0.14 ± 0.01 (12)	0.13 ± 0.01 (31)
Neuronal Number (E5)	APN	3.26 ± 0.22 (14)	3.73 ± 0.30 (13)	2.94 ± 0.33 (14)	2.47 ± 0.28 (10)	2.91 ± 0.46 (23)	3.25 ± 0.33 (13)
	CMN	2.07 ± 0.21 (14)	2.15 ± 0.11 (13)	1.73 ± 0.19 (14)	1.63 ± 0.24 (10)	1.73 ± 0.12 (22)	1.81 ± 0.10 (13)
Neurons per cc (E6)	APN	2.15 ± 0.10 (14)	2.34 ± 0.16 (13)	2.11 ± 0.14 (14)	1.96 ± 0.15 (10)	2.21 ± 0.13 (23)	2.41 ± 0.18 (13)
	CMN	1.55 ± 0.11 (14)	1.78 ± 0.13 (13)	1.37 ± 0.12 (14)	1.36 ± 0.16 (10)	1.39 ± 0.10 (22)	1.41 ± 0.14 (13)
Oligodendrocyte Number (E5)	APN	8.27 ± 1.66 (14)	7.31 ± 1.67 (13)	4.79 ± 0.73 (15)	4.23 ± 0.92 (11)	4.40 ± 0.55 (24)	4.54 ± 0.69 (13)
	CMN	5.86 ± 1.10 (14)	2.90 ± 0.38 (14)	3.63 ± 0.50 (14)	3.41 ± 0.66 (10)	3.45 ± 0.44 (22)	3.49 ± 0.62 (13)
Oligodendrocytes per cc (E6)	APN	5.40 ± 0.95 (14)	4.52 ± 0.96 (13)	3.59 ± 0.38 (15)	3.48 ± 0.49 (11)	3.38 ± 0.32 (24)	3.31 ± 0.43 (13)
	CMN	4.33 ± 0.75 (14)	2.41 ± 0.32 (14)	2.98 ± 0.42 (14)	2.93 ± 0.55 (10)	2.73 ± 0.36 (22)	2.56 ± 0.48 (13)

Bolded values represent significant main effects for diagnostic group. There were no nucleus by diagnostic group interactions. SZ, MDD, BPD(all) and BPD (psychotic) groups were tested against NCs. The group comprising all subjects with a history of psychosis was tested only against the groups comprising all subjects with no psychotic history, including NC. The main effect of SZ was significant for both number ($z = 2.47$, $p = .01$) and density ($z = 2.89$; $p = .004$) of oligodendrocytes. When all subjects were categorized on the basis of history versus no history of psychosis there was a main effect of psychosis for number ($z = -2.92$, $p = .03$) and density ($z = -2.55$, $p = .01$) of oligodendrocytes and number of neurons ($z = -2.51$, $p = .01$). In the comparison of BPD(psychotic) vs NC, the main effect of psychosis remained significant for the number of oligodendrocytes which was decreased relative to NCs in ($z = -2.35$, $p = .02$) and approached significance for number of neurons ($z = -1.81$, $p = .07$).