Postmortem Study Methods and Results

Methods

Characteristics of Subjects

Human brain specimens from the prefrontal cortex (PFC) of patients with MDD and matched controls were obtained from the Dallas Brain Collection (1). Briefly, after obtaining next of kin permission, brain tissue was collected from cases at the Dallas County Medical Examiners Office and Transplant Service Center at the University of Texas Southwestern Medical Center. Blood toxicology screens were conducted in each case. We excluded subjects with known history of neurological disorders or head injury. Clinical records and collateral information from telephone interviews with a primary caregiver was obtained for each case. Two board certified psychiatrists carried out an extensive review of the clinical information and made independent diagnoses followed by a consensus diagnosis using DSM-IV criteria. There were no significant differences in clinical or demographic parameters between the two groups. Seven of the control subjects and 4 of the depressed subjects were smokers at the time of death. Smoking status for 1 control and 2 depressed subjects were unknown. Seven out of 14 subjects were on antidepressants at the time of death, 6 of them were on SSRIs. Information about the onset of the illness were obtained for only 8 of the subjects out of 14 and 3 out of 8 subjects had an early onset of depressive symptoms (<age 25). The collection of human brain specimens was approved by the Institutional Review Board of University of Texas Southwestern Medical Center. The groups were matched as closely as possible for race, gender, age, pH and postmortem interval (PMI) (see Table 1S for details).

Tissue preparation

In each case, cerebral hemispheres were cut coronally into 1-2 cm blocks. Prefrontal cortex samples from Brodman's area 9 (BA9) were dissected and immediately placed in a

mixture of dry ice and isopentane (1:1, v:v). Tissue blocks were kept frozen at -80^{o} C until they were cryostat sectioned in the coronal plane at 14 µm at -20^{o} C**,** thaw-mounted onto gelatin-coated microscope slides, then stored at -80° C.

Tissue processing and quantification of [^{125}I]A85380 binding

The day of the binding experiments, slides were allowed to warm to room temperature and equilibrium binding was performed as described previously (2). [^{125}I]A85380 was purchased from Perkin Elmer (Waltham, MA, USA) and diluted (200 pM) in TRIS-buffer (50 mM, pH=7.4) and placed on slices for 30 min. Slides were then rinsed briefly 3 times in chilled buffer and one time in milliQ water. After drying, slices were exposed for 24 hr to MR film (KODAK). Films were digitized, analyzed using Adobe Photoshop, and mean gray value was then averaged at three focal points in each brain regions. The entire gray matter region and the white matter immediately below this region were analyzed. The regions were differentiated based on cellular density which was consistent with a distinct pattern of higher binding density in the gray matter areas.

Statistical analysis

Postmortem data were analyzed using ANOVA and correlations were analyzed using appropriate correlation coefficients. For these data, each cell was an average of three measurements performed for each brain region for each patient. Data were then analyzed by ANOVA with "group" (Control or MDD) as between-factor and "brain region" (grey or white matter) as within-factor. Alpha was set at 5%.

Results

Demographic data for the sample of patients with a diagnosis of MDD and matched controls are provided on Table 1S. The groups were matched for age and gender and there was

no statistically significant difference in the postmortem interval (PMI) or RNA integrity number (RIN) between the two groups.

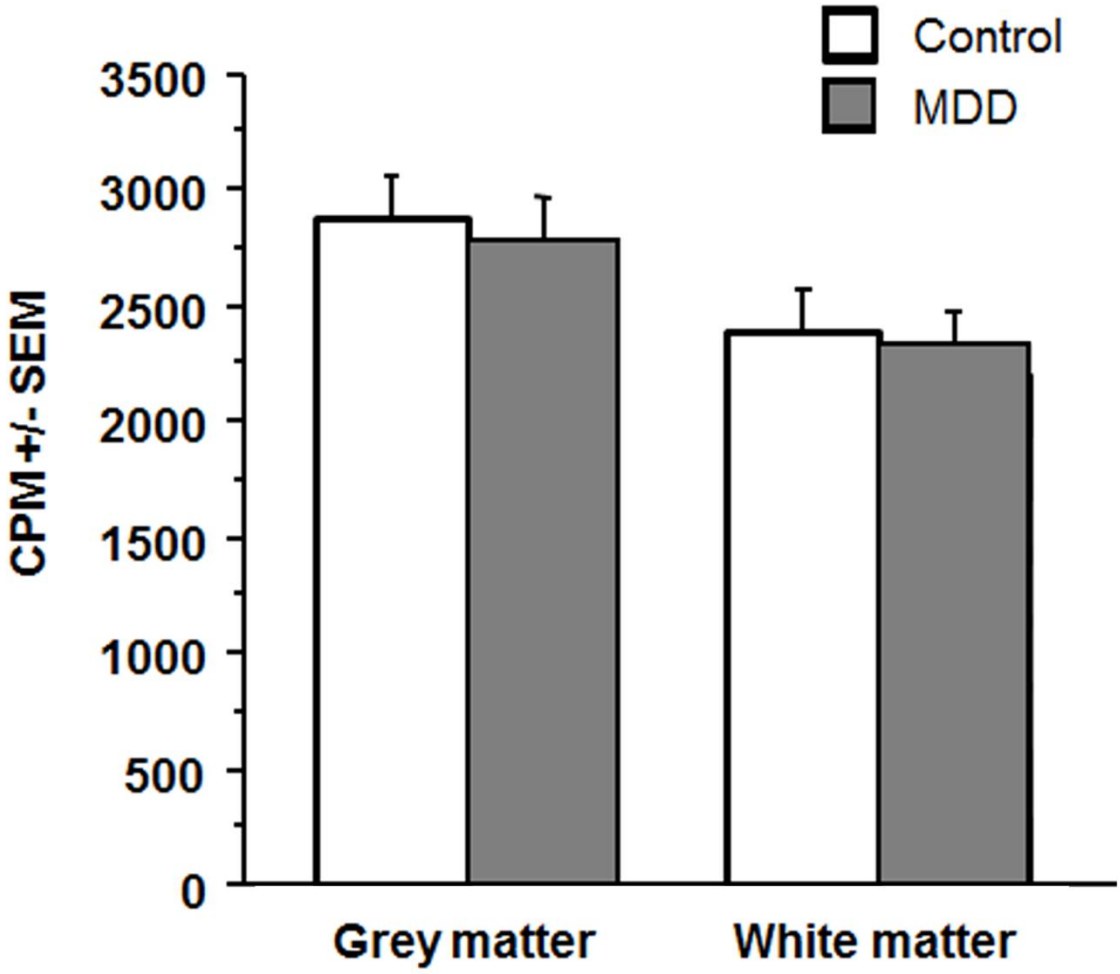
There was an effect of region (grey matter vs white matter; $F=15.25$, df1, $p<0.01$) but no effect of diagnosis or diagnosis*region interaction (all F's <1).

Equilibrium binding studies of [^{125}I]A85380 (the same compound used for in vivo studies) were performed in postmortem tissue, under conditions in which all endogenous ligand binding was washed out. In the postmortem study there were no statistically significant differences in β2*-nAChR number in patients with MDD compared with healthy controls (Figure 2; main effect of group $F(1, 23) = 0.46$, $p = 0.52$; for interaction region by group: $F(1, 46) = 0.23$, $p = 0.78$).

Table 1S. Demographic and postmortem variables on sample of patients with MDD (n=14) and matched controls (n=14). There were no statistically significant differences between groups in age or gender and there were no statistically significant correlations between β_2*-nAChR binding and postmortem interval (PMI) or RNA integrity number (RIN).

Characteristics	**Depressed (N=14)**	**Controls (N=14)**	**Statistics**
Age (Mean, SD)	47.6 (14.0)	49.1 (14.4)	p=0.78
Male (N, %)	10 (71.4)	12 (85.7)	X^2_1 =0.85, p=0.36
Caucasian (N, %)	13 (92.9)	13 (92.9)	X^2_1 =0.00, p=1.0
PMI (Mean, SD)	18.7 (6.1)	18.0 (8.4)	p=0.78
RIN (Mean, SD)	8.1 (1.1)	8.0 (1.0)	p=0.70

Figure 1S. Comparison of β_2*-nAChR number in postmortem samples of the prefrontal cortex from patients with MDD and controls ($F_{1, 23} = 0.46$, $p = 0.52$; for interaction group*brain region: $F_{1, 46} = 0.23$, $p = 0.78$)



References

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2. Rollema H, Guanowsky V, Mineur YS, Shrikhande A, Coe JW, Seymour PA, Picciotto MR: Varenicline has antidepressant-like activity in the forced swim test and augments sertraline's effect. Eur J Pharmacol 2009; 605(1-3):114-6