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## Reciprocal Alterations in Cortical Cannabinoid Receptor 1 Binding Relative to Protein Immunoreactivity and Transcript Levels in Schizophrenia

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### Abstract

The deleterious effects of cannabis use in schizophrenia have been linked, in part, to underlying disturbances in endogenous cannabinoid signaling in the prefrontal cortex. However, while receptor autoradiography studies of the primary cannabinoid receptor (CB1R) have consistently found higher CB1R binding in the prefrontal cortex in schizophrenia, deficits in CB1R mRNA levels and protein immunoreactivity have also been reported in the illness. To investigate this apparent discrepancy, we quantified CB1R binding using receptor autoradiography with the selective CB1R ligand [<sup>3</sup>H]-OMAR in the prefrontal cortex of 21 subjects with schizophrenia that were previously found to have lower levels of both CB1R mRNA using in situ hybridization and CB1R protein using radioimmunochemistry relative to matched healthy comparison subjects. We observed higher levels of [<sup>3</sup>H]-OMAR binding in the prefrontal cortex of schizophrenia subjects that did not appear to be attributable to psychotropic medications or substance abuse. The combination of lower levels of CB1R mRNA and immunoreactivity with higher CB1R receptor binding may reflect either altered trafficking of the receptor resulting in higher levels of

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#### Contributors

Dr. Volk oversaw all aspects of the design and implementation of the study and was the primary author of the manuscript. Drs. Eggan, Horti, Wong, and Lewis contributed to the design of the study and interpretation of the data. All authors contributed to and have approved the final manuscript.

#### Disclosures

David A. Lewis currently receives investigator-initiated research support from Bristol-Myers Squibb and Pfizer and in 2012-2014 served as a consultant in the areas of target identification and validation and new compound development to Autifony, Bristol-Myers Squibb, Concert Pharmaceuticals, and Sunovion. All other authors have nothing to disclose.

membrane-bound CB1R or higher CB1R affinity. In either case, greater CB1R receptor availability may contribute to the increased susceptibility of schizophrenia subjects to the deleterious effects of cannabis use.

## Keywords

CB1R; cannabis; cannabinoid; marijuana; PET; rimonabant; radioligand; prefrontal cortex; schizophrenia

## 1. Introduction

Cannabis use during early adolescence has been linked to a higher risk of developing schizophrenia, an earlier age of onset, and greater illness severity (D'Souza et al. 2005; Compton et al. 2009; Foti et al. 2010; Casadio et al. 2011; Galvez-Buccollini et al. 2012). These associations may indicate that the disease process of schizophrenia involves disturbances in endogenous cannabinoid signaling which may in turn predispose at-risk adolescents to greater debilitating effects of cannabis use. However, at the present time, studies of the primary endogenous cannabinoid receptor (CB1R) in the brains of individuals with schizophrenia have yielded apparently conflicting results. Receptor autoradiography studies using a CB1R agonist ( $[^3\text{H}]$ -CP55940) or antagonists/inverse agonists ( $[^3\text{H}]$ -SR141716 and  $[^3\text{H}]$ -MePPEP) have consistently found higher CB1R binding to receptor protein across multiple brain regions, including the prefrontal cortex, in schizophrenia subjects (Dean et al. 2001; Zavitsanou et al. 2004; Newell et al. 2006; Dalton et al. 2011; Jenko et al. 2012). In contrast, we previously reported lower CB1R mRNA levels by *in situ* hybridization and CB1R protein levels using radioimmunocytochemistry in the prefrontal cortex of subjects with schizophrenia (Eggan et al. 2008; Eggan et al. 2010b). Furthermore, other groups have reported either lower or unchanged levels of CB1R immunoreactivity and mRNA in prefrontal cortex and anterior cingulate cortex (Koethe et al. 2007; Uriguen et al. 2009). The reason for this discrepancy in CB1R measures in schizophrenia has remained unclear.

Interestingly, binding of an allosteric modulation site has been reported to induce a conformational change in CB1R, which in turn increases the affinity of ligands such as  $[^3\text{H}]$ -CP55940 for the orthosteric binding site on CB1R (Price et al. 2005). Thus, reports of higher CB1R binding in the prefrontal cortex in schizophrenia may reflect greater receptor affinity, perhaps even in the presence of fewer CB1Rs. The recent development of a novel analog of the selective CB1R inverse agonist rimonabant, OMAR (JHU75528; 4-cyano-1-(2,4-dichlorophenyl)-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide), that displays a high binding affinity for CB1R (Fan et al. 2006) provides an opportunity to examine this idea. Indeed, a pilot PET study employing radiolabeled  $[^{11}\text{C}]$ -OMAR reported evidence of higher brain CB1R receptor binding in some subjects with schizophrenia (Wong et al. 2010) and of an inverse correlation between CB1R receptor binding and negative symptoms assessed using the Brief Psychiatric Rating Scale (Wong et al. 2012). Consequently, we conducted a receptor autoradiography study using  $[^3\text{H}]$ -OMAR in the same schizophrenia subjects in whom we had previously found lower CB1R mRNA levels

by in situ hybridization and protein levels by radioimmunocytochemistry. Employing a highly similar film-based quantification approach across studies, we sought to determine the relationship between CB1R binding and CB1R mRNA and immunoreactivity levels in nearby tissue sections from the same subjects with schizophrenia.

## 2. Materials and methods

### 2.1. [<sup>3</sup>H]-OMAR ([Methoxy-3H]JHU75528)

The radiotracer was purchased from PerkinElmer (Shelton, Connecticut). The specific radioactivity was > 80 Ci/mmol and radiochemical purity was 97.0%.

### 2.2. Human subjects

Brain specimens were obtained during routine autopsies conducted at the Allegheny County Office of the Medical Examiner (Pittsburgh, PA) after consent was obtained from next-of-kin. An independent committee of experienced research clinicians made consensus DSMIV (American Psychiatric Association 1994) diagnoses for each subject using structured interviews with family members and review of medical records, and the absence of a psychiatric diagnosis was confirmed in healthy comparison subjects (Volk et al. 2011; Volk et al. 2012). To control for experimental variance, subjects with schizophrenia or schizoaffective disorder (n=21) were matched individually to one healthy comparison subject for sex and as closely as possible for age and postmortem interval (Table 1; Supplemental Table S1) as previously described (Eggen et al. 2008), and samples from subjects in a pair were processed together throughout all stages of the study. The mean age, postmortem interval, brain pH, and tissue freezer storage time did not differ between subject groups ( $t_{(40)} = 0.67$ ,  $p = 0.51$ ) (Table 1). In the right hemisphere of all subject pairs, CB1R mRNA and protein levels were previously quantified by in situ hybridization and radioimmunocytochemistry, respectively (Eggen et al. 2008). (Two subject pairs from this previous study of CB1R mRNA and protein levels were not included in the present study due to lack of tissue availability). All procedures were approved by the University of Pittsburgh's Committee for the Oversight of Research Involving the Dead and Institutional Review Board for Biomedical Research.

### 2.3. Tissue processing

For each subject, coronal blocks through the right prefrontal cortex were frozen and stored at  $-80^{\circ}\text{C}$ . Cryostat sections (20  $\mu\text{m}$ ) from the middle portion of the superior frontal sulcus were thaw mounted on Superfrost slides (VWR Scientific, West Chester, Pennsylvania) and stored at  $-80^{\circ}\text{C}$ . Cytoarchitectonic criteria (Rajkowska and Goldman-Rakic 1995) were used to identify the location of area 9 in Nissl-stained sections (Volk et al. 2000). For each subject, 3 sections separated by at least 320  $\mu\text{m}$  and at a similar rostral-caudal level to the matched subject within the pair were processed for quantitative receptor autoradiography. The selected sections were located within the same tissue block as the tissue sections previously studied for CB1R mRNA using in situ hybridization and protein using radioimmunocytochemistry.

## 2.4. CB1R autoradiography with [<sup>3</sup>H]-OMAR

To our knowledge, [<sup>3</sup>H]-OMAR has not been employed previously in radiolabeled ligand binding studies of human prefrontal cortex tissue. Consequently, we tested a wide range of concentrations of [<sup>3</sup>H]-OMAR (1.25 nM - 140 nM) in a series of pilot studies including prefrontal cortex tissue sections from 3-4 healthy comparison subjects. We found that 10 nM [<sup>3</sup>H]-OMAR provided the clearest and most specific signal as indicated by a sharp and clearly demarcated gray matter-white matter border (Figure 1A-B), and this specific signal was greatly reduced by competition with 1000× fold excess cold CP55940 (a CB1R agonist), cold SR141716 (a CB1R antagonist/inverse agonist), and cold OMAR in separate pilot studies. Consequently, a concentration of 10 nM [<sup>3</sup>H]-OMAR was used in the final study.

Glass slides containing tissue sections were removed from the freezer and allowed to thaw and dry at room temperature. Slides were placed in a pre-incubation buffer containing 50 mM Tris-HCl (pH 7.4) for 30 min at room temperature, then incubated in 10 nM [<sup>3</sup>H]-OMAR (specific activity 81.8 Ci/mmol) in 50 mM Tris-HCl containing 1% bovine serum albumin (pH 7.4) for one hour at room temperature. Slides were then placed through a series of post-incubation rinses in 50 mM Tris-HCl/1% bovine serum albumin (pH 7.4) at 4°C, rinsed briefly in ice-cold ultrapure water, and air dried in front of a fan. Tissue sections were then exposed to BioMax MR film (Kodak, Sigma-Aldrich, St. Louis, Missouri) along with tritium standards (μCi/g) on glass slides (American Radiolabeled Chemicals, St. Louis, Missouri) for 4.5 weeks. All three tissue sections from each of the 21 schizophrenia and 21 healthy comparison subjects were incubated in [<sup>3</sup>H]-OMAR on the same day and exposed to film on the same day, and later the films were developed on the same day.

## 2.5. Quantification of [<sup>3</sup>H]-OMAR binding

[<sup>3</sup>H]-OMAR binding was quantified using a Microcomputer Imaging Device system (Imaging Research Inc, London, Ontario, Canada) without knowledge of diagnosis or subject number by random coding of film autoradiograms in a manner highly similar to our previous studies of CB1R mRNA using in situ hybridization and protein using radioimmunocytochemistry conducted in nearby tissue sections from the same subjects (Eggan et al. 2008). Briefly, prefrontal cortex area 9 gray matter optical density was measured in each tissue section by drawing contours of the full thickness of the cortex exclusively in the zones where the cortex was cut perpendicular to the pial surface. The mean (SD) total area of gray matter sampled in each subject was 277 (109) mm<sup>2</sup> for control subjects and 291 (141) mm<sup>2</sup> for subjects with schizophrenia. Standardization curves derived from the tritium standards exposed on the same film were used to convert optical density measures of the autoradiograms into fmol [<sup>3</sup>H]-OMAR/mg estimated tissue equivalents using the specific activity of [<sup>3</sup>H]-OMAR (81.8 Ci/mmol). Next, to determine differences in [<sup>3</sup>H]-OMAR binding across lamina, optical density was measured in approximately 1 mm-wide cortical traverses extending from the pial surface to the white matter (Eggan et al. 2008). Three cortical traverses per section (9 traverses per subject) were placed in locations where the tissue section was cut perpendicular to the pial surface as determined by the presence of pyramidal neurons with vertically oriented apical dendrites in adjacent Nissl-stained sections. Within each traverse, the optical density in each layer was determined by

dividing the total cortical thickness from the pial surface to white matter into zones of 1% to 10%, 10% to 30%, 30% to 50%, 50% to 60%, 60% to 80%, and 80% to 100% approximating layers 1, 2 to superficial 3, deep 3, 4, 5, and 6, respectively (Pierri et al. 1999; Eggan et al. 2008).

## 2.6. Statistical analysis

Analyses of covariance (ANCOVA) were first conducted to determine whether [<sup>3</sup>H]-OMAR binding measures were related to sex, race, age at death, postmortem interval, brain pH, and/or freezer storage time. No effect of these covariates was found on [<sup>3</sup>H]-OMAR binding (all  $F < 2.3$ ;  $p > 0.14$ ), and Pearson correlation analyses also found no relationship between the continuous covariates and [<sup>3</sup>H]-OMAR binding (all  $r < |.24|$ ,  $p > .13$ ). Consequently, these covariates were not included in the final analysis. However, subject pairing, which accounts for the parallel processing of tissue samples from a pair and balances diagnostic groups for sex, age, and postmortem interval, had a significant effect on [<sup>3</sup>H]-OMAR binding ( $F_{(1,20)}=3.30$ ;  $p=0.005$ ). The significant effect of pair may be thought of as validating the paired approach as the matching of subjects as pairs captured a substantial portion of the variance across all subjects due to factors other than the effect of diagnosis. Therefore, the ANCOVA model we report includes [<sup>3</sup>H]-OMAR binding as the dependent variable, diagnostic group as the main effect, and subject pair as a blocking factor. The reported  $p$  values for comparisons of [<sup>3</sup>H]-OMAR binding are one-tailed because CB1R receptor binding has been repeatedly shown to be higher in the prefrontal cortex in schizophrenia (Dean et al. 2001; Zavitsanou et al. 2004; Newell et al. 2006; Dalton et al. 2011; Jenko et al. 2012).

## 3. Results

### 3.1. [<sup>3</sup>H]-OMAR binding in the prefrontal cortex in schizophrenia

Horti, Wong, and colleagues previously demonstrated a high degree of specificity of [<sup>11</sup>C]-OMAR binding for CB1R including: 1) mouse, baboon, and human studies that found a regional distribution of OMAR binding (e.g., high levels in cerebral cortex gray matter, globus pallidus, hippocampus and cerebellum and low levels in cerebral cortex white matter and thalamus) (Horti et al. 2006; Wong et al. 2010) consistent with the reported distribution of CB1R by radioimmunocytochemistry (Eggen and Lewis 2007); and 2) blocking of specific OMAR binding by a selective CB1R inverse agonist rimonabant (SR141716) (Horti et al. 2006). Consistent with this evidence of specific binding to CB1R, we also found that [<sup>3</sup>H]-OMAR binding is much greater in prefrontal cortex gray matter than in white matter (Figure 1).

Quantitative receptor autoradiography with the highly selective CB1R ligand [<sup>3</sup>H]-OMAR revealed higher binding (+8%;  $F_{(1,20)}=2.99$ ,  $p<.05$ ) in the gray matter of schizophrenia subjects ( $213.3 \pm 43.1$  fmol/mg) relative to healthy comparison subjects ( $197.5 \pm 43.8$  fmol/mg). Laminar analysis revealed that [<sup>3</sup>H]-OMAR binding was increased in all cortical layers in schizophrenia subjects relative to healthy comparison subjects (Figure 2). These differences were statistically significant in layer 1 (+14.5%;  $F_{(1,20)}=3.90$ ,  $p=0.031$ ), layer deep 3 (+8.2%;  $F_{(1,20)}=3.01$ ,  $p=0.049$ ), and layer 5 (+8.2%;  $F_{(1,20)}=2.97$ ,  $p=0.05$ ), but did

not reach significance in layer 2/superficial 3 (+8.8%;  $F_{(1,20)}=2.57$ ,  $p=0.063$ ), layer 4 (+8.2%;  $F_{(1,20)}=2.85$ ,  $p=0.054$ ), or layer 6 (+6.2%;  $F_{(1,20)}=1.98$ ,  $p=0.088$ ). Furthermore, we found that [ $^3\text{H}$ ]-OMAR binding did not differ in schizophrenia subjects with versus without a history of suicide as manner of death, cannabis use, smoking or use of antipsychotic, antidepressant, or benzodiazepine or anticonvulsant medications at time of death (all  $F < 2.39$ ,  $p > 0.148$ ).

### 3.2. Relationship between [ $^3\text{H}$ ]-OMAR binding and mRNAs for CB1R and endogenous cannabinoid synthesizing and metabolizing enzymes

In the prefrontal cortex of the present cohort of schizophrenia subjects, we previously reported lower CB1R mRNA levels and lower levels of CB1R radioimmunoactivity (Eggen et al. 2008). In the same schizophrenia subjects, we also previously reported higher mRNA levels for serine hydrolase  $\alpha$ - $\beta$ -hydrolase domain 6 (ABHD6) (Volk et al. 2013), which metabolizes the major cortical endocannabinoid 2-arachidonoylglycerol and tightly regulates 2-arachidonoylglycerol signaling in the prefrontal cortex (Marrs et al. 2010). In contrast, mRNA levels for the synthesizing (diacylglycerol lipase  $\alpha$ ) and for other metabolizing (monoglyceride lipase) enzymes for 2-arachidonoylglycerol were not altered in these subjects with schizophrenia (Volk et al. 2010).

Therefore, we sought to determine whether [ $^3\text{H}$ ]-OMAR binding is associated with CB1R mRNA levels, CB1R radioimmunoactivity levels, or mRNA levels for 2-arachidonoylglycerol synthesizing and metabolizing enzymes in the same subjects. Interestingly, as shown in Figure 3, [ $^3\text{H}$ ]-OMAR binding was negatively correlated with mRNA levels for CB1R ( $r=-0.30$  [95% confidence interval -0.60, 0.01],  $p=0.029$ ) and positively correlated with mRNA levels for ABHD6 ( $r=0.30$  [95% confidence interval -0.01, 0.60],  $p=0.029$ ). In contrast, no relationship was found between [ $^3\text{H}$ ]-OMAR binding and CB1R radioimmunoactivity levels ( $r=.003$ ;  $p=.49$ ) or mRNA levels for diacylglycerol lipase  $\alpha$  ( $r=-0.16$ ,  $p=0.16$ ) or monoglyceride lipase ( $r=0.04$ ,  $p=0.41$ ).

## 4. Discussion

Elucidating the nature of the deleterious relationship between cannabis use and schizophrenia requires an investigation into underlying disturbances in the endogenous cannabinoid system in the disorder. However, prior studies of CB1R in the prefrontal cortex of subjects with schizophrenia have reported apparently conflicting findings of higher CB1R receptor binding (Dean et al. 2001; Zavitsanou et al. 2004; Newell et al. 2006; Dalton et al. 2011; Jenko et al. 2012) and lower or unchanged CB1R mRNA and protein immunoreactivity levels (Koethe et al. 2007; Eggen et al. 2008; Uriguen et al. 2009; Eggen et al. 2010b). We sought to explore this apparent discrepancy by conducting CB1R receptor binding studies with a new ligand and comparing the results to measures of CB1R mRNA and protein (assessed by radioimmunocytochemistry) in nearby prefrontal cortex tissue sections from the same cohort of schizophrenia and comparison subjects. Using the novel CB1R selective ligand [ $^3\text{H}$ ]-OMAR, we report that schizophrenia subjects with lower CB1R mRNA and protein immunoreactivity levels also have higher levels of [ $^3\text{H}$ ]-OMAR binding to CB1R. The magnitude of higher [ $^3\text{H}$ ]-OMAR binding in the prefrontal cortex in

schizophrenia that we report (+8%) is consistent with, though slightly smaller, than the reported increases in binding previously reported using other CB1R ligands in the same brain region (+19-25% using [<sup>3</sup>H]-MePpP and [<sup>3</sup>H]-CP55940) (Dean et al. 2001; Dalton et al. 2011; Jenko et al. 2012). Furthermore, we report for the first time that [<sup>3</sup>H]-OMAR receptor binding was increased across all cortical layers in schizophrenia subjects and achieved statistical significance in layers 1, deep 3, and 5.

The combination of lower CB1R mRNA and protein immunoreactivity with higher CB1R receptor binding in the same schizophrenia subjects may reflect a redistribution of the receptor due to altered trafficking of the receptor and higher levels of membrane-bound CB1R. Thus, while the total amount of CB1R may be reduced, as suggested by lower CB1R mRNA and protein immunoreactivity levels, levels of functional CB1R that are present on the membrane and accessible to ligand binding may actually be increased. Alternatively, the presence of higher levels of [<sup>3</sup>H]-OMAR receptor binding in the face of lower CB1R protein immunoreactivity levels might reflect greater CB1R affinity which could lead to a reduced need for CB1R receptors resulting in lower CB1R transcription and translation in schizophrenia. Unfortunately, we could not directly assess CB1R affinity in this study due to the apparent receptor saturation that occurred at higher doses of OMAR ligand. Furthermore, while binding of an allosteric modulation site can induce a conformational change in CB1R which increases the affinity of an agonist ligand (i.e. [<sup>3</sup>H]-CP55940) for the orthosteric binding site on CB1R (Price et al. 2005), evidence supporting a similar affinity change for antagonist/inverse agonist ligands such as [<sup>3</sup>H]-OMAR is not currently available. As a third interpretation, lower CB1R mRNA and protein immunoreactivity levels may instead reflect a failure of CB1R-containing axon terminals to fully develop in schizophrenia. Consequently, the remaining axon terminals that contain CB1R may have a higher affinity as a compensatory response in an attempt to maintain homeostatic levels of endogenous cannabinoid signaling. Each of these interpretations is supported by the negative correlations between [<sup>3</sup>H]-OMAR receptor binding and CB1R mRNA levels in the same subjects. As a fourth interpretation, the lack of a correlation between [<sup>3</sup>H]-OMAR binding and CB1R radioimmunoreactivity levels in our study may reflect the fact that our previous radioimmunocytochemistry study employed an antibody that primarily targets a CB1R epitope present on inhibitory axon terminals and not on excitatory axon terminals (Eggan et al. 2010a) while the [<sup>3</sup>H]-OMAR ligand presumably binds to all available CB1R regardless of cell-type localization. Consequently, it may also be that CB1R levels are lower in inhibitory axons but higher in excitatory axons in schizophrenia. However, additional studies that quantify CB1R levels specifically in pyramidal neurons in schizophrenia are required to further test this interpretation. As a final interpretation, we also previously reported higher mRNA levels for ABHD6, but not diacylglycerol lipase or monoglyceride lipase, in this cohort of schizophrenia subjects (Volk et al. 2010; Volk et al. 2013). As described previously, higher levels of ABHD6, which metabolizes 2-arachidonoylglycerol, may lead to lower 2-arachidonoylglycerol levels, and, consequently, a compensatory up-regulation in the membrane localization or affinity of locally affected CB1R that does not necessitate a corresponding upstream change in CB1R transcription and translation.

Discriminating among these possible interpretations requires additional testing through animal models and additional cell-type specific measures of CB1R levels in schizophrenia.

However, our findings of higher CB1R binding, even in the presence of deficits in CB1R mRNA and protein immunoreactivity levels, may have important relevance for cannabis use in schizophrenia. The consistent finding of higher CB1R binding in the present study and many others (Dean et al. 2001; Zavitsanou et al. 2004; Newell et al. 2006; Dalton et al. 2011; Jenko et al. 2012) suggests that schizophrenia subjects may be more susceptible and have a amplified response to the effects of cannabis, even in the face of lower numbers of receptors. Furthermore, if the number of CB1R-containing axon terminals is indeed reduced and higher CB1R receptor binding is a compensatory response in individuals with schizophrenia, then cannabis use may interfere with the functioning of an already disturbed signaling pathway.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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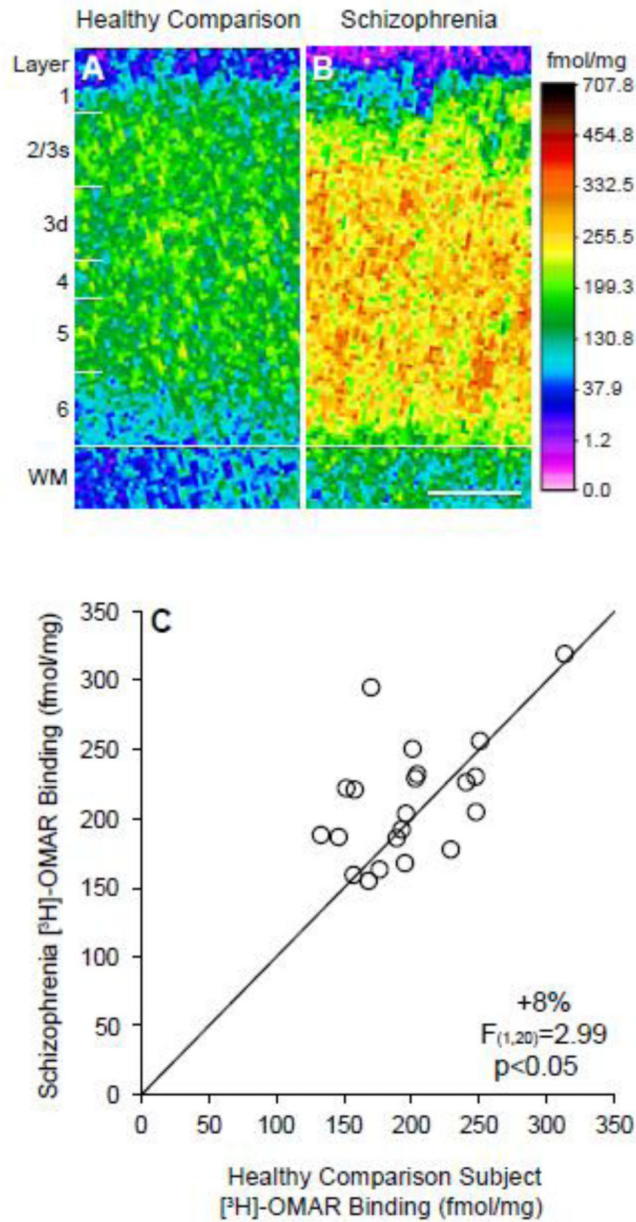
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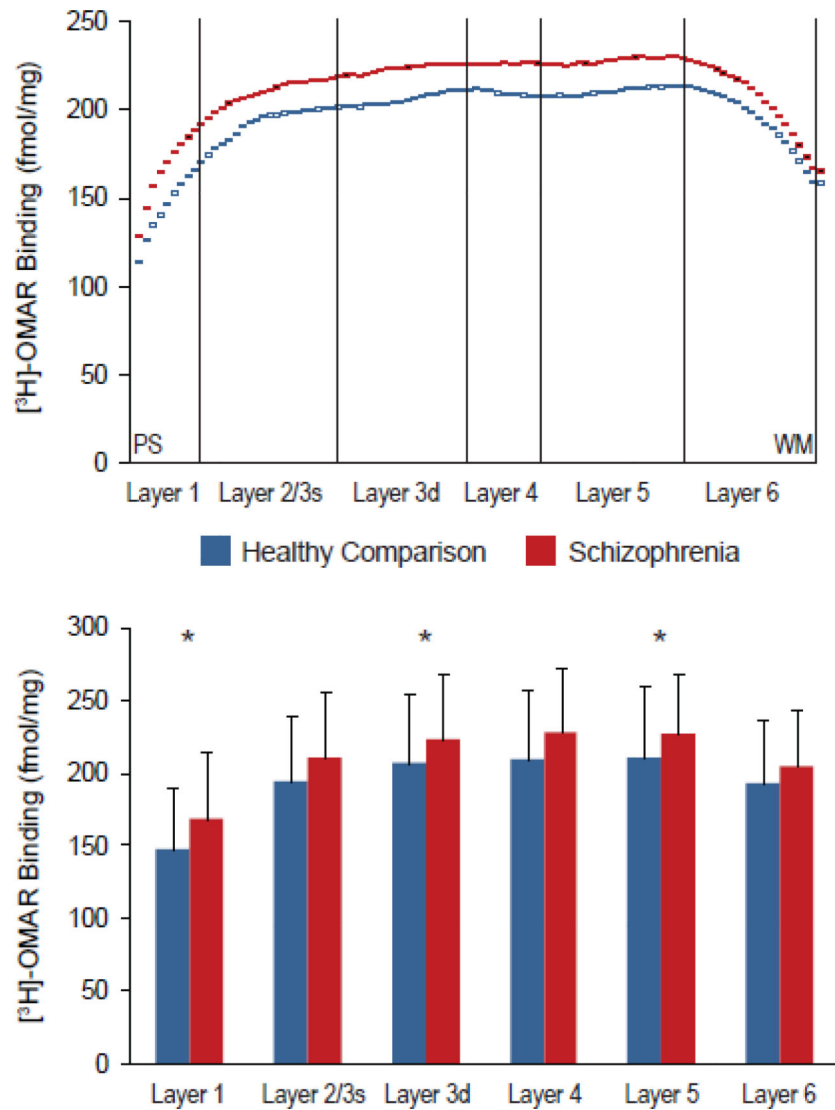
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**Figure 1. Receptor autoradiography for [<sup>3</sup>H]-OMAR binding in the prefrontal cortex in schizophrenia**

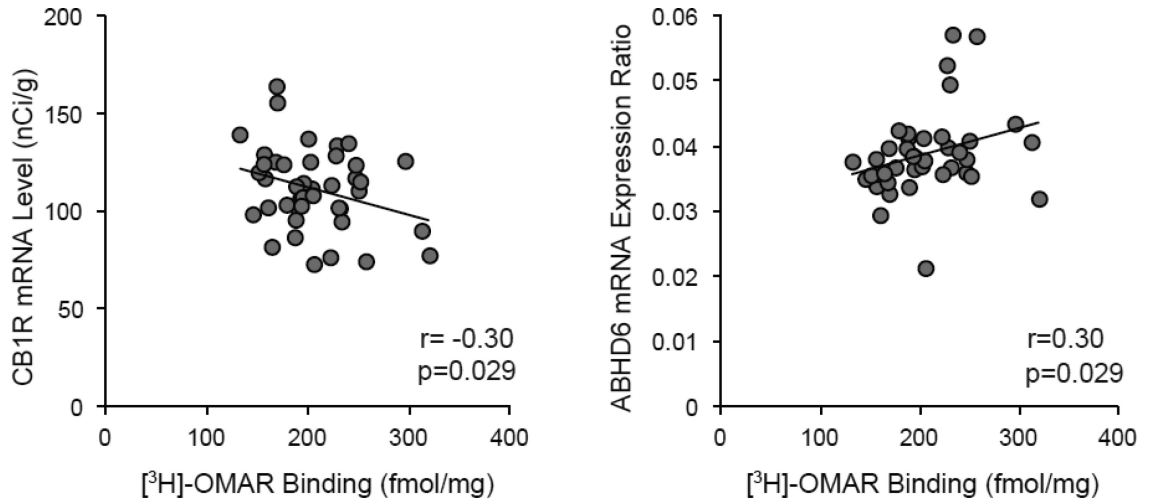
**A-B.** Pseudocolored film autoradiographs of prefrontal cortical sections processed by receptor autoradiography demonstrate higher [<sup>3</sup>H]-OMAR binding in a schizophrenia subject (**B**) relative to the matched comparison subject (**A**). Solid white line indicates the layer 6/white matter (WM) border; white distance calibration bar = 1 mm. **C.** Average [<sup>3</sup>H]-OMAR binding levels across gray matter of prefrontal cortical area 9 for schizophrenia subjects relative to matched healthy comparison subjects in a pair are indicated by open circles. Data points to the left of the unity line indicate higher [<sup>3</sup>H]-OMAR binding levels in the schizophrenia subject relative to the healthy comparison subject and vice versa. Mean

[<sup>3</sup>H]-OMAR binding was 8% higher in schizophrenia subjects relative to matched healthy comparison subjects.



**Figure 2. Laminar analysis of [<sup>3</sup>H]-OMAR binding in the prefrontal cortex of schizophrenia subjects**

**A.** Mean [<sup>3</sup>H]-OMAR binding levels from all schizophrenia subjects (red) and healthy comparison subjects (blue) measured in cortical traverses from the pial surface (PS) to the white matter (WM) border. **B.** Mean [<sup>3</sup>H]-OMAR binding levels from all schizophrenia and healthy comparison subjects were determined in each cortical layer. Error bars indicate standard deviations in each cortical layer, and asterisks indicate statistically significant differences between schizophrenia and healthy comparison subjects.



**Figure 3. Relationship between [<sup>3</sup>H]-OMAR binding and mRNA levels for CB1R and an endogenous endocannabinoid synthesizing enzyme**

In prefrontal cortex gray matter from the same cohort of subjects, [<sup>3</sup>H]-OMAR binding is negatively correlated with mRNA levels for CB1R ( $r = -0.30$ ,  $p = 0.029$ ) that were previously quantified using in situ hybridization and a highly similar film analysis (Eggan et al. 2008). In contrast, [<sup>3</sup>H]-OMAR binding is positively correlated with mRNA levels for ABHD6 ( $r = 0.30$ ,  $p = 0.029$ ) that were previously quantified by quantitative PCR (Volk et al. 2013) in the same subjects.