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Density of metabotropic glutamate receptors 2 and 3 (mGluR2/3) in the dorsolateral prefrontal cortex does not differ with schizophrenia diagnosis but decreases with age

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Density of metabotropic glutamate receptors 2 and 3 (mGluR2/3) in the dorsolateral prefrontal cortex does not differ with schizophrenia diagnosis but decreases with age

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Abstract

Metabotropic glutamate receptors 2 and 3 (mGluR2/3) have been shown as efficient targets for antipsychotic intervention. We therefore investigated the receptor density of mGluR2/3 in the dorsolateral prefrontal cortex (dlPFC; Brodman area 46) of schizophrenia/schizoaffective patients (n=37) and matched controls (n=37) using receptor autoradiography. No difference in mGluR2/3 density was identified in relation to schizophrenia diagnosis. Overall and in individual groups, a negative correlation of mGluR2/3 density and age at death has been found. These and previous results suggest that density of mGluR2/3 in the dlPFC is less likely to impact on the efficiency of the mGluR2/3 agonist in treating schizophrenia symptoms.

1. Introduction

Schizophrenia is a highly complex brain disorder and its underlying mechanisms are still not understood. With the strong implication of glutamatergic neurotransmission, metabotropic glutamate receptors 2 and 3 (mGluR2/3) have been examined as potential new targets for antipsychotic treatment (Harrison et al. 2008; Krivoy et al. 2008; Swanson et al. 2005). In both animal and human studies, the highly selective agonist for mGluR2/3, LY404039, was shown to have antipsychotic potential (Patil et al. 2007; Rorick-Kehn et al. 2007b). Intriguingly, no appreciable affinities for other glutamate receptor subtypes and transporters neither for non-glutamate receptors, such as dopamine or serotonin, were found for this compound (Rorick-Kehn et al. 2007a). The efficiency and specificity of the mGluR2/3 agonist make alterations in the density of mGluR2/3 in schizophrenia-relevant brain regions highly likely.

Cognitive impairments in schizophrenia have been associated with a dysfunction of the dorsolateral prefrontal cortex (dlPFC) (Callicott et al. 2000; Eisenberg and

Berman 2010); increased prefrontal glutamate concentrations have been found in a subgroup of schizophrenia patients (Olbrich et al. 2008). Using post-mortem brain tissue of schizophrenia patients an earlier study did not find changes in the protein expression of mGluR2/3 in the dlPFC (Crook et al. 2002). A recent study, however, showed a higher expression of mGluR2 than mGluR3 in the dlPFC and a lower expression of mGluR3 in the dlPFC of patients (Ghose et al. 2009); receptor binding density, however, has not been studied.

We therefore studied mGluR2/3 density in post-mortem brain tissue of the patient cohort of the schizophrenia research institute (SRI) comprising 37 patients with schizophrenia diagnosis (including 7 schizoaffective patients) and 37 matched controls to identify the potential role of dlPFC mGluR2/3 in schizophrenia.

2. Methods

2. 1. Post-mortem brain tissue

For the present study, post-mortem brain tissue of the dlPFC (Brodman Area 46) of 37 schizophrenia/schizoaffective patients and 37 matched controls was used. All research was approved and conducted under the guidelines of the Human Research Ethics Committee at the University of Wollongong (HE99.222) and University of New South Wales (HREC 07261). Clinical assessments, selection of cases and matched controls, assessment of tissue quality and preparation of slide-mounted coronal tissue sections (14µm) were performed by the Tissue Resource Centre and the Schizophrenia Research Institute (Weickert et al. 2010). All experiments and analysis was done blind to the clinical details of each case.

2.2. Receptor Autoradiography for mGluR2/3 density

Receptor autoradiography on mGluR2/3 was performed using [^{3}H]LY354740 based on the protocol described previously (Richards et al. 2005). Two slides per case were pre-incubated for 2 x 10 min in a buffer solution (50mM Tris buffer, 2mM $MgCl_{2,}$ 2mM $CaCl_{2;}$ pH=7) at room temperature (RT). Thereafter, the slides were incubated for 60 min at RT in buffer solution containing 50nM [^{3}H]LY354740. As controls, adjacent brain sections of 6 selected cases (3 patients vs. 3 matched controls) were incubated with 50nM [^{3}H]LY354740 in the presence of 0.01 mM DCG-IV to determine non-specific binding. Following incubation the slides were washed in cold (4^{o}C) washing buffer (50mM Tris buffer, pH7) for 2 x 30sec and 1 x 1 min.

2. 3. Receptor density quantification

Quantification was performed using the Beta-Imager and B vision+ program (BioSpace, France). Cortical regions were analysed according to histological standards (provided by Weickert et al, see Figure 1).

Four schizophrenia cases (3 schizophrenia, 1 schizoaffective diagnosis) and 1 control case were excluded from analysis as binding data deviated more than 30% of overall standard deviation.

2. 4. Statistical Analysis

All data was analysed using SPSS (17.0). Data was tested for normality (Lilliefors test) and homogeneity (Levene's test). Student's t-test was used to compare the levels of radioligand binding for schizophrenia/schizoaffective patients vs controls, schizophrenia patients vs controls, schizoaffective patients vs controls, schizoaffective vs schizophrenia patients as well as gender differences and manner of death. Bonferroni correction was used to correct for multiple testing in case of significance. Spearman's correlation was used to test for any effects of continuous descriptive variables including age at death, pH (prefrontal cortex), post mortem

interval (PMI), RNA integrity (RIN), freezer storage time, brain weight, brain volume, chlorpromazine equivalent (mg) of lowest, highest, lifetime and last recorded antipsychotic dose, antidepressant treatment, daily ethanol intake, smoking quantity, age of onset and duration of illness on receptor binding (for clinical data see (Weickert et al. 2010). ANCOVA analysis, controlling for age of death, was performed for obtained significances when using the the Student's t-test; levels of radioligand binding in suicidal vs naturally deceased schizophrenia/schizoaffective patients as well as patients on last recorded high (>450mg/day chlorpromazine equivalent) or low (<450mg/day chlorpromazine equivalent) dose of antipsychotics were therefore studied. Significance was accepted with $p < 0.05$. Data is presented as mean ± SEM.

3. Results

Independent of diagnosis, no difference was found between patients and controls in mGluR2/3 receptor density in the dlPFC.

In all groups, schizophrenia patients ($R=-0.62$; $p<0.01$), schizoaffective patients ($R=-0.92$:$p<0.01$), controls ($R=-0.65$; $p<0.01$) and overall cases ($R=-0.66$; $p<0.01$), there was a strong negative correlation between mGluR2/3 binding and age at death (Figure 2).

In all cases and schizophrenia/schizoaffective patients only, mGluR2/3 binding additional correlated with pH, RIN, time of storage, brain weight, manner of death, antipsychotic dose (as chlorpromazine equivalent), duration of illness and smoking quantity. None of these correlations remained, however, significant after correcting these variables for age at death. The correlation of mGluR2/3 density and age at death remained significant when correcting for any of these variables.

Whereas suicide victims amongst schizophrenia/schizoaffective patients showed higher (17.1 ± 0.5; n=8) mGluR2/3 levels than patients dying from natural death (14.3 ± 0.7; n=26), this was only due to the young age when committing suicide rather than the manner of death (uncorrected $F(32,1)=5.99$; $p<0.05$; corrected for age at death $F(30,1)=0.70$; $p=0.41$). Similarly, whereas users of (on average in this cohort) low doses of antipsychotics (>450mg/day chlorpromazine equivalent) showed higher levels of mGluR2/3 binding (16.8 ± 0.1; n=13) than users of high doses of antipsychotics (>450mg/day chlorpromazine equivalent) (13.5 ± 0.7; n=16), no significance was given after correction for age at death (uncorrected $F(27,1)=7.72$; $p<0.05$; corrected for age at death $F(26,1)=2.27$; $p=0.14$).

4. Discussion

Metabotropic glutamate receptors 2 and 3 have been shown as efficient targets for antipsychotic intervention (Patil et al. 2007). We therefore investigated the receptor density of mGluR2/3 in the dlPFC, which has been implicated in the symptomatology of schizophrenia, in patients with schizophrenia/schizoaffective diagnosis and matched control subjects (Figure 1). No difference in receptor density was found due to schizophrenia/schizoaffective diagnosis (Table 1). A strong correlation of mGluR2/3 binding density with age at death was found overall and separately for patient and control groups (Figure 2). No other variables were correlated after correcting for age at death, with and without consideration of DSM-IV diagnosis.

Our data are consistent with previous results, which showed no changes in protein or mRNA expression of mGluR2/3 in the dlPFC of schizophrenia patients compared to controls (Crook et al. 2002; Gupta et al. 2005). No differences in mGlu2/3R binding

levels between schizophrenia or schizoaffective diagnosed patients additionally suggests that this might be common to psychotic illnesses.

We also did not observe any changes correlated to antipsychotic or antidepressant drug treatment. This is in accordance with findings of Gupta et al (2005) and Crook et al (2002), who neither detected changes in medicated vs unmedicated schizophrenia patients. All studied patient cohorts, including our cohort, showed, however, either variations in treatment history or age at death as a confounding factor so that drug effects cannot be fully excluded. Still, these data are supported by earlier studies on rats, which showed that treatment with haloperidol, clozapine or olanzapine did little or not affect mGluR2/3 expression in the frontal cortex (Tascedda et al. 2001).

Age-related reduction in glutamate receptor binding in post-mortem human brain tissues has been demonstrated before (Newell et al. 2005). Specifically for mGluR2/3 receptors, reduced mRNA and protein expression levels have been shown in healthy controls, however, not schizophrenia patients (Colantuoni et al. 2008; Crook et al. 2002). This is contrasted by other studies on mGluR2/3, which did not observe age-related effects (Ghose et al. 2009; Gupta et al. 2005). Studies on rats, in comparison, have shown rather an increase in mGluR2/3 expression levels with older age (Simonyi et al. 2005). Considering the modulatory properties of mGluR2/3 receptors on the glutamatergic synapse, these results require further attention. The synaptic localization of mGluR2 and mGluR3 can be both pre- and postsynaptic, where they function primarily as autoreceptors and are involved in regulation of neurotransmitter release and ion channel openings (Swanson et al. 2005; Wieronska and Pilc 2009). It was speculated that mGluR2/3 receptors thereby protect neurons from excitotoxicity in suppressing excitatory neurotransmission (Swanson et al. 2005). With mGluR2/3 most likely attenuating synaptic transmission, a reduced availability with increasing

age might require other compensatory mechanisms to maintain appropriate glutamatergic transmission. No matter if in relation to physiological or pathological conditions, receptor densities of other mGluR and modulators of synaptic glutamatergic transmission remain therefore to be studied and correlated in the present cohort.

While mGluR2 and mGluR3 receptors have a quite similar distribution in the brain, and are believed to have similar modulatory roles in glutamatergic neurotransmission, subtype specific receptor changes might be relevant for drug action. Whereas the problem of selective measurement of mGluR2 and mGluR3 protein expression has been resolved (Ghose et al. 2009), selective measurement of receptor density remains problematic due to a lack of specific ligands (Harrison et al. 2008). The radioligand used in our study, [^{3}H]LY354740, targeted both mGluR2 and mGluR3. Ghose et al (2009) showed, however, when, using subtype specific antibodies, a higher expression of mGluR2 than mGluR3 in the dlPFC of healthy controls. In animal studies on mGluR2 and mGluR3 receptor knock-out mice, it was additionally found that mGluR2 rather than mGluR3 mediate the actions of the therapeutically active mGluR2/3 receptor agonist LY379268 (Woolley et al. 2008); indicating that the mGluR2 is more likely to be involved in the therapeutic efficiency in schizophrenia pathology. We therefore used a protocol favouring binding to mGluR2 over mGluR3 (Richards et al. 2005). Whereas this confirmed the data of Crook et al (2002), who did not find any differences in mGluR2/3 protein expression, the decrease mGluR3 receptor levels observed by Ghose et al (2009) needs to be confirmed using a more mGluR3 specific protocol or a specific ligand for receptor binding; particularly considering genetic human population studies that suggested

that the mGluR3 rather than the mGluR2 are associated with schizophrenia (Harrison and Weinberger 2005).

Our study and studies of others have shown that mGluR2/3 receptors are abundant in the dlPFC, where mGluR2/3 agonists might exhibit their potential to treat schizophrenia symptoms particularly related to cognitive deficits (Crook et al. 2002; Ghose et al. 2009; Gupta et al. 2005). Though, overall findings to date for expression levels of mGluR2/3 and receptor density of mGluR2/3, with a bias for mGluR2, in the dlPFC showed little or no changes (our results and (Crook et al. 2002; Ghose et al. 2009)). Corti et al (2007) showed, however, decreased dimerisation of mGluR3 in Brodmann area 10 of schizophrenic patients while total mGluR3 expression remained unchanged. This indicates that also functional receptor binding for both mGluR2 and mGluR3 and their interaction needs to be explored in schizophrenia patients as well as in response to mGluR2/3 agonist drug treatment before final conclusions can be drawn. On the other hand, considering recent studies on mGluR2 and mGluR3 mutant mice, limbic rather than cortical brain areas might be of relevance to schizophrenia pathology and mGluR2/3 agonist efficiency (Hetzenauer et al. 2008). Altogether, the mGluR2/3 receptors are a novel and highly promising target for antipsychotic intervention, their way of action, however, remains to be uncovered for a better understanding of their potency to treat schizophrenia.

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Legends

Figure 1: (A) Receptor autoradiographs using [3H]LY354740 to label metabotropic glutamate receptors 2 and 3 (mGluR2/3) in the dorsolateral prefrontal cortex (dlPFC) of control subjects and schizophrenia/schizoaffective patients, obtained by beta imager scanning. No difference in mGluR2/3 density in the dlPFC was found due to diagnosis. (B) Addition of the selective mGlu2/3 agonist DCG-IV prevented radioactive labelling of mGluR2/3 evidencing the specificity of [3H]LY354740 binding to mGluR2/3. (C) Analysis was based on histological identification of the dlPFC provided by Weickert et al (for reference see Weickert et al. (2010)).

Figure 2: Correlation of metabotropic glutamate receptor 2 and 3 (mGluR2/3) density in the dorsolateral prefrontal cortex (dlPFC) of control subjects (CON; open squares) and patients with schizophrenia/schizoaffective diagnosis (SCZ, black diamonds) with the respective age at death. mGluR2/3 was found lower in older subjects independent of schizophrenia diagnosis ($R=0.66$; $p<0.01$).

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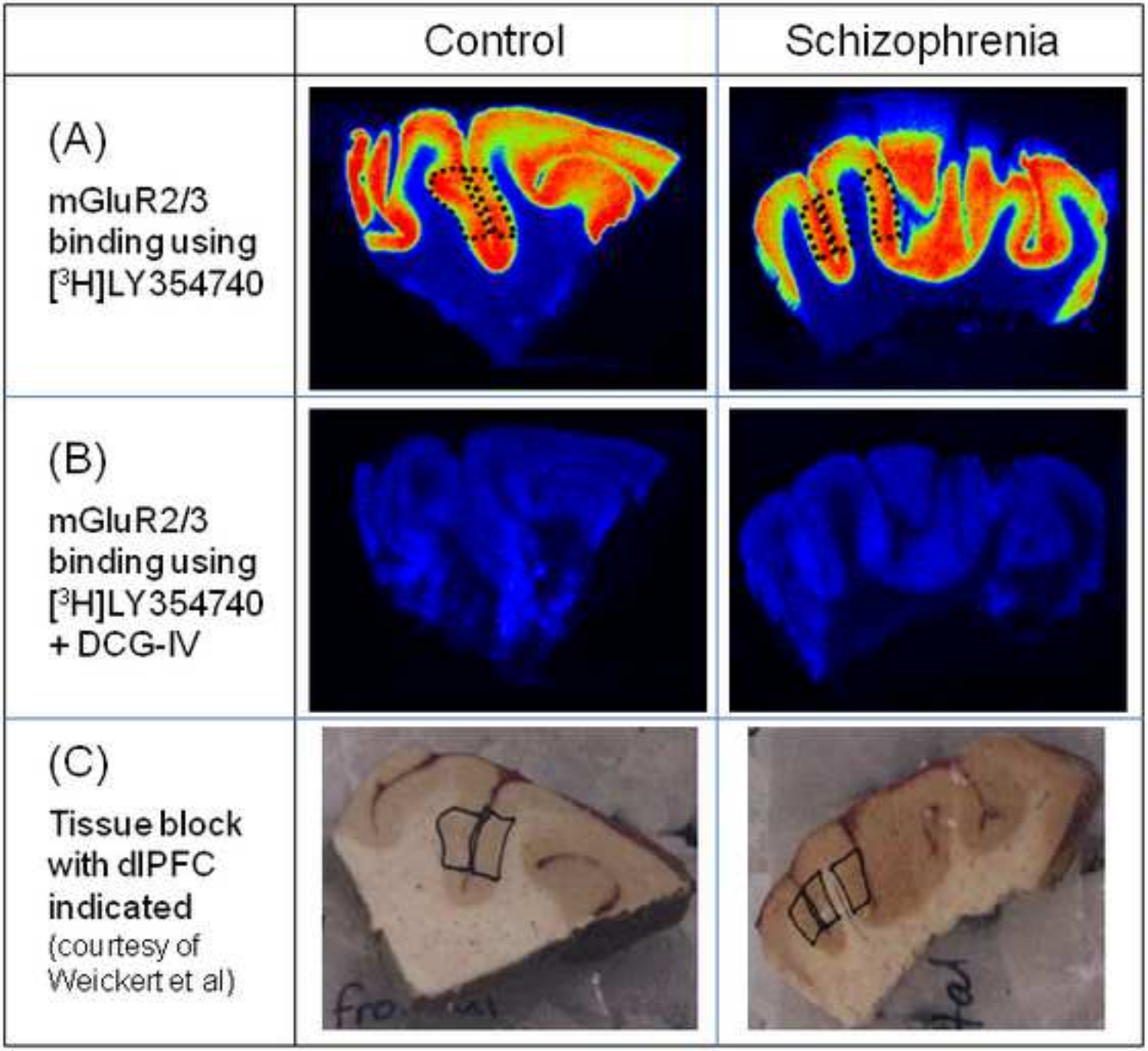

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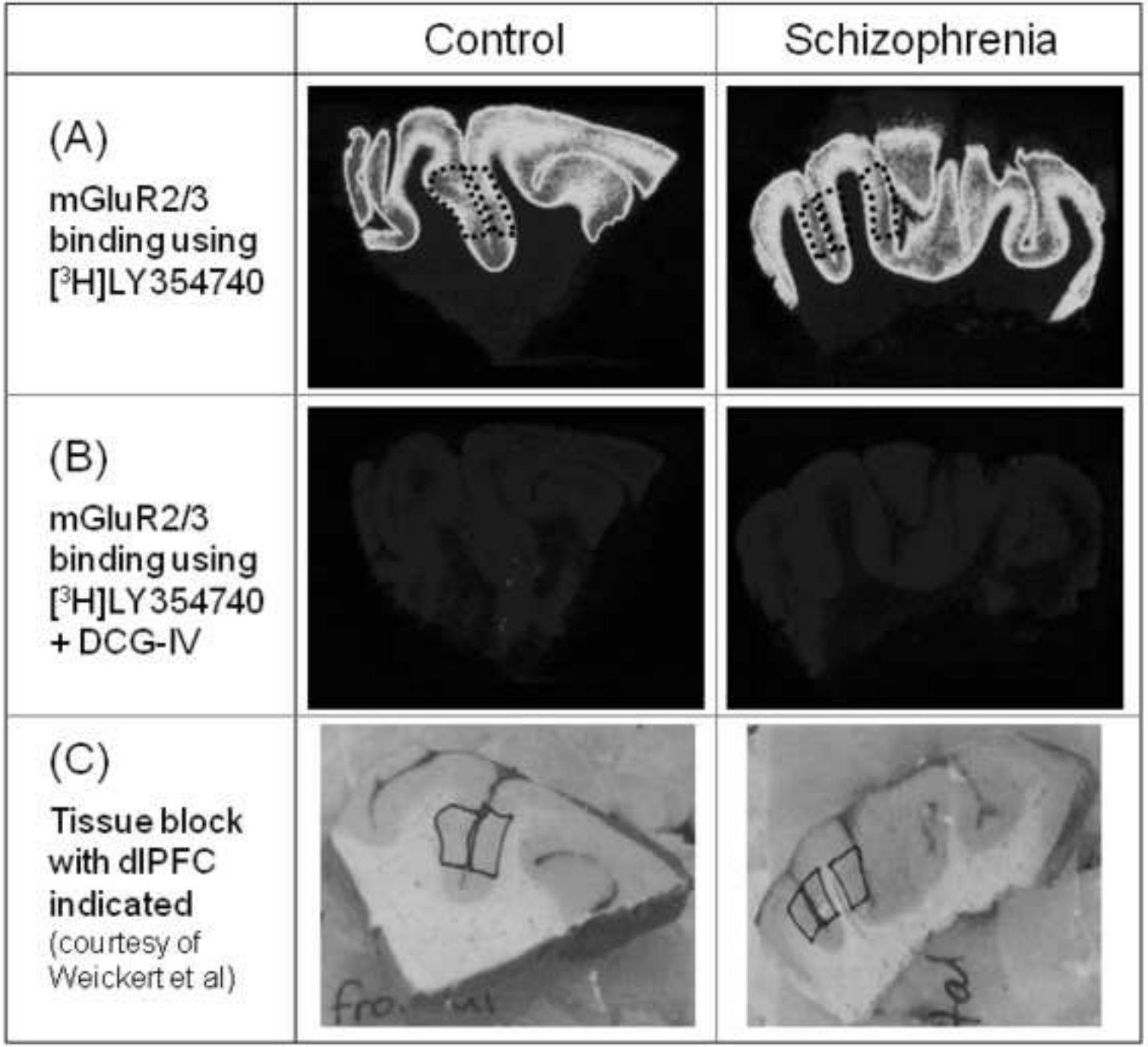

Figure 2

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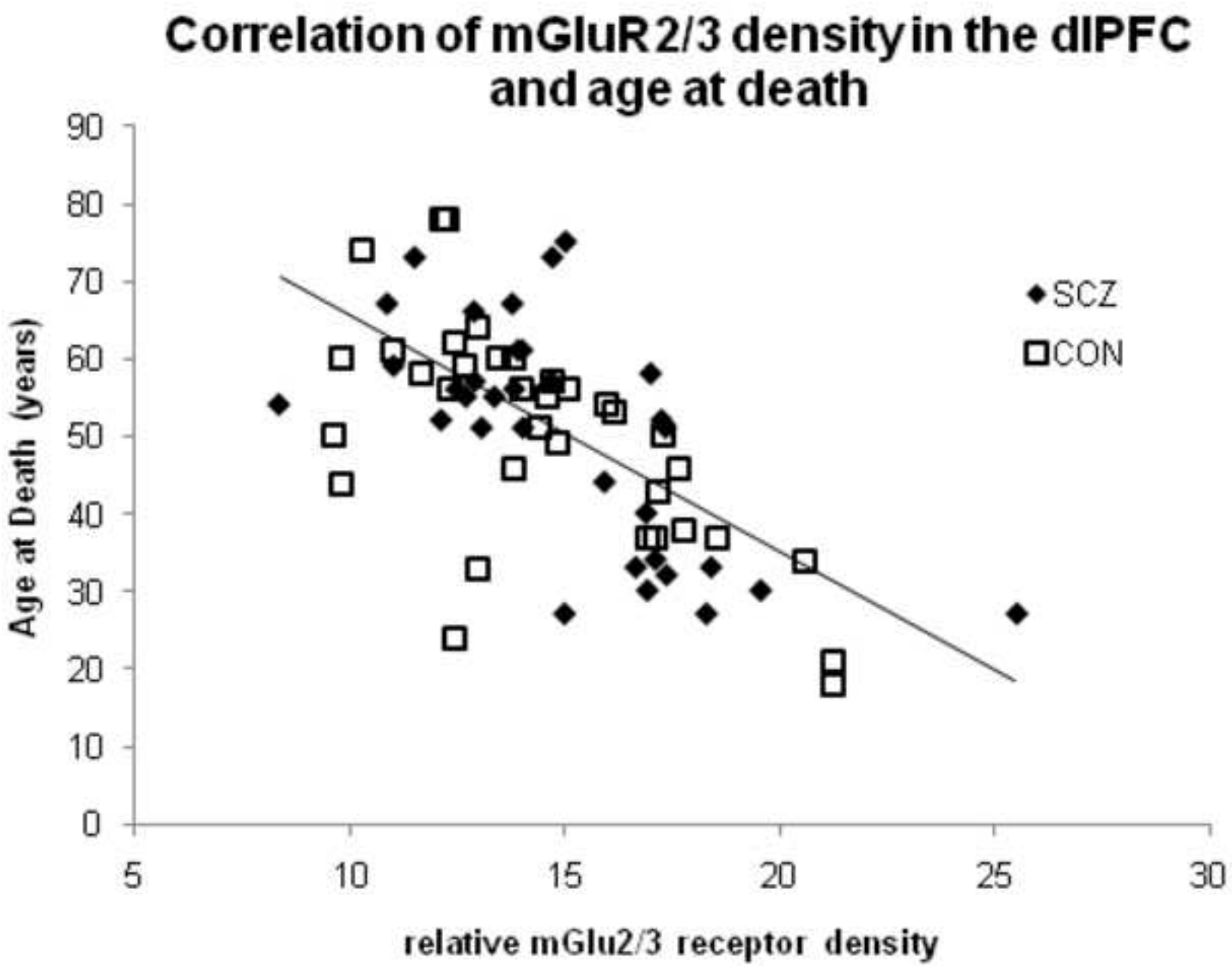

Table 1

Table 1: Relative metabotropic glutamate receptor 2 and 3 (mGluR2/3) density in the dorsolateral prefrontal cortex (dlPFC) of control subjects vs patients with schiozphrenia/schizoaffective diagnosis.

	Controls	All patients with schizophrenia or schizoaffective diagnosis		
			Patients with schizophrenia diagnosis only	Patients with schizoaffective diagnosis only
Relative mGluR2/3 density (means±SEM)	14.41 ± 0.05	14.99 ± 0.5	14.79 ± 0.6	15.87 ± 1.2
Number of cases	36	33	27	6

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Contributors

All authors contributed to the study design. Elisabeth Frank and Kelly Newell established the binding protocol. Elisabeth Frank performed the study, was responsible for the data analysis and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

Role of Funding Source

The funding sources had no role in this study, including study design, data collection and publication decisions.