$GABA_A$ RECEPTORS EXPRESSED IN OLIGODENDROCYTES CULTURED FROM THE NEONATAL RAT CONTAIN α3 AND γ1 SUBUNITS AND PRESENT DIFFERENTIAL FUNCTIONAL AND PHARMACOLOGICAL PROPERTIES

by

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MOLPHARM-AR-2020-000091 - Supplemental Information

Supplementary Figure 1 (S1)

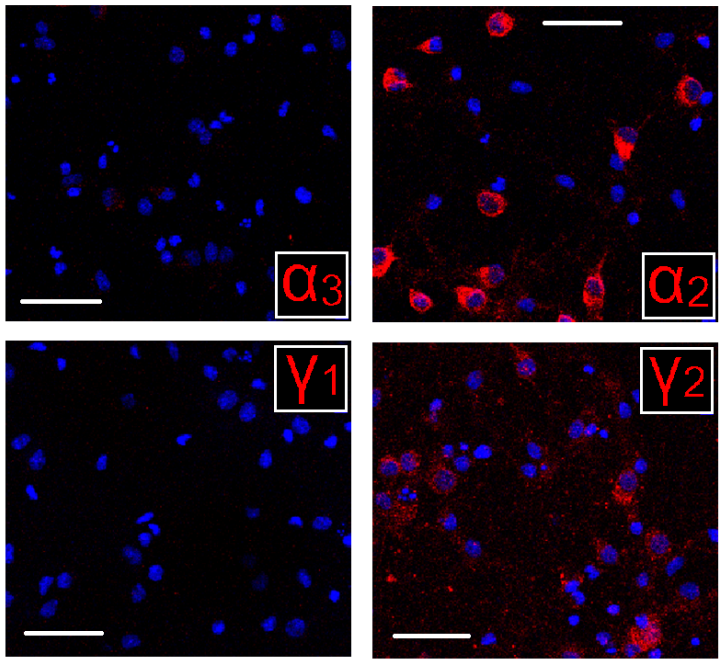

Figure S1. Expression of $GABA_AR$ subunits in cortical neurons. Analysis by immunocytochemistry of rat cortical neurons maintained in culture. Panels show images from confocal microscopy of the fluorescence signal for a specific antibody against the $GABA_AR$ subunit protein (in red) indicated in each panel, and nuclei labeling with DAPI (blue). Bars = 50 μm.

Supplementary Figure 2 (S2)

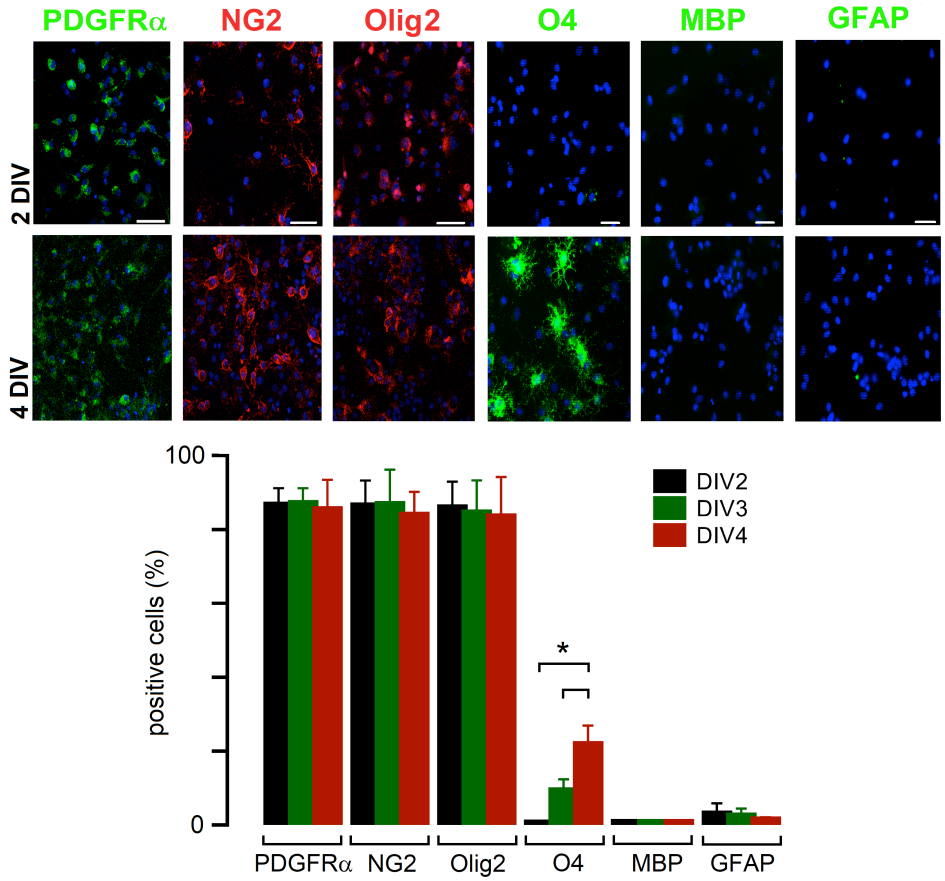

Figure S2. Expression of glial markers in OPCs from 2 DIV to 4 DIV maintained in proliferative medium. Images in the upper panels show representative fields of OPCs maintained in culture and processed for immunocytochemistry at 2 DIV (first row) or 4 DIV (second row). The markers used (indicated at the top) were visualized (either in red or green) by confocal microscopy or epifluorescence. Positive cells for each marker were counted and stated as % of cells (mean ± S.D.) with respect to the total number of cells (nuclei labeled with DAPI) in representative fields. The analysis of expression (% of cells) from 2 DIV to 4 DIV in the column graph indicated that specific markers for OPCs, such as PDGFRα, NG2, and Olig2, maintained expression levels close to 85% throughout the period in culture, while MBP and GFAP, markers for myelinating OLs and astrocytes, respectively, were not detected. Also, O4 showed an increase in its expression that became statistically significant towards 4 DIV. *$p < 0.0001$ 4 DIV compared to 2 DIV and 3 DIV, one-way ANOVA followed by a Tukey's *post hoc* Test.

Supplementary Figure 3 (S3)

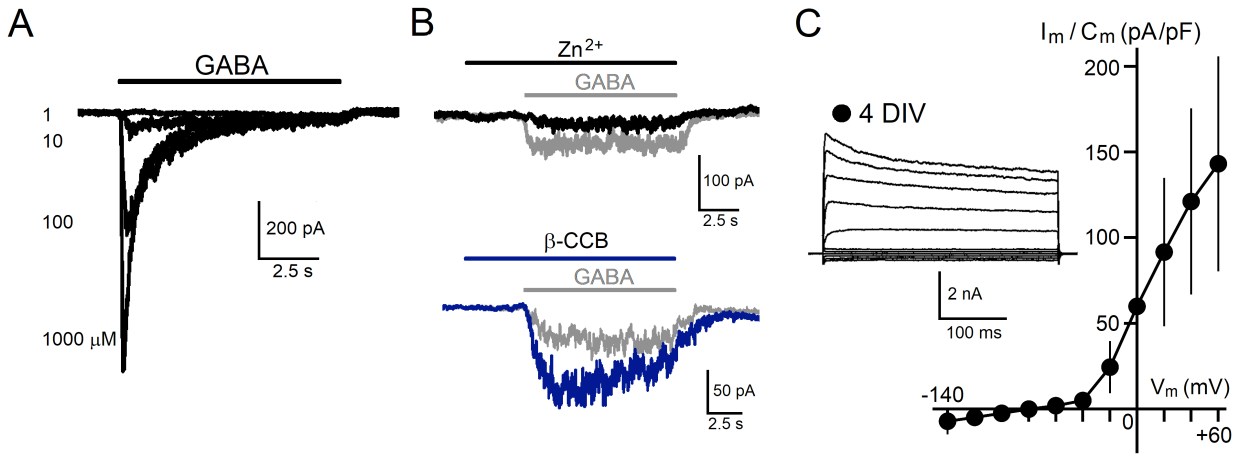

Figure S3. $GABA_AR$ pharmacological profile in OPCs at 4 DIV and their I/V relationship. A) Traces illustrate GABA responses in an OPC at 4 DIV (held at -80 mV) to distinct neurotransmitter concentrations as indicated. D-R curves built with the peak amplitude response in each concentration gave an EC_{50} of 85.2 ± 4.42 µM (n=15 cells). B) Traces illustrate the modulatory effect of either (10 µM) Zn^{2+} or β-CCB on the (10 µM) GABA response. Each set includes the control GABA response in gray and the response in the presence of a modulator (black traces; Zn^{2+} IC_{50} of 20.1 ± 4.24 µM (n=10 cells); β-CCB enhancement of 227.3 ± 59.1% (n=10 cells)). C) OPC I/V relationship at 4 DIV in proliferative medium. Traces illustrate the normalized current membrane response to a voltage-step protocol applied from -140 to +60 mV in cells held at -80 mV. Data points are the normalized (I_m/C_m) peak current (± S.D.) recorded in 30 cells.