Clemastine and metformin extend the window of NMDA receptor surface expression in ageing oligodendrocyte precursor cells

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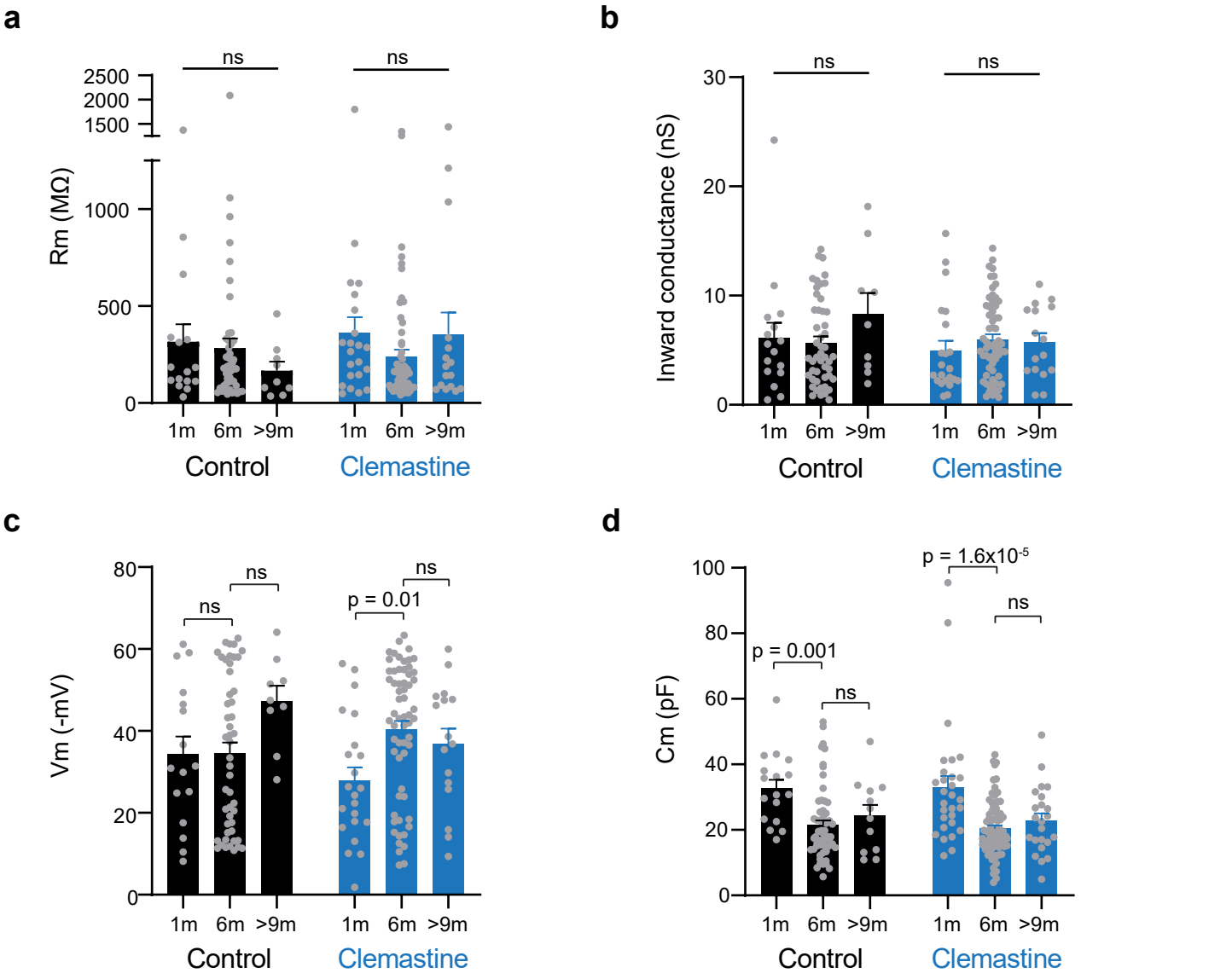
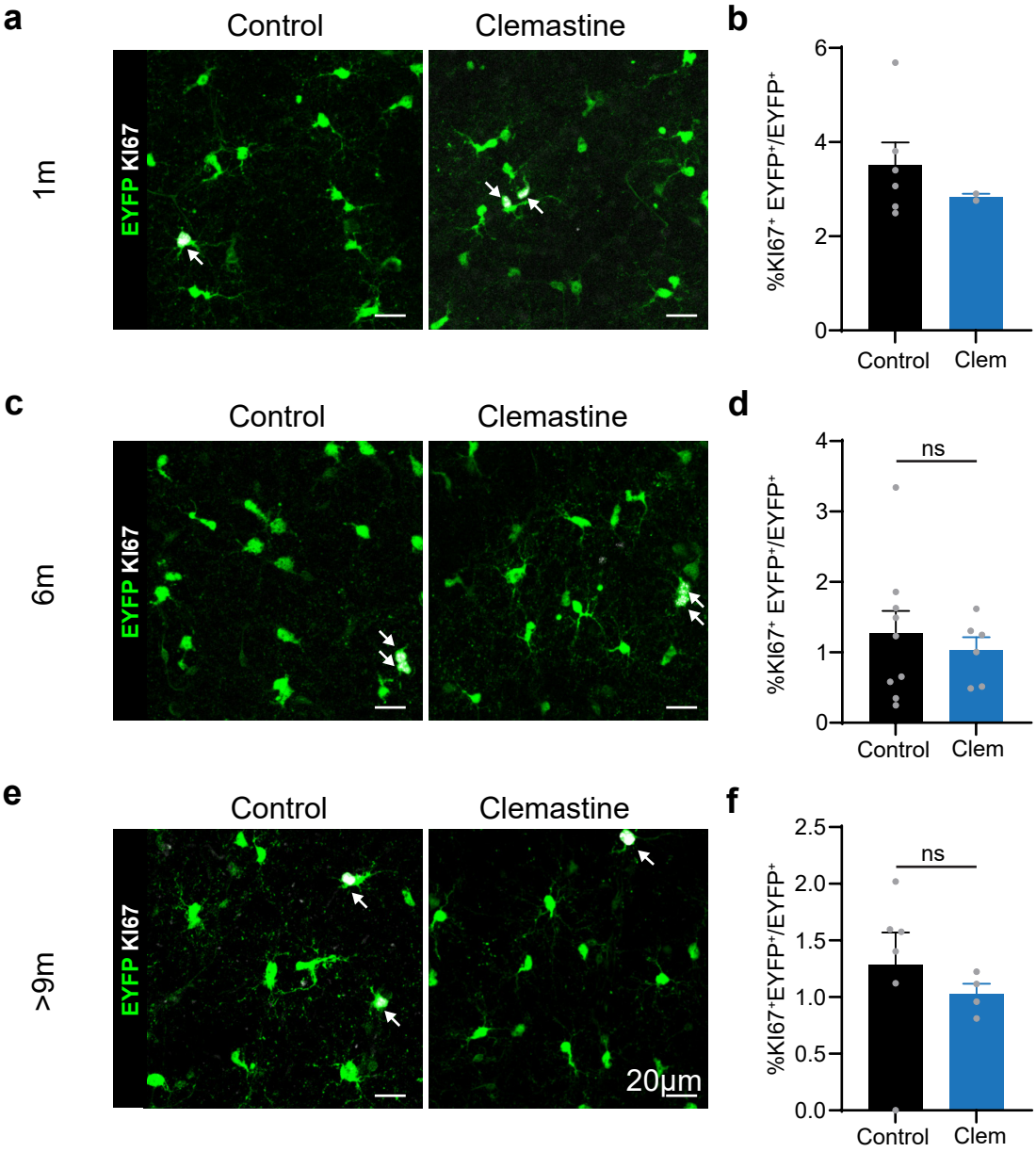
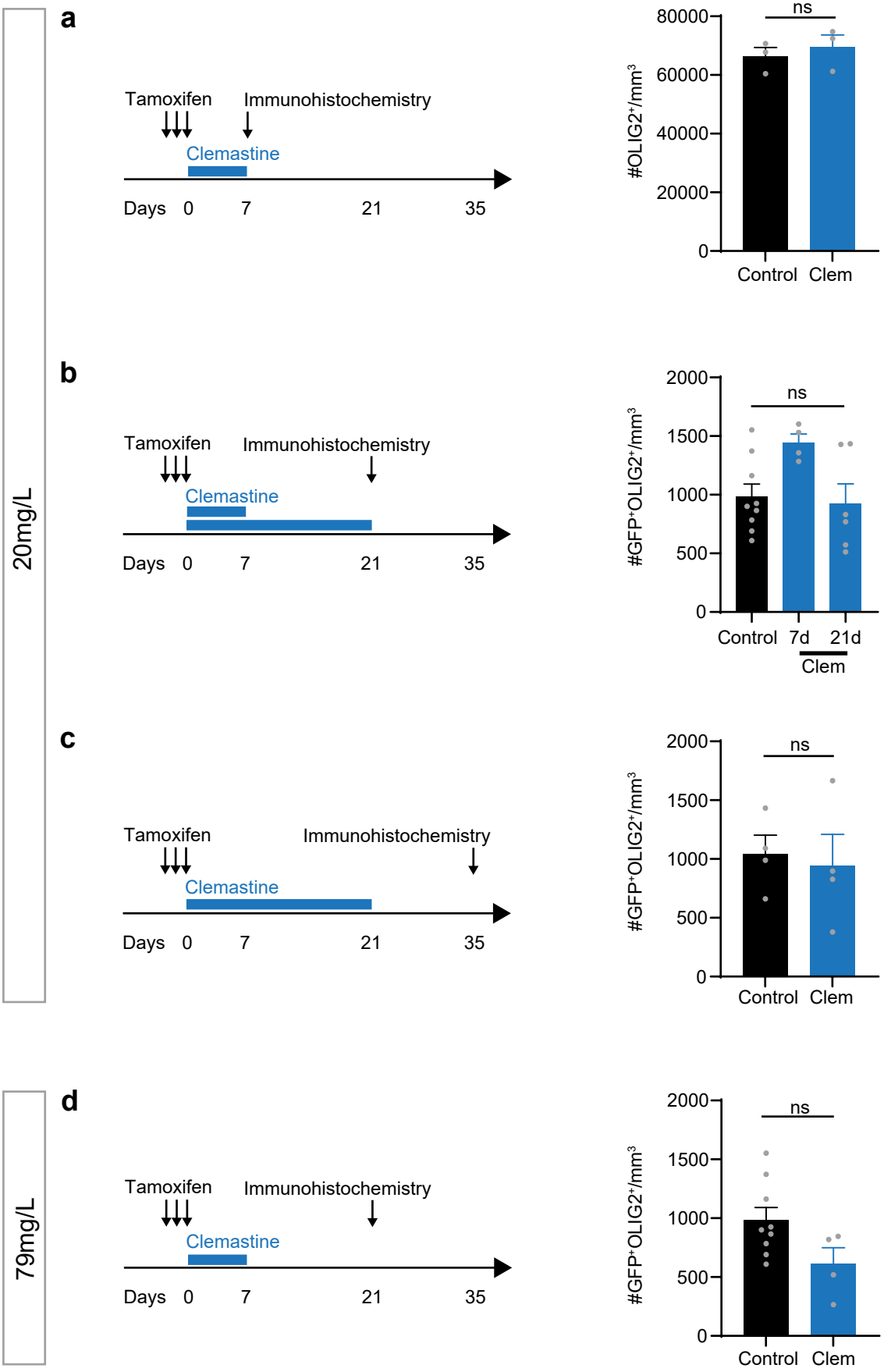


Supplementary Figure S1. Clemastine does not alter OPC passive membrane properties
a Membrane resistance (Rm) does not differ with age or clemastine treatment in adult cortical OPCs. A two-way ANOVA was run to compare control and clemastine-treated OPCs across ages: condition main effect, p=0.3; age main effect, p=0.5; interaction, p=0.3. 1m control: n=16, 6m control: n=49, 9m control: n=9, 1m clemastine: n=22, 6m clemastine:n=61, 9m clemastine: n=16. p-values on the bar graph are from the condition and age main effects. **b** Inward rectifying K^+ conductance (inward conductance) does not differ with age or clemastine treatment in adult cortical OPCs. A two-way ANOVA was run to compare control and clemastine-treated OPCs across ages: condition main effect, p=0.1; age main effect, p=0.4; interaction, p=0.3; 1m control: n=16, 6m control: n=49, 9m control: n=9; 1m clemastine: n=22, 6m clemastine:n=61; 9m clemastine: n=16. p-values on the bar graphs are from condition and age main effects. **c** Resting membrane potential (Vm) was more negative in 6m clemastine-treated mice. In contrast, in control mice, Vm did not change with age. A two-way ANOVA was run to compare control and clemastine-treated OPCs across ages: condition main effect, p=0.2; age main effect, p=0.04; interaction, p=0.04, indicating no difference in Vm between conditions, but a difference in how Vm changes with age with clemastine treatment. 1m control: n=16, 6m control: n=49, 9m control: n=9; 1m clemastine: n=22, 6m clemastine:n=61; 9m clemastine: n=16. p-values on the bar graph are from Holm-Bonferroni post-hoc tests. **d** Membrane capacitance (Cm), a proxy for cell surface area, decreases with age in adult cortical OPCs in both control and clemastine-treated mice. A two-way ANOVA was run to compare control and clemastine-treated OPCs across ages: condition main effect, p=0.7; age main effect, $p=6.5x10^{-8}$; interaction, p=0.9. 1m control: n=17, 6m control: n=60, 9m control: n=12; 1m clemastine: n=29, 6m clemastine:n=77; 9m clemastine: n=23. p-values on the bar graph are from Holm-Bonferroni post-hoc tests. Data are shown as mean±SEM, with grey dots indicating individual recorded cells.



Supplementary Figure S2 Clemastine does not alter OPC proliferation
a Cortical slices from 1m NG2-EYFP mice were immulabelled against EYFP and KI67, a proliferation marker. EYFP⁺KI67⁺ cells are indicated with white arrows. **b** 7-10 days clemastine treatment is unlikely to alter OPC proliferation in 1m mice, but this was not tested statistically due to a small sample size: control: n=6; clemastine: n=2. **c** Cortical slices from 6m NG2-EYFP mice were immulabelled against EYFP and KI67. EYFP⁺KI67⁺ cells are indicated with white arrows. **d** 7-10 days clemastine treatment did not alter OPC proliferation in 6m mice. p=0.6, unpaired two-tailed t-test; control: n=9, clemastine: n=6. **e** Cortical slices from >9m NG2-EYFP mice were immulabelled against EYFP and KI67. EYFP⁺KI67⁺ cells are indicated with white arrows. **f** 7-10 days clemastine treatment did not alter OPC proliferation in >9m mice. p=0.5, unpaired two-tailed t-test; control: n=6, clemastine: n=4. Data are shown as mean ±SEM, with grey dots indicating individual animals.



Supplementary Figure S3. Different clemastine administration protocols and doses do not promote differentiation

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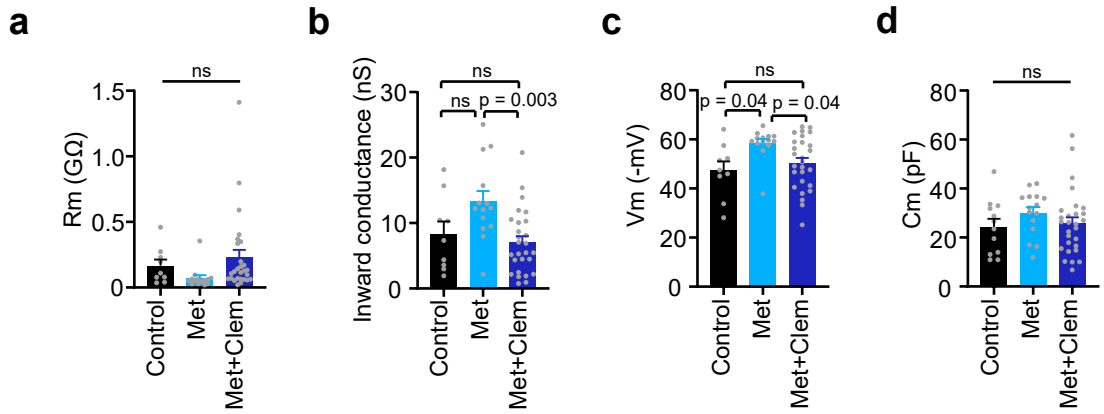
a Following 3 days of tamoxifen administration to induce Cre activity, 6m PdgfrαCreER^{T2}:Tau-mGFP mice were given 20mg/L clemastine for 7 days in their drinking water before perfusion-fixation for immunohistochemical analysis. 7 days clemastine treatment did not alter the number of oligodendrocyte lineage cells (OLIG2^{+}). p=0.6, unpaired two-tailed t-test; n=3 for both conditions. **b** Following 3 days of tamoxifen administration to induce Cre activity, 6m PdgfrαCreER^{T2}:Tau-mGFP mice were given 20mg/L clemastine for 7 or 21 days in their drinking water. All mice were perfused-fixed after 21 days for immunohistochemical analysis. Clemastine treatment did not alter the number of newly differentiated oligodendrocytes in the cingulate cortex. p=0.054, one-way ANOVA, control: n=9, 7 days clemastine: n=4, 21 days clemastine: n=6. **c** Following 3 days of tamoxifen administration to induce Cre activity, 6m PdgfrαCreER^{T2}:Tau-mGFP mice were given 20mg/L clemastine for 21 days in their drinking water before perfusion-fixation at 35 days for immunohistochemical analysis. This longer treatment did not alter differentiation in the cingulate cortex. p=0.8, unpaired two-tailed t-test; n=4 for both conditions. **d** Following 3 days of tamoxifen administration to induce Cre activity, 6m PdgfrαCreER^{T2}:Tau-mGFP mice were given 79mg/L clemastine for 7 days in their drinking water before perfusion-fixation after 21 days for immunohistochemical analysis. Differentiation in clemastine-treated animals did not differ from control animals in the cingulate cortex. p=0.07, unpaired two-tailed t-test; control: n=9, clemastine: n=4. Data are shown as mean ±SEM, with grey dots indicating individual animals.



Supplementary Figure S4. Metformin alters passive membrane properties in OPCs
a Membrane resistance (Rm) does not differ with metformin or metformin+clemastine treatment in adult cortical OPCs. p=0.1, one-way ANOVA, control: n=9, metformin: n=14, metformin+clemastine: n=27. **b** Inward rectifying K+ conductance (inward conductance) was increased by metformin treatment alone, but not by metformin+clemastine treatment. p=0.004, one-way ANOVA, control: n=9, metformin: n=14, metformin+clemastine: n=27. p values on the bar graph are from Holm-Bonferroni post-hoc tests. **c** Metformin treatment alone hyperpolarized OPCs, but metformin+clemastine administration did not alter resting membrane potential (Vm). p=0.007, one-way ANOVA, control: n=9, metformin: n=14, metformin+clemastine: n=27. p values on the bar graph are from Holm-Bonferroni post-hoc tests. **d** Membrane capacitance (Cm), a proxy for cell surface area, did not differ between conditions. p=0.4, one-way ANOVA, control: n=12, metformin: n=15; metformin+clemastine: n=28.