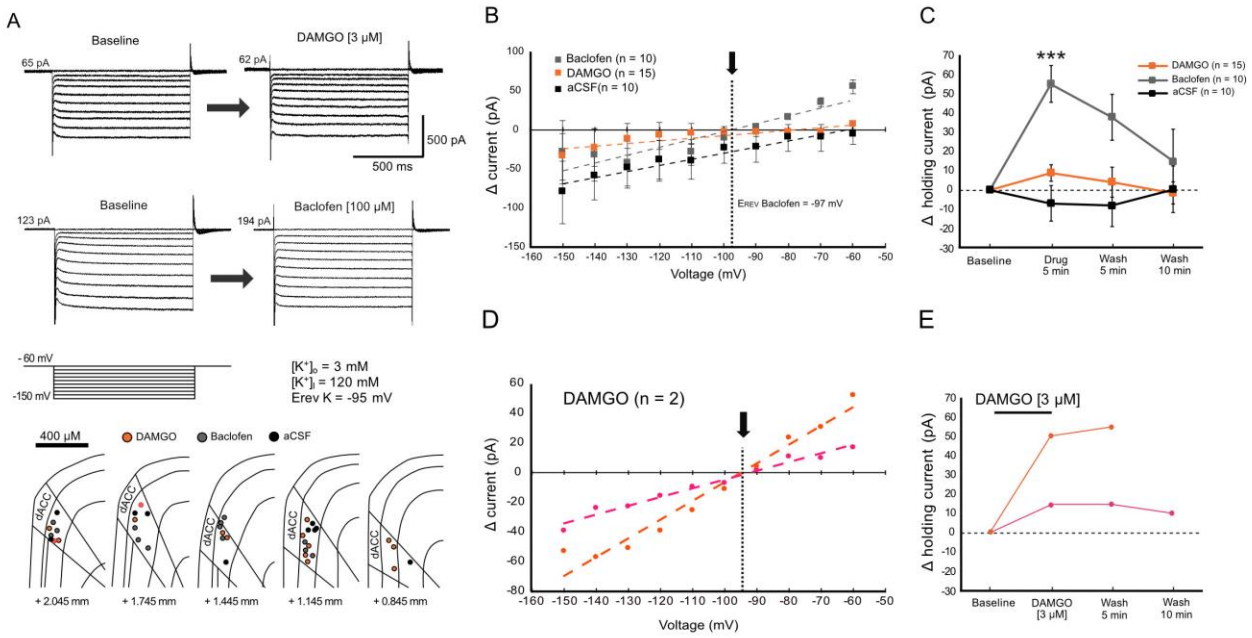
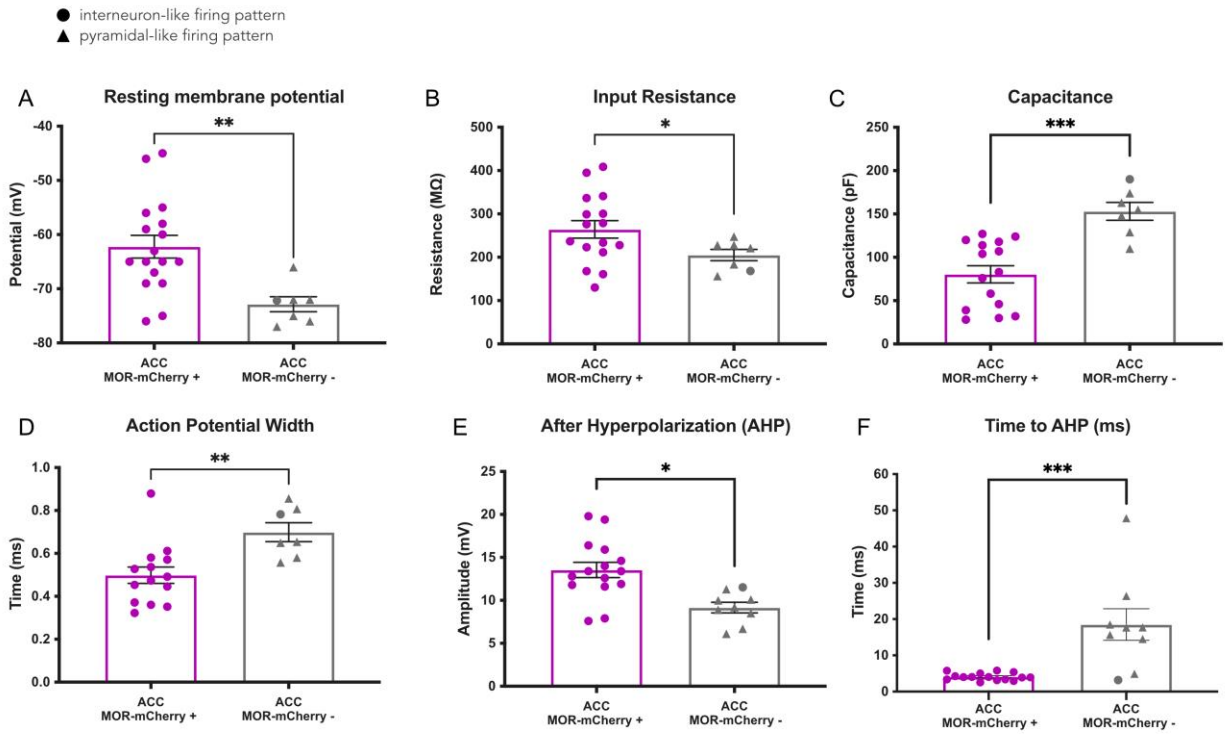
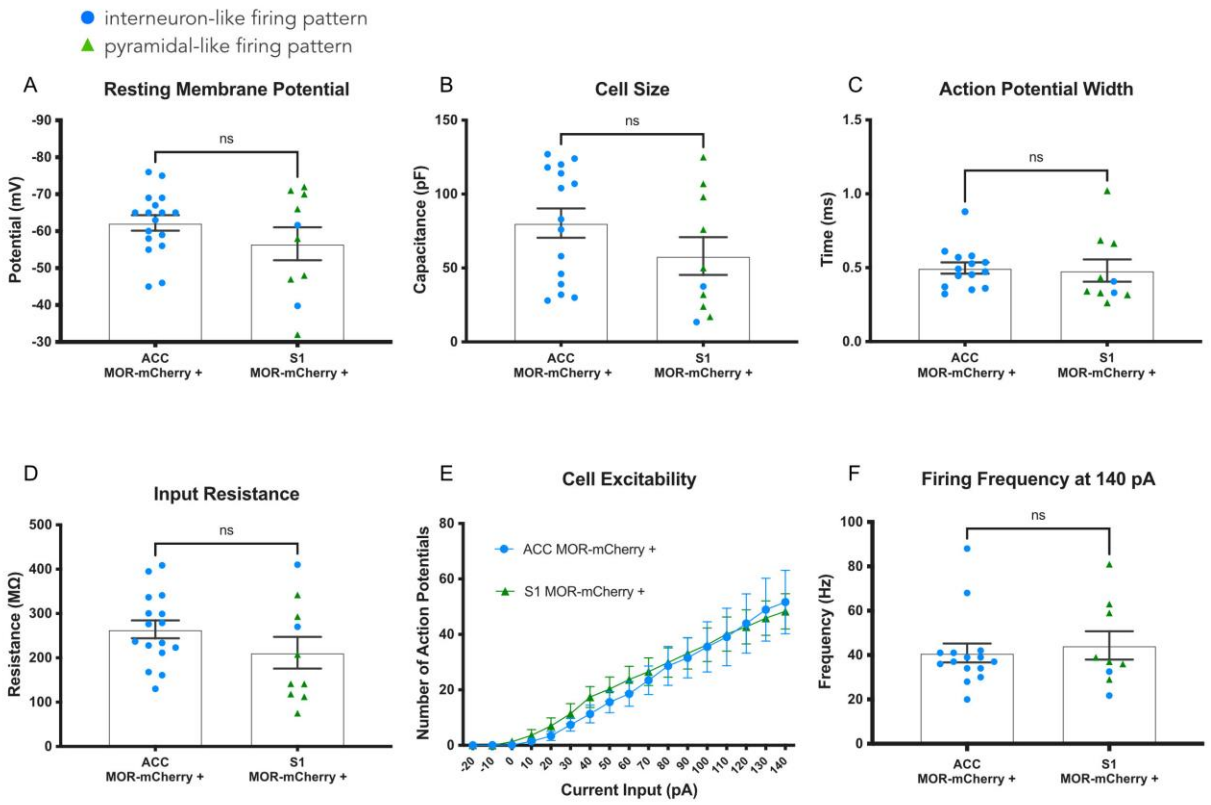
Supplemental Information



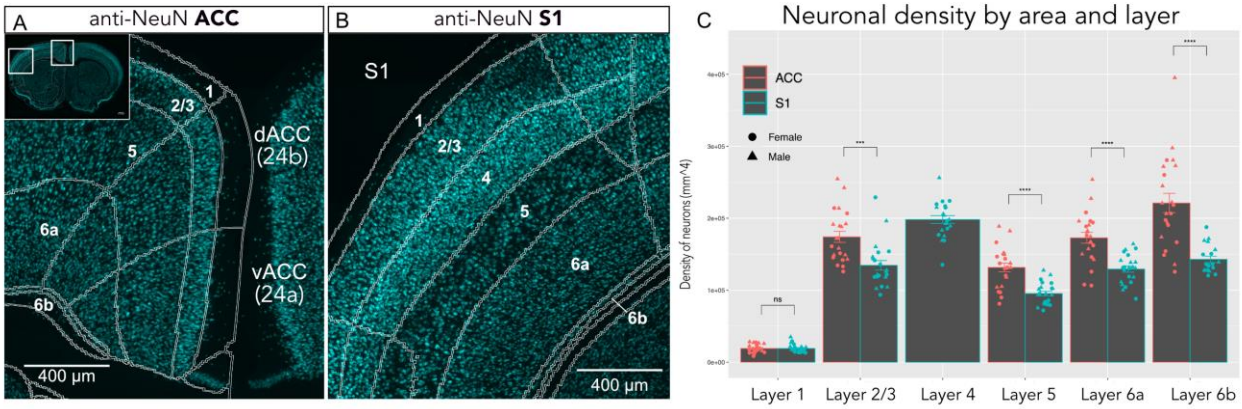
Supplemental Figure 1 - DAMGO induces hyperpolarizing potassium currents in a subpopulation of neurons in ACC in wildtype mice. A) Voltage clamp traces from -150 mV to -60 mV square pulses in 10 mV increments. Neuron recorded at baseline and after 5 minutes of DAMGO application (top traces). Current needed to keep neuron at Vh= -60 mV is indicated. Neuron recorded at baseline and after baclofen (bottom traces). Lower panel shows location of recorded neurons, and all neurons displayed pyramidal-like firing patterns. B) Differential (Delta) current (drug minus baseline). C) Quantification of holding currents following baclofen (55.3 ± 9.6 pA), DAMGO (8.8 ± 4.3 pA), and aCSF (-7.0 ± 9.3 pA). Two-way mixed ANOVA $F(2,35) = 17.04$, $p < 0.0001$. Tukey's multiple comparisons tests. Baclofen group was significantly greater than DAMGO and aCSF, which did not significantly differ from each other. For B) & C) Baclofen group (n= 10 cells, 4 mice), DAMGO (n= 15 cells, 4 mice), aCSF group (n= 10 cells, 4 mice). All recordings were performed in male mice. D) Neurons (2/15) in the DAMGO group have delta currents with reversal near -95 mV, suggesting that these excitatory neurons express MOR. E) Holding currents for DAMGO-sensitive neurons. Data are presented as mean ± SEM in B, C. *** $p<0.001$.



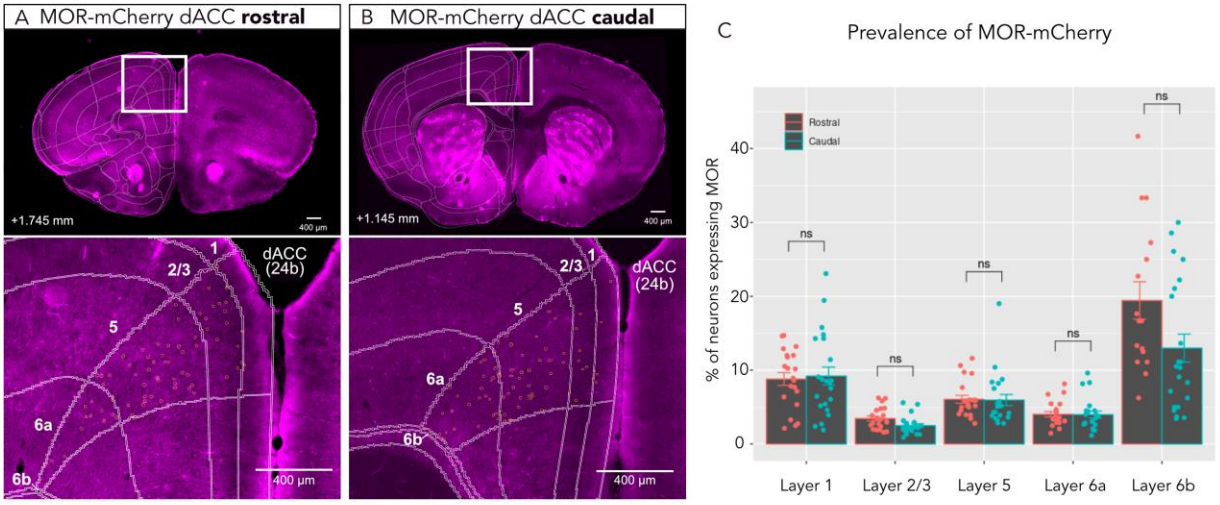
Supplemental Figure 2 - Electrophysiological differences between MOR-mCherry+ and MOR-mCherry-negative neurons. Interneuron-like firing patterns are represented with circles and pyramidal-like firing patterns are represented with triangles. A) MOR-mCherry+ neurons had higher resting membrane potential (RMP) (mean = -62.24mV) compared to the MOR-mCherry-negative group (mean = -72.86 mV), t(22) = 3.120 p <0.01. B) Input resistance (Rin) was significantly high in MOR-mCherry+ neurons (mean = 264.2 MΩ) compared to MOR-mCherry-negative neurons (mean = 205.1 MΩ), t(21), = 1.850, p < 0.05. (one tailed t-test performed). C) MORmCherry+ group had a significantly lower capacitance (mean = 80.40 pF) compared to MOR-mCherry-negative group (mean = 153 pF), t(20) = 4.484, p <0.001. D) Action potential width was significantly narrower in MOR-mCherry+ neurons (mean = 0.50 ms) compared to MOR-mCherry-negative (mean = 0.70 ms) t(20) = 3.203 , p < 0.01. E) AHP amplitude was significantly higher in MOR-mCherry+ neurons (mean = 13.54 mV) compared to MOR-mCherry-negative neurons (mean = 9.156 mV) t(22)=3., p < 0.01. F) Time to AHP, was significantly shorter in MOR-mCherry+ neurons (mean = 4.10 ms) compared to MOR-mCherry-negative neurons (mean = 18.49 ms), t(22) = , p < 0.001. ACC MOR-mCherry+ group (n= 17 cells, 9 mice), ACC MOR-mCherry-ve group (n= 7 cells, 4 mice). All recordings were performed in male mice. Two-tailed student t-test were performed except where mentioned otherwise. Data are presented as mean ± SEM with dots showing individual neurons, * p < 0.05, ** p < 0.01, *** p < 0.001.



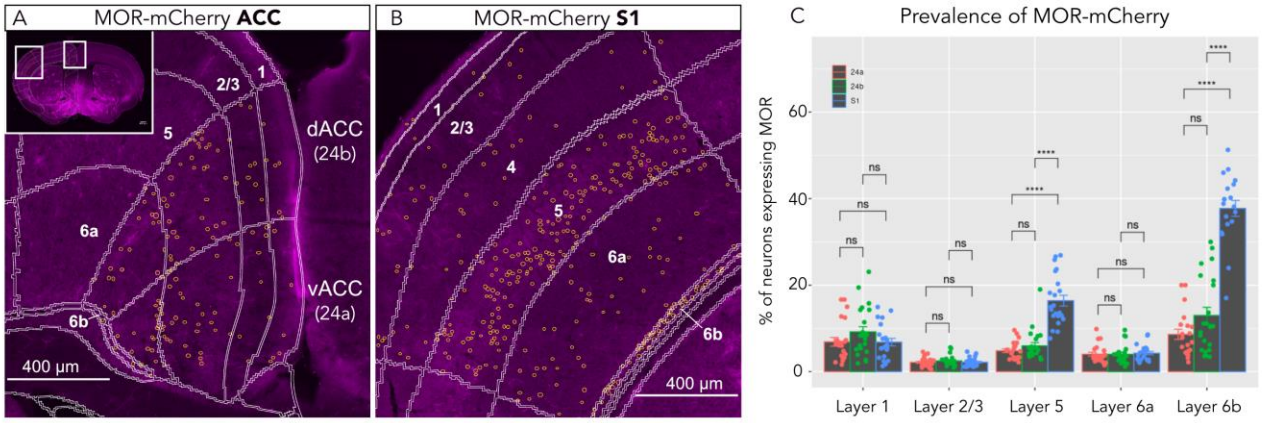
Supplemental Figure 3 – Electrophysiological properties of MOR-mCherry+ neurons in ACC and S1. A) Resting membrane potential, t(25) = 1.297, p = 0.2066. B) Cell size t(23) = 1.396, p = 0.1761. C) Action potential width, t(22) = 0.2257, p = 0.8235. D) Input resistance t(24) =1.387, p = 0.1781 E) Cell excitability - the number of action potentials induced by increasing current steps from -20 pA to + 140 pA, two-way mixed ANOVA F(16,391) = 0.08002, p > 0.999. F) Firing frequency at 140 pA t (22) = 0.4602, p = 0.6499. ACC MOR-mCherry+ group (n= 17 cells, 9 mice), S1 MOR-mCherry-ve group (n= 10 cells, 7 mice). All recordings were performed in male mice. Data are presented as mean ± SEM with dots showing individual neurons. Interneuron-like firing pattern (blue circles) and pyramidal-like firing pattern (green triangles).



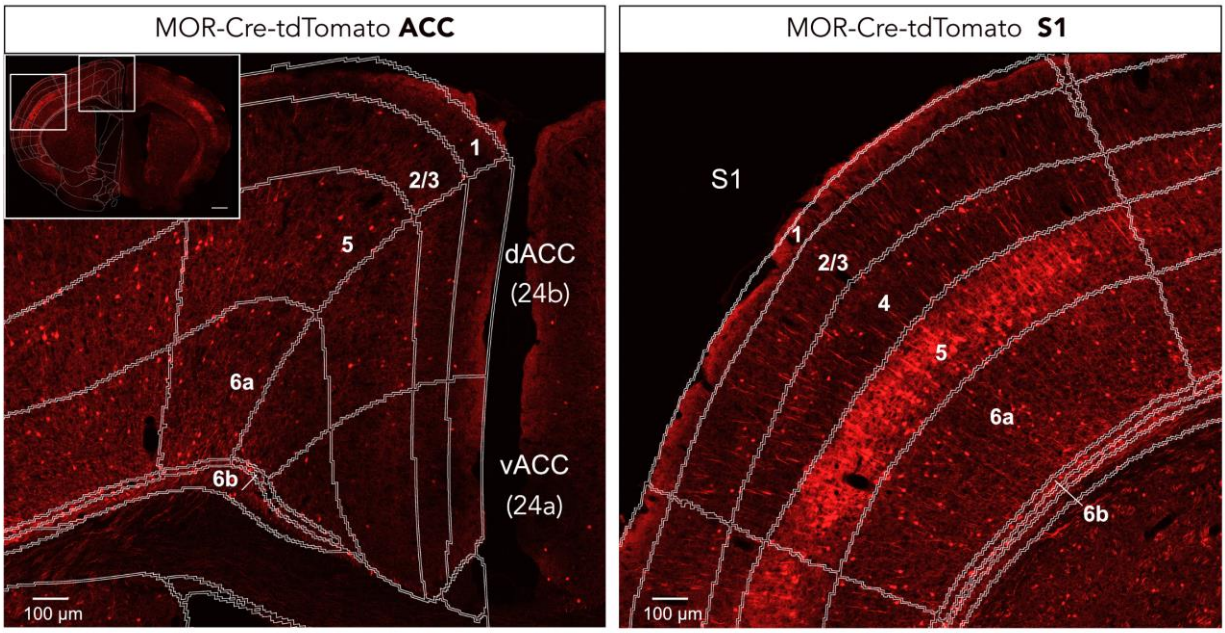
Supplemental Figure 4 – Neuronal density by cortical area and layer. A) Sample images of NeuN staining in ACC (left) and S1 (right), atlas is overlaid in white. B) Quantification of NeuN staining. Two-way mixed ANOVA $F(1.68,30.33) = 26.01$, $p < 0.0001$. Wilcoxon signed-rank test. For both the ACC and S1 data, we analyzed the male group (n= 12 sections, 3 mice) and the female group (n= 10 sections, 3 mice). Data are presented as mean ± SEM with dots showing individual data points. *** $p < 0.001$, **** $p < 0.0001$.



Supplemental Figure 5 – Comparison of MOR expression on a rostro-caudal axis of dorsal ACC (area 24b). A) Typical whole brain images of rostral (top, bregma +1.745 mm to +1.445 mm) and caudal section (bottom, bregma +1.145 mm to -0.055 mm) of MOR-mCherry mice. B) Magnification of dACC in rostral (left) and caudal section (right). Atlas is overlaid in white, and MOR-mCherry+ neurons are outlined in yellow. C) Quantification of MOR-mCherry expression in dACC. Two-way mixed ANOVA $F(1.43, 21.47) = 3.08$, $p = 0.081$. Wilcoxon signed-rank test. Rostral sections – pooled male group (n= 12 sections, 3 mice) and female group (n= 11 sections, 3 mice). Caudal sections – pooled male group (n= 12 sections, 3 mice) and female group (n= 10 sections, 3 mice). Data are presented as mean ± SEM with dots showing individual data points.



Supplemental Figure 6 – Comparison of MOR expression between dACC, vACC and S1. A-B) Representative whole mouse brain section with MOR-mCherry staining (top corner). Magnification of dACC and vACC (left) and S1 (right). Atlas is overlaid in white and MOR-mCherry+ neurons outlined in yellow circles. C) Quantification of MOR-mCherry expression by region and layer. Two-way mixed ANOVA $F(3.54, 56.62) = 72.19$, $p < 0.0001$. Wilcoxon signed-rank test. For dACC (24b), vACC (24a), and S1 the entire area was analyzed in pooled male group (n= 12 sections, 3 mice) and female group (n= 10 sections, 3 mice). Data are presented as mean ± SEM with dots showing individual data points. **** $p < 0.0001$.



Supplemental Figure 7 - Qualitative cross-validation of MOR expression with the *Oprm1*^{Cre} – tdTomato reporter line. MOR-expressing tdTomato+ neurons visualized at the whole brain level (top corner), in ACC (left) and S1(right). Similar experiments were performed on a male group (n= 3) and female group (n= 4), data not shown.