and Δg (C) showing how U73122 (20μM) but not U73343 (20μM) abrogates the E_2-induced attenuation of the OFQ/N-induced outward current in ARH POMC neurons.*, P<0.05, Mann Whitney U-Test.

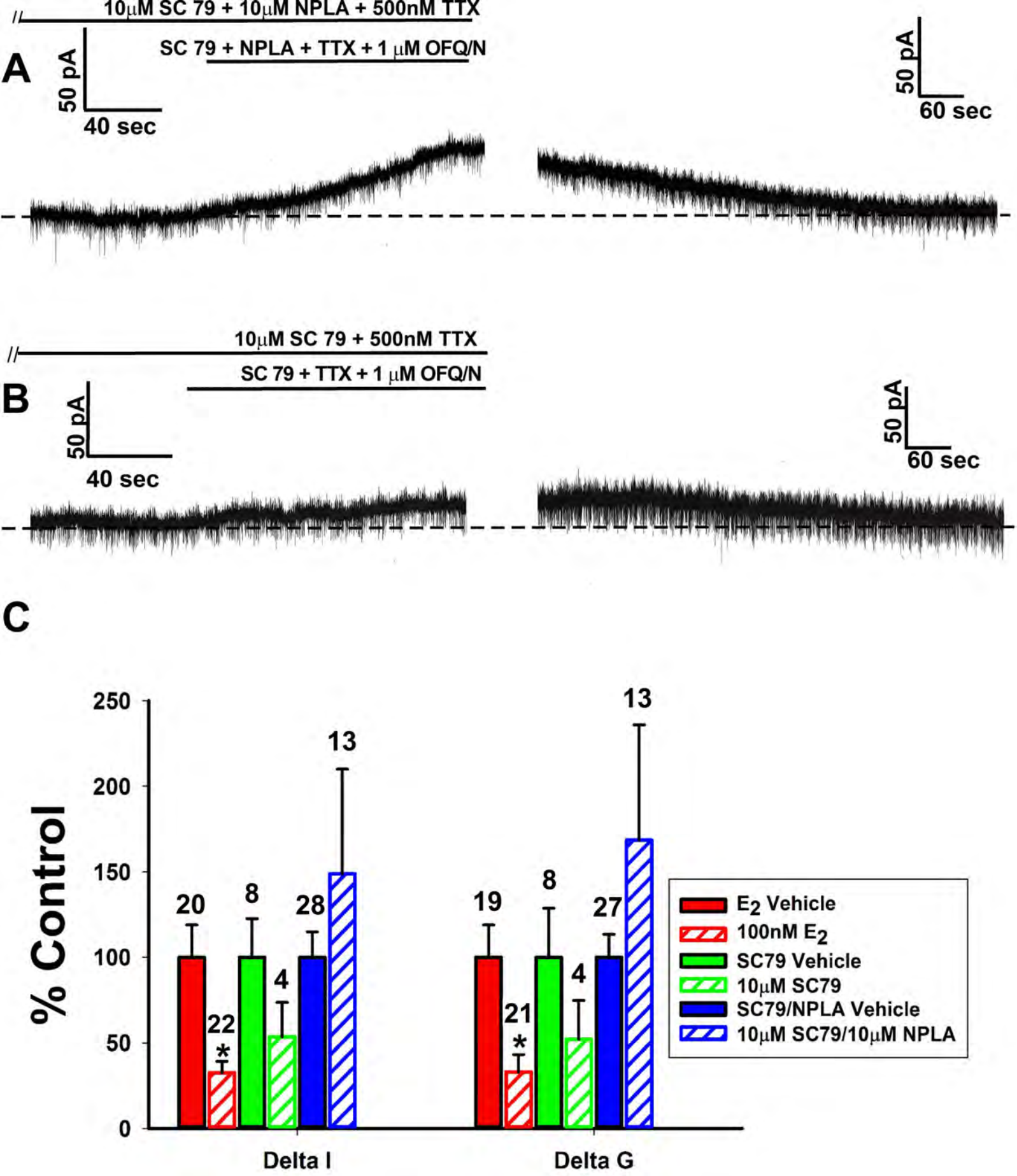
Figure 9. The estrogenic modulation of the OFQ/N-induced outward current in POMC neurons is blocked by inhibition, and mimicked by activation, of PKC. Membrane current traces (A) and (B) and composite bar graph of ΔI and Δg (C) showing the restoration of the OFQ/N-induced outward current caused by the PKC inhibitor NPC 15437 (30μM) in E_2-treated slices, and the attenuation caused by the PKC activator PDBu (1μM) *per se*.*, P<0.05, Mann Whitney U-Test.

Figure 10. The estrogenic modulation of the OFQ/N-induced outward current in POMC neurons is blocked by inhibition, and mimicked by activation, of PKA. Membrane current traces (A) and (B) and composite bar graph of ΔI and Δg (C) showing that the estrogenic attenuation of the OFQ/N-induced outward current is reversed by the PKA inhibitor KT 5720 (300nM) and mimicked by the PKA activator Sp-cAMP (100μM).*, P<0.05, Mann Whitney U-Test.

Figure 11. The estrogenic modulation of the OFQ/N-induced outward current in POMC neurons is blocked by inhibition, and mimicked by activation, of nNOS. Membrane current traces (A) and (B) and composite bar graph of ΔI and Δg (C) showing that the estrogenic diminution of the OFQ/N-induced outward current is blocked by the nNOS inhibitor NPLA (10μM) and mirrored by the NOS substrate L-Arginine (30μM).*, P<0.05, Mann Whitney U-Test.

Figure 12. A Schematic representation of how E2 attenuates ORL-1/GIRK coupling in MPN-projecting, ARH POMC neurons. In the absence of ER activation, ORL-1/GIRK coupling is fully functional; resulting in the robust inhibition of MPN-projecting ARH POMC neurons. **B.** E_2 activation of ERα or G_q-mER causes the disassociation of the α_q subunit to activate PLC. PLC activates PKC, which then phosphorylates adenylyl cyclase that converts ATP to cAMP. cAMP then activates PKA, which could phosphorylates either the ORL-1 receptor, RGS proteins (not shown) and/or GIRK channels. Additionally, ERα can activate PI3K, which stimulates the nNOS enzyme and subsequently guanylate cyclase that converts GTP to cGMP. cGMP activates PKG, which may also contribute to the phosphorylation of either the ORL-1 receptor, RGS proteins or GIRK channels. Collectively, this increases the excitability of MPN-projecting, ARH POMC neurons and thus the release of β-endorphin.

Supplemental Figure 1. The estrogenic modulation of the OFQ/N-induced outward current in POMC neurons is mimicked by the activation of Akt, and blocked by the concomitant inhibition of nNOS. Membrane current traces (A) and (B) and composite bar graph of ΔI and Δg (C) showing that the estrogenic diminution of the OFQ/N-induced outward current is mimicked by the Akt activator SC 79 (10μM) and rescued by the nNOS inhibitor NPLA (10μM).*, P<0.05, Mann Whitney U-Test, n = 4 - 27.


A
10μM SC 79 + 10μM NPLA + 500nM TTX
SC 79 + NPLA + TTX + 1 μM OFQ/N
50 pA
40 sec
50 pA
60 sec
B
10μM SC 79 + 500nM TTX
SC 79 + TTX + 1 μM OFQ/N
50 pA
40 sec
50 pA
60 sec
C
% Control
250
200
150
100
50
0
20
22
8
4
28
13
19
21
8
4
27
13
*
*
Delta I
Delta G
E2 Vehicle
100nM E2
SC79 Vehicle
10μM SC79
SC79/NPLA Vehicle
10μM SC79/10μM NPLA