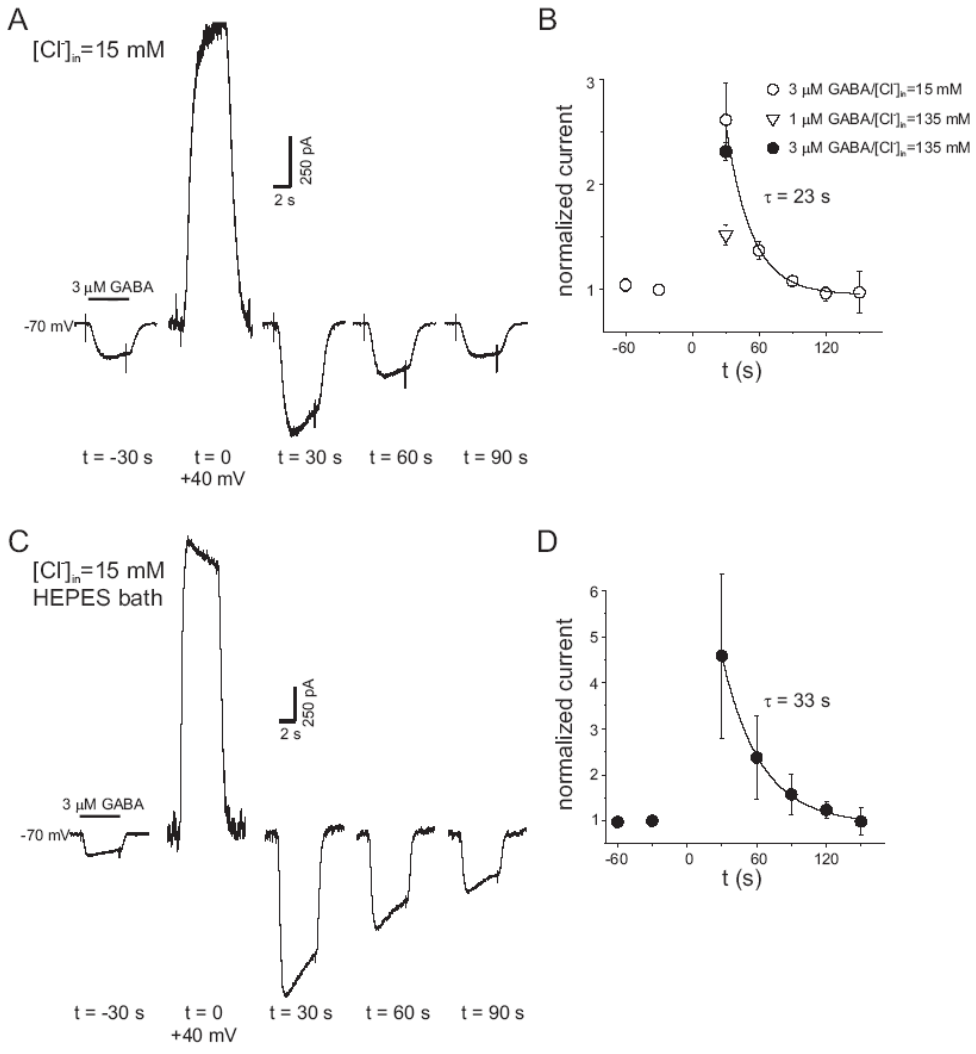
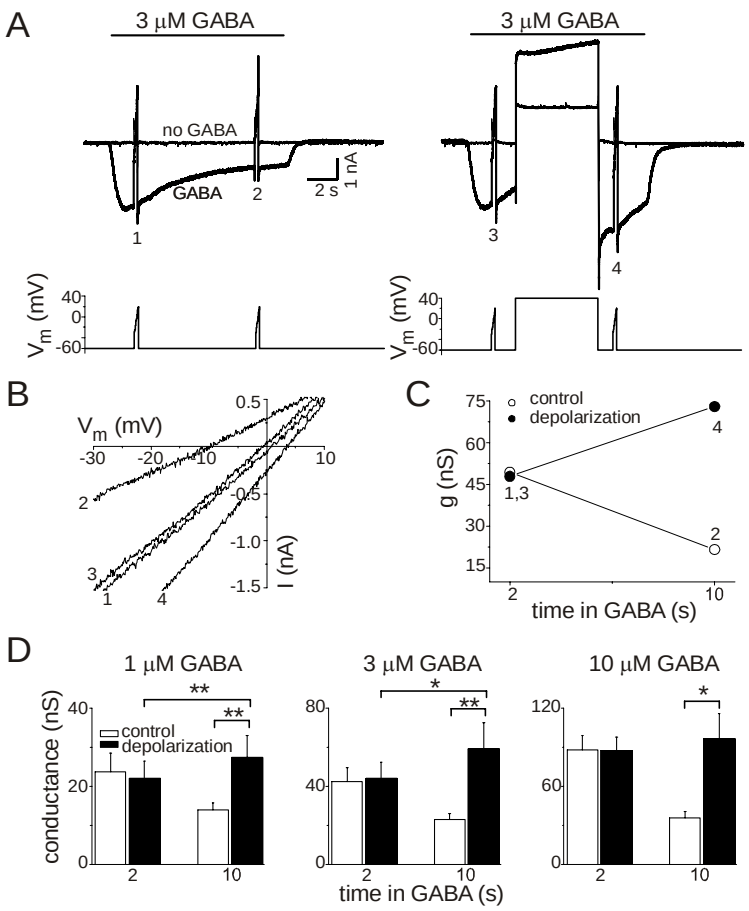


Supplemental Figure 1

Supplemental Figure 1 – PDP of GABA-evoked currents occurred with low intracellular Cl^- and HCO_3^--free bath solution. A: Currents evoked with serial applications of GABA (3 μM) every 30 s in a neuron recorded with Cs-methanesulfonate pipette solution ($[Cl^-]_i$ = 15 mM). At t=0 GABA was applied after depolarization to +40 mV from the holding potential of –70 mV. Currents were potentiated 30 s after this transient depolarization. Currents are baseline-adjusted to allow comparison. B: Mean normalized current in neurons with low $[Cl^-]_i$, measured as in panel A. The average potentiation of currents measured 30 s after repolarization was 161±35% (n=5 cells). For comparison the peak PDP seen with high $[Cl^-]_i$ is plotted for experiments with 1 μM GABA (down triangles, n=5) and 3 μM GABA (filled circles, n=4). PDP with 3 μM GABA was not affected by $[Cl^-]_i$ (p=0.48). C: PDP of GABA-evoked current in HCO_3^--free bath solution (HEPES). D: Mean normalized current from 3 neurons studied as in panel C.

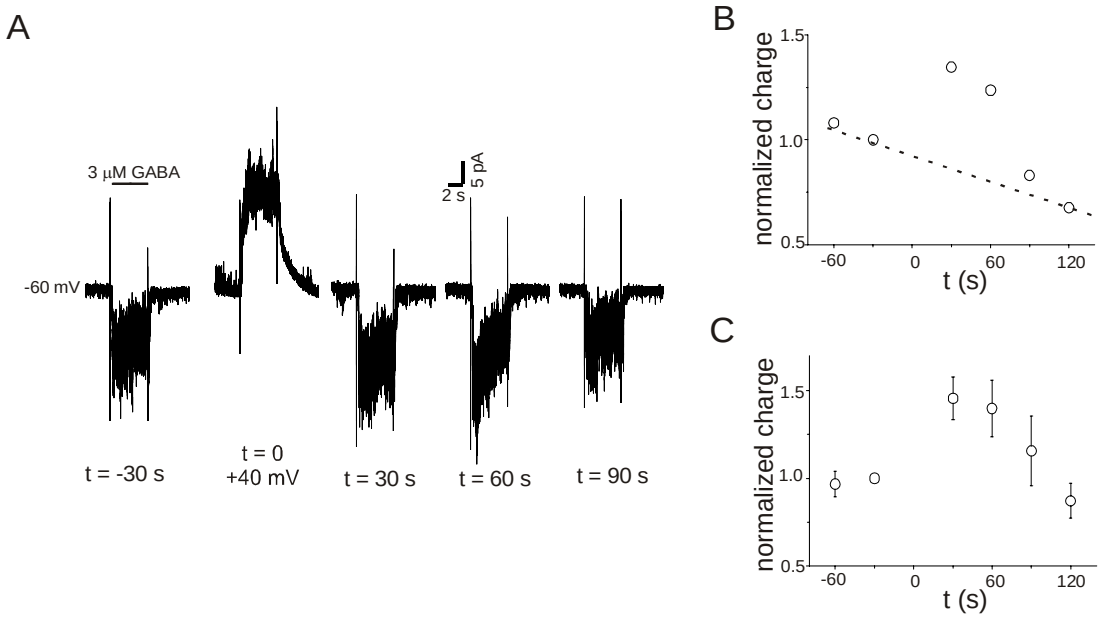
Because PDP of GABA current was observed within seconds of repolarization (see Fig. 5A), it is possible that anion accumulation at these time points could be larger than that measured 30 s after repolarization. To evaluate this, we applied voltage ramps (-30 mV to +30 mV over 250 ms) at two different time points during GABA application (Supplemental Fig. 2A). Difference currents from records obtained in the presence and absence of GABA (1-10 μM) were used to determine E_{GABA} when cells were held at -60 mV or transiently depolarized to +40 mV for 5 s (Supplemental Fig. 2A-B). Chord conductance was calculated using experimentally determined E_{GABA} and current amplitude at -60 mV (measured immediately prior to voltage ramps). As expected, the reversal potential shifted towards more negative values during GABA application at -60 mV as intracellular anion concentration dropped during the sustained inward current (Supplemental Fig. 2B). With an intervening depolarization, E_{GABA} shifted towards positive values, due to intracellular anion accumulation. For the experiment illustrated in Supplemental Fig. 2A, chord conductance without depolarization was 49 nS after 2 s of GABA application and 22 nS after 10 s in GABA (-66% conductance change, Supplemental Fig. 2C). In contrast, when the neuron was depolarized conductance went from 48 nS before depolarization to 73 nS after depolarization (+51% conductance change, Supplemental Fig. 2C). Thus, chord conductance measured after depolarization was increased by 48% compared to the control value at 2 s (point 4 vs. point 3), and was increased by 218% compared to conductance at 10 s without depolarization (point 4 vs. point 2). The conductance changes seen with different concentrations of GABA are summarized in Supplemental Fig. 2D. On average, depolarization increased conductance (compared to conductance at 10 s without depolarization) by 85±15%, 150±35%, and 174±35% with GABA concentrations of 1, 3, and 10 μM, respectively (n=4-9 cells at each concentration).



Supplemental Figure 2

Supplemental Figure 2 - GABA conductance increased immediately after transient depolarization. A: Current responses to GABA (3 μM) with voltage ramps given at 2 s and 10 s into GABA application. Voltage ramps were from –30 to +30 mV over 250 ms. In the right hand panel the neuron was depolarized to +40 mV between the two voltage ramps. Point by point subtractions were made between traces recorded in the presence (thick lines) or absence (thin lines) of GABA to yield the GABA-evoked current. B: Difference currents from data in panel A. Numbers refer to time points shown in panel A. Under control conditions reversal potentials were +0.6 mV and -9.7 mV at 2 s and 10 s after GABA application, respectively (traces 1 and 2). With depolarization reversal potentials were -0.7 mV and +3.4 mV at 2 s and 10 s after GABA application, respectively (traces 3 and 4). C: GABA conductance at -60 mV for experiment in panel A. Chord conductance was calculated using measured reversal potentials and current values immediately prior to voltage ramps. Conductance decreased by 66% during GABA application at –60 mV (control). In contrast, conductance increased by 51% after depolarization. Numbers refer to time points in panel A. D: Summary data of conductance values at 2 s and 10 s after GABA application (determined as in panels A-C) with different GABA concentrations. n=4-9 cells at each concentration. * - $p<0.05$, ** - $p<0.01$.

If PDP depended critically on anion accumulation or cellular factors this type of voltage-dependent modulation should be absent in excised membrane patches, a situation with optimal experimental control of voltage and ion concentrations. To explore this we recorded macroscopic GABA currents (1-3 μM) at –60 every 30 s in outside-out patches before and after depolarization to +40 mV. These experiments were complicated by the frequent appearance of high conductance channels that appeared within minutes of patch excision and rapid seal breakdown. In a small number of stable patches receptor activation was quantified by integrating the current during the period of GABA application to obtain total charge transfer. PDP was observed in outside-out patches. On average, transient depolarization increased charge movement by 46±13% (n=4 patches, Supplemental Fig. 3A-B). In 3 additional stable patches no potentiation was seen, but in those cases the GABA currents were inwardly-rectifying, suggesting that PDP may require specific $GABA_A$ receptor subunit combinations (data not shown). Although a small sample, these results support the conclusion that PDP resulted from intrinsic receptor properties independent of anion redistribution or cellular factors.



Supplemental Figure 3

Supplemental Figure 3 – GABA currents in outside-out patches underwent PDP. A: PDP in an outside-out patch. GABA application was made every 30 s. At t=0 GABA was applied to depolarized patch. Currents were integrated to obtain charge transfer produced by GABA. B: Time course for normalized charge transfer from data in panel C. Charge transfer was increased 35% by transient depolarization and this recovered over 90 s. Note current rundown seen over course of experiment (dashed line). E: Time course for mean data from 4 outside-out patches studied as in A-B.