Supporting Information

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Hippocampal Slice Preparation

Following isoflurane anesthesia, young adult male Sprague–Dawley rats (2–4 mo of age) were rapidly decapitated, and the brain was placed in cold, high-sucrose, low-Na^+ aCSF containing the following: 85 mM NaCl, 2.5 mM KCl, 4 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 25 mM glucose, and 75 mM sucrose. Coronal slices (400 μm) were cut from dorsal hippocampus in cold, high-sucrose, low-Na^+ aCSF equilibrated with 95% O_2 and 5% CO_2 using a vibratome (VT100P; Leica Biosystems). Following their preparation, slices were allowed to recover for 1 h at room temperature in standard aCSF containing the following: 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, 26 mM $NaHCO_3$, and 11 mM glucose equilibrated with 95% O_2 and 5% CO_2.

Whole-Cell Recordings

Slices were placed in a submersion chamber and continuously perfused (2 $mL \cdot min^{-1}$) with modified aCSF (5 mM K^+ instead of 2.5 mM K^+) equilibrated with 95% O_2 and 5% CO_2 and maintained at 27–29 °C. Elevated K^+ has been previously used to enhance neuronal firing and facilitate detection of spontaneous synaptic events and APs (41, 56). To test for potential selective effects of NMDAR antagonists on GABAergic and/or glutamatergic transmission, sIPSCs and sEPSCs were recorded from CA1 pyramidal cells that were blind-patched using a pipette solution containing the following: 120 mM cesium gluconate, 0.3 mM EGTA, 2.8 mM NaCl, 5 mM $MgCl_2$, 2 mM ATP, 0.3 mM GTP, 20 mM Hepes, and 5 mM QX-314, with pH adjusted to 7.2 with CsOH. sEPSC and sIPSCs were recorded at V = −50 mV and V = 0 mV, respectively. Throughout each experiment, series resistance was monitored. An experiment was discarded if the series resistance change was >15%.

In current-clamp recordings, CA1 pyramidal cells were blind-patched using pipettes with 3–6 MΩ resistance and an intracellular solution containing the following: 120 mM K-gluconate, 0.3 mM EGTA, 2.8 mM NaCl, 5 mM $MgCl_2$, 2 mM ATP, 0.3 mM GTP, and 20 mM Hepes, with pH adjusted to 7.2 with KOH. Input resistance was monitored throughout the experiment, and any cells in which the resistance change was >15% were discarded.

Extracellular Recordings

Slices were placed in a submersion chamber and continuously perfused (3–4 $mL \cdot min^{-1}$) with aCSF equilibrated with 95% O_2 and 5% CO_2 and maintained at 26–28 °C. The CBI, which measures the length of the waveform, was used to analyze changes in excitability (53, 54). The equation $CBI = \sum(|V_{\chi+1} - V_{\chi}|)$ summates the voltage differential between each point in the physiological waveform (sampled at 10 kHz) and is sensitive enough to detect voltage changes due to differences in excitability.

For both whole-cell and extracellular recordings, control experiments were performed where the aCSF perfusion was switched between two different reservoirs to control for possible flow effects that could cause an increase or decrease in AP probability or PS amplitude and to demonstrate stability of the recording over time. Control experiments were interleaved with all drug experiments.

Drugs

Ketamine was purchased from MWI Animal Health. The GluN2B NMDAR subunit-selective antagonist Ro 25-6981 and the NMDAR glycine site partial agonist GLYX-13 were purchased from Tocris, and scopolamine was purchased from Sigma–Aldrich. All drugs were bath-applied at the stated concentration. Ketamine was made fresh daily using serial dilutions in water to obtain a final working concentration of 1 μM. Stock concentrations of Ro 25-6981, GLYX-13, and scopolamine were made, and aliquots were frozen at −20 °C. Each day, a stock aliquot was thawed and diluted to the desired working concentration and was used for no longer than 6 h once prepared.

Statistics

For sEPSCs and sIPSCs, events were detected during 5 min of baseline monitoring and during drug exposure. An averaged example trace (30–50 sEPSCs or sIPSCs) was created to use as a template for analyzing sEPSCs or sIPSCs during baseline and drug conditions for each neuron, and sEPSCs and sIPSCs were manually accepted based on a match to the template.

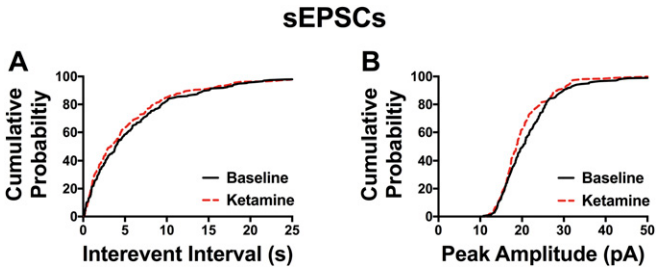

Fig. S1. Ketamine does not change pharmacologically isolated sEPSC frequency but does decrease amplitude. (*A*) Cumulative probability showing no significant change in sEPSC IEI with application of 1 μM ketamine in the presence of 100 μM picrotoxin ($P = 0.64$, $n = 5$, 235 baseline events, 260 ketamine events). (*B*) Cumulative probability indicating that in the presence of 100 μM picrotoxin, ketamine significantly decreases sEPSC peak amplitude ($P < 0.01$, $n = 5$, 235 baseline events, 260 ketamine events).

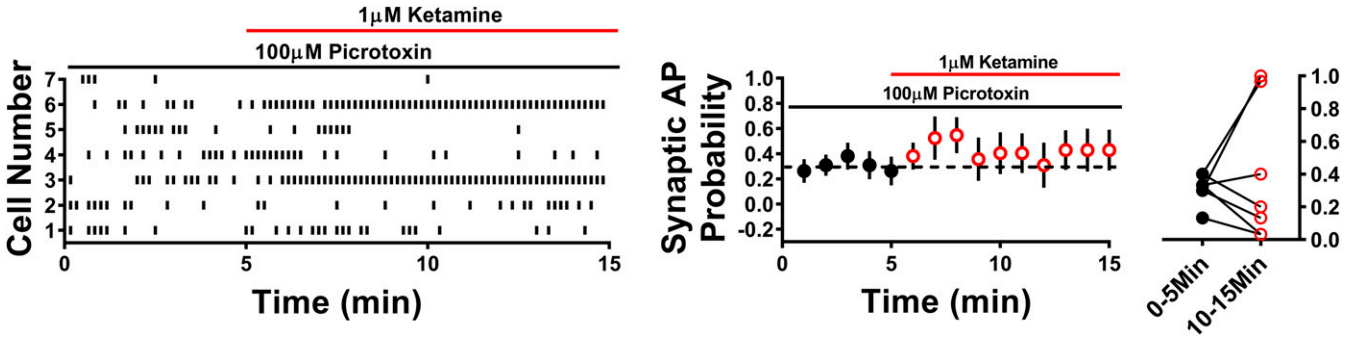

Fig. S2. Ketamine does not increase synaptic AP probability when inhibition is blocked. In the presence of 100 μM picrotoxin, bath application of 1 μM ketamine had no significant effect on synaptic AP probability (0–5 min: 0.31 ± 0.03, 10–15 min: 0.40 ± 0.16; $n = 7$; paired t test, $P = 0.44$). All values are mean ± SEM.

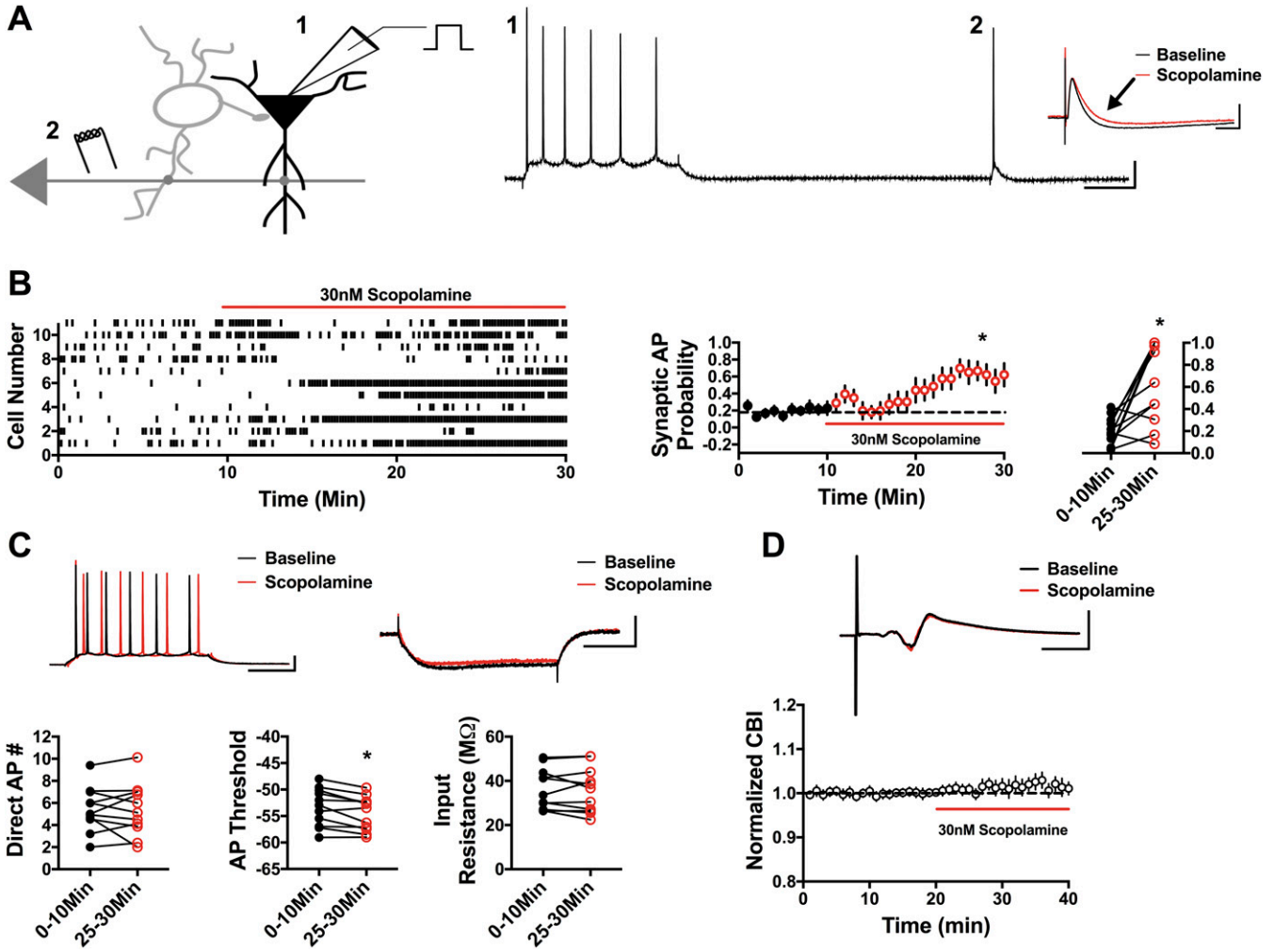

Fig. S3. Scopolamine (30 nM) increases synaptic AP probability in individual CA1 pyramidal cells but not at the population level. (*A*) Schematic represents the recording configuration during whole-cell experiments in CA1 pyramidal cells where APs were elicited with a direct depolarizing current injection (1) and electrical stimulation of Schaffer collaterals (2). (Scale bars: 20 mV and 100 ms.) (*Inset*) EPSP-IPSP sequence in baseline (black) and scopolamine (red) from cell 7. Bath application of scopolamine decreases the evoked IPSP decreases (black arrow). (Scale bars: 2 mV and 50 ms.) (*B*) Raster plot shows synaptic APs at baseline and in the presence of 30 nM scopolamine in each cell. The summary plot shows a significant increase in AP probability with bath application of scopolamine (0–10 min: 0.197 ± 0.04, 25–30 min: 0.63 ± 0.11; $n = 11$; paired t test, $^*P = 0.004$). (*C*) AP threshold is significantly hyperpolarized in 30 nM scopolamine (0–10 min: −53.29 ± 1.08 mV, 25–30 min: −54.51 ± 0.97; $n = 11$; paired t test, $^*P = 0.01$), while no change is observed with direct AP number (0–10 min: 5.44 ± 0.60, 25–30 min: 5.36 ± 0.71; $n = 11$; paired t test, $P = 0.85$) or input resistance (0–10 min: 36.41 ± 2.80 MΩ, 25–30 min: 35.75 ± 3.07 MΩ; $n = 11$; paired t test, $P = 0.55$). (Scale bars: 20 mV and 100 ms, 4 mV and 100 ms.) (*D*) Extracellular recordings in CA1 stratum pyramidale show no change in excitability in the presence of scopolamine (baseline: 0.9996 ± 0.002, 35–40 min: 1.016 ± 0.01291; $n = 7$; paired t test, $P = 0.20$). All values are mean ± SEM. (Scale bars: 1 mV and 10 ms.)