Supporting Information

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SI Materials and Methods

Chronic Drug Treatment. Sprague–Dawley rats (~3 wk old) were injected s.c. with PBS, nicotine (1 mg/kg), donepezil (2 mg/kg), galantamine (3 mg/kg), or pirenzepine (20 mg/kg) twice daily for at least 10 d and up to 15 d. RS86 (1–2 mg/kg) was administered i.p. to rats twice daily for at least 7 d and up to 15 d. When pirenzepine (20 mg/kg) was coadministered with nicotine (1 mg/kg) or donepezil (2 mg/kg), nicotine or donepezil was injected into a different site 10–15 min after injection of pirenzepine. Pirenzepine passes the blood–brain barrier, but only to a small extent (1). However, pirenzepine (20–75 mg/kg, i.p.) was shown to block the ameliorative effect of acetylcholinesterase inhibitors on spatial memory deficits (2). Thus, pirenzepine (20 mg/kg, i.p., twice daily for 10 d) was administered alone or together with nicotine (1 mg/kg, s.c., twice daily for 10 d) to rats. Donepezil at 2.5 mg/kg causes a significant increase in the levels of synaptic ACh in the hippocampus of rats (3). Donepezil is a more potent drug than galantamine in the inhibition of rat brain acetylcholinesterase after in vivo administration (4, 5). However, galantamine, but not donepezil, also acts as an allosteric potentiating ligand at nicotinic ACh receptors (5, 6). This modulation occurs only within a specific concentration range (0.02–2.0 μM). Previous studies suggest that 0.1–1.0 μM concentrations of brain galantamine are expected 2–3 h after s.c. injection of galantamine at 3.0 mg/kg (5). Thus, rats were injected with donepezil (2 mg/kg, s.c., twice daily for 10 d) or galantamine (3.0 mg/kg, s.c., twice daily for 10 d). RS86 was used as an m1 muscarinic receptor agonist (7, 8). Because i.p. administration of 1–1.5 mg/kg of RS86 twice daily for 7 d was shown to induce significant molecular and behavioral changes in rats (7, 8), we used the same drug regime to treat rats.

Slice Preparation. Ninety minutes or, in some experiments, ~16 h after the last injection, the animals were decapitated under anesthesia with urethane. These different procedures did not change outcomes. Transverse hippocampal slices (300 μm) were then prepared using a vibrating blade microtome (VT1000S; Leica); maintained at 30–32 °C in artificial cerebrospinal fluid (ACSF) containing 124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 22 mM $NaHCO_3$, and 10 mM glucose; and oxygenated with 95% (vol/vol) O_2 and 5% (vol/vol) CO_2.

Electrophysiological Recording. Excitatory postsynaptic currents (EPSCs) were recorded using the whole-cell patch clamp technique as described previously (9). Slices were placed in a recording chamber (capacity of 0.3–0.4 mL), submerged, and continuously perfused at 1–2 mL/min with oxygenated ACSF at 30 °C. Neurons were visualized for whole-cell recording using a 40× water-immersion objective and a differential interference contrast system under IR light (Axioskop; Zeiss). Voltage-clamp recordings were then made from the somatic region of CA1 pyramidal cells at a holding potential of +40 mV in the presence of the $GABA_A$ receptor antagonist bicuculline (10 μM), which blocks GABAergic synaptic transmission. The patch electrodes were pulled from borosilicate glass (World Precision Instrument) using a micropipette puller (P-97; Sutter Instrument). The patch pipettes (5–7 MΩ) were filled with solution containing 117 mM Cs-methanesulfonate, 10 mM Hepes, 0.5 mM EGTA, 2.8 mM NaCl, 5 mM tetraethylammonium chloride, 5 mM QX-314, 2.5 mM Mg-ATP, and 0.3 mM Na-GTP, adjusted to pH 7.3 with CsOH. Series resistances were monitored throughout experiments by application of hyperpolarizing pulses through the patch pipette; if the series resistances changed more than 20%, the experiment was stopped and the data were eliminated. Afferent fibers (Schaffer collateral/commissural afferents) were stimulated once every 30 s (duration of 0.15–0.2 ms) using a bipolar electrode placed in the stratum radiatum. Stimulation intensity (<50 μA for 50-μs pulse) was adjusted to evoke EPSC amplitude in the range of 100–200 pA. EPSCs were amplified and filtered (2–5 kHz) using an Axopatch-1D amplifier (Axon Instruments), digitized at 10 kHz using Digidata 1200 (Axon Instruments), stored on a microcomputer, and analyzed using pCLAMP7 (Axon Instruments). NMDA receptor (NMDAR)/AMPA receptor (AMPAR) ratios were calculated by measuring the average peak EPSC at +40 mV (EPSCs recorded over 5 min) before and after application of the NMDAR antagonist 2-amino-5-phosphopentanoate (AP5; 40 μM). The NMDAR EPSC amplitude was obtained by digital subtraction of the AMPAR EPSC amplitude (peak current in the presence of AP5) from the initial EPSC. NMDAR-mediated responses were recorded from pyramidal cells voltage-clamped at −30 to −40 mV in the presence of a non-NMDAR antagonist, 6,7- dinitroquinoxalline-2,3-dione (20 μM), and a $GABA_A$ receptor antagonist, bicuculline (10 μM). Src (30 U/mL; Upstate) was directly applied into pyramidal cells by diffusional exchange through patch pipettes. Src was stored as 100× single-use stocks and prepared immediately before use. To determine the change of the synaptic NMDAR EPSCs, mean amplitudes recorded 25–30 min after establishment of whole-cell configuration were calculated and expressed as a ratio of the mean amplitudes during first 5–10 min. If current responses did not stabilize within 10 min after achievement of whole-cell configuration, the experiments were stopped and the data were discarded. Focal application of NMDA (1 mM dissolved in ACSF) was performed in the presence of tetrodotoxin (1 μM) by pressure ejection (6–10 ms, 15–20 psi) into the proximal region of the apical dendrites of pyramidal cells using a Picospritzer II (General Valve).

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