Supporting Information

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SI Methods

Measurement of Acetylcholinesterase Activity. Brain sections were preincubated in 10 mL medium [7.4 mL 0.1 M Tris-maleate buffer, pH 5.1; 1.0 mL 0.4 M sodium citrate; 1.0 mL 0.12 M copper sulfate; 0.5 mL 0.16 M potassium ferricyanide; and 0.1 mL 10–3 M iso-tetraisopropylpyrophosphoramide (CAS#: 513-00-8; OMPA)] for 20 min at room temperature. Sections were then incubated in the same medium containing 2 nM acetylthiocholine for 1 h at 37 °C. Brain sections were rinsed in medium, mounted on slides, and coverslipped. Specificity of acetylcholinesterase (AChE) measurements were confirmed by including inhibitors of AChE (either 10 μM A9013 (Sigma-Aldrich) or 10 μM physostigmine) or by omitting acetylthiocholine from the incubation medium.

shRNA Design. Three sequences targeting AChE were chosen in the consensus coding region of the mRNA encoding the different isoforms of AChE (GenBank accession NM_009599.3) using methods described previously (1): 5′-3′-GAGTGGAGTGAAA-AGCTGCCTGTATA; GAGCTGATAGCCTGCTTGAGGACA; and GAGCCTGAACTGGACCCCTTATA.

For the scrambled shRNA, we selected a random sequence of 24 bases with no similarity to any known mRNA. Synthetic oligonucleotides were designed by adding antisense sequences directed against the selected mRNA region, followed by a miR23 loop of 10 nucleotides (CCTCTGTGCA) to the 5′ end of the sequences above and by adding overhanging ends identical to those created by SapI and XbaI restriction enzyme digestion. Annealed oligonucleotides were ligated into a pAAV-EGFP-shRNA vector as described previously (1), and positive clones were verified by sequencing. In vitro efficacy of AChE knockdown was assessed in N2a cells (see below).

In Vitro Efficacy of AChE Knockdown in N2a Cells. N2a cells were transfected with plasmids carrying AChE-shRNA-1, -2, or -3 alone or in combination. Five days after transfection, AChE activity in transfected with plasmids carrying AChE-shRNA-1, -2, or -3 alone was assessed in N2a cells (see below).

Quantitative Real-Time PCR for in Vivo Knockdown. Quantification of mRNA was performed as described previously (4). Following viral infusions, mice were decapitated and the hippocampus was quickly removed before total RNA was extracted using the RNeasy Lipid tissue kit (Qiagen). Primer design, reverse transcription, and quantitative real-time PCR (qPCR) was performed exactly as described previously (1). β-Glucuronidase (Gusb) was used to normalize across samples, and amplification products were quantified using the ΔΔCt method. Primer sequences were as follows: AChE (NM_009599.3)—forward, GC-AATTGACACCGGAGCTGTA; reverse, CTACCAGGCAAGGAAAGGA; and Gusb (NM_010368.1)—forward, AACCTCTGTGGGCTTACCT; reverse, TCCCGATAAGGAGGTGTAGA; and EGFP (EU056362.1)—forward, GGGCATTCAAGGTTACTT; reverse, TCACGAACTCCAGGAGAC.

Only samples with detectable EGFP expression were included in the analysis. qPCR identified a significant reduction in AChE mRNA levels in hippocampus of AAV-shAChE–injected compared with AAV-Scr–injected mice [Δ(9) = 1.89, P < 0.05], which could be observed visually in brain slices (Fig. 3). In contrast, no gross morphological differences were observed between neurons infected with AAV and controls.

Validation of AAV Infusion Site. Mice were anesthetized with chloral hydrate and perfused intracardially with PBS (~75 mL) followed by 4% (wt/vol) PFA in PBS (~75 mL) for 5 min each. Brains were then removed, postfixed for 24 h in 4% PFA at 4 °C, then placed in 30% (wt/vol) sucrose in PBS for cryoprotection, and stored at 4 °C. Brain sections (40 μm) were cut with a sliding microtome and placed in PBS before injection sites were examined by visual confirmation of EGFP expression using a Nikon fluorescence microscope.

Fig. S1. AChE activity measured in brain slices following acute physostigmine administration. The y axis represents integrated density of the colorimetric reaction used to measure AChE activity. n = 8–10 per group. Data are expressed as mean ± SEM. *P < 0.05; ***P < 0.001
Fig. S2. AChE activity measured in brain slices following 2 wk of chronic treatment with fluoxetine. The y axis represents integrated density of the colorimetric reaction used to measure AChE activity. \( n = 8-10 \) per group. Data are expressed as mean ± SEM. **\( P < 0.01 \).

Fig. S3. AChE activity measured in brain slices following 2 wk of chronic treatment with physostigmine. The y axis represents integrated density of the colorimetric reaction used to measure AChE activity. \( n = 8-10 \) per group. Data are expressed as mean ± SEM. *\( P < 0.05 \).
In vitro knockdown of AChE in N2a cells. Representative histochemical staining of AChE activity in N2A cells (mouse neuroblastoma) showing reduced AChE activity in cells transfected with plasmids expressing shRNA-AChE-GFP. Three days after transfection, cells were evaluated with the same colorimetric assay used to detect AChE activity in tissue (observed as black staining). The merged picture shows that the cells transfected with the shRNA construct (green) had decreased AChE activity compared with nontransfected cells. Insets highlight the decreased AChE activity in a transfected cell (Inset 1), relative to a nontransfected cell (Inset 2).
Fig. S5. Infusion of AAV-hAChE into the hippocampus. hAChE expression in hippocampus on its own induced no significant effects in the tail suspension test, forced-swim test, or chronic social defeat paradigm. $n = 8–15$ per group. Data are expressed as mean ± SEM.