Supplementary Materials

Induction of neuronal VEGF expression by cAMP in the dentate gyrus of the hippocampus is required for antidepressant-like behaviors

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Supplementary Table 1. Primer sequences used.
**Suppl Figure 1. Ap oa\textsubscript{1} transgenic mice exhibit decreased immobility in the FST in response to acute octopamine.** Immobility time was measured at two time points after acute octopamine treatment. (a) At 30 min, immobility time was decreased in Ap oa\textsubscript{1} transgenic mice treated once with octopamine (1 mg/kg). Significant effects of genotype are seen (Ap oa\textsubscript{1}, 139.0 ± 15.62 s versus WT mice, 187.66 ± 12.29 s; **p < 0.01, one-way ANOVA; n = 6-9 animals per group). (b) When immobility time was measured after 2 weeks (i.p. injection 2 weeks before test), there was no significant difference between Ap oa\textsubscript{1} transgenics (167.66 ± 21.35 s) and WT mice given octopamine (168.81 ± 21.80 s) or vehicle (166.21 ± 19.43 s) (**p > 0.05; n = 6 animals per group), indicating that the effect of acute Ap oa\textsubscript{1} activation on immobility time in the FST does not last long.

**Suppl Figure 2. The number of BrdU\textsuperscript{+} cells is higher in the hippocampi of Ap oa\textsubscript{1} mice than in those of their littermate controls.** (a) Representative immunostaining for BrdU (red) in littermate wild-type, tet-O-Ap oa\textsubscript{1}, tTA and Ap oa\textsubscript{1} mice. (b) Chronic octopamine (1 mg/kg) treatment increased the number of BrdU\textsuperscript{+} cells in Ap oa\textsubscript{1} mice compared to vehicle. Octopamine at 2 mg/kg was not more effective than at 1 mg/kg. There were no differences in the number of BrdU\textsuperscript{+} cells between Ap oa\textsubscript{1} transgenic mice given vehicle and littermate controls given octopamine (1 mg/kg) or vehicle. Data are numbers of BrdU\textsuperscript{+} cells in the
hippocampus, expressed as means ± s.e.m. Ap oa₁ mice given octopamine, 2611 ± 323 versus single transgenics given octopamine, 1007 ± 190 or vehicle, 901 ± 49 (two-way ANOVA, $F_{(2,22)} = 6.882$: Tukey’s multiple comparison, *** $p < 0.001$; $n=10-13$ animals per genotype).

**Suppl Figure 3. Increased survival of BrdU⁺ cells in Ap oa₁ transgenic mice.**

(a) Experimental design for long-term survival studies.

(b) Octopamine (1 mg/kg) increased survival of BrdU⁺ cells in Ap oa₁ mice. (Top) BrdU (red) and DAPI (blue) immunoreactivities. (Bottom) Quantification of BrdU⁺ cell in the DG. Ap oa₁ mice given octopamine, 1275 ± 66 versus WT, 666 ± 88; versus Ap oa₁ mice given vehicle, 726 ± 109 (two-way ANOVA, $F_{(2,20)} = 5.155$: Tukey’s multiple comparison, ** $p < 0.01$; $n=7-12$ animals per genotype).

**Suppl Figure 4. Conditional activation of Ap oa₁ expression in mouse forebrain neurons.**

RT-PCR reveals expression of the Ap oa₁ transgene (315 bp, arrow) in the hippocampus, cortex, but not cerebellum, of Ap oa₁ transgenic mice. The transgene was not seen in any of these brain regions in inbred WT, tTA single, tet-O-Ap oa₁ single mutant littermates, indicating that the amplified band originates from Ap oa₁ messenger RNA specifically present in the Ap oa₁ transgenic mice. The lower panel shows the RT-PCR product corresponding to the GAPDH
control in these regions. Lane 1, cerebellum; lane 2, hippocampus; lane 3, cortex.

**Suppl Figure 5. Acute octopamine treatment increases pCREB, but not VEGF, levels in the hippocampi of Ap oα1 mice.**

(a) pCREB immunostaining in the hippocampus 2 h after a single injection of octopamine or vehicle (n = 3 animals per group).

(b) Densitometric analyses of DG granule cells (arrows). Significant effects are seen for genotype (Ap oα1 versus single transgenic controls) [OD (percentage of control): 100 ± 3.12 % in controls versus 223.43 ± 8.96 % in Ap oα1, ***p < 0.001, Student’s t test; n = 4 animals per group]. Data are shown relative to the level in single transgenic mice given octopamine.

(c) Quantitative immunoblot analysis of pCREB in whole hippocampi. (Top) Representative immunoblots. (Bottom) Densitometric analyses of total immunoreactivity. Data are normalized to total CREB in each group and are shown relative to the level in single transgenic control mice given octopamine [OD (percentage of control): 100 ± 2.11 % in controls versus 105.64 ± 5.63 % in Ap oα1, p > 0.05, Student’s t test; n = 4 per group].

(d) Specificity of VEGF antibody. Immunoreactivity of VEGF was detected only in the presence of VEGF antibody when tested in the brain tissues of Ap oα1 mice.

(e) VEGF immunostaining in the hippocampus 24 h after a single injection of octopamine or
vehicle. (f) Densitometric analyses in the DG (arrows). Significant effects of genotype are not seen after acute octopamine treatment [OD (percentage of control): 100 ± 6.20 % in single transgenic controls versus 103.33 ± 10.24 % in Ap oa,  p > 0.05, Student’s t test; n = 5 per group]. (Top) Representative immunoblots. (Bottom) Densitometric analyses of total immunoreactivity. Data are normalized to control mice.

(g) VEGF protein expression shown by western blotting. Significant effects of genotype are not seen after acute octopamine [OD (percentage of control): 100 ± 5.45 % in single transgenic controls versus 102.11 ± 28.74 % in Ap oa,  p > 0.05, Student’s t test]. Data are normalized to β-actin in each group and are shown relative to the level in control mice.

Suppl Figure 6. BDNF expression is increased in Ap oa, mice.

(a) BDNF levels in the DG of Ap oa mice and WT littermates receiving acute or chronic octopamine treatment.

(b) Densitometric analyses of DG granule cells in (a). Significant effects on genotype are seen after chronic octopamine treatment (two-way ANOVA,  F(3,17) = 18.00; ***p < 0.001; Tukey’s post hoc; n = 6-9 animals per group). Data are normalized to WT mice given octopamine.

(c) Quantitative immunoblot analysis of BDNF in whole hippocampi. (Top) Densitometric analyses of total immunoreactivity. Data are normalized to total β-actin in each group, and are
shown relative to the level in WT mice given octopamine (two-way ANOVA, $F_{(3,14)} = 21.82$; ***$p < 0.001$; Tukey’s post hoc; $n = 5-7$ animals per group). (Bottom) Representative immunoblots.

(d) BDNF mRNA expression by quantitative RT-PCR. Significant effects on treatment are seen after chronic octopamine (Ap oao_1 versus WT littermates, two-way ANOVA, $F_{(3,14)} = 20.23$; ***$p < 0.001$; Tukey’s post hoc; $n = 5-7$ animals per group). Data are normalized to GAPDH in each group and are shown relative to the level in WT mice.

Suppl Figure 7. Knocking down VEGF does not have effects on BDNF expression in Ap oao_1 mice.

(a) BDNF immunostaining in the DG. Lenti-shVEGF did not reduce BDNF protein level in the DG of Ap oao_1 transgenic mice

(b) BDNF mRNA expression by RT-PCR. (Top) Representative RT-PCR. (Bottom) Densitometric analyses. Lenti-shVEGF did not reduce BDNF mRNA level in the whole hippocampus of Ap oao_1 transgenic mice ($p > 0.05$ versus lenti-EGFP; ###$p < 0.001$ versus WT controls treated with lenti-EGFP or lenti-shVEGF, two-way ANOVA, Tukey’s post hoc; $n = 5$ animals per group) and WT mice ($p > 0.05$, lenti-EGFP versus lenti-shVEGF in WT mice, two-way ANOVA, Tukey’s post hoc, $n = 5$ animals per group). Data are normalized to GAPDH in
each group and are shown relative to the level in WT mice given lenti-EGFP.

**Suppl Figure 8. Effects of VEGF knockdown on the proliferation and survival of neural progenitor cells in the SGZ of DG in Ap oa₁ mice.**

(a, b) Lenti-shVEGF decreased the number of Ki67⁺ and BrdU⁺ cells compared to lenti-EGFP. Ki67⁺ (green), BrdU⁺ (red) and DAPI (blue) immunoreactivities.

(c) Quantification of Ki67⁺ and BrdU⁺ cells (**p < 0.01, *p < 0.05, Student’s t test; n = 5-7 animals per group).

**Suppl Figure 9. The distribution of BrdU immunoreactivity throughout the DG in the hippocampi of Ap oa₁ mice injected with lentivirus.**

(a) Representative immunostaining for BrdU (red) and DAPI (blue) in Ap oa₁ mice that were injected with lenti-EGFP and lenti-shVEGF.

(c) BrdU immunoreactivities shown in (b). The designated area is DG. Brain sections shown in (a) and (b) were selected from tissues at 180 µm intervals obtained by coronal section and are displayed from the anterior to posterior portion of the brain.

**Suppl Figure 10. Levels of anxiety are normal in Ap oa₁ transgenic mice after chronic**
octopamine treatment.

(a) Open field test. The total distance moved in a box was measured for WT and Ap \( \text{oa}_1 \) transgenic mice treated with lent-EGFP or lenti-shVEGF. All groups moved similar distances in the box (WT: \( 3819 \pm 89 \) cm in lenti-EGFP treated, \( 3921 \pm 345 \) cm in lenti-shVEGF treated; Ap \( \text{oa}_1 \): \( 3808 \pm 419 \) cm in lenti-EGFP treated, \( 3922 \pm 407 \) cm in lenti-shVEGF treated; \( p > 0.05 \); two-way ANOVA; Tukey’s multiple comparison; \( n = 6 \) animals per group).

(b) Elevated arm maze test. The time spent in the open arm, expressed as a percent of the total time spent in the open and closed arms, was measured for WT and Ap \( \text{oa}_1 \) transgenic mice treated with lent-EGFP or lenti-shVEGF. All four groups spent similar amounts of time in the open arm (WT: \( 22.94 \pm 11.00 \) % in lenti-EGFP treated, \( 21.30 \pm 7.39 \) % in lenti-shVEGF treated; Ap \( \text{oa}_1 \): \( 35.63 \pm 19.51 \) % in lenti-EGFP treated, \( 32.77 \pm 7.04 \) % in lenti-shVEGF treated; \( p > 0.05 \); two-way ANOVA; Tukey’s multiple comparison; \( n = 6 \) animals per group).

All data are presented as mean \( \pm \) s.e.m.

Suppl Figure 11. Fluoxetine-induced proliferation of neural progenitor cells.

(a) Experimental design. (b) VEGF protein levels in the hippocampus following fluoxetine (10 mg/kg) treatment for 21 days. Western blots of two representative mice showing that fluoxetine increased total VEGF protein in the hippocampus. (c) Increased number of BrdU\(^+\)
cells in mice treated with fluoxetine (3598 ± 233) compared to vehicle control (2637 ± 91) (p < 0.05, one way-ANOVA; n = 6 animals per group). BrdU (red) and DAPI (blue) immunoreactivities. Data are number of BrdU⁺ cells in the hippocampus, expressed as means ± s.e.m.

Suppl Figure 12. CaMKIIα expression in BrdU⁺ cells in Ap oa₁ mice.

(a) Experimental designs.

(b) Newborn BrdU⁺ cells did not express CaMKIIα 3 days after birth, as determined by double IHC with antibodies against CaMKIIα and BrdU.

Suppl Figure 13. HIF-1α expression at the mRNA and protein levels in Ap oa₁ mice.

(a) Quantitative RT-PCR. Hippocampal HIF1α mRNA expression in WT and Ap oa₁ mice subjected to acute (100 ± 1.12 % versus 110.34 ± 23.54 %, p > 0.05, Two-way ANOVA; n = 3 animals per group) or chronic (100 ± 2.47 % versus 102.78 ± 13.45 %, p > 0.05, two-way ANOVA; n = 4 animals per group) octopamine treatment. Data are normalized to the amount of GAPDH in each group and are shown relative to the level in WT mice given octopamine. (b) Quantitative immunoblot analysis of HIF1α in whole hippocampi. (Top) Representative immunoblots. (Bottom) Densitometric analyses of total immunoreactivity. Data are normalized
to total β-actin in each group, and are shown relative to the level in WT mice given octopamine (Acute: $100 \pm 1.07\%$ versus $117.39 \pm 11.37\%$, $p > 0.05$; Chronic: $102 \pm 3.45\%$ versus $103.34 \pm 9.21\%$, $p > 0.05$, two-way ANOVA; $n = 4$ animals per group).

**Supplementary Table 1: Primer sequences used**