# Figures and figure supplements

Translational control by eIF2$\alpha$ phosphorylation regulates vulnerability to the synaptic and behavioral effects of cocaine

**Wei Huang *et al***

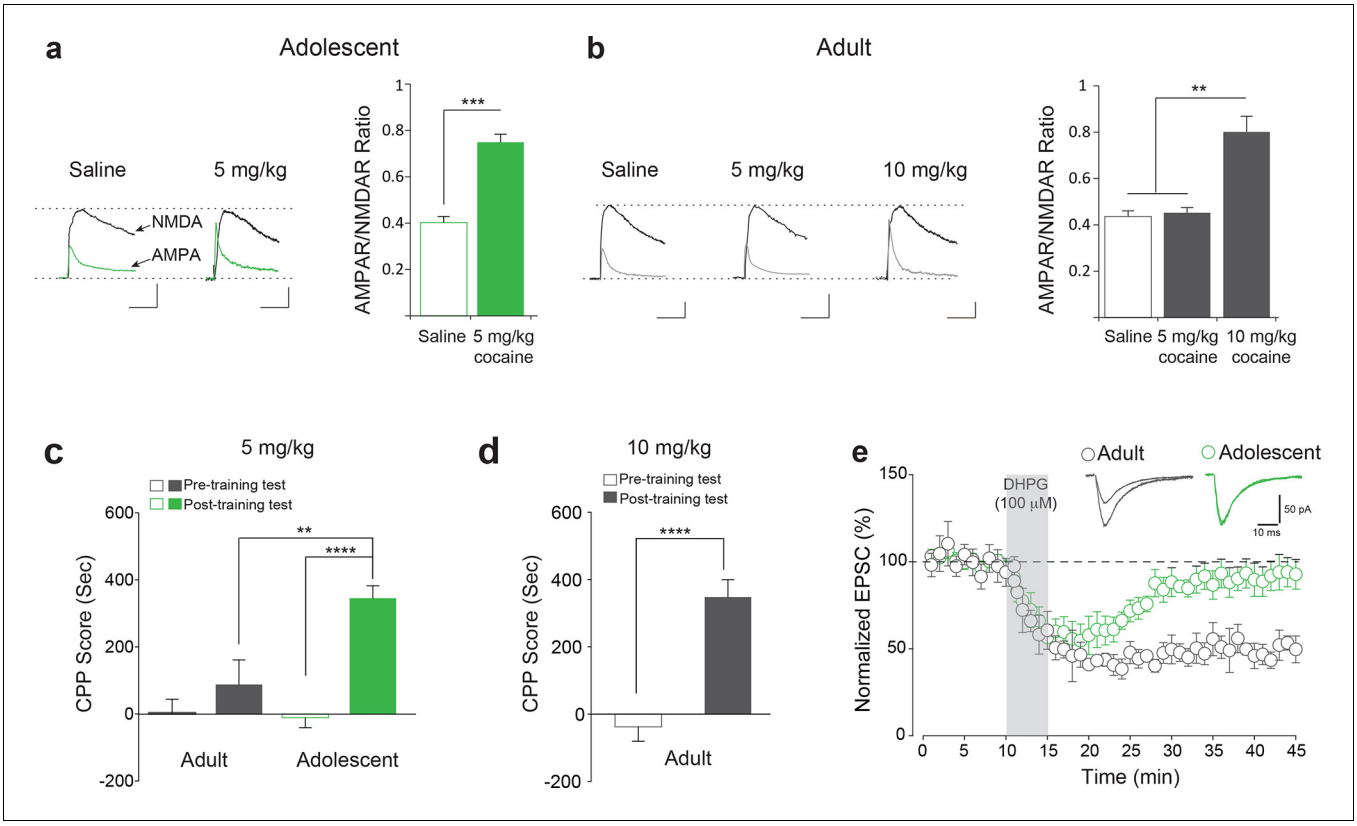


**Figure 1.** Enhanced susceptibility of adolescent mice to cocaine-induced synaptic potentiation and behavior. (**a–b**) Left, Representative traces of AMPAR and NMDAR EPSCs recorded in VTA DA neurons 24 hr after a single i.p. injection of saline or cocaine. A low dose of cocaine (5 mg/kg) induced LTP, as determined by the increase in the AMPAR/NMDAR ratio (**a**, Right, p<0.001, n=11/10 saline/cocaine, $t_{19}$=8.09) as well as CPP (**c**, p<0.0001, n=11, $t_{20}$=7.487) in adolescent mice (5 weeks old), but not in adult mice (3–5 months old, **b**, Right, p=0.951, n=8/9/7 saline/5 mg/kg cocaine/10 mg/kg cocaine, $F_{2,22}$=27.20; **c**, p=0.3289, n=9, $t_{16}$=1.007). A higher dose of cocaine (10 mg/kg) induced LTP in VTA DA neurons (b, Right, p<0.01 vs. saline or 5 mg/kg cocaine, n=8/9/7 saline/5 mg/kg cocaine/10 mg/kg cocaine, $F_{2,22}$=27.20) and CPP in adult mice (**d**, p<0.0001, n=15, $t_{28}$=5.750). (**e**) DHPG (100 μM, 5 min) evoked LTD in VTA DA neurons of adult mice (p<0.001, n=6, $t_{10}$=19.38), but not in adolescent mice (p=0.10, n=7, $t_{12}$=1.76).
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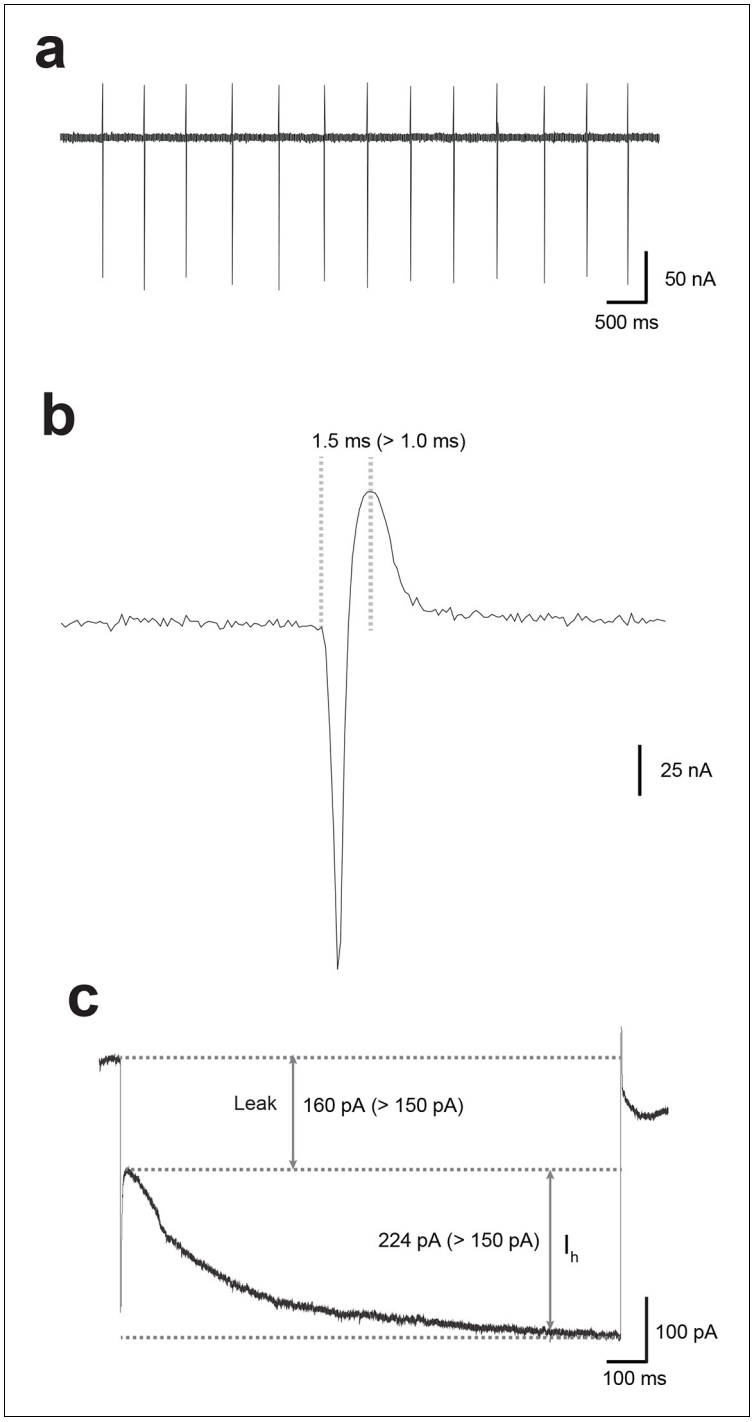


**Figure 1—figure supplement 1.** Identification of lateral VTA DA neurons in mouse midbrain slices. (**a**) Stable pacemaker firing at 1–5 Hz was recorded from neurons in the lateral VTA in cell-attached mode. (**b**) At Vh=-55 mV, spike width was measured from the start of the inward deflection to the outward peak. Cells with spike widths >1.0 ms were taken as dopaminergic. (**c**) Cells only in the ventrolateral VTA with a large (>150 pA) hyperpolarization-activated current (Ih), and a large (>150 pA) leak current were studied.
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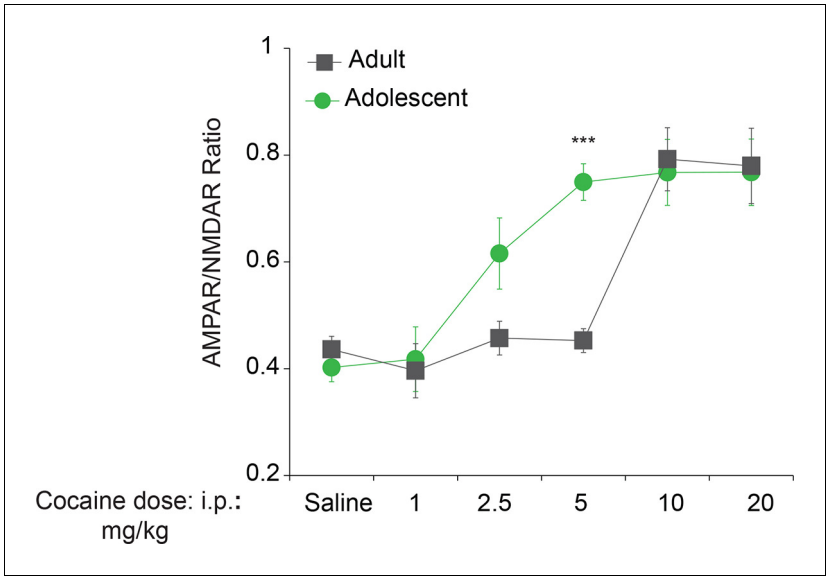


**Figure 1—figure supplement 2.** Adolescent mice are more susceptible than adult mice to cocaine-induced LTP in VTA DA neurons. Adolescent (5 weeks old, n=6-11 per group) or adult mice (3–5 months old, n=6-9 per group) were i.p-injected with saline or cocaine at indicated doses and whole-cell recording were performed in VTA DA neurons. LTP, manifested by an increase in AMPAR/NMDAR ratio, was induced at a lower dose of cocaine (5 mg/kg, $F_{5,77}=22.15$, $p<0.001$ vs. saline) in adolescent mice than in adults (10 mg/kg, $F_{5,77}=22.15$, $p<0.01$ vs. saline or 5 mg/kg cocaine).

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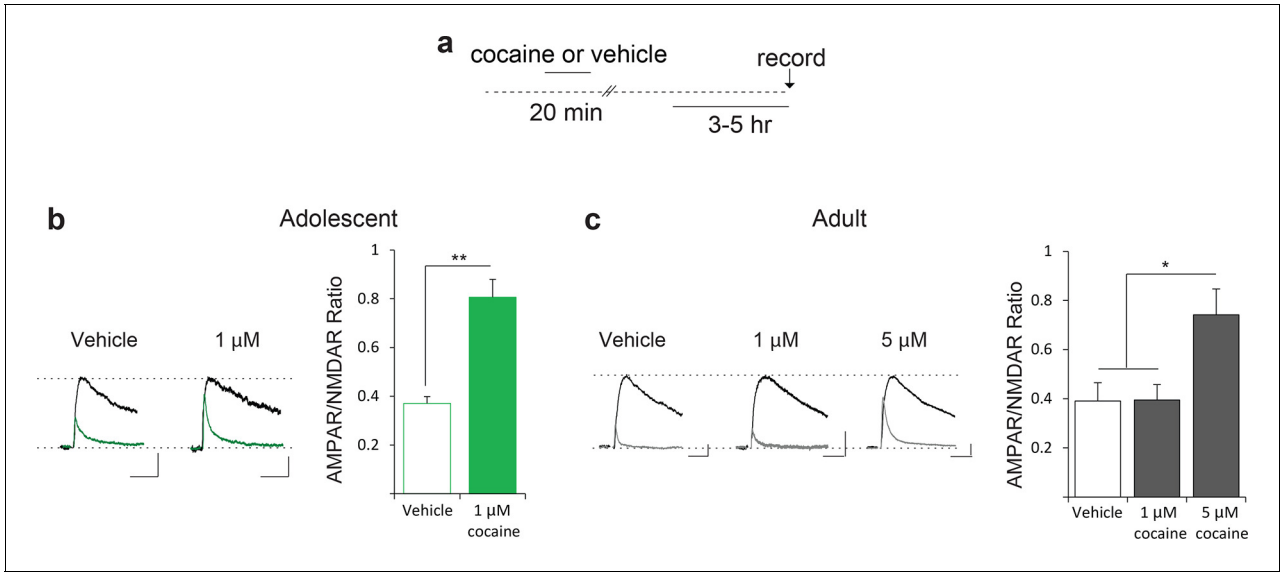


**Figure 1—figure supplement 3.** VTA slices from adolescent mice more susceptible to cocaine-induced LTP in vitro. (**a**) Scheme of experimental procedure (**b**) Direct application of a low concentration of cocaine (1 μM) increased AMPAR/NMDAR ratio 3–5 hr post-treatment in VTA DA neurons of adolescent mice, as compared to adult mice (n=5-11 per group, $F_{1,32}=6.56$, $p>0.01$ Eif2s1S/A vs. wild-type control).

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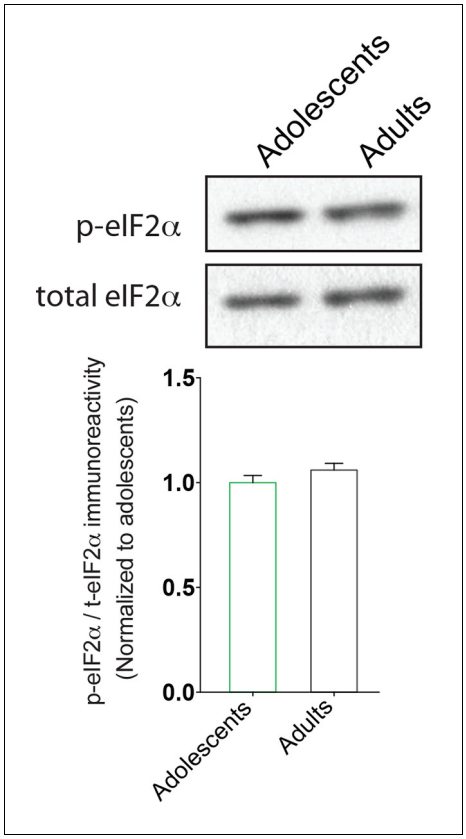


**Figure 1—figure supplement 4.** Basal p-eIF2$\alpha$ phosphorylation levels are similar in the VTA of adult and adolescent mice. Western blots are shown on top and quantification of eIF2$\alpha$ levels is shown below (n=4, p>0.05).

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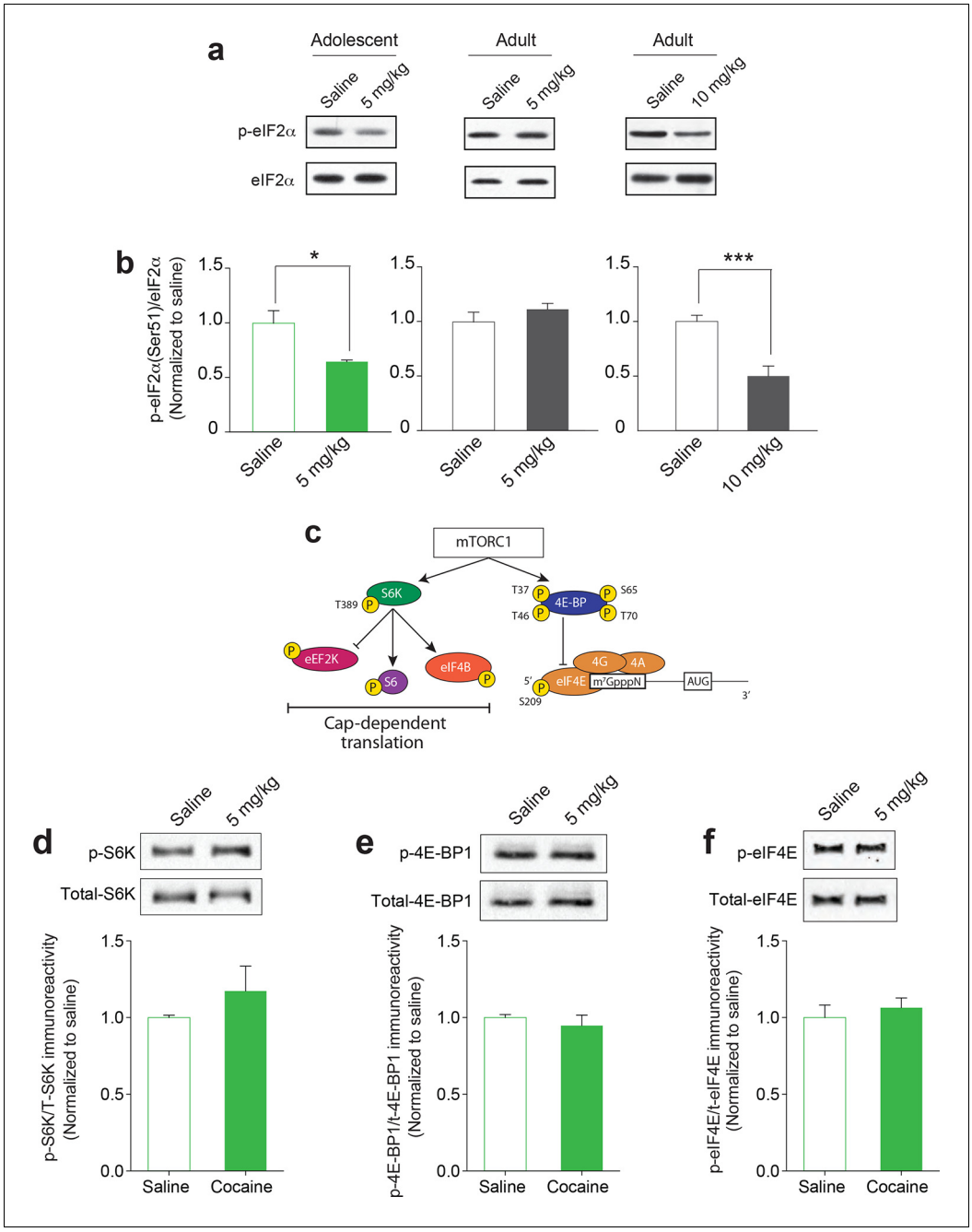


**Figure 2.** A low dose of cocaine selectively reduces p-eIF2$\alpha$ in the VTA of adolescent mice. (**a–b**) A low dose of cocaine (5 mg/kg) reduced p-eIF2α in the VTA of adolescent ($p<0.05$, n=5 per group, $t_8=3.029$), but not adult mice ($p=0.329$, n=3 per group, $t_4=1.110$). A higher dose of cocaine (10 mg/kg) was needed to reduce p-eIF2α in VTA of adult mice ($p<0.001$, n=6 per group, $t_{10}=4.640$). (**c**) Schematic of mTORC1- and eIF4E-mediated translation. In abilescnt mice, a low dose of cocaine (5 mg/kg) did not significantly alter phosphorylation of S6K at Thr-389 (**d**), 4E-BP1 at Thr-37 and Thr-46 (**e**) and eIF4E at Ser209 (**f**). Western blots are shown on top and quantification for each phospho-protein/total-protein is shown at the bottom (n=3/3 saline/cocaine; S6K, $p=0.3467$, $t_4=01.066a$; 4E-BP1, $p=0.5031$, $t_4=0.7351$; eIF4E, $p=0.5669$, $t_4=0.6233$). Plots are mean ± s.e.m.

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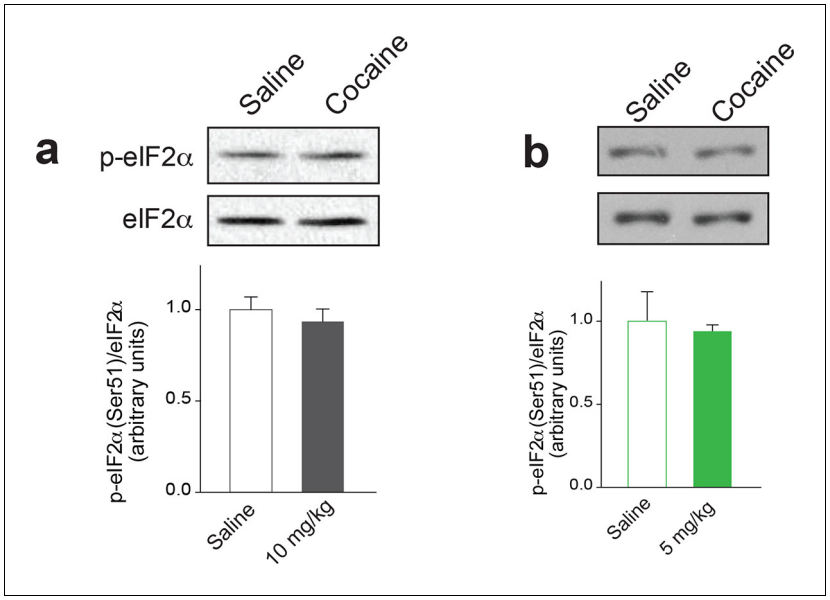


**Figure 2—figure supplement 1.** Doses of cocaine which lower p-eIF2$\alpha$ in the VTA have no effect in nucleus accumbens (NAc). (**a**) Scheme of the experimental procedure (**b**) A low dose of cocaine (5mg/kg) or a higher dose of cocaine (10 mg/kg) had no effect on p-eIF2 in the NAc of adolescent (p=0.678, n=3 per group, t4=0.4) or adult mice (p=0.18, n=3 per group, t4=1.6), respectively. Plots are mean ± s.e.m.

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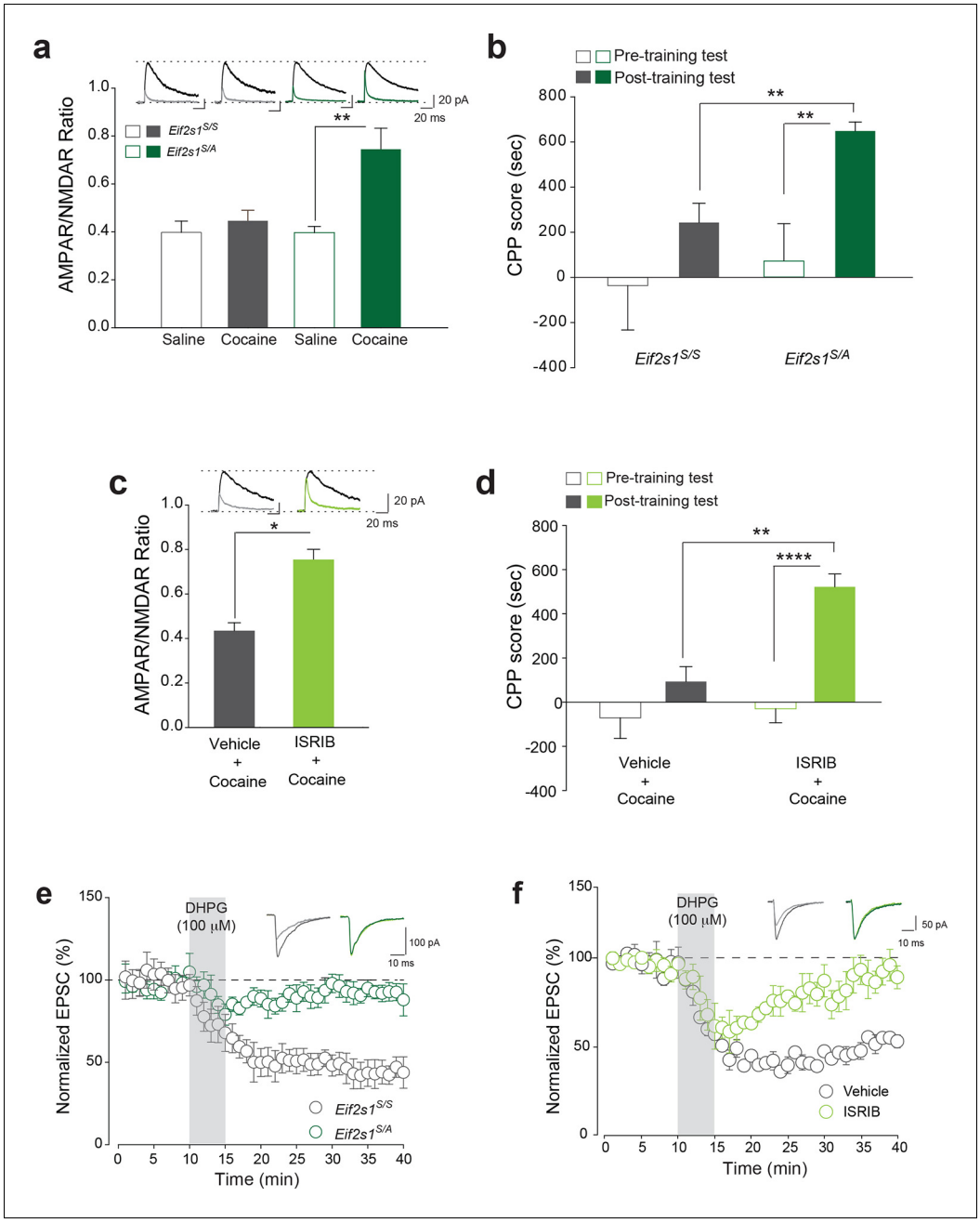


**Figure 3.** Decreasing p-eIF2$\alpha$ makes adult mice more susceptible to cocaine-induced LTP and behavior. (**a**–**b**) A low dose of cocaine (5 mg/kg) induced both LTP in VTA DA neurons (a, p<0.05, n=5, $t_8$=4.193) and CPP in adult *Eif2s1*$^{S/A}$ mice (b, p<0.01, n=7, $t_{12}$=3.411) compared to *Eif2s1*$^{S/S}$ mice (a, p=0.89, n=5, $t_8$=0.14; b, p=0.2170, n=7, $t_{12}$=1.303). (**c**–**d**) A low dose of cocaine (5 mg/kg) elicited LTP (c, p<0.001, n=6, $t_{10}$=3.43) and CPP (d, p=0.1761, n=8 vehicle+cocaine, $t_{14}$=1.425; p<0.0001, n=16 ISRIB+cocaine, $t_{30}$=2.433) in ISRIB-injected adult mice compared to vehicle-injected mice. (**e**–**f**) DHPG (100 μM, 5 min) induced LTD in WT adult VTA DA neurons (e, p<0.001, n=5, $t_8$=20.3) and vehicle-injected WT adult mice (f, p<0.001, n=5, $t_8$=5.17), but not in *Eif2s1*$^{S/A}$ mice (e, p=0.26, n=7, $t_{12}$=1.2) and ISRIB-injected mice (f, p=0.42, n=4, $t_6$=0.86).

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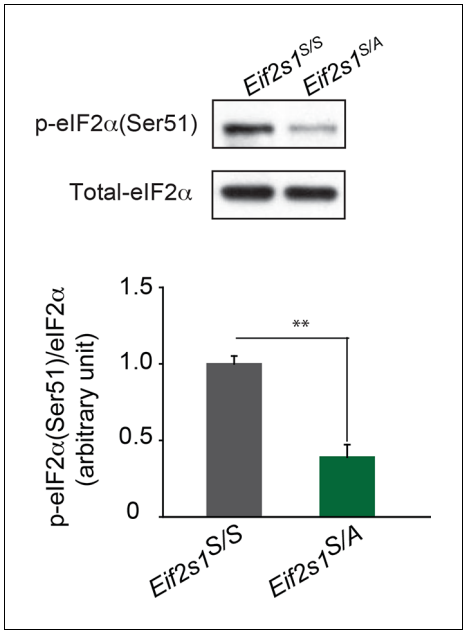


**Figure 3—figure supplement 1.** eIF2α phosphorylation is reduced in VTA from adult *Eif2s1*$^{S/A}$ mice. Western blots (top) show reduction in p-eIF2α in *Eif2s1*$^{S/A}$ mutant mice compared to wild-type littermates (*Eif2s1*$^{S/S}$). Quantification of eIF2α phosphorylation vs. total-eIF2α is shown below ($p<0.01$, $n=3$ per group, $t_4=6.67$).

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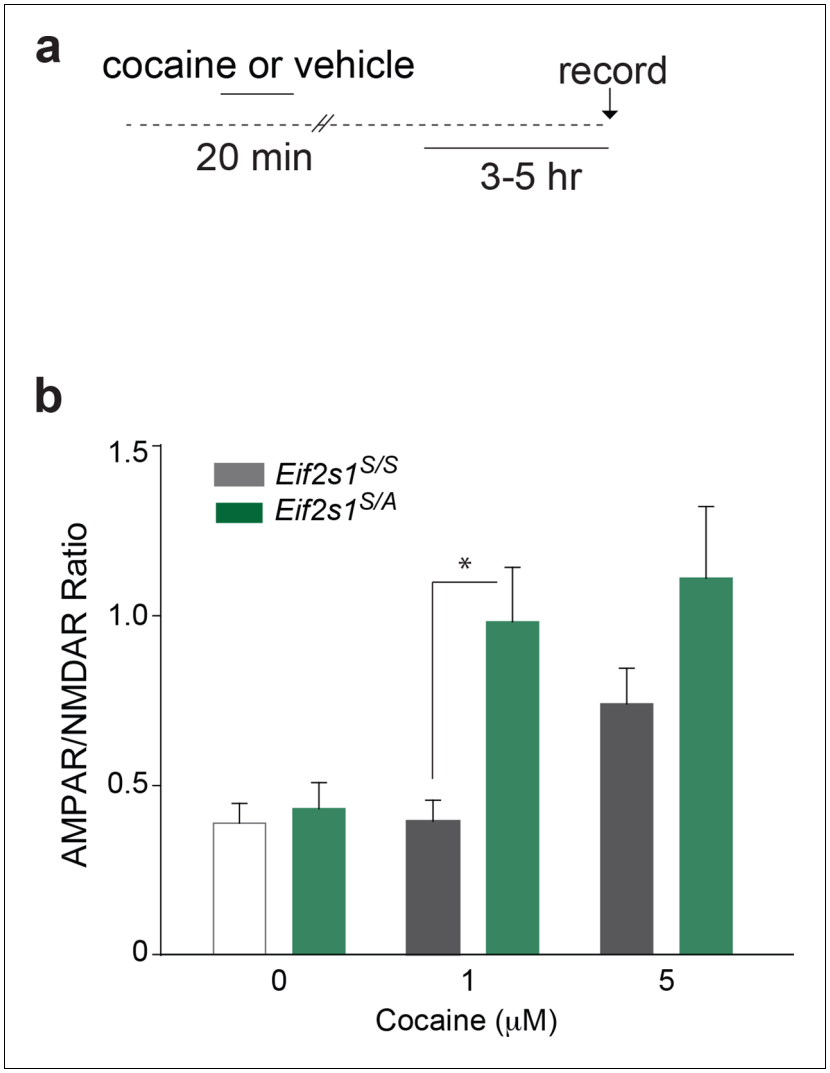


**Figure 3—figure supplement 2.** Decreasing p-eIF2$\alpha$ makes VTA slices from adult mice more susceptible to cocaine-induced LTP in vitro. Direct application of a low concentration of cocaine (1 μM) increased AMPAR/NMDAR ratio 3–5 hr post-treatment in VTA DA neurons of *Eif2s1*$^{S/A}$ mice, as compared to wild-type controls (n=5-11 per group, $F_{1,32}$=6.56, p<0.01 *Eif2s1*$^{S/A}$ vs. wild-type control).

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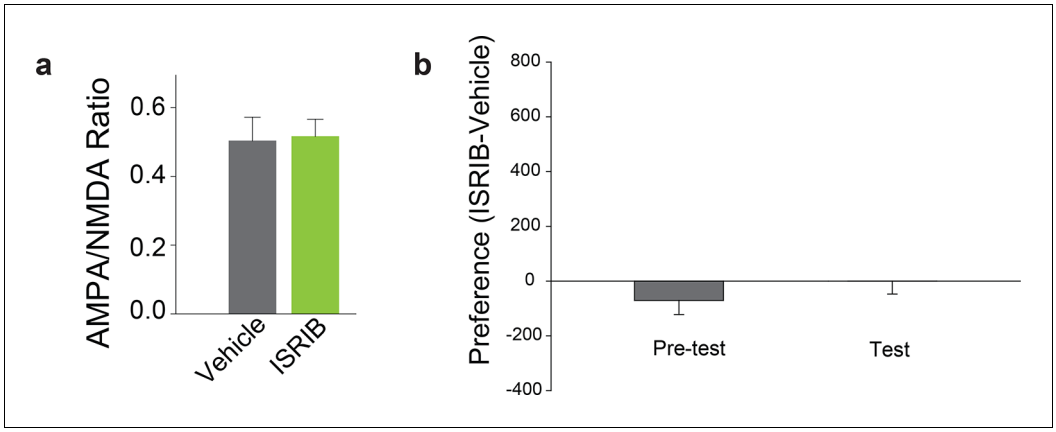


**Figure 3—figure supplement 3.** In adult mice, systemic administration of ISRIB alone failed to induce LTP in VTA DA neurons and CPP. **a**, **b**. i.p. injection of ISRIB (2.5 mg/kg) alone did not induce LTP (a, p=0.79, n=6/3 ISRIB/vehicle, t7=0.28) or CPP (b, p=0.329, n=9, t16=1.008), as indicated by the lack of potentiation of VTA DA neurons and difference between average pre- and post-test preference scores, respectively.
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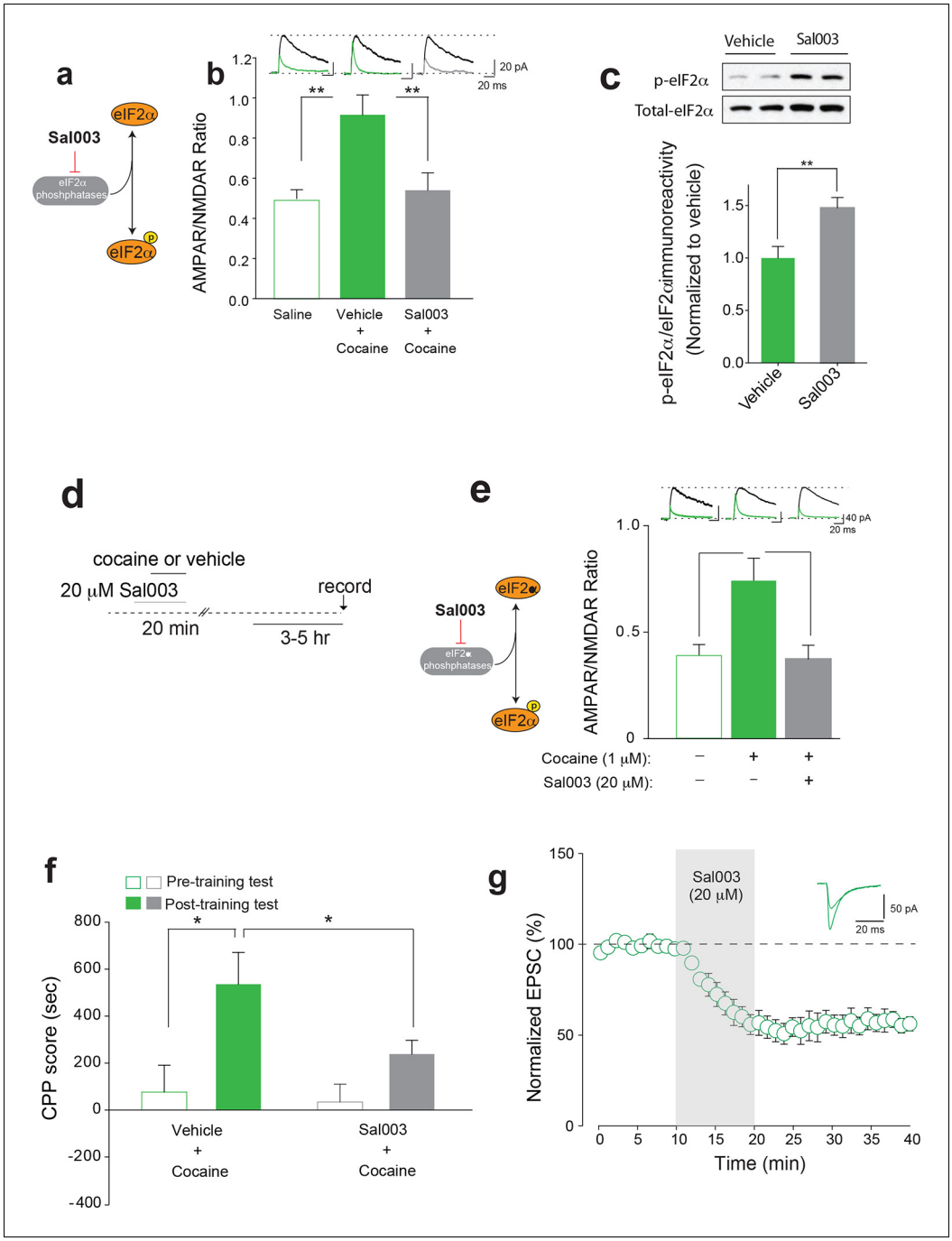


**Figure 4.** Increasing p-eIF2$\alpha$ in young mice blocks cocaine-induced LTP and behavior. (**a**) Schematic showing Sal003 mechanism of action. (**b–c**) Infusion of Sal003 into the VTA blocked cocaine-induced potentiation (c, p<0.001, n=5 per group, $t_8$=3.81) and increased p-eIF2α in the VTA (p<0.01, n=7/6 vehicle/Sal003, $t_{11}$=3.172). (**d**) Schematic of experimental design. (**e**) Direct application of cocaine (1 μM) induced LTP 3–5 hr post-treatment (p<0.05, n=11/6 vehicle/cocaine, $F_{2,20}$=7.48), whereas Sal003 prevented it (p<0.05, n=11/6, vehicle/cocaine+Sal003, $F_{2,20}$=7.48, cocaine vs. cocaine+Sal003). Representative traces of AMPAR and NMDAR EPSCs (top). (**f**) Infusion of Sal003 into the VTA blocked CPP (p<0.05, n=7 vehicle+cocaine, $t_{12}$=2.592; p=0.1147, n=10 Sal003+cocaine, $t_{18}$=1.892) in adolescent mice. (**g**) Application of Sal003 (20 μM, 10 min), a selective inhibitor of eIF2α phosphatases, induced LTD in VTA DA neurons from adolescent mice (p<0.001, n=6, $t_{10}$=9.517). Plots are mean ± s.e.m.

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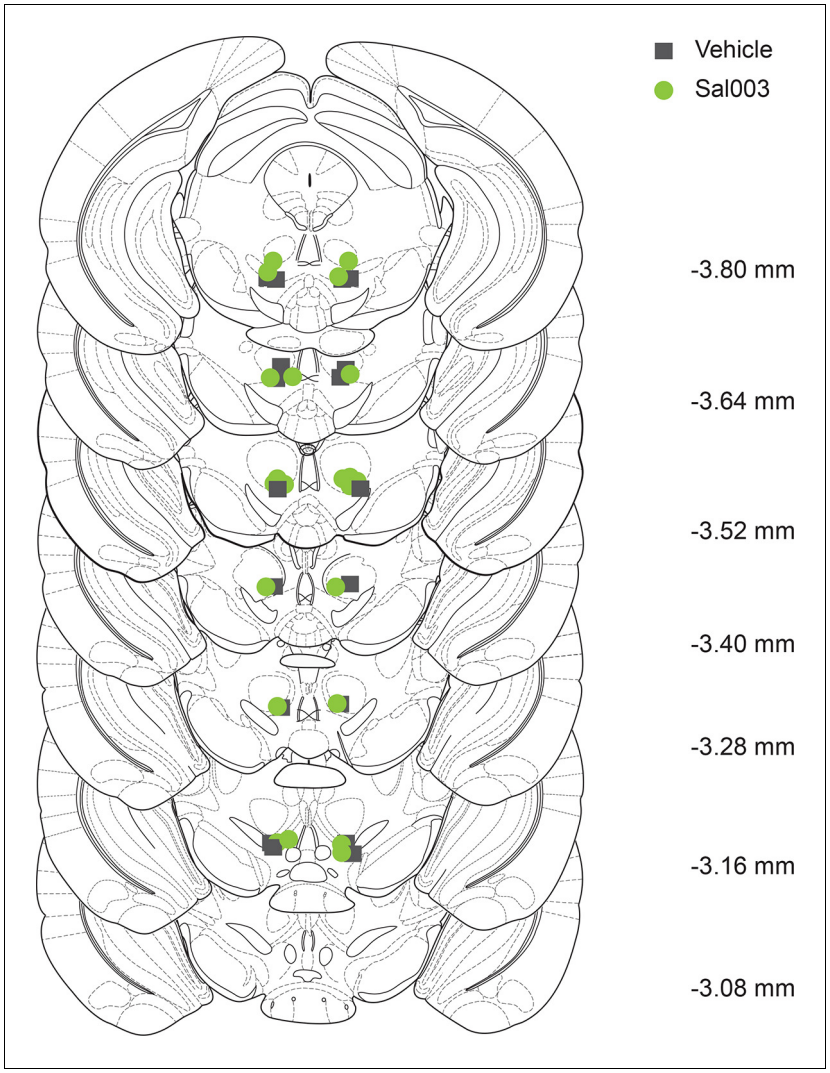


**Figure 4—figure supplement 1.** Sites of Sal003 infusions into VTA at seven rostrocaudal planes and corresponding increase in p-eIF2$\alpha$. Coordinates are posterior to bregma and cannula tip placements are from mice infused with Sal003 (1 µl; 20 µM) and vehicle (1 µl).
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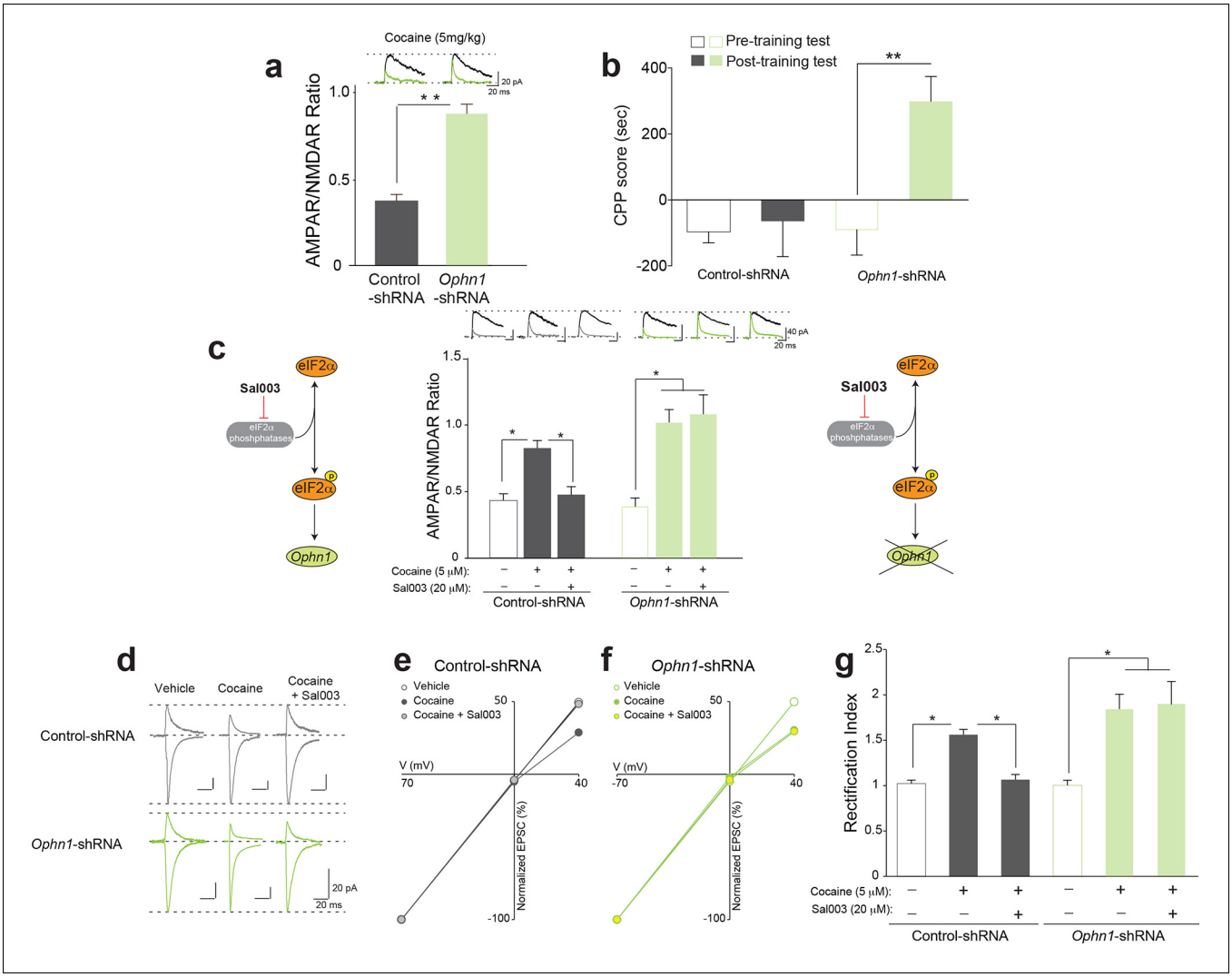


**Figure 5.** Decreasing OPHN1 levels in VTA DA neurons makes adult mice more susceptible to cocaine-induced LTP. (**a**) A low dose of cocaine (5 mg/kg) induced LTP in adult *Ophn1*-shRNA injected VTA DA neurons (a, Right, p<0.01, n=5, $t_8$=5.464); above representative traces of AMPAR and NMDAR EPSCs (top). (**b**) Low doses of cocaine (5 mg/kg) induced CPP in mice locally injected with *Ophn1*-shRNA (p<0.01, n=14, $t_{26}$=3.600), but not in control mice injected with scrambled shRNA (p=0.7829, n=4, $t_6$=0.2882). (**c**) Sal003 (20 μM) blocked the cocaine-induced LTP in the VTA of control shRNA-injected mice (p<0.01, n=6/6/7 vehicle/cocaine/cocaine+Sal003, $F_{2,16}$=13.03), but failed to do so in *Ophn1*-shRNA VTA DA neurons (p=0.29, n=6/6/11, vehicle/cocaine/cocaine+Sal003, $F_{2,20}$=4.29, cocaine vs. cocaine+Sal003; p<0.05 vehicle vs. cocaine or cocaine+Sal003). (**d**) Representative sample traces of AMPAR EPSCs. (**e–f**) *I-V* plots. (**g**) Cocaine increased the rectification index in control-shRNA injected VTA neurons while Sal003 blocked it (p<0.001, n=6/6/7 vehicle/cocaine/cocaine+Sal003, $F_{2,16}$=30.30, cocaine vs. vehicle or cocaine vs. cocaine+Sal003), whereas both cocaine and cocaine +Sal003 increased the rectification index in VTA DA neurons from *Ophn1*-shRNA-injected mice (p<0.05, n=6/6/11 vehicle/cocaine/cocaine+Sal003, $F_{2,20}$=3.92, vehicle vs. cocaine or cocaine+Sal003; p=0.80 cocaine vs. cocaine+Sal003). Plots are mean ± s.e.m.

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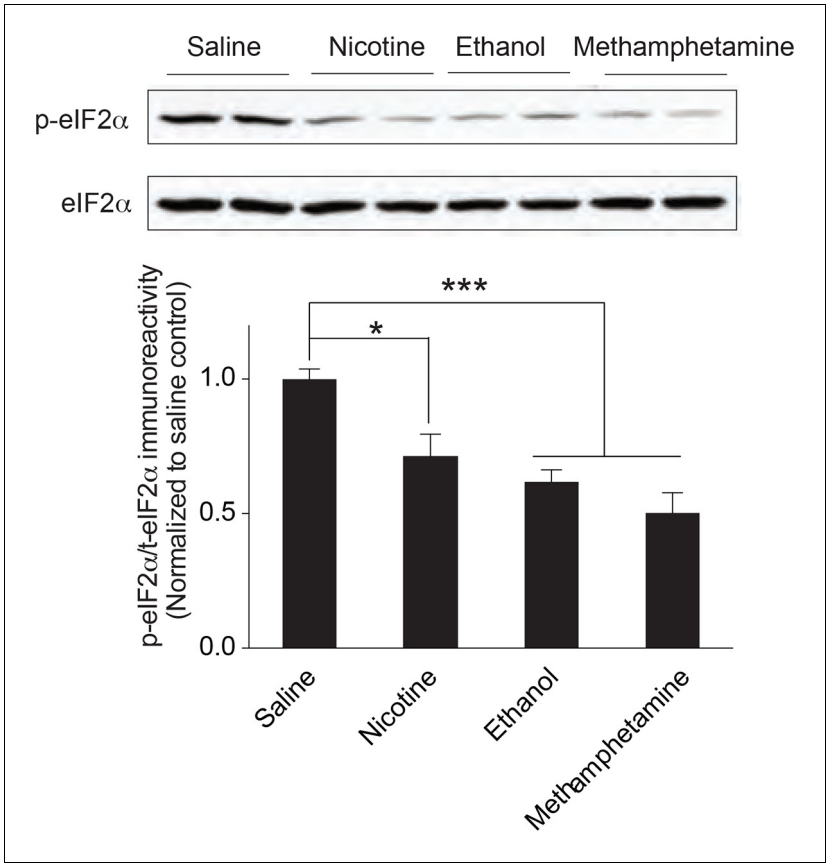


**Figure 6.** Multiple drugs of abuse reduce p-eIF2$\alpha$ in VTA of adult mice. (**a**) i.p. injection of nicotine (1 mg/kg), ethanol (2 g/kg), or methamphetamine (1 mg/kg) reduces p-eIF2$\alpha$ in VTA (n=5 per group; Saline vs. nicotine, $p<0.05$, $t_8=2.879$; ethanol, $p<0.001$, $t_8=6.278$ methamphetamine, $p<0.001$, $t_8=5.449$).
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