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SHORT REPORT

Figures and figure supplements

Translational control of nicotine-evoked synaptic potentiation in mice and neuronal responses in human smokers by eIF2α

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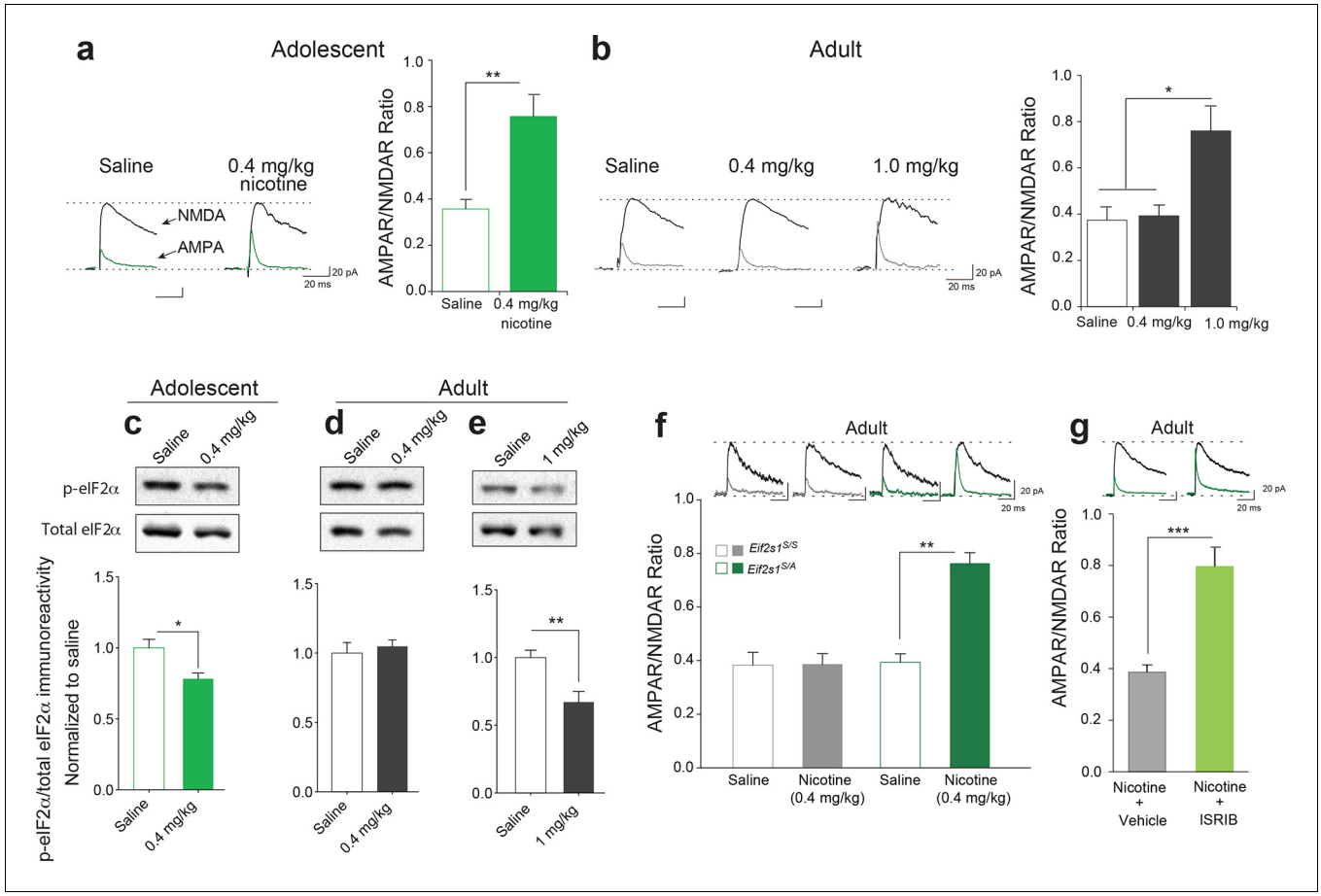

Figure 1. Reduced p-eIF2α-mediated translational control increases the susceptibility to nicotine-induced LTP. (**a-b**) Left, Representative traces of AMPAR and NMDAR EPSCs recorded from VTA DA neurons 24 hr after i.p. injection of saline or the indicated dose of nicotine. A relatively low dose of nicotine (0.4 mg/kg) induced LTP, shown by an increase in AMPAR/NMDAR ratio in VTA DA neurons (**a**, Right, $P<0.01$, $n=6,6$ saline/0.4 mg/kg nicotine, $t_{10}=4.026$) from adolescent mice (5 weeks old), but not in those from adult mice (3–5 months old, b, Right, $P=0.802$, $n=6/7/6$ saline/0.4 mg/kg nicotine/1.0 mg/kg nicotine, $F_{2,16}=9.029$). A higher dose of nicotine (1.0 mg/kg) was required to increase the AMPAR/NMDAR ratio in VTA DA neurons from adult mice (**b**, Right, $P<0.05$ vs. saline or 1.0 mg/kg nicotine, $n=6/7/6$ saline/0.4 mg/kg nicotine/1.0 mg/kg nicotine, $F_{2,16}=9.029$). (**c-d**) A low dose of nicotine (0.4 mg/kg) reduced p-eIF2α in the VTA of adolescents (**c,** $P<0.05$, $n=9/5$ saline/0.4 mg/kg nicotine, $t_{12}=2.479$), but not adult mice (**d,** $P=0.5710$, $n=7/11$ saline/0.4 mg/kg nicotine, $t_{16}=0.5784$). (**e**) A higher dose of nicotine (1 mg/kg) was required to reduce p-eIF2α in VTA of adult mice ($P<0.01$, $n=11/5$ saline/1 mg/kg nicotine, $t_{14}=3.428$). (**f**) A low dose of nicotine (0.4 mg/kg) failed to induce LTP in VTA DA neurons from adult WT (*Eif2s1*$^{S/S}$) mice (Left, $P=0.964$, $n=5$ per group, $t_8=0.05$), but elicited significant LTP in adult *Eif2s1*$^{S/A}$ mice (Right, $P=0.003$, $n=5$ per group, $t_8=6.73$). (**g**) A low dose of nicotine (0.4 mg/kg) induced LTP in ISRIB-injected adult mice compared to vehicle-injected mice ($P<0.001$, $n=7/7$ nicotine+vehicle/nicotine+ISRIB, $t_{12}=5.222$).

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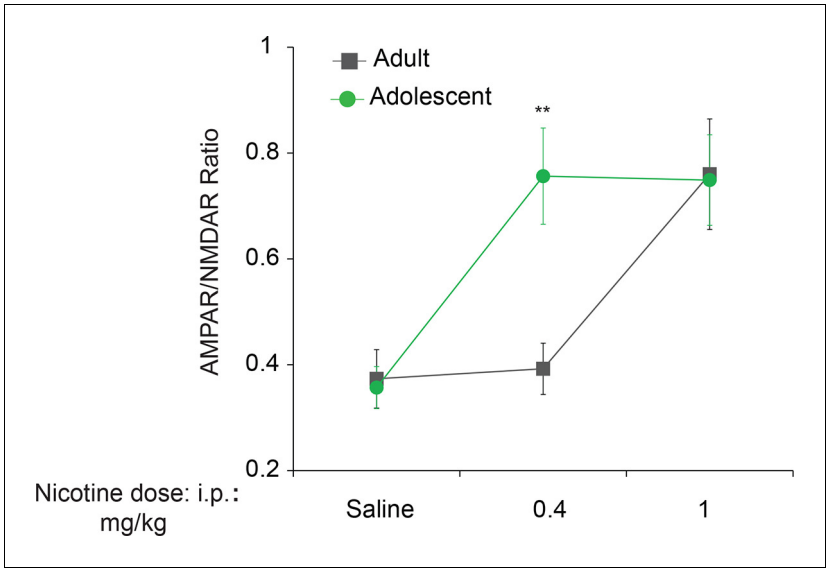

Figure 1—figure supplement 1. Adolescent mice are more susceptible than adult mice to nicotine-induced synaptic potentiation. Adolescent (5 weeks old, n=6–7 per group) or adult mice (3–5 months old, n=6–7 per group) were i.p-injected with saline or nicotine at indicated doses and whole-cell recordings were performed in VTA DA neurons. An increase in the AMPAR/NMDAR ratio (an index of LTP) was induced with the 0.4 mg/kg dose of nicotine ($F_{2,32}$=4.34, P<0.01 vs. saline) in adolescent mice, whereas 1.0 mg/kg was required for a significant increase in adults ($F_{2,32}$=4.34, P<0.05 vs. saline or 0.4 mg/kg nicotine).

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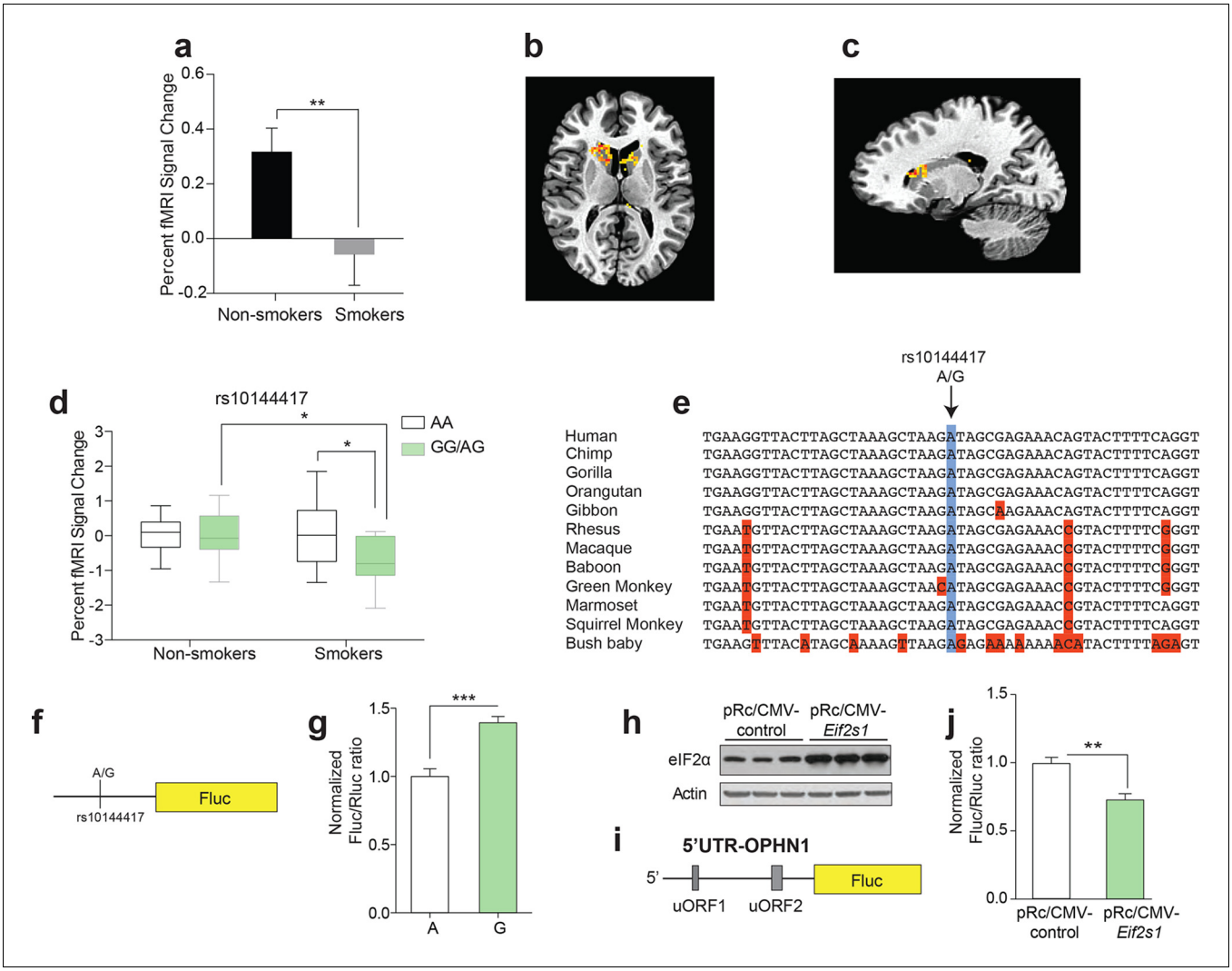

Figure 2. The effect of a single nucleotide polymorphism (SNP) in the promoter of the *Eif2s1* gene on reward-dependent striatal activity in human tobacco smokers. (**a-c**) Reward-related activity in caudate/putamen is lower in smokers than non-smokers (**b,** $P<0.01$, n=33/55, $t_{86}=2.678$). (**b-c**) Transverse (**b**) and sagittal (**c**) views of significant fMRI BOLD signal in caudate/putamen of non-smokers compared to smokers in response to juice reward. (**d**) Interaction between smoking and rs10144417 genotype ($P<0.05$, $F_{1,86}=5.836$). (**e**) Partial alignment of *Eif2s1* promoter sequences in human and related animals. Note high level of nucleotide conservation: the rs10144417 SNP is indicated in blue and the non-conserved nucleotides in red. (**f**) Schematic of firefly luciferase reporter constructs, in which a 5-kb *Eif2s1* promoter fragment containing either the A or G allele was cloned upstream of the firefly luciferase gene in the p15Amp reporter vector. A renilla luciferase reporter was co-transfected with reporters containing the A or G variant and firefly luciferase (Fluc) activity was normalized to renilla (Rluc) activity. (**g**) Effects of A and G variants of SNP rs10144417 on the transcriptional activity of the *Eif2s1* promoter as assessed by a luciferase reporter assay in HEK-293 cells. The data are from three independent experiments ($P<0.001$, $n=6$ per group, $t_{10}=5.405$). (**h**) Western blotting showing that overexpression of *Eif2s1* (pRc/CMV-*Eif2s1*) increased eIF2α levels compared to control (vector alone pRc/CMV). (**i**) Diagram of the 5′ UTR-*Ophn1*-Fluc reporter, which consists of the 5′UTR of *Ophn1* mRNA fused to the coding region of Firefly luciferase (Fluc). A renilla luciferase (Rluc) reporter vector was co-transfected into HEK293T as a transfection control. (**j**) Overexpression of *Eif2s1* reduced expression of 5′ UTR-*Ophn1*-Fluc ($P<0.01$, n=6, $t_{10}=3.9425$).

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a

	Gender	AA			GG		
		N	Age	StDev	N	Age	StDev
Control	Female	16	32.56	13.58	14	27.21	9.47
	Male	14	32.64	10.02	10	33.50	13.17
Sated	Female	8	44.13	10.25	0	-	-
	Male	18	32.22	13.57	9	42.56	12.13

b

Ethnicity	N
African American	26
American Indian	1
Asian	5
Caucasian	37
Hispanic	17
Mixed (Caucasian/Hispanic)	1
Did not specify	2

Figure 2—figure supplement 1. Demographic information of human participants involved in fMRI studies. (**a**) Table showing the number of participants by gender, age, and smoking status carrying the A or the G variant in the *Eif2s1* gene. (**b**) Table showing the number of participants by their self-reported ethnicities The participants have no history of any other drug dependance.

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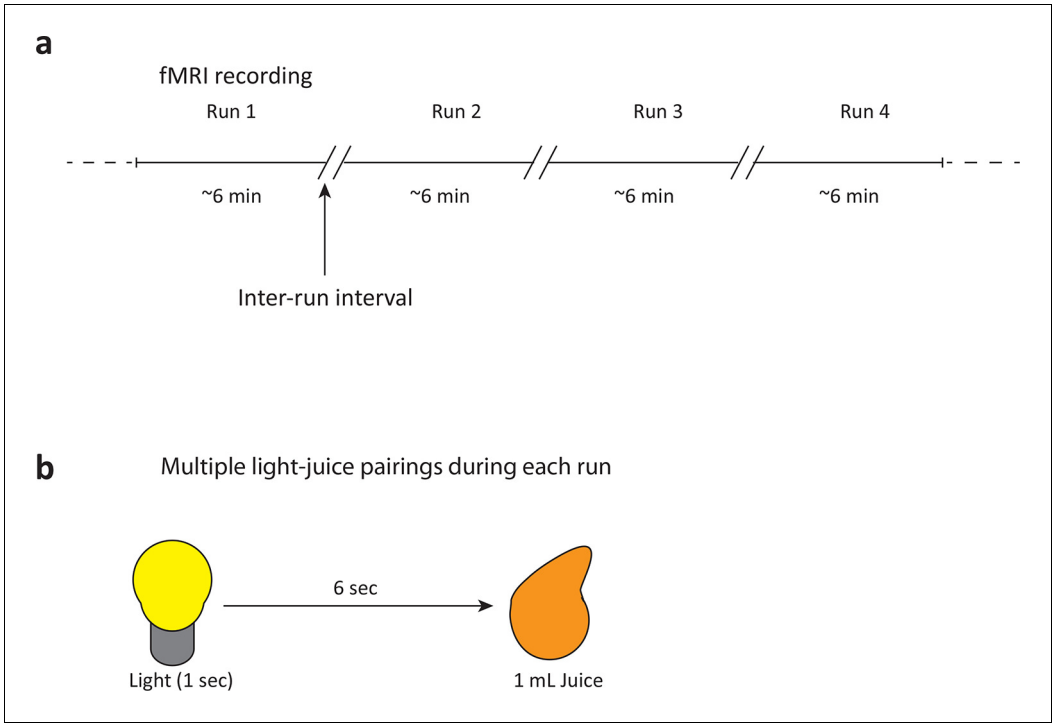

Figure 2—figure supplement 2. fMRI recording paradigm and reward-stimulus pairing in human smokers. (**a**) The fMRI recording session consisted of four 5–7 min blocks of light-juice pairings. A self-paced break ("inter-run interval") was included between runs to allow participants to ask any questions and to allow the investigators to provide feedback on participant motion within the scanner. (**b**) A total of fifty-five light-juice (1 mL) pairings were presented. The delay between light and juice was 7 s.

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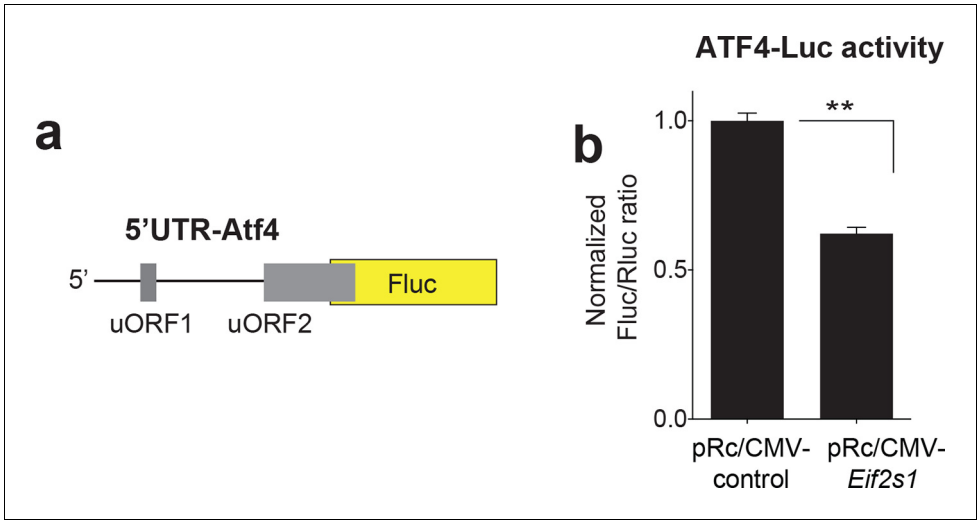

Figure 2—figure supplement 3. ATF4-Luciferase construct design and activity with *Eif2s1*. (**a**) Diagram of the 5 UTR-ATF4 Fluc reporter, which consists of the 5'UTR of ATF4 mRNA fused to the coding region of Firefly luciferase (Fluc). A renilla luciferase (Rluc) reporter vector was co-transfected into HEK293T as a transfection control. (**b**) Overexpression of Eif2s1 reduced expression of 5 UTR-ATF4-Fluc ($P<0.0001$, $n=4$ per group, $t_6=11.33$).
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