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Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read Reporting Life Sciences Research.

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported (section, e.g. Results, & paragraph number).
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the paragraph number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process; it is misleading not to state this clearly.

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
example	1a	one-way ANOVA	Fig. legend	9, 9, 10, 15	mice from at least 3 litters/group	Methods para 8	error bars are mean +/- SEM	Fig. legend	p = 0.044	Fig. legend	F(3, 36) = 2.97	Fig. legend
example	results, para 6	unpaired t-test	Results para 6	15	slices from 10 mice	Results para 6	error bars are mean +/- SEM	Results para 6	p = 0.0006	Results para 6	t(28) = 2.808	Results para 6

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
+ -	1a	Repeated Measures Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Cocaine 1WD = 7 Saline 1WD = 7 Cocaine 7WD = 7 Saline 7WD = 7	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats randomly assigned to cocaine/saline groups	Online Method: Regulation of Activin/ Smad3 signaling following withdrawal from cocaine self-administration Page 11-12	Data expressed as mean +/- SEM	Fig. legend	Interaction P < 0.0001	Fig. legend	Interaction (drug x WD) F(27,240) = 4.697	Fig. legend
+ -	1b	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Cocaine 1WD = 6 Saline 1WD = 6 Cocaine 7WD = 6 Saline 7WD = 6	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced on SA performance before divided into withdrawal (1/7WD) groups	Fig. Legend	Data expressed as mean +/- SEM	Fig. Legend	Interaction: P = 0.0081 Follow-up: p = 0.9525 for Saline 1 WD vs Saline 7 WD p = 0.6256 for Saline 1 WD vs Cocaine 1 WD p = 0.0018 for Saline 1 WD vs Cocaine 7 WD p = 0.6680 for Saline 7 WD vs Cocaine 1 WD p = 0.0015 for Saline 7 WD vs Cocaine 7 WD p = 0.0006 for Cocaine 1 WD vs Cocaine 7 WD	Fig. Legend	Interaction (drug x WD) F(1,20) = 8.648	Fig. Legend

+ -	1c	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Cocaine 1WD = 5 Saline 1WD = 5 Cocaine 7WD = 6 Saline 7WD = 6	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced on SA performance before divided into withdrawal (1/7WD) groups	Fig. legend	Data expressed as mean +/- SEM	Fig. legend	Interaction: P = 0.0154 Follow-up: p = 0.6964 for Saline 1 WD vs Saline 7 WD p = 0.5852 for Saline 1 WD vs Cocaine 1 WD p = 0.0117 for Saline 1 WD vs Cocaine 7 WD p = 0.8561 Saline 7 WD vs Cocaine 1 WD p = 0.0035 for Saline 7 WD vs Cocaine 7 WD p = 0.0033 for Cocaine 1 WD vs Cocaine 7 WD	Fig. legend	Interaction (drug x WD) F(1,18) = 7.157	Fig. legend
+ -	1d	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Cocaine 1WD = 5 Saline 1WD = 5 Cocaine 7WD = 6 Saline 7WD = 6	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced on SA performance before divided into withdrawal (1/7WD) groups	Fig. legend	Data expressed as mean +/- SEM	Fig. legend	Main Effect (Drug) P = 0.0407 (Withdrawal) P = 0.0418 Follow-up: p = 0.8102 for Saline 1WD vs Saline 7WD p = 0.8111 for Saline 1WD vs Cocaine 1WD p = 0.0061 for Saline 1WD vs Cocaine 7WD p = 0.9925 for Saline 7WD vs Cocaine 1WD p = 0.0076 for Saline 7WD vs Cocaine 7WD p = 0.0105 for Cocaine 1WD vs Cocaine 7WD	Fig. legend	Main Effect (Drug) F(1,18) = 4.864 (Withdrawal) F(1,18) = 4.805	Fig. legend

+ -	2a micr oinje ction dose resp onse	Repeated Measures Two-Factor ANOVA; Tukey's multiple comparison test	Online Metho ds: Statisti cal analys es Page 18	Vehicle = 18 (Activin A Vehicle = 9; SB43154 2 Veh = 9) Activin A = 7 SB43152 = 8 Complet e descripti on of group allocatio n found in: Pharmac ological manipula tion of Activin-receptor signaling in the NAc Cocaine dose-response Methods Page 12 - 13	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced based on baseline within-session dose response performance before divided into microinjection groups	Fig. Legend Online Methods: Pharmac ological manipula tion of Activin-receptor signaling in the NAc Cocaine dose-response Methods Page 12 - 13	Data expressed as mean +/- SEM	Fig. legend	Interaction p = 0.0015 Main Effect: Dose p < 0.001 Virus p < 0.001 Follow-up: Dose 0.03: SB431542 vs vehicle = 0.9205 SB431542 vs Activin A = 0.5160 Activin A vs Veh = 0.3957 Dose 0.1: SB431542 vs vehicle = 0.0015 SB431542 vs Activin A = 0.0001 Activin A vs Veh = 0.0001 Dose 0.3: SB431542 vs vehicle = 0.0107 SB431542 vs Activin A = 0.0019 Activin A vs Veh = 0.0373 Dose 1.0: SB431542 vs vehicle = 0.1234 SB431542 vs Activin A = 0.0026 Activin A vs Veh = 0.0381	Fig. legend	Interaction (dose x drug) F (6, 120) = 3.858 Main Effect (Dose) F(3,120) = 36.62 (Virus) F(2,120) = 17.00	Fig. legend

+ -	2b (10 mg/kg) microinject drug reinstatement	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	Vehicle = 8 SB43152 = 8	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced on SA and extinction performance before divided into microinjection drug groups (Veh/SB431542)	Fig. Legend Online Methods: Pharmacological manipulation of Activin-receptor signaling in the NAc Drug-induced reinstatement Page 13	Data expressed as mean +/- SEM	Fig. legend	p = 0.0363	Fig. legend	t(14) = 2.316	Fig. legend
+ -	2b (5 mg/kg) microinject drug reinstatement	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	Vehicle = 9 Activin A = 9	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced on SA and extinction performance before divided into microinjection drug groups (Veh/Activin A)	FIg. Legend Online Methods: Pharmacological manipulation of Activin-receptor signaling in the NAc Drug-induced reinstatement Page 13	Data expressed as mean +/- SEM	Fig. legend	p = 0.0010	Fig. legend	t(16) = 4.013	Fig. legend
+ -	2c microinjection rate of responding food activin	One factor ANOVA	Online Methods: Statistical analyses Page 18	Vehicle (BSA) = 7 Activin A = 7 Vehicle (DMSO) = 7 SB43152 = 7	Naive animals randomly assigned to groups	Online Method: Food reinforcement Page 13-14	Data expressed as mean +/- SEM	Fig. legend	p = 0.7036	Fig. legend	F(3,24) = 0.4735	Fig. legend

+ -	2d viral dose response	Repeated Measures Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	dnSmad3 = 10 wtSmad3 = 11 GFP = 18 Complete description of group allocation found in: Online Methods: Alteration of Smad3 signaling in the NAc Cocaine dose-response Page 14-15	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced based on baseline within-session dose response performance before divided into groups	Fig. Legend Online Methods: Alteration of Smad3 signaling in the NAc Cocaine dose-response Page 14-15	Data expressed as mean +/- SEM	Fig. legend	Interaction P = 0.001 Main Effect: Dose p < 0.001 Virus p < 0.001 Follow-up: Dose 0.03: dnSmad3 vs GFP = 0.3974 dnSmad3 vs wtSmad3 = 0.1900 wtSmad3 vs GFP = 0.5209 Dose 0.1: dnSmad3 vs GFP = 0.0009 dnSmad3 vs wtSmad3 < 0.0001 wtSmad3 vs GFP = 0.0015 Dose 0.3: dnSmad3 vs GFP = 0.0320 dnSmad3 vs wtSmad3 = 0.0003 wtSmad3 vs GFP = 0.0124 Dose 1.0: dnSmad3 vs GFP = 0.5346 dnSmad3 vs wtSmad3 = 0.2510 wtSmad3 vs GFP = 0.5011	Fig. legend	Interaction (dose x virus) F(6,144) = 5.007 Main Effect (Dose) F (3,144) = 32.57 (Virus) F(2,144) = 11.42	Fig. legend
+ -	2e viral(10mg/kg) drug reinstatement	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	GFP = 10 dnsmad3 = 9	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced on SA and extinction performance before divided into virus groups (GFP/dnSmad3)	Fig. Legend Online Methods: Alteration of Smad3 signaling in the NAc Drug-induced reinstatement Page 15	Data expressed as mean +/- SEM	Fig. legend	p = 0.0139	Fig. legend	t(17) = 2.742	Fig. legend

+ -	2e (5mg /kg) viral drug reinstatement	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	GFP = 11 wtSmad3 = 10	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats counterbalanced on SA and extinction performance before divided into virus groups (GFP/wtSmad3)	Fig. legend Online Methods: Alteration of Smad3 signaling in the NAc Drug-induced reinstatement Page 15	Data expressed as mean +/- SEM	Fig. legend	p = 0.0479	Fig. legend	t(19) = 2.115	Fig. legend
+ -	2f viral food rate responding	One factor ANOVA	Online Methods: Statistical analyses Page 18	GFP = 7 dnSmad3 = 7 wtSmad3 = 8	Naive animals randomly assigned to groups	Online Method: Food reinforcement Page 13-14	Data expressed as mean +/- SEM	Fig. legend	p = 0.6447	Fig. legend	F(2,19) = 0.4493	Fig. legend
+ -	3b dnSmad3 spine density	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Saline GFP = 5 Saline dnSmad3 = 4 Cocaine GFP = 4 Cocaine dnSmad3 = 4	An average was obtained from 6 – 10 neurons per rat	Fig. legend Online Methods: Dendritic spine analysis Page 16-17	Data expressed as mean +/- SEM	Fig. legend	Interaction P = 0.0003 Saline GFP vs Saline dnSmad3 = 0.0334 Saline GFP vs Cocaine GFP = 0.0002 Saline GFP vs Cocaine dnSmad3 = 0.7284 Saline dnSmad3 vs Cocaine GFP = 0.0234 Saline dnSmad3 vs Cocaine dnSmad3 = 0.0772 Cocaine GFP vs. Cocaine dnSmad3 = 0.0006	Fig. legend	Interaction: F(1,13) = 23.92	Fig. legend

+ -	3c dnSmad3 thin spine	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Saline GFP = 4 Saline dnSmad3 = 4 Cocaine GFP = 4 Cocaine dnSmad3 = 4	6-8 neurons/ animal from 3-4 sections averaged per subject	Fig. legend Online Methods: Dendritic spine analysis Page 16-17	Data expressed as mean +/- SEM	Fig. legend	Interaction: p = 0.0074 Saline GFP vs Saline dnSmad3 = 0.0281 Saline GFP vs Cocaine GFP = 0.0009 Saline GFP vs Cocaine dnSmad3 = 0.0381 Saline dnSmad3 vs Cocaine GFP = 0.0834 Saline dnSmad3 vs Cocaine dnSmad3 = 0.8711 Cocaine GFP vs. Cocaine dnSmad3 = 0.0624	Fig. legend	Interaction F (1,12) = 10.35	Fig. legend
+ -	3d dnSmad3 mushroom spine	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Saline GFP = 4 Saline dnSmad3 = 4 Cocaine GFP = 4 Cocaine dnSmad3 = 4	6-8 neurons/ animal from 3-4 sections averaged per subject	Fig. legend Online Methods: Dendritic spine analysis Page 16-17	Data expressed as mean +/- SEM	Fig. legend	Main Effect (Drug) p = 0.0478 (Withdrawal) p = 0.0017 Saline GFP vs Saline dnSmad3 = 0.1875 Saline GFP vs Cocaine GFP = 0.9783 Saline GFP vs Cocaine dnSmad3 = 0.0010 Saline dnSmad3 vs Cocaine GFP = 0.1958 Saline dnSmad3 vs Cocaine dnSmad3 = 0.0124 Cocaine GFP vs. Cocaine dnSmad3 0.0010	Fig. legend	Main Effect (Drug) F (1,12) = 4.398 (Withdrawal) F (1,12) = 16.28	Fig. legend

+ -	3f wtSmad3 spine density	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Saline GFP = 5 Saline wtSmad3 = 5 Cocaine GFP = 5 Cocaine wtSmad3 = 5	An average was obtained from 6–10 neurons per rat	Fig. legend Online Methods: Dendritic spine analysis Page 16-17	Data expressed as mean +/- SEM	Fig. legend	Main Effect Drug < 0.0001 Virus = 0.0060 Saline GFP vs Saline wtSmad3 = 0.2374 Saline GFP vs Cocaine GFP = 0.0003 Saline GFP vs Cocaine wtSmad3 < 0.0001 Saline wtSmad3 vs Cocaine GFP = 0.0039 Saline wtSmad3 vs Cocaine wtSmad3 < 0.0001 Cocaine GFP vs. Cocaine wtSmad3 = 0.0050	Fig. legend	Main Effect: (Drug) F (1,16) = 62.85 (Virus) F (1,16) =10.01	Fig. legend
+ -	3g wtSmad3 thin spine	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Saline GFP = 4 Saline wtSmad3 = 4 Cocaine GFP = 4 Cocaine wtSmad3 = 4	6-8 neurons/ animal from 3-4 sections averaged per subject	Fig. legend Online Methods: Dendritic spine analysis Page 16-17	Data expressed as mean +/- SEM	Fig. legend	Interaction & Main Effects P > 0.5	no effect of thin spines	Interaction F(1,12) = 0.2684 (no effect)	no effect of thin spines

+ -	3h wtSmad3 mushroom spine	Two-Factor ANOVA; Tukey's multiple comparison test	Online Methods: Statistical analyses Page 18	Saline GFP = 4 Saline wtSmad3 = 4 Cocaine GFP = 4 Cocaine wtSmad3 = 4	6-8 neurons/ animal from 3-4 sections averaged per subject	Fig. legend Online Methods: Dendritic spine analysis Page 16-17	Data expressed as mean +/- SEM	Fig. legend	Main Effects: Drug = 0.0049 Virus = 0.0001 Saline GFP vs Saline wtSmad3 = 0.0007 Saline GFP vs Cocaine GFP 0.0090 Saline GFP vs Cocaine wtSmad3 < 0.0001 Saline wtSmad3 vs Cocaine GFP = 0.1749 Saline wtSmad3 vs Cocaine wtSmad3 = 0.1052 Cocaine GFP vs. Cocaine wtSmad3 = 0.0077	Fig. legend	Main Effect (Drug) F(1,12) = 11.82 (Virus) F(1,12) = 30.01	Fig. legend
+ -	3i pcr	One factor ANOVA; multiple comparison test	Online Methods: Statistical analyses Page 18	5-6 for saline and cocaine Ctnnb1 Grin2a Mef2d Cap2 Dbn1 Pdyn Cfl1	1 punch from each rat	Fig. legend Online Method: Chromatin immunoprecipitation (ChIP) followed by qPCR Page 17-18	Data expressed as mean +/- SEM	Fig. legend	Main effect: p < 0.0001 Follow-up: 0.0104 for Ctnnb1; 0.0131 for Grin2a; 0.0087 for Mef2d; 0.0129 for Cap2; 0.0003 for Dbn1; 0.0334 for Pdyn; 0.1053 for Cfl1	Fig. legend	Main effect: F(13,66)=5.523	Fig. legend
+ -	3j smad3 chip	One factor ANOVA; multiple comparison test	Online Methods: Statistical analyses Page 18	5-7 for saline and cocaine Ctnnb1 Grin2a Mef2d Cap2 Dbn1 Pdyn Cfl1	7 punches from 2 rats equals one ChIP sample	Fig. legend Online Method: Chromatin immunoprecipitation (ChIP) followed by qPCR Page 17-18	Data expressed as mean +/- SEM	Fig. legend	Main effect: p < 0.0001 Follow-up: 0.0235 for Ctnnb1; 0.0026 for Grin2a; 0.0290 for Mef2d; 0.0045 for Cap2; 0.0205 for Dbn1; 0.4940 for Pdyn; 0.9986 for Cfl1	Fig. legend	Main effect: F(13,66)=3.897	Fig. legend

+ -	In main text: Nac acvr 1b	Students t-test (unpaired)	t-test stated in main text page 2 Online Methods: Statistical analyses Page 18	cocaine (7WD) = 8 saline (7WD) = 8	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Main Text Page 2	Data expressed as +/- SEM	Main Text Page 2	p = 0.7826	Fig. legend	t(14) = 0.2813	Fig. legend
+ -	Suppl. Fig 2a CPU acvr 2a 7WD	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	cocaine (7WD) = 6 saline (7WD) = 7	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Supplemental Figure Legend	Data expressed as +/- SEM	Supplemental Figure Legend	p = 0.66	Supplemental Figure Legend	t(11) = 0.4485	Supplemental Figure Legend
+ -	Suppl. Fig 2b CPu psmad3/ tsmad3 7WD	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	cocaine (7WD) = 6 saline (7WD) = 8	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Supplemental Figure Legend	Data expressed as +/- SEM	Supplemental Figure Legend	p = 0.1	Supplemental Figure Legend	t(12) = 1.501	Supplemental Figure Legend
+ -	Suppl. Fig 2c NAC CORE acvr 2a 7WD	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	cocaine (7WD) = 6 saline (7WD) = 6	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Supplemental Figure Legend	Data expressed as +/- SEM	Supplemental Figure Legend	p = 0.01	Supplemental Figure Legend	t(10) = 2.801	Supplemental Figure Legend
+ -	Suppl. Fig 2d NAC CORE psmad3/ tsmad3 7WD	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	cocaine (7WD) = 5 saline (7WD) = 5	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Supplemental Figure Legend	Data expressed as mean +/- SEM	Supplemental Figure Legend	p = 0.3	Supplemental Figure Legend	t(8) = 1.001	Supplemental Figure Legend

+ -	Suppl. Fig. 3a 1 hr acvr 2a	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	AcvR2a: cocaine (1 hr) = 5 saline (1hr) = 6	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Supplemental Figure Legend	Data expressed as +/- SEM	Supplemental Figure 1 Legend	p = 0.59	Supplemental Figure Legend	t(9) = 0.5546	Supplemental Figure Legend
+ -	Suppl. Fig. 3b 1 hr psmad3/smad3	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	cocaine (1 hr) = 5 saline (1hr) = 6	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Supplemental Figure Legend	Data expressed as +/- SEM	Supplemental Figure Legend	p = 0.6544	Supplemental Figure Legend	t(9) = 0.4629	Supplemental Figure Legend
+ -	Suppl. Fig. 3d reexposure acvr 2a	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	cocaine = 6 saline = 6	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Supplemental Figure Legend	Data expressed as +/- SEM	Supplemental Figure Legend	p = 0.06	Supplemental Figure Legend	t(10) = 2.1	Supplemental Figure Legend
+ -	Suppl. Fig. 3e reexposure psmad3/smad3	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	cocaine = 6 saline = 5	Heterogeneous Outbred non-littermate Male Sprague Dawley (Charles River) rats	Supplemental Figure Legend	Data expressed as +/- SEM	Supplemental Figure Legend	p = 0.02	Supplemental Figure Legend	t(9) = 2.745	Supplemental Figure Legend
+ -	Suppl. 4a food sb431452	Students t-test (unpaired)	Students t-test (unpaired)	Vehicle (DMSO) = 7 SB431542 = 7	Naive animals randomly assigned to groups	Supplemental Figure Legend Online Method: Food reinforcement Page 12	Data expressed as +/- SEM	Supplemental Figure Legend	p > 0.9999	Supplemental Figure Legend	t(12) = 0.0	Supplemental Figure Legend
+ -	Suppl. 4b loco sb431542	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	Vehicle (DMSO) = 7 SB431542 = 7	Naive animals randomly assigned to groups	Supplemental Figure Legend Online Method: Locomotor Activity Page 13	Data expressed as +/- SEM	Supplemental Figure Legend	p = 0.59	Supplemental Figure Legend	t(12) = 0.4833	Supplemental Figure Legend

+ -	Suppl. 4c food acta	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	Vehicle (BSA) = 7 Activin A = 7	Naive animals randomly assigned to groups	Supplemental Figure Legend Online Method: Food reinforcement Page 12	Data expressed as mean +/- SEM	Supplemental Figure Legend	p = 0.7714	Supplemental Figure Legend	t(12) = 0.2972	Supplemental Figure Legend
+ -	Suppl 4d loco acta	Students t-test (unpaired)	Online Methods: Statistical analyses Page 18	Vehicle (BSA) = 8 Activin A = 8	Naive animals randomly assigned to groups	Supplemental Figure Legend Online Method: Locomotor Activity Page 13	Data expressed as mean +/- SEM	Supplemental Figure Legend	p = 0.98	Supplemental Figure Legend	t(14) = 1.093	Supplemental Figure Legend
+ -	Suppl 4e food viral	One factor ANOVA	Online Methods: Statistical analyses Page 18	GFP = 7 dnSmad3 = 7 wtSmad3 = 8	Naive animals randomly assigned to groups	Supplemental Figure Legend Online Method: Food reinforcement Page 12	Data expressed as mean +/- SEM	Supplemental Figure Legend	p = 0.5629	Supplemental Figure Legend	F(2,19) = 0.5923	Supplemental Figure Legend
+ -	Suppl 4f viral loco	One factor ANOVA	Online Methods: Statistical analyses Page 18	GFP = 7 dnSmad3 = 7 wtSmad3 = 8	Naive animals randomly assigned to groups	Supplemental Figure Legend Online Method: Locomotor Activity Page 13	Data expressed as mean +/- SEM	Supplemental Figure Legend	p = 0.6501	Supplemental Figure Legend	F(2,19) = 0.4405	Supplemental Figure Legend

Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

   If so, what figure(s)?

Yes.
Figures 1c, 1d, 3a, 3e
Suppl. Figure 5-7

2. For each representative image, is there a clear statement of how many times this experiment was successfully repeated and a discussion of any limitations in repeatability?

   If so, where is this reported (section, paragraph #)?

Yes.
Histology Figures (Supplemental 5b) showing localization of viral-mediated expression do not depend on repetitions, but rather are representations of the experiments conducted within the manuscript. The experiment was repeated independently at least two times (described in Online Methods: Dendritic Spine Analysis).

Western Blotting: (Figures 1c, 1d, and Suppl. Fig 6-7) were repeated twice across 2 gels.

Statistics and general methods

1. Is there a justification of the sample size? If so, how was it justified? Where (section, paragraph #)? Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.	No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous works (Gancarz-Kausch et al., 2014; Gancarz-Kausch et al., 2013; Dietz et al., 2012) and based on expected effect sizes and power analyses. Further, as required by University at Buffalo's Institutional Animal Care and Use Committee, we use discretion in animal use. Will add statement of sample size justification in revised manuscript.
2. Are statistical tests justified as appropriate for every figure? Where (section, paragraph #)?	Yes.
a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?	Yes. All statistical tests are described in detail in Online Methods: Statistical Analyses (Page 18)
b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)? Where is this described (section, paragraph #)?	Yes. As part of statistical tests, Shapiro-Wilks test of normality were conducted. In events that normal distribution could not be assumed, non-parametric tests were utilized (now described in Online Methods: Statistical Analyses).
c. Is there any estimate of variance within each group of data? Is the variance similar between groups that are being statistically compared? Where is this described (section, paragraph #)?	Yes. Barlett's Test of Homogeneity of variance was assessed (now described in Online Methods: Statistical Analyses, Page 18).
d. Are tests specified as one- or two-sided?	Yes, two-sided.
e. Are there adjustments for multiple comparisons?	Yes. Bonferroni Corrections/Tukey's/Sidak Post Hoc corrections for multiple comparisons (described in Online Methods: Statistical Analyses).
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	Yes. Only rats with patent catheters were used in data analyses of self-administration experiments (Online Methods: Jugular catheterization and patency testing; Page 10-11) The criterion for acquisition of cocaine self-administration was an average of ten infusions per day (Online Methods: Regulation of Activin/Smad3 signaling following withdrawal from cocaine self-administration; Page 8-9). Viral and cannula targeting to NAc shell was confirmed for all animals; Anything outside of this area were excluded for anatomically incorrect placements (Online Methods: Page 10 & 11). For rt-PCR and ChIP, if melt curve did not produce 1 distinct peak, samples were removed from analysis.

4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data. If no randomization was used, state so. Where does this appear (section, paragraph #)?	Rats counterbalanced based on SA performance before divided into groups to control for history of drug intake (Online Methods: Regulation of Activin/Smad3 signaling following withdrawal from cocaine self-administration; Page 11-12)
5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included? If no blinding was done, state so. Where (section, paragraph #)?	Yes. All confocal acquisition and analyses of spines were conducted by investigators blind to the experimental conditions (Online Methods: Dendritic Spine Analyses; Page 16-17).
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	Yes. This study was conducted in accordance with the guidelines set up by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo (Online Methods: Subjects; Page 10)
7. Is the species of the animals used reported? Where (section, paragraph #)?	Yes. Naïve Male Sprague-Dawley rats (Online Methods: Subjects; Page 10)
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	Yes. Naïve Male Sprague-Dawley rats (275–350 g) (Online Methods: Subjects; Page 10)
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	Yes. Naïve Male Sprague-Dawley rats (275–350 g) (Online Methods: Subjects; Page 10)
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	n/a Animals were approximately age-matched when purchased from Vendor (ordered based on weight: 250-275 g)
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	Yes. Behavioral testing took place 7 d/wk during the dark phase of the 12 h light-dark cycle. (Online Methods: Subjects; Page 10)
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	Yes. Singly housed following surgery and for the duration of the self-administration phase of the experiments in order to protect the catheter/harness assembly. (Online Methods: Subjects; Page 10)
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	Yes. Behavioral testing took place 7 d/wk during the dark phase of the 12 h light-dark cycle. (Online Methods: Subjects; Page 10)
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)?	Yes. Naïve Male Sprague-Dawley rats (275–350 g) (Online Methods: Subjects; Page 10)

a. If multiple behavioral tests were conducted in the same group of animals, is this reported?

Where (section, paragraph #)?

n/a

15. If any animals/subjects were excluded from analysis, is this reported?

Where (section, paragraph #)?

Yes.
Loss of patency, failure to acquire self-administration (see Online Methods: Jugular catheterization and patency testing (page 10-11).

Viral and cannula targeting to NAc was confirmed for all animals; Animals excluded for anatomically incorrect placements (Page 12-13).

a. How were the criteria for exclusion defined?

Where is this described (section, paragraph #)?

- Loss of patency, failure to acquire self-administration
- Viral and cannula targeting to NAc was confirmed for all animals; Animals excluded for anatomically incorrect placements

b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

Where is this described (section, paragraph #)?

- Loss of patency, failure to acquire self-administration
- Viral and cannula targeting to NAc was confirmed for all animals; Animals excluded for anatomically incorrect placements

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

Yes.

a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

Yes. Online Methods, Western Blotting; Page 16

b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

Where does this appear (section, paragraph #)?

Validation data were not reported in the submitted manuscript. The citations listed below are validation of the antibodies used in both Western Blotting and ChIP. These citations can be added to the revised manuscript.

Western Blotting:
- Smad3:
Li, Q, et al. (2008). Mol Cell Bio 28, 7001-11
Louafi, F., et al. (2010) J Biol Chem 285, 41328 - 36.

- pSmad3:
Attisano, L., et al. (2002) Science, 296, 1646
Moustakas, A., et al. (2001) J. Cell Sci. 114, 4359.

- AcvR2a:
Gold, EJ et al. (2003). Changes in activin and activin receptor subunit expression in rat liver during the development of CCl4-induced cirrhosis. Mol. Cell., Endocrinol.
Leal AM et al. (2002) Effect of adenovirus-mediated overexpression of follistatin and extracellular domain of activin receptor type II on gonadotropin secretion in vitro and in vivo. Endocrinol.

ChIP:
- Smad3
Zhou, B. et al. (2012). J. Biol. Chem. 2(287): 7026-38.
Estaras, C., et al. (2013) Mol. Biol. Cell. 24(3): 351-60

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?

   Where (section, paragraph #)?

   n/a

   a. Were they recently authenticated?

      Where is this information reported (section, paragraph #)?

   n/a

Data deposition

Data deposition in a public repository is mandatory for:

a. Protein, DNA and RNA sequences
b. Macromolecular structures
c. Crystallographic data for small molecules
d. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available here. We encourage the provision of other source data in supplementary information or in unstructured repositories such as Figshare and Dryad.

We encourage publication of Data Descriptors (see Scientific Data) to maximize data reuse.

1. Are accession codes for deposit dates provided?

   Where (section, paragraph #)?

   n/a

Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

   n/a

2. If computer code was used to generate results that are central to the paper's conclusions, include a statement in the Methods section under "**Code availability**" to indicate whether and how the code can be accessed. Include version information as necessary and any restrictions on availability.

   n/a

Human subjects

1. Which IRB approved the protocol?

   Where is this stated (section, paragraph #)?

   n/a

2. Is demographic information on all subjects provided?

   Where (section, paragraph #)?

   n/a

3. Is the number of human subjects, their age and sex clearly defined?

   Where (section, paragraph #)?

   n/a

4. Are the inclusion and exclusion criteria (if any) clearly specified?

   Where (section, paragraph #)?

   n/a

5. How well were the groups matched?

   Where is this information described (section, paragraph #)?

   n/a

6. Is a statement included confirming that informed consent was obtained from all subjects?

   Where (section, paragraph #)?

   n/a

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?

   Where (section, paragraph #)?

   n/a

fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?

   n/a

   a. If yes, is the number rejected and reasons for rejection described?

      Where (section, paragraph #)?

      n/a

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

   Where (section, paragraph #)?

   n/a

3. Is the length of each trial and interval between trials specified?

   n/a

4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.

   n/a

5. Is the task design clearly described?

   Where (section, paragraph #)?

   n/a

6. How was behavioral performance measured?

   n/a

7. Is an ANOVA or factorial design being used?

   n/a

8. For data acquisition, is a whole brain scan used? If not, state area of acquisition.	n/a
a. How was this region determined?	n/a
9. Is the field strength (in Tesla) of the MRI system stated?	n/a
a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?	n/a
b. Are the field-of-view, matrix size, slice thickness, and TE/TR/ flip angle clearly stated?	n/a
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?	n/a
11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)?	n/a
12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)?	n/a
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?	n/a
14. Were any additional regressors (behavioral covariates, motion etc) used?	n/a
15. Is the contrast construction clearly defined?	n/a
16. Is a mixed/random effects or fixed inference used?	n/a
a. If fixed effects inference used, is this justified?	n/a
17. Were repeated measures used (multiple measurements per subject)?	n/a
a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?	n/a
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?	n/a
19. Are statistical inferences corrected for multiple comparisons?	n/a

a. If not, is this labeled as uncorrected?	n/a
20. Are the results based on an ROI (region of interest) analysis?	n/a
a. If so, is the rationale clearly described?	n/a
b. How were the ROI's defined (functional vs anatomical localization)?	n/a
21. Is there correction for multiple comparisons within each voxel?	n/a
22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?	n/a

Additional comments

Additional Comments	