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#### Supplementary information

# Transient nicotine exposure in early adolescent male mice freezes their dopamine circuits in an immature state

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6 Supplementary Figure 1. Supplementary data for Figure 1. (A) Individual weight measurements across the 7 entire oral self-administration task for adolescent-pretreated (Left) and adult-pretreated mice (Right). (B) 8 Actograms of drinking behavior over the duration of the Two Bottle Choice task for adult mice pre-treated in 9 adolescence (left) or in adulthood (right). Shaded regions represent mean volumes across the groups for 10 each solution tested. (C) Sucrose solution and overall liquid consumption per day for adolescent-pretreated 11 (Left) and adult-pretreated mice (Right) during the Sucrose nicotine oral self-administration testing. (D) 12 Nicotine solution and overall liquid consumption per day for adolescent-pretreated (Left) and adult-13 pretreated mice (*Right*) during the nicotine oral self-administration testing. (E) Individual data for Figure 1B 14 center, % nicotine of total intake for mice treated with NIC or SAC in adolescence. (F) Individual data for Figure 1C center, % nicotine of total intake for mice treated with NIC or SAC in adulthood. (G) Percent 15 16 preference for left side bottle across the entire oral self-administration task for adolescent-pretreated (Left) 17 and adult-pretreated mice (*Right*). (H) Individual data for Figure 1E, solid lines indicate group means while dotted lines are individual animals for mice treated with NIC or SAC in adolescence. (I) Open arm entries 18 19 (Left) and Distance traveled (cm, Right) in the EOM task for adolescent-pretreated mice. (J) Individual data 20 for Figure 1H, solid lines indicate group means while dotted lines are individual animals for mice treated with 21 NIC or SAC in adulthood. (K) Open arm entries (Left) and Distance traveled (cm, Right) in the EOM task for adult-pretreated mice. All line graphs are presented as mean values ± SEM. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 22 23 0.01, ns = not significant. Detailed Statistics are available in Supplementary Table 4. Source data are 24 provided as a Source Data file.



**Supplementary Figure 1** 

Detailed Statistics for Supplementary Figure 1

Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure
weight - adolesc	ents					
SAC = 21, NIC = 24	Shapiro-Wilk normality test		W	0.9723	0.0000	Fig S1A
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 0%	W	237.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 0.5%	W	250.5000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 1%	W	256.5000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 10µg/mL	W	264.5000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 50µg/mL	W	256.5000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 100µg/mL	W	249.5000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 200µg/mL	W	251.5000	1.0000*	
weight - adults						
SAC = 12, NIC = 12	Shapiro-Wilk normality test		W	0.9771	0.0070	Fig S1A
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 0%	W	60.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 0.5%	W	60.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 1%	W	60.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 10µg/mL	W	71.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 50µg/mL	W	75.5000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 100µg/mL	W	81.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 200µg/mL	W	75.0000	1.0000*	

sucrose consumption (mL/day) - adolescent pretreatment

\* Holm correction for multiple comparisons

Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure
SAC = 21, NIC = 24	Shapiro-Wilk normality test		W	0.9223	0.5218	Fig S1C
	Two-way mixed ANOVA	Interaction	F	0.9197	0.4025	
		Treatment (between subj)	F	1.4590	0.2337	
		Sucrose conc (within subjects)	F	119.6943	0.0000	
total liquid intake	e (mL/day) - adolescent pre	etreatment				
SAC = 21, NIC = 24	Shapiro-Wilk normality test		W	0.8878	0.5218	Fig S1C
	Two-way mixed ANOVA	Interaction	F	1.9069	0.4025	
		Treatment (between subj)	F	1.0621	0.2337	
		Sucrose conc (within subjects)	F	12.6504	0.0000	
sucrose consum	ption (mL/day) - adult pret	reatment				
SAC = 12, NIC = 12	Shapiro-Wilk normality test		W	0.9088	0.4287	Fig S1C
	Two-way mixed ANOVA	Interaction	F	0.5978	0.5531	
		Treatment (between subj)	F	2.5198	0.1174	
		Sucrose conc (within subjects)	F	0.0534	0.9480	
total liquid intake	e (mL/day) - adult pretreatr	ment				
SAC = 12, NIC = 12	Shapiro-Wilk normality test		W	0.8711	0.2307	Fig S1C
	Two-way mixed ANOVA	Interaction	F	0.4784	0.6220	
		Treatment (between subj)	F	3.1207	0.0821	
		Sucrose conc (within subjects)	F	1.3045	0.2785	
nicotine consum	ption (mL/day) - adolescer	nt pretreatment				
SAC = 21, NIC = 24	Shapiro-Wilk normality test		W	0.9888	0.0769	Fig S1D
	Two-way mixed ANOVA	Interaction	F	1.7830	0.1344	
* Holm correction f	or multiple comparisons					

Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure
		Treatment (between subj)	F	5.8579	0.0198	
		nicotine conc (within subjects)	F	62.9866	0.0000	
total liquid intake	(mL/day) - nic choice - ac	lolescent pretreatm	nent			
SAC = 21, NIC = 24	Shapiro-Wilk normality test		W	0.9841	0.0127	Fig S1D
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 0µg/mL	W	243.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 10µg/mL	W	205.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 50µg/mL	W	274.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 100µg/mL	W	312.5000	0.8610*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 100µg/mL	W	259.0000	1.0000*	
nicotine consum	otion (mL/day) - adult preti	reatment				
SAC = 12, NIC = 12	Shapiro-Wilk normality test		W	0.9664	0.0043	Fig S1D
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 0µg/mL	W	243.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 10µg/mL	W	205.0000	0.3130*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 50µg/mL	W	274.0000	0.0829*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 100µg/mL	W	312.5000	0.8564*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 100µg/mL	W	259.0000	1.0000*	
total liquid intake	(mL/day) - nic choice - ac	lult pretreatment				
SAC = 12, NIC = 12	Shapiro-Wilk normality test		W	0.9770	0.0374	Fig S1D
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 0µg/mL	W	67.0000	1.0000*	
* Holm correction f	or multiple comparisons					

Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 10µg/mL	W	44.0000	0.4494*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 50µg/mL	W	37.0000	0.2319*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 100µg/mL	W	49.0000	0.5818*	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC at 100µg/mL	W	73.0000	1.0000*	
Open arm entries	s - adolescent pretreatme	nt				
SAC + SAL = 12, SAC + NIC = 16,	Shapiro-Wilk normality test		W	0.9280	0.0000	Fig S1I
NIC + SAL = 11, NIC + NIC = 14	Kruskal-Wallis rank sum test	All groups, 0-3 min	Kruskal-Wallis chi- squared	11.1952	0.0107	
	Kruskal-Wallis rank sum test	All groups, 3-6 min	Kruskal-Wallis chi- squared	11.7069	0.0085	
	Kruskal-Wallis rank sum test	All groups, 6-9 min	Kruskal-Wallis chi- squared	14.1702	0.0027	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 0-3 min	W	53.5000	0.5292*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 0-3 min	W	38.0000	0.0279*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 0-3 min	W	48.0000	0.5292*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 0-3 min	W	144.5000	0.5292*	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 3-6 min	W	35.0000	0.0707*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 3-6 min	W	45.5000	0.0707*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 3-6 min	W	77.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 3-6 min	W	122.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 6-9 min	W	49.5000	0.4067*	
* Holm correction f	or multiple comparisons					

Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 6-9 min	W	26.5000	0.0043*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 6-9 min	W	83.5000	0.4663*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 6-9 min	W	140.5000	0.4663*	
Distance traveled	d EOM - adolescent pretre	eatment				
SAC + SAL = 12, SAC + NIC = 16,	Shapiro-Wilk normality test		W	0.9809	0.0265	Fig S1I
NIC + SAL = 11, NIC + NIC = 14	Kruskal-Wallis rank sum test	All groups, 0-3 min	Kruskal-Wallis chi- squared	14.4259	0.0024	
	Kruskal-Wallis rank sum test	All groups, 3-6 min	Kruskal-Wallis chi- squared	8.5321	0.0362	
	Kruskal-Wallis rank sum test	All groups, 6-9 min	Kruskal-Wallis chi- squared	6.7473	0.0804	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 0-3 min	W	40.0000	0.1371*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 0-3 min	W	30.0000	0.0094*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 0-3 min	W	54.0000	0.8837*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 0-3 min	W	131.0000	0.8837*	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 3-6 min	W	54.0000	0.6541*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 3-6 min	W	39.0000	0.0349*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 3-6 min	W	79.0000	0.8834*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 3-6 min	W	130.0000	0.8834*	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 6-9 min	W	57.0000	0.8572*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 6-9 min	W	49.0000	0.1235*	
* Holm correction f	or multiple comparisons					

Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 6-9 min	W	71.0000	0.8572*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 6-9 min	W	138.0000	0.8572*	
Open arm entries	s - adult pretreatment					
SAC + SAL = 15, SAC + NIC = 15,	Shapiro-Wilk normality test		W	0.8975	0.0000	Fig S1K
NIC + SAL = 15, NIC + NIC = 16	Kruskal-Wallis rank sum test	All groups, 0-3 min	Kruskal-Wallis chi- squared	25.7673	0.0000	
	Kruskal-Wallis rank sum test	All groups, 3-6 min	Kruskal-Wallis chi- squared	20.4812	0.0001	
	Kruskal-Wallis rank sum test	All groups, 6-9 min	Kruskal-Wallis chi- squared	22.0303	0.0001	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 0-3 min	W	100.5000	0.0792*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 0-3 min	W	47.0000	0.0792*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 0-3 min	W	149.5000	0.3767*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 0-3 min	W	284.5000	0.0938*	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 3-6 min	W	86.5000	0.0172*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 3-6 min	W	78.5000	0.0020*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 3-6 min	W	181.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 3-6 min	W	223.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 6-9 min	W	69.0000	0.0043*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 6-9 min	W	86.0000	0.0043*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 6-9 min	W	188.5000	1.0000*	
* Holm correction f	or multiple comparisons					

N	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 6-9 min	W	224.0000	1.0000*	
Distance travele	d EOM - adult pretreatme	nt				
SAC + SAL = 15, SAC + NIC = 15,	Shapiro-Wilk normality test		W	0.9697	0.0001	Fig S1K
NIC + SAL = 15, NIC + NIC = 16	Kruskal-Wallis rank sum test	All groups, 0-3 min	Kruskal-Wallis chi- squared	26.7687	0.0000	
	Kruskal-Wallis rank sum test	All groups, 3-6 min	Kruskal-Wallis chi- squared	21.1452	0.0001	
	Kruskal-Wallis rank sum test	All groups, 6-9 min	Kruskal-Wallis chi- squared	9.8571	0.0198	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 0-3 min	W	87.0000	0.0205*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 0-3 min	W	42.0000	0.0000*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 0-3 min	W	173.0000	0.8493*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 0-3 min	W	260.0000	0.3934*	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 3-6 min	W	86.0000	0.0188 <sup>*</sup>	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 3-6 min	W	74.0000	0.0016*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 3-6 min	W	189.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 3-6 min	W	223.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	NIC-SAL vs NIC-NIC, 6-9 min	W	118.0000	0.2165 <sup>*</sup>	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs SAC- NIC, 6-9 min	W	114.0000	0.0510*	
	Wilcoxon rank sum test with continuity correction	SAC-SAL vs NIC- SAL, 6-9 min	W	194.0000	1.0000*	
	Wilcoxon rank sum test with continuity correction	SAC-NIC vs NIC-NIC, 6-9 min	W	216.0000	1.0000*	
* Holm correction f	for multiple comparisons					

- 25 Supplementary Figure 2. Supplementary data for Figure 2. (A) Experimental strategy. (B) Volcano plots
- showing differential cFos expression across brain regions following an injection of saline for adolescent-
- 27 pretreated (*Left*) and adult-pretreated mice (*Right*). (**C-D**) Correlation matrices for relationships between
- 28 cFos expression in brain regions following an injection of saline in adolescent-pretreated (C) and adult-
- 29 pretreated mice (**D**). (**E**) Correlation matrices for relationships between cFos expression in brain regions
- 30 following an injection of nicotine in adult-pretreated mice. (F) Voxel-by-voxel analysis reveals no change in
- 31 reactivity to nicotine within the VTA and DA terminal regions in adult-pretreated mice. (G) Full networks
- 32 derived from correlation matrices in Fig 2C and organized into communities with Louvain community
- analysis. Source data are provided as a Source Data file.



# **Supplementary Figure 2**

Supplementary Figure 3. Supplementary data for Figure 3. (A) Experimental design for DA terminal 34 35 density analysis. (B) Tyrosine Hydroxylase (TH)+ labeling in DA terminal regions. (Core = NAc Core, MShell 36 = NAc Medial Shell, LShell = NAc Lateral Shell, CeA = Central Amygdala, BLA = Basolateral Amygdala, 37 CPu = Caudate-Putamen). (C) Confocal image processing for bouton segmentation. (D) Bouton density 38 counts across the NAc (Left) and the AMG (Right). (E) Left : Nicotine current traces were analyzed and neurons were found to show two types of responses to nicotine: monophasic (one component) and bi-39 40 phasic (with a fast and slow component). More neurons from adult mice pretreated with NIC in adolescence 41 showed a biphasic response than those treated with SAC. Center. Nicotine currents in neurons from mice 42 treated with NIC in adolescence showed a lower T indicative of a faster current decay. *Right*: Less charge is transferred in response to a nicotine puff in neurons from mice treated with NIC in adolescence. (F) Average 43 change in NMDAR currents following application of Ifenprodil (5 mM) to VTA DA neurons (SAC N = 4, n = 7; 44 NIC N = 4, n = 7). (G) Paired pulse ratio measurements for VTA DA neurons of adolescent-pretreated (Top) 45 and adult-pretreated mice (Bottom). (H) sEPSCs onto DA neurons measured before and after nicotine puff 46 47 (Left) sEPSC frequency was reduced at baseline and after nicotine puff in adult mice pretreated with NIC in 48 adolescence in comparison to mice pretreated with SAC (Top row), however NIC pretreated mice showed a 49 greater augmentation (% of baseline) in response to the nicotine puff (*Right*). Mice pretreated with NIC in adulthood showed no differences from mice pretreated with SAC in adulthood (Bottom row). All bar graphs 50 are presented as mean values ± SEM. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.01. Detailed Statistics are available in 51 52 Supplementary Table 5. Source data are provided as a Source Data file. 53



Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure	
NAc boutons							
SAC = 8, NIC = 8	Shapiro-Wilk normality test		W	0.9719	0.3007	Fig S3D	
	Two-way mixed ANOVA	Interaction	F	1.1643	0.3268		
		Treatment (between subj)	F	3.0443	0.1029		
		Region (within subjects)	F	74.5824	0.0000		
Amg boutons							
SAC = 7, NIC = 8	Shapiro-Wilk normality test		W	0.8895	0.0040	Fig S3D	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC in the BLA	W	34.0000	1.00001		
	Wilcoxon rank sum test with continuity correction	SAC vs NIC in the CeA	W	31.0000	1.00001		
	Wilcoxon rank sum test with continuity correction	BLA vs CeA in SAC	W	11.0000	0.16861		
	Wilcoxon rank sum test with continuity correction	BLA vs CeA in NIC	W	0.0000	0.00381		
Weighted Tau, nicotine cu	rrent						
NIC = 2 mice, 14 neurons; SAC = 2 mice, 13 neurons	Shapiro-Wilk normality test		W	0.9567	0.3102	Fig S3E	
	Welch Two Sample t-test	SAC vs NIC	t	-6.7097	0.0000		
Charge Transferred - nicol	ine current						
NIC = 2 mice, 14 neurons; SAC = 2 mice, 13 neurons	Shapiro-Wilk normality test		W	0.8268	0.0004	Fig S3E	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC	W	141.0000	0.0163		
% NMDA current - ifenproo	dil						
NIC = 4 mice, 7 neurons; SAC = 4 mice, 7 neurons	Shapiro-Wilk normality test		W	0.9679	0.8470	Fig S3F	
	Welch Two Sample t-test	SAC vs NIC	t	-1.3859	0.1912		
<sup>1</sup> Holm correction for multiple comparisons							

Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure	
PPR - adolescent pretreatment							
NIC = 5 mice, 20 neurons; SAC = 5 mice, 19 neurons	Shapiro-Wilk normality test		W	0.9746	0.5122	Fig S3G	
	Welch Two Sample t-test	SAC vs NIC	t	0.3017	0.7653		
PPR - adult pretreatment							
NIC = 4 mice, 12 neurons; SAC = 4 mice, 14 neurons	Shapiro-Wilk normality test		W	0.9705	0.6154	Fig S3G	
	Welch Two Sample t-test	SAC vs NIC	t	-0.0377	0.9702		
Baseline sEPSCs - adoles	cent pretreatment						
NIC = 2 mice, 14 neurons; SAC = 2 mice, 14 neurons	Shapiro-Wilk normality test		W	0.9020	0.0127	Fig S3H	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC	W	18.0000	0.0003		
After sEPSCs - adolescent	t pretreatment						
NIC = 2 mice, 14 neurons; SAC = 2 mice, 14 neurons	Shapiro-Wilk normality test		W	0.9656	0.4681	Fig S3H	
	Welch Two Sample t-test	SAC vs NIC	t	-1.9394	0.0635		
Percent sEPSCs - adolesc	cent pretreatment						
NIC = 2 mice, 14 neurons; SAC = 2 mice, 14 neurons	Shapiro-Wilk normality test		W	0.7356	0.0000	Fig S3H	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC	W	183.0000	0.0001		
Baseline sEPSCs - adult p	retreatment						
NIC = 5 mice, 8 neurons; SAC = 5 mice, 10 neurons	Shapiro-Wilk normality test		W	0.6963	0.0001	Fig S3H	
	Wilcoxon rank sum test with continuity correction	SAC vs NIC	W	39.0000	0.6534		
After sEPSCs - adult pretre	eatment						
NIC = 5 mice, 8 neurons; SAC = 5 mice, 10 neurons	Shapiro-Wilk normality test		W	0.9171	0.1001	Fig S3H	
<sup>1</sup> Holm correction for multiple	comparisons						

Ν	Test	Factor	Statistic	Statistic Value	p value	Corresponding Figure			
	Welch Two Sample t-test	SAC vs NIC	t	-0.6067	0.5536				
Percent sEPSCs - adult pr	Percent sEPSCs - adult pretreatment								
NIC = 5 mice, 8 neurons; SAC = 5 mice, 10 neurons	Shapiro-Wilk normality test		W	0.8870	0.0343	Fig S3H			
	Wilcoxon rank sum test with continuity correction	SAC vs NIC	W	52.0000	0.3069				
<sup>1</sup> Holm correction for multiple comparisons									