SUPPLEMENTARY DATA

Supplementary Methods

Across-Session Dose-Response determinations for nicotine SA

After one week of recovery from IV surgery NOP $^{(+/+)}$ (n=9) and NOP $^{(-/-)}$ (n=8) rats were trained in 2 h daily nicotine (30μg/kg/0.1ml infusion) self-administration sessions (5 days/week). The response requirement for each infusion was incremented from a fixed ratio 1 (FR1) to fixed ratio 3 (FR3) until baseline was reached. Pressing the active (right) lever resulted in the infusion of 0.1ml of nicotine followed by the activation of the cue light above the lever and a 20 second time-out period during which responses at the active lever had no programmed consequences. Inactive lever presses were recorded but resulted in no reinforcer delivery.

When responding stabilized on the FR3 rats self-administered different unit dose of nicotine (10.0, 30.0 and 60.0 μg/kg/0.1ml infusion) in a within subject Latin square counterbalancing using Williams' design. Rats had access to each nicotine dose for four consecutive sessions.

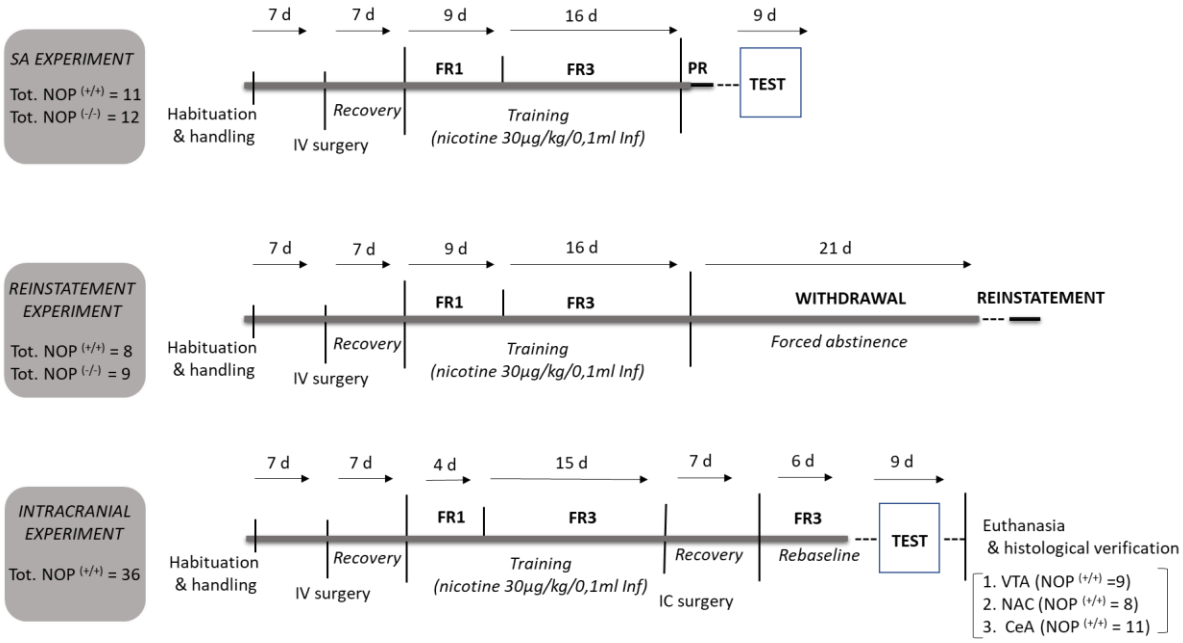
Data were analyzed by analysis of variance (ANOVA) followed by post hoc tests when appropriate. In particular, the difference between the NOP $^{(+/+)}$ and NOP $^{(-/-)}$ in the number of infusions and lever presses were analyzed by two-way ANOVA with one factor between (rat line) and one factor within (nicotine doses). Post hoc comparisons were carried out by Dunnett´s. The data are presented as the mean ± SEM. Statistically significant difference was set at $P< .05$. Prior to ANOVA we examined for significant violations for assumptions of homogeneity of variance by using Levene's test Mauchly's test of sphericity was used to test if assumption of sphericity had been violated when using repeated measures ANOVA. Data were analyzed using STATISTICA, Stat Soft 13.0 (RRID:SCR_014213).

Supplementary results

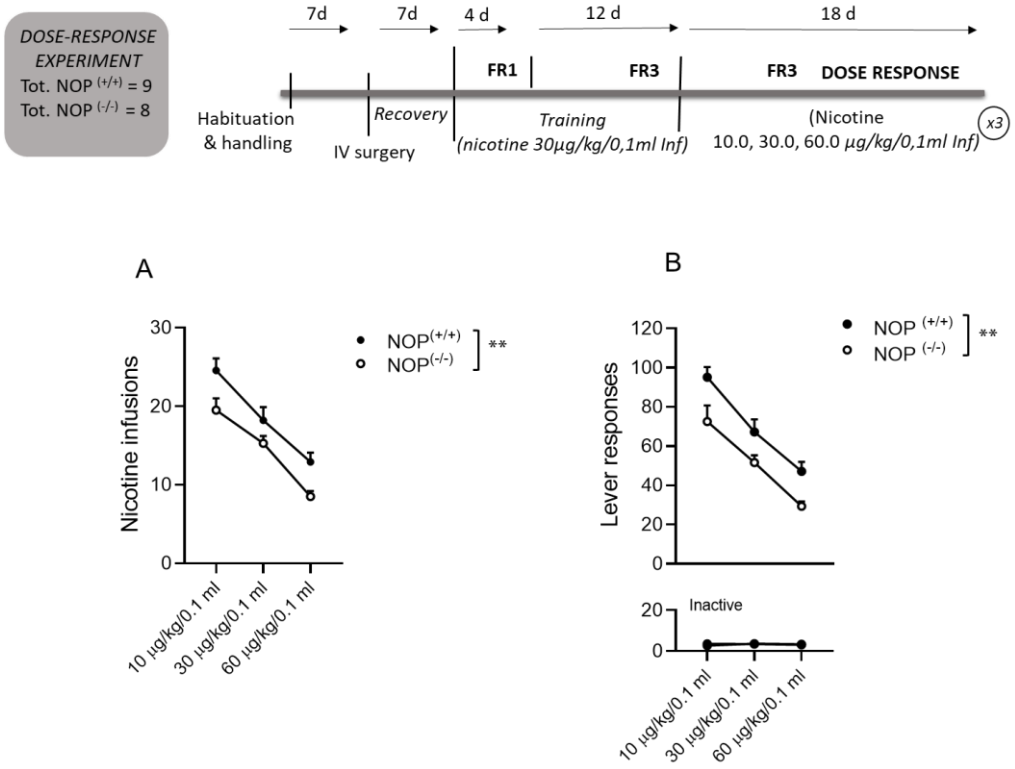
Reduced motivation for nicotine in NOP $^{(-/-)}$ rats does not depend upon altered drug sensitivity

After establishment of stable responding for nicotine NOP $^{(+/+)}$ (n=9) and NOP $^{(-/-)}$ (n=8) rats were tested for multiple drug concentrations. In here the mean intake over the four dose-response sessions was averaged to obtain individual subject means. As shown in figure S2 NOP $^{(+/+)}$ rats reached a significant higher number of infusions and increased active lever responding for nicotine than the NOP $^{(-/-)}$ rats. No differences were detected in the inactive lever press between the two lines.

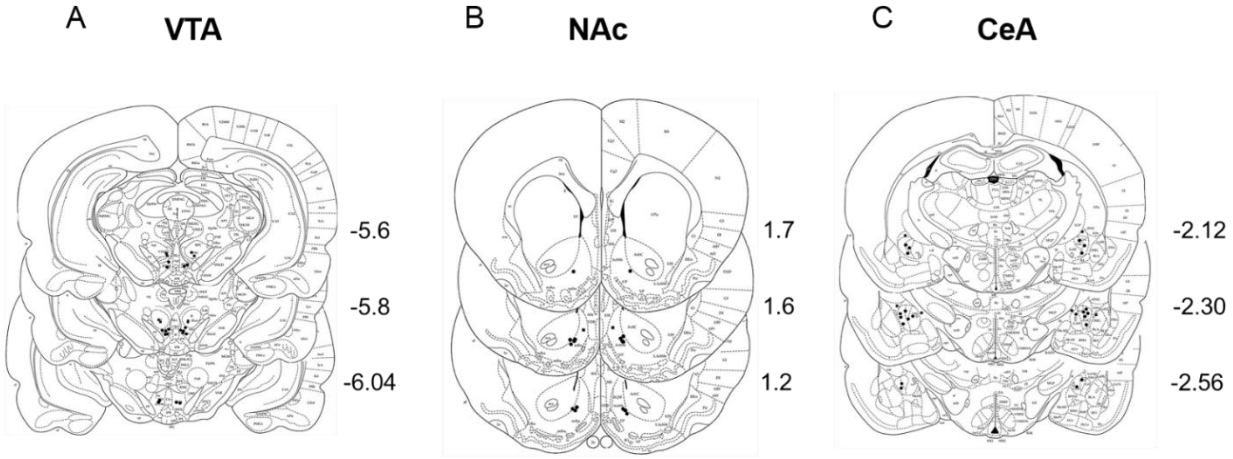
SUPPLEMENTARY FIGURES



Supplementary Fig.S1. Schematic representation of the behavioural testing



Supplementary Fig.S2. Pilot study to determine the self-administration behaviour of NOP $^{(+/+)}$ and NOP $^{(-/-)}$ in response to different concentrations of nicotine. Number of responses A) infusions B) Active and Inactive lever presses for three different concentrations of nicotine (10.0, 30.0 and 60.0 µg/kg/0.1ml infusion) in NOP $^{(+/+)}$ (n = 9) and NOP $^{(-/-)}$ (n = 8) rats. **p < 0.01 *vs*. NOP $^{(+/+)}$ and NOP $^{(-/-)}$ rats.



Supplementary Fig.S3. Illustration of LY2817412 and vehicle injection sites in the VTA (A), NAc (B) and CeA (C). NOP $^{(+/+)}$ rats (n = 36) were implanted with bilateral cannulas in the VTA (n= 9), NAc (n= 8) and CeA (n= 11). Black dots represent the cannula placement after histological verification. Only data resulting from correct cannula placement were included in the statistical analysis.

	Line	Session	Line x Session	Lever	Lever x session	Lever x line	Lever x line x session
Nicotine infusions Fig.1A	FR1 $F_{(1,21)} = 2.83$; p= NS FR3 $F_{(1,21)}=7.33$; p<0.01	FR1 $F_{(8,168)} = 13.2$; p<0.000 FR3 $F_{(15,315)}=6.64$; p<0.000	FR1 $F_{(8,168)} = 0.39$; p= NS FR3 $F_{(15,315)}=2.47$; p<0.01				
Nicotine lever responses Fig.1A	FR1 $F_{(1,21)} = 2.63$; p= NS FR3 $F_{(1,21)=}6.48$; p<0.05	FR1 $F_{(8,168)} = 6.05$; p<0000 FR3 $F_{(15,315)}=4.65$; p<0.0000	FR1 $F_{(8,168)} = 0.67$; p=NS FR3 $F_{(15,315)}=2.06$; p≤0.01	FR1 $F_{(1,21)} = 102.34$; p<0.0000 FR3 $F_{(1,21)}=250.02$; p<0.0000	FR1 $F_{(8,168)} = 5.80$; p<0.0000 FR3 $F_{(15,315)}=5.73$; p<0000	FR1 $F_{(1,21)} = 2.98$; p=NS FR3 $F_{(1,21)}=12.35$; p<0.01	FR1 $F_{(8,168)} = 0.11$; p=NS FR3 $F_{(15,315)}=1.66$; p≤0.05
Progressive ratio Fig.1B	BP $t_{(21)}=3.08$; p<0.01						
Reinstatement Fig.1C	$F_{(1,15)} =27.27$; p<0.0001		$F_{(1,15)} = 8,03$; p<0.01	$F_{(1,15)} = 12,49$; p<0.01			

Table S1. Corresponding degrees of freedom, F, t and P- values of the of the statistical results for figure 1 represented in the Supporting Information

LY2817412	**NOP $^{(+/+)}$**	**NOP $^{(-/-)}$**
Fig. 2 A-B	Infusions $F_{(2,20)}=19.8$; p<0.000 Active l.presses $F_{(2,20)}=19.01$; p<0.000 Inactive L.presses $F_{(2,20)}= 0.04$; p=NS	Infusions $F_{(2,22)}=1.01$; p=NS Active l.presses $F_{(2,22)}=0.60$; p=NS Inactive l.presses $F_{(2,22)}=1.29$; p=NS
Fig. 3. A	Infusions $F_{(2,16)}=12.92$; p<0.001 Active l.presses $F_{(2,16)}=12.36$; p<0.000 Inactive l.presses $F_{(2,16)}=0.61$; p=NS	
B	Infusions $F_{(2,14)}=0.12$; p=NS Active l.presses $F_{(2,14)}=0.01$; p=NS Inactive l.presses $F_{(2,14)}= 2.05$; p=NS	
C	Infusions $F_{(2,20)}=1.19$; p=NS Active l.presses F(2,20)=0.86; p=NS Inactive l.presses F(2,20)= 0.20; p=NS	

Table S2. Corresponding degrees of freedom, F, t and P- values of the statistical results for Figures 2-3 represented in the Supporting Information

FIG.S2	Line	Dose	Line x Dose
Nicotine infusions	$F_{(1,15)} = 8,417$; $p<0.01$	$F_{(2,30)} = 56.66$; $p<0.000$	$F_{(2,30)} = 0.52$; p= NS
Nicotine active responses	$F_{(1,15)} = 10.28$; $p<0.01$	$F_{(2,30)} = 53.55$; $p<0.000$	$F_{(2,30)} = 0.33$; p= NS
Nicotine inactive responses	$F_{(1,15)} = 0.12$; p= NS	$F_{(2,30)} = 0.65$; p= NS	$F_{(2,30)} = 1.06$; p= NS

Table S3. Corresponding degrees of freedom, F, t and P- values of the statistical results for Figure S2 represented in the Supporting Information