Supplementary Information

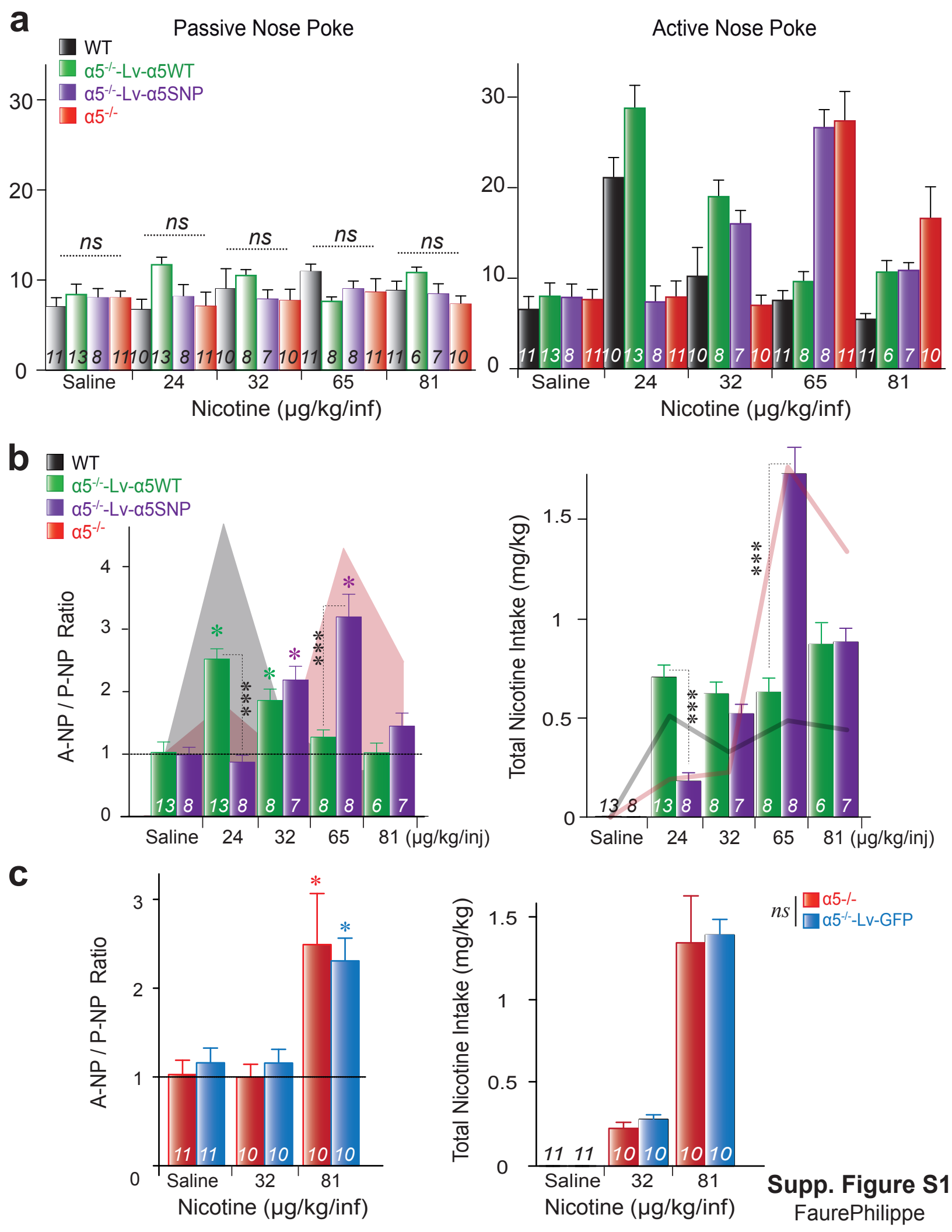
1. Supplementary Data

1.1 Sup. Fig. S1: IVSA profiles.

(a) Mean±SEM Passive (*left*) and Active (*right*) nose poke activity for WT (black), $\alpha5^{-/-}$-Lv-α5WT (green) and $\alpha5^{-/-}$-Lv-α5SNP (purple) and $\alpha5^{-/-}$ (red) mice acutely self-administering different doses of nicotine (μg/kg/infusion). The number of mice tested is indicated in black (*left*) or white (*right*) for each corresponding group.

(b) IVSA is restored in $\alpha5^{-/-}$-Lv-α5WT, but not in $\alpha5^{-/-}$-Lv-α5SNP mice. *Right*: Mean±SEM *A-NP/P-NP* ratio for $\alpha5^{-/-}$-Lv-α5WT (green) and $\alpha5^{-/-}$-Lv-α5SNP (purple) mice acutely self-administering different doses of nicotine (μg/kg/infusion). *Left*: Mean±SEM of total nicotine intake (μg/kg) by WT (black) and $\alpha5^{-/-}$ (red) mice. ***p<0.005, *p<0.05, Student *t*-test. The number of mice tested is indicated in white for each corresponding group.

(c) GFP lentiviral re-expression does not alter the $\alpha5^{-/-}$ mouse IVSA profile. *Left:* Mean±SEM A-NP/P-NP ratio for $\alpha5^{-/-}$ (red) and $\alpha5^{-/-}$-Lv-GFP (blue) mice self-administering different nicotine concentrations (μg/kg/infusion). Number of mice tested is indicated in white for each corresponding group. *p<0.05 vs corresponding yoked passive, Student *t*-test. *Right:* Mean±SEM of total nicotine intake (μg/kg).


a
Passive Nose Poke
Active Nose Poke
WT
α5-/--Lv-α5WT
α5-/--Lv-α5SNP
α5-/-
ns
Saline
24
32
65
81
Nicotine (µg/kg/inf)
b
A-NP / P-NP Ratio
Total Nicotine Intake (mg/kg)
81 (µg/kg/inj)
c
α5-/-
α5-/--Lv-GFP
Supp. Figure S1
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1.2 Sup. Fig. S2: Electrophysiological analysis of VTA DAergic neurons.

(a, b) Spontaneous activity is not altered in any group analyzed.

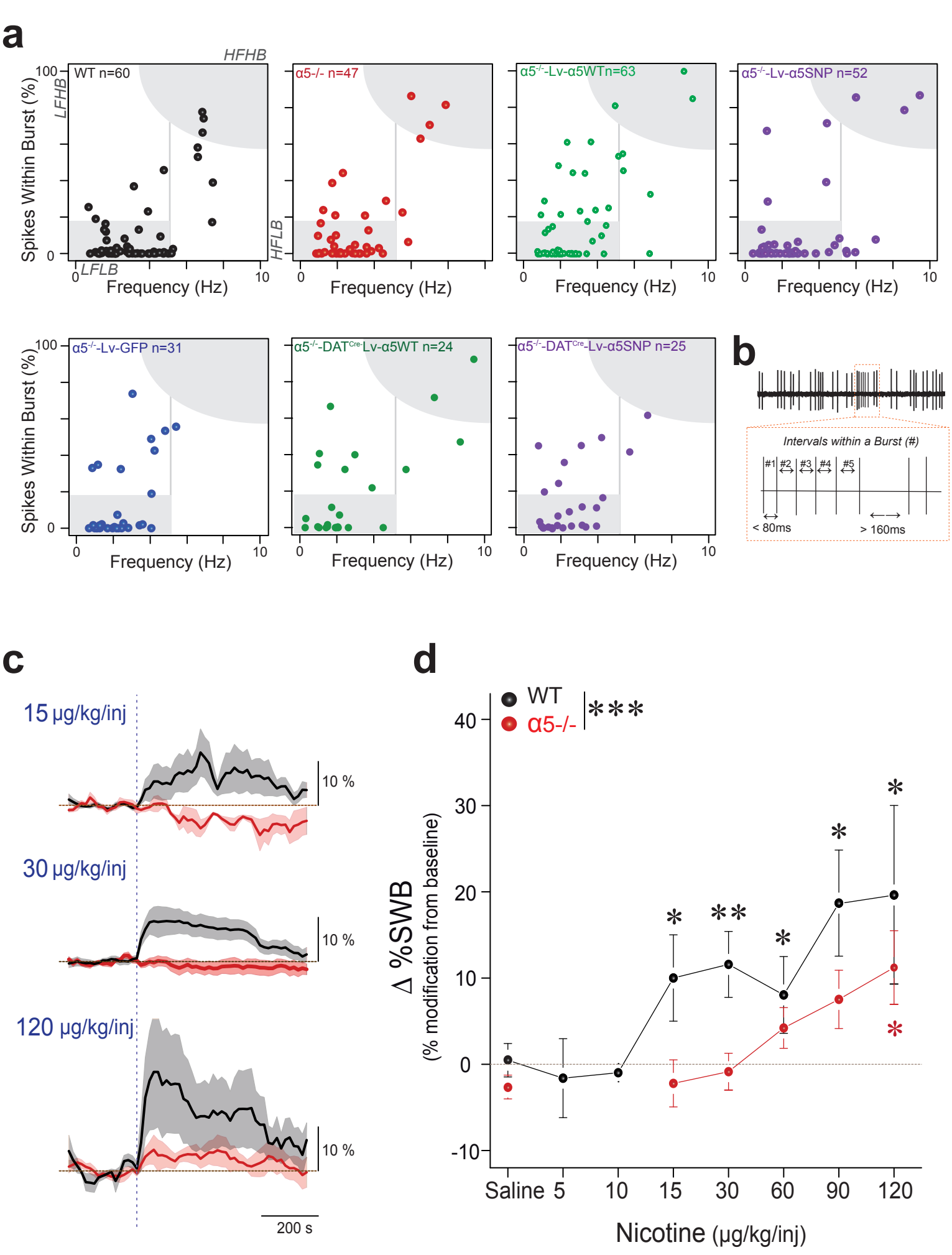
(a) Plot of mean firing frequency (Hz) against percentage of spikes within a burst (%SWB) for n=60 individual cells in WT (black), n=58 in $\alpha5^{-/-}$ mice (red), n=61 in $\alpha5^{-/-}$-Lv-α5WT (Top, green), n=52 in $\alpha5^{-/-}$-Lv-α5SNP (Top, purple) and n=31 in $\alpha5^{-/-}$-Lv-GFP (blue), n=24 in $\alpha5^{-/-}$-DATCre-Lv-α5WT (*Bottom*, green), n=25 in $\alpha5^{-/-}$-Lv-α5SNP (*Bottom*, purple) mice. Four main functional sub-populations *(LFLB, LFHB, HFLB* and *HFHB)* are delineated by grey masks.

(b) Principle of burst structure analysis.

(c, d) Shift of dose-response in nicotine-elicited DA cell bursting activation in $\alpha5^{-/-}$ mice.

(c) Mean±SEM of increased response from baseline in bursting activity of WT (black) and $\alpha5^{-/-}$ (red) mice for indicated nicotine concentrations (time of injection indicated by vertical dashed blue lines).

(d) Dose-response curve of DA cell bursting activity as a function of nicotine dose (in μg/kg/injection) for WT (black) and $\alpha5^{-/-}$ (red) mice. ***$p<0.0005$, Kruskall Wallis; *$p<0.05$, **$p<0.01$ vs baseline, post-hoc Wilcoxon test; 0% indicated by horizontal dashed black line.



Supp. Figure 2
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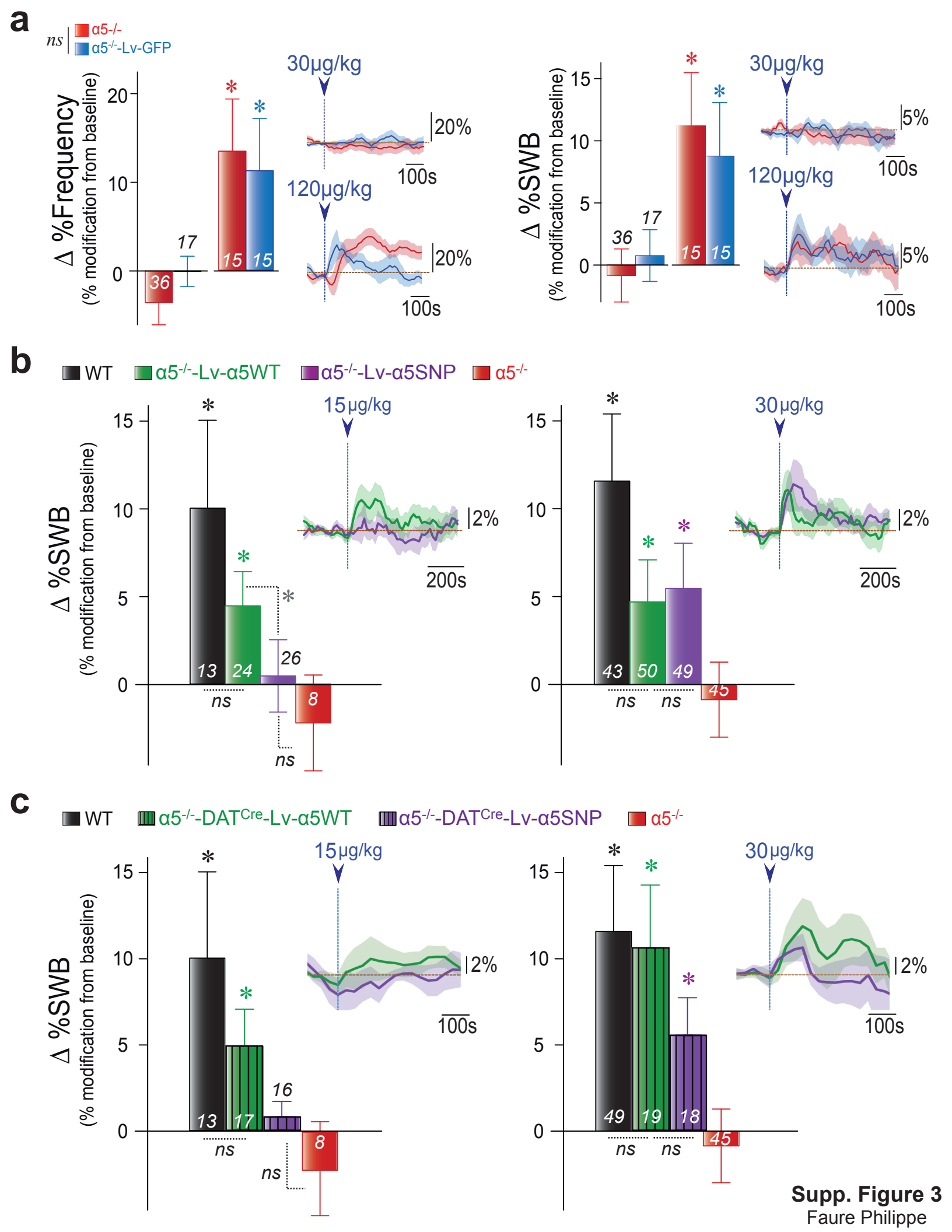
1.3 Sup. Fig. S3: VTA DAergic neurons activity in respons to an acute nicotine injection.

(a) $\alpha5^{-/-}$ and $\alpha5^{-/-}$-Lv-GFP mice displayed similar basal and nicotine-induced VTA DA cells firing frequency and bursting. *Left: Firing frequency*. Top: Mean±SEM of maximum response elicited by indicated nicotine concentrations in $\alpha5^{-/-}$ (red) and $\alpha5^{-/-}$-Lv-GFP (blue) mice. *p<0.05 vs corresponding baseline, Wilcoxon test; ns=no significant nicotine response differences between $\alpha5^{-/-}$ and $\alpha5^{-/-}$-Lv-GFP mice. Number of recorded neurons is indicated in white for each corresponding group. *Bottom*: Mean±SEM of variation in percentage from baseline elicited by nicotine injection (dashed blue lines) in $\alpha5^{-/-}$ (red) and $\alpha5^{-/-}$-Lv-GFP (blue) mice. Nicotine concentrations (µg/kg) as indicated. *Rigth*: *Bursting*. Top: Mean±SEM of maximum modification (%SWB) elicited by indicated nicotine concentrations in $\alpha5^{-/-}$ (red) and $\alpha5^{-/-}$-Lv-GFP (blue) mice. *p<0.05 vs corresponding baseline, Wilcoxon test; ns=no significant nicotine elicited response differences between $\alpha5^{-/-}$ and $\alpha5^{-/-}$-Lv-GFP mice. *Bottom*: Mean±SEM of variation of %SWB from baseline elicited by nicotine injection (dashed blue lines) in $\alpha5^{-/-}$ (red) and $\alpha5^{-/-}$-Lv-GFP (blue) mice. Nicotine concentrations (µg/kg) as indicated. Number of recorded neurons is indicated in white for each corresponding group.

(b) Mean±SEM of maximum bursting response (%SWB) elicited by two different nicotine concentrations in WT (black), $\alpha5^{-/-}$-Lv-α5WT (green), $\alpha5^{-/-}$-Lv-α5SNP (purple) and $\alpha5^{-/-}$ (red) mice. *p<0.05 vs baseline or between each group, Wilcoxon test; ns=not-significant differences. Inset: Mean±SEM of variation of the %SWB from baseline elicited by nicotine injection (dashed blue lines) in $\alpha5^{-/-}$-Lv-α5WT and $\alpha5^{-/-}$-Lv-α5SNP

mice. Number of recorded neurons is indicated in white for each corresponding group.

(c) Mean±SEM of maximum bursting response (%SWB) elicited by two different nicotine concentrations in WT (black), $\alpha5^{-/-}$-DAT^{Cre}-Lv-α5WT (green), $\alpha5^{-/-}$-DAT^{Cre}-Lv-α5SNP (purple) and $\alpha5^{-/-}$ (red) mice. *$p<0.05$ vs baseline or between each group, Wilcoxon test; ns=not- significant differences. Inset: Mean±SEM of variation of the %SWB from baseline elicited by nicotine injection (dashed blue lines) in $\alpha5^{-/-}$-DAT^{Cre}-Lv-α5WT and $\alpha5^{-/-}$-DAT^{Cre}-Lv-α5SNP mice. Number of recorded neurons is indicated in white for each corresponding group.



Supp. Figure 3
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1.5 Supplementary Text

Spontaneous DA cell activity is not altered.

Dopamine (DA) cells fire in different modes of activity[15]. They were classified into four main groups as the LFLB, LFHB, HFLB and HFHB modes. The low-frequency and low-burst firing LFLB is characterized by a firing rate lower than 5Hz and a percentage of spikes within bursts (%SWB) lower than 20%. The low-frequency and high-burst firing LFHB is characterized by a firing rate lower than 5Hz and a high %SWB. The high-frequency and low-burst firing HFLB corresponds to a high regular firing rate and a small %SWB. Finally, the high-frequency and high-burst firing HFHB is characterized by a high firing rate and a high %SWB. We analyzed these DA subpopulations in both WT and $\alpha5^{-/-}$ mice and found no differences in firing patterns (firing frequency (Ff) and %SWB, with Kolmogorov-Smirnov statistical test, $p>0.05$) between WT (mean Ff=3.29; mean %SWB=10.67, n=60) and $\alpha5^{-/-}$ mice (mean Ff=2.91; mean %SWB=12.50, n=47). We also analyzed the organization into subpopulations in $\alpha5^{-/-}$-Lv-α5WT (mean Ff=2.95; mean %SWB=16.45, n=63), $\alpha5^{-/-}$-Lv-α5SNP (mean Ff=2.92; mean %SWB=11.89, n=52) and $\alpha5^{-/-}$-Lv-GFP mice (mean Ff=2.68; mean %SWB=16.48, n=31), and also in $\alpha5^{-/-}$-DAT^{Cre}-Lv-α5WT (mean Ff=3.04; mean %SWB=21.34, n=24) and $\alpha5^{-/-}$-DAT^{Cre}-Lv-α5SNP (mean Ff=2.12; mean %SWB=17.64, n=25), and again observed no differences (**Figure S1a**). Finally, no statistical differences between any subpopulation organization was observed (Chi square test, $p>0.05$). We conclude that the same spontaneous firing patterns are conserved in all genotypes studied.

2. Supplementary Methods

2.1 Subjects. Male C57BL/6J (Charles River, France), α5 nAChR KO mice[25] ($\alpha5^{-/-}$ mice; Charles River, France) and their corresponding WT controls were used, weighing 24-28g at the time of experiments. The animals were housed eight per cage with food and water available *ad libitum*. Animals were kept under standard conditions (temperature 21±1°C, 60-65% relative humidity) on a reversed 12h light/dark cycle (light on 7.00 p.m.) and left undisturbed for at least ten days before starting the experimental procedure. All experiments were performed in strict accordance with both the Guide for the Care and Use of Laboratory Animals (NIH) and the European Commission regulations for animal use in research (CEE n° 86/609).

2.2 Drugs. For all experiments, (-)-nicotine bitartrate (Sigma, Italy) was freshly dissolved in 0.9% saline, and the pH adjusted to 7.2±0.1 with NaOH (0.1N). For behavioral experiments the drug doses used in this study ranged from 24 to 81µg/kg/inj (free base). For electrophysiological recordings the drug doses used were 5, 10, 15, 30, 60, 90 and 120µg/kg/inj (free base).

2.3 Nicotine self-administration task (IVSA). Nicotine naïve mice are tested in pairs as previously described[16,17]. IVSA experiments took place during the dark phase of the cycle, between 9.00-12.00 am. Mice were tested in pairs of identical test cages (8*8*8cm inner size), each presenting a central frontal hole (diameter: 25mm) 1cm above the box floor

fitted with an infrared sensor interfaced to an operating computer that controlled an automatic syringe pump (PHM-100A, Med Associates). A rear vertical chink (5mm wide) was made on the opposite wall through which the tail was extended outside the box and taped to a horizontal surface allowing access to the lateral tail veins with a 27G winged needle (external diameter: 0.4mm), connected to the syringe through Teflon tubing.

Since mice were housed on a reversed light/dark cycle, they were kept in the dark during transportation from the housing to the experimental room. This room was always the same, exclusively dedicated to self-administration experiments in mice, and had the same environmental conditions as the housing room (temperature 21±1°C; 65% humidity).

Experiments were conducted in the dark. A dim light located above the apparatus was switched on for a few minutes for incannulation of the tail vein only, whereas the box containing the animal was covered with black cloth so that mice were never exposed to the light. No infrared lighting or other expedients were used to provide vasodilation of the tail vein or to facilitate needle insertion. No detergents, soap or alcohol were used between sessions, distilled water only being used to clean boxes at the end of each session.

Animals were first placed in the test cage for 10min of habituation (pre-test) with their tails taped but no needle inserted. Pairs of animals were selected on the basis of an approximately equal level of nose-poking activity during the pre-test. Thereafter, the matched pairs were placed into the experimental boxes, one mouse defined as active (A) and the other one as passive (P), and needles inserted in the lateral tail vein. Animals

were randomly allocated to the different experimental groups and allowed access to nicotine under a continuous reinforcement (FR-1) schedule. Each nose-poke (NP) of the active (A) mouse activated the computer-operated syringe pump delivering either nicotine or saline infusion (volume of injection: 1.0µl, infusion time: 1s) both to the A and the passive (P) mouse, so that animals received the same amount of the drug simultaneously. A white cue light was activated by each NP of the A mouse for 1s, i.e., during nicotine delivery, thus serving as a drug-associated cue along with the noise of the activated syringe pump. No cue light was activated by NPs of the yoked P mouse.

NPs of the P mouse were recorded but had no scheduled consequences. A short time-out period (i.e., inactivation of NP responses) of 2s was imposed after each nicotine infusion. Each treatment included at least 6 pairs of animals. IVSA sessions lasted 30min.

To obtain gradual measurements of the reinforcing effects of nicotine, the mean ratio (or Reinforcement Index) R[16,17,42] between the number of responses (NPs) of the active (A) and passive (P) mice during the 30 min session was calculated, nicotine effect being considered rewarding, neutral or aversive when R was greater than, equal to, or less than 1, respectively.

2.4 *In vivo* electrophysiological recordings

Mice were anesthetized with an intraperitoneal injection of chloral hydrate (8%), 400 mg/kg, supplemented as required to maintain optimal anesthesia throughout the experiment, and positioned in a stereotaxic frame (David Kopf). Body temperature was kept at 37°C by means of a thermostatically controlled heating blanket. All animals had a catheter inserted into their saphenous vein for *i.v.* administrations of drugs.

An incision was made in the midline to expose the skull. A hole was drilled above the VTA (coordinates: between 3.5±0.3 mm posterior to Bregma and 0.5±0.3 mm lateral to the midline[14,21]). Recording electrodes were pulled with a Narishige electrode puller from borosilicate glass capillaries (with outer and inner diameters of 1.50 and 1.17mm, respectively; Harvard Apparatus). The tips were broken under microscope control and filled with 1.5% neurobiotin in 0.5% sodium acetate. These electrodes had tip diameters of 1-2 mm and impedances of 4-8 MΩ. A reference electrode was placed into the subcutaneous tissue. The recording electrodes were lowered vertically through the hole with a micro drive. Electrical signals were amplified by a high-impedance amplifier (Axon Instruments) and monitored visually with an oscilloscope (Tektronix TDS 2002), and audibly through an audio monitor (A.M. Systems). When a single unit was well isolated, the oscilloscope sweep was triggered from the rising phase of the action potential and set so as to display the action potential over the entire screen (usually 0.5 ms/division). Such continuous observation of the expanded action potential provided assurance that the same single unit was being monitored throughout the experiment. The unit activity digitized at 25 kHz was stored in Spike2 program (Cambridge Electronic Design, UK).

The electrophysiological characteristics of VTA DA neurons were analyzed in the active cells encountered by systematically passing the microelectrode in a stereotaxically defined block of brain tissue including the VTA. Its margins ranged from 3 to 3.8mm posterior to Bregma, 0.25 to 0.8mm mediolateral with respect to the Bregma point, and 4.0 to 4.8mm ventral to the cortical surface according to the coordinates of Paxinos and Franklin[43]. Sampling was initiated on the right side, and then further on the left side.

After a baseline recording of 5-10min, 10µl saline (0.9% NaCl) was injected into the saphenous vein, and after 5-10min, 10µl of nicotine. In paired injection protocols, nicotine concentrations were injected in a random sequence, with a delay of 15-30min observed between consecutive injections.

Cell identification. Extracellular identification of DA neurons was based on their location as well as on the set of unique electrophysiological properties that characterize these cells *in vivo*: (1) a typical triphasic action potential with a marked negative deflection; (2) a characteristic long duration (>2.0 ms); (3) an action potential width from start to negative trough >1.1 ms; (4) a slow firing rate (between 1 and 10Hz) with an irregular single spiking pattern and occasional short, slow bursting activity. These electrophysiological properties distinguish DA from non-DA neurons[23,24,44].

Immunohistochemical identification of some recorded neurons was performed following the recordings. At the end of the recording period, the neurons were stimulated by application of positive current steps to electroporate neurobiotin onto the neurons. Following the sacrifice of the animal, the brain was rapidly removed and fixed by immersion in 4% paraformaldehyde. Following a period of at least 3 days of fixation at 4°C, serial 60µm sections were cut from the midbrain with a vibratome (Leica Microsystems). These sections were transferred to chilled buffered saline (PBS), before being pre-blocked and permeabilized for 1hr in a solution of 3% bovine serum albumen (BSA) and 0.2% Triton-100x in PBS. The sections were then incubated overnight at 4°C in a primary antibody solution containing the relevant antibodies, 1.5% BSA and 0.2% Triton-100x in PBS with 1/200 mouse anti-Tyrosine Hydroxylase (TH) antibodies and

1/1000 rabbit anti-GFP antibodies (Monoclonal anti-tyrosine hydroxylase produced in mouse, Sigma-Aldrich; Polyclonal anti-GFP rabbit serum, Invitrogen). Following three 5min rinses in PBS, the sections were incubated at room temperature with appropriate secondary antibodies (1/200 Cy3 Donkey anti-mouse IgG, Jackson ImmunoResearch + 1/200 FITC Donkey anti-rabbit, Jackson ImmunoResearch) and streptavidin-AMCA 1/200 (AMCA-conjugated Streptavidin, Jackson ImmunoResearch) for neurobiotin visualization, in the same solution as for the pr 1x phosphate imary antibodies. Sections were then rinsed a further three times for 5min, mounted on slides (SuperFrostSlide, Menzel Gläser), and cover-slipped with ProLong Gold antifade reagent (Invitrogen). Microscopy was carried out with a fluorescence microscope, and images captured using a CoolSNAP Fx camera (Photometrics) and MetaMorph imaging software (Molecular Devices). Neurons labeled positive for TH and neurobiotin within the VTA depending on the Paxinos area definition were considered confirmed DA cells.

In some cases, we performed pharmacological identification (typically, the last recorded neurons of the experiments) by using the DA receptor agonist quinpirole (1mg/kg *i.v.*, Tocris) followed by the D2 DA receptor antagonist eticlopride (1 mg/kg *i.v.*; Tocris). Thereby, with immuno-histochemistry or pharmacological techniques, 13.3% of WT VTA cells were confirmed DA, like 19.1% of the $\alpha5^{-/-}$ cells, 23.8% of the $\alpha5^{-/-}$-Lv-α5WT cells, 25% of the $\alpha5^{-/-}$-Lv-α5SNP cells, and 19.4% of the $\alpha5^{-/-}$-Lv-GFP cells.

2.5 Slice preparation and whole-cell recordings

Male adult mice were deeply anesthesized with ketamine and xylasine. Coronal midbrain sections (250µm) were sliced using a vibratome (VT1000S; Leica) after intracardial

perfusion of with cold (4°C) artificial cerebrospinal fluid (ACSF) containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 6 MgCl2, 26 NaHCO3, 25 sucrose, 2.5 Glucose, 1 Kynurenate (pH 7.2, 325 mosM). After 1h at 37°C for recovery, individual slices were transferred to a recording chamber and continuously superfused at 2 ml/min with oxygenated ACSF containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 26 $NaHCO_3$, 15 sucrose, 10 Glucose (pH 7.2, 325 mosM) at room temperature. Whole-cell recordings were performed with a KGlu based intra-pipette solution containing (in mM): 144 KGlu, 3 $MgCl_2$, 10 HEPES, 0.5 EGTA and 3 mg/ml biocytin (pH7 7.2, 295mOsm) using a patch-clamp amplifier (Multiclamp 700B, Molecular Devices, Sunnyvale, CA) connected to a Digidata 1440A interface board (Molecular Devices). Signals were amplified and collected using the data-acquisition software pClamp 10.2 (Molecular Devices). DMPP (Dimethylphenylpiperazinium, 100µM, Sigma) was applied through a local perfusion system in the presence of a cocktail of antagonists: 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10µM), 2-amino-5-phosphonovalerate (APV, 50µM), Gabazine (GBZ, 1µM), haloperidol (2µM), atropine (8µM) from Sigma (Saint Louis, MO, USA) and CGP55845 (4µM) from Tocris (Bristol, GB).

2.6 Construction of lentiviral vectors and lentiviral re-expression

Construction of lentiviral vectors. The lentiviral expression vectors are derived from the pHR' expression vectors first described by Naldini et al.[20], with several subsequent modifications. In the lentiviruses used in this study, the bi-cistronic expression of mouse wild-type or mutant α5 nAChR subunit cDNAs and the eGFP cDNA are under the

control of the mouse phosphoglycerate kinase (PGK) promoter. Further details can be found in Maskos et al.[21].

To create the pTripΔU3-PGK-α5-IRES2-eGFP, a two step strategy was used. First, the α5 cDNA from pCS2+ plasmid (a kind gift of Dr. Jerry Stitzel), was cloned into the pIRES2-eGFP expression plasmid (Clontech) between XhoI and EcoRI of the multiple cloning site. Then, in the pre-existing pTRIP-β2-IRES2-eGFP lentiviral vector[21] the fragment coding for β2-IRES2-eGFP was replaced by the fragment α5-IRES2-eGFP between the XhoI and BsrGI sites. The same strategy was used to create the PGK-α5SNP-IRES2-eGFP. Before sub-cloning the whole fragment α5SNP- IRES2-eGFP into the lentiviral vector, a mutagenesis (QuickChange, Stratagene) was carried out on the plasmid α5-IRES2-eGFP to introduce the α5SNP which consists of a substitution of the acid aspartic in position 397 by an asparagin.

Sequences of the primers using to introduce the α5SNP:

Forward : $^{5'}$GAGGCCGCGCTCAATTGCATTCGCTACATC$^{3'}$

Reverse : $^{5'}$GATGTAGCGAATGCAATTGAGCGCGGCCTC$^{3'}$

Lentiviral re-expression. Vectors carrying either α5-IRES2-eGFP, α5D397N-IRES2-eGFP (SNP) or only eGFP under control of the PGK promotor (phosphoglycerate kinase) were used to generate viral particules in HEK-293T cells. Immediately before stereotaxic injections, lentiviruses were diluted in PBS to achieve a dose of injection of 1.7-1.9 x10^6 TU in 2µl (equivalent to 150ng of p24 protein for bi-cistronic viruses).

To create the conditional lentivectors the same sub-clonning strategy was used. In the previously describe lentivector pTrip PDGF-floxed-mCherry-eGFP-WPRE [22]we took advantage of the compatibility between SalI and XhoI restriction sites to replaced the

eGFP cDNA by the fragment coding either for the WT or the polymorphic version of α5 subunit.

Lentivirus stereotaxic injections. Mice aged 10 to 12 weeks were anaesthetized using 250µl of ketamine 1.5% (Merial, France)/xylazine 0.05% (Bayer Healthcare, France) in PBS. The mouse was introduced into a stereotaxic frame adapted for use with mice[20]. Lentivirus (2µl at 75ng p24 protein per µl for bi-cistonic lentiviral vectors and 2µl at 25ng p24 protein per µl for eGFP vector) was injected bilaterally at: antero-posterior –3.4mm, lateral ±0.5mm from Bregma, and –4.4mm from the surface for VTA injection. All procedures were carried out in accordance with European Commission directives 219/1990 and 220/1990, and approved by Animalerie centrale and Médecine du travail, Institut Pasteur. The mice were tested after 6 to 8 weeks of viral expression.

We generated α5KO-DATCre mice coupled with a Cre recombinase-activated lentiviral expression vector to drive α5-WT or α5-SNP exclusively in VTA DA neurons. There is no basal expression of DAT in IPN in DATCre mice[45]. Therefore, lentiviral injection into the VTA guarantees that α5-WT or α5-SNP were exclusively expressed in VTA DA neurons, and not in the IPN or other brain areas.

2.7 Data analysis

Behavioral data. The number of NPs for both A and P mice in each treatment group was analyzed, first with a Shapiro test to certify the normality of distributions, then with two-way ANOVA to evaluate effects of the drug delivery mode ("contingently" versus "non-contingently"), unit dose (including vehicle), and interactions between group and drug dose. For *post-hoc* comparisons, a Student *t*-test was used to compare single groups of

mice, and respective vehicle controls. Statistical significance was set at $p<0.05$. The whole study was designed as a between-subjects (independent groups) experiment, because each treatment was performed on a single set of animals. To study differences between WT and $\alpha5^{-/-}$ mice, two-way ANOVA was used and statistical significance was set at $p<0.05$. To evaluate differences between the self-administration profile of the $\alpha5^{-/-}$-Lv-α5WT and $\alpha5^{-/-}$-Lv-α5SNP mice we used the Student *t*-test with repeated Bonferroni corrections.

Electrophysiological data. Statistics and electrophysiological recordings of DA cell activity were analyzed using R (*http://www.r-project.org*.) a language and environment for statistical computing. DA cell firing was analyzed with respect to the average firing rate and the percentage of spikes within a burst (%SWB, number of spikes within a burst divided by the total number of spikes). Bursts were identified as discrete events consisting of a sequence of spikes which onset is defined by two consecutive spikes within an interval <80 ms, and that terminate with an interval >160 ms[23, 24, 46]. Firing rate x and %SWB were evaluated on successive windows of 60s, with a 45s overlapping period. To quantify nicotine effects, each cell's activity was rescaled by its averaged baseline value x0 (for the firing frequency) and %SWB0 (for the bursting), estimated over a 3min period before nicotine injection. For the firing frequency x, the rescaling X, expressed as % of the baseline, is defined by $X=x*100/x0$. For the busting parameter, we used the difference from the value during baseline $Y=\%SWB-\%SWB0$. For each neuron, we determined Xbef, the maximum of fluctuation before substance injection (during 3min used as baseline), and Xaft the maximum of fluctuation after injection (during 3min after injection). Because of non- normal distributions of data (Shapiro test), effects of

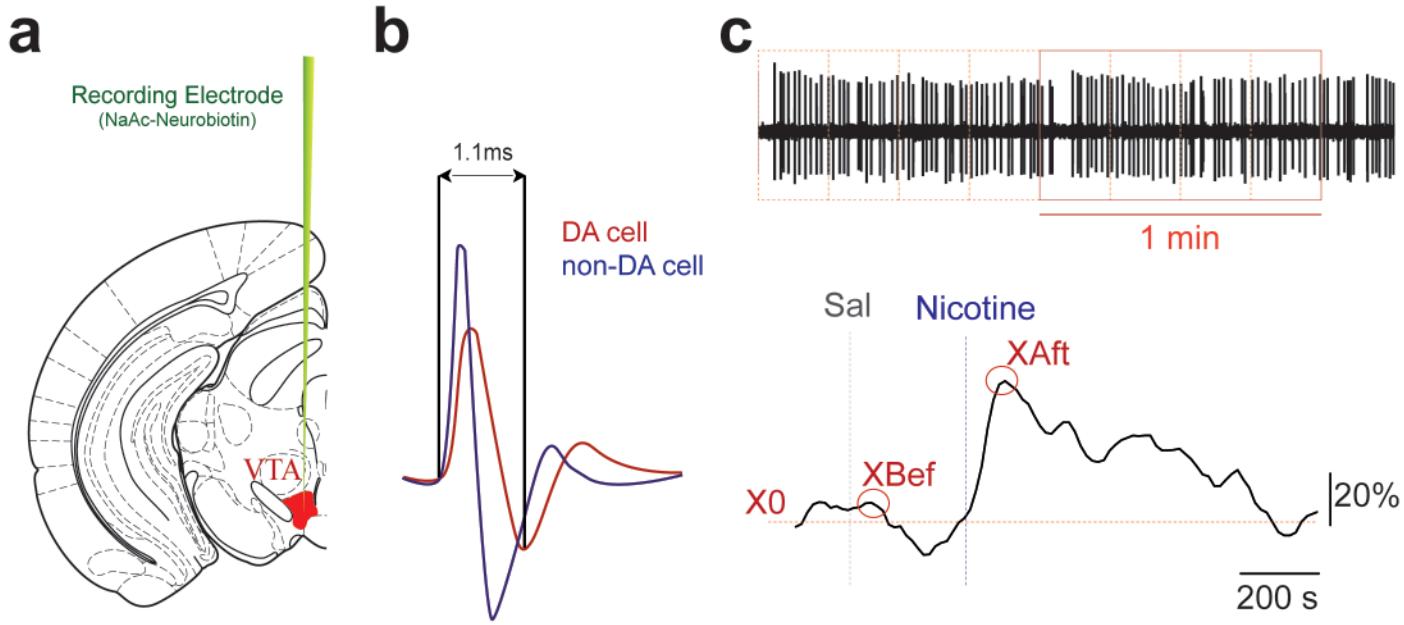
nicotine were analyzed using a paired non-parametric Wilcoxon test to compare Xbef (Ybef) with Xaft (Yaft) for firing frequency and %SWB, respectively. Differences were considered significant if $p<0.05$. Data are expressed as mean ± standard deviation of the mean (SEM). For each nicotine injection using the Wilcoxon paired test, the null hypothesis is that the location parameters of the distribution are the same before and after nicotine injection. Differences were considered significant if $p<0.05$. To study differences between WT and $\alpha5^{-/-}$ mouse dose-response curves, we used the Kruskall Wallis non-parametric test, and statistical significance was set at $p<0.05$. For $\alpha5^{-/-}$-Lv-α5WT / $\alpha5^{-/-}$-Lv-α5SNP and $\alpha5^{-/-}$-DAT^{Cre}-Lv-α5WT / $\alpha5^{-/-}$-DAT^{Cre}-Lv-α5SNP analyses we used a Wilcoxon non-paired test with Bonferroni corrections. For all analysis statistical significance was set at $p<0.05$.

2.8 Sup. Fig. S4, related to Figure 2, 4 and 5:

(a) The recording electrodes were lowered vertically through the hole with a micro drive into the VTA.

(b) Extracellular distinction between DA neurons (red) with a long duration (>2.0 ms) and an action potential width from start to negative trough >1.1 ms and a non-DA cell (blue).

(c) *Top*: Estimation of firing frequency. Red squares delimit 60 second windows with a 15 second lag used to calculate the firing frequency. *Bottom*: For each cell x0 mean of firing frequency is calculated during 5 minutes of baseline, then Xbef (red circle) is calculated, the maximum of firing frequency variation in the 3 minutes before the nicotine injection. XBef is compared with XAft (red circle), the maximum of firing frequency modulation during the 3 minutes after injection.



Supp. Figure 4
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3. Supplementary references

42. Kuzmin A, Semenova S, Zvartau EE, Van Ree JM. Enhancement of morphine self-administration in drug naive, inbred strains of mice by acute emotional stress. *Eur Neuropsychopharmacol* 1996; **6**: 63–68.

43. Paxinos G, Franklin KBJ. *The mouse brain in stereotaxic coordinates*. Academic Press 2004.

44. Ungless MA, Grace AA. Are you or aren't you? Challenges associated with physiologically identifying dopamine neurons. *TINS* 2012; **35**: 422–430.

45. Turiault M, Parnaudeau S, Milet A, Parlato R, Rouzeau J-D, Lazar M *et al.* Analysis of dopamine transporter gene expression pattern -- generation of DAT-iCre transgenic mice. *FEBS J* 2007; **274**: 3568–3577.

46. Floresco SB, West AR, Ash B, Moore H, Grace AA. Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat Neurosci* 2003; **6**: 968–973.