Median and Dorsal Raphe Serotonergic Neurons Control Moderate Versus Compulsive Cocaine Intake

Supplementary Information

Supplementary Methods and Materials

Animals

All rats used were adult male Wistar rats. They were housed in groups of 2-3 in Macrolon type III cages (42 x 26 x 15 cm) under a 12 h / 12 h reversed day/night cycle (lights off at 8:00 AM) in a temperature-controlled room (22±2°C). Food pellets and water were available *ad libitum*, except during the cocaine self-administration sessions. All procedures were carried out in agreement with the current National Research Council Guide for the Care and Use of Laboratory Animals and were approved by local Institutional Animal Care and Use Committees. All efforts were made to reduce the number of animals used and their suffering.

Drug

Cocaine was provided by National Institute on Drug Abuse (NIDA), Rockville, MD, and was dissolved in saline 0.9%.

Experimental approaches

Two approaches were used to study the effects of serotonin transporter (SERT) reduction on the voluntary intake of cocaine. In the first approach (A, performed at The Scripps Research Institute), rats were locally infused with viral vectors encoding a short hairpin RNA (shRNA) directed against the SERT transcript. In the second approach (B, performed at Radboud University Medical Centre), constitutive SERT knockout (KO) rats were used. A schematic representation of the experimental procedures can be found in Supplementary Figure S1.

Approach A: local SERT knockdown (KD)

Intravenous catheterization. Male Wistar rats (Charles River) weighing 250-300 g were implanted with a micro Renathane catheter (0.3 mm i.d. × 0.64 mm o.d.; MRE037, Braintec scientific Inc, Braintree, MA) into the right external jugular vein according to previously reported procedures (1). This aseptic surgery procedure was performed under isoflurane anesthesia (2-3%). After surgery, rats were given analgesics (Flunixin®, 2.5 mg/kg, s.c., Merck Animal Health, Madison, NJ) and antibiotics (Cefazolin®, 0.033 mg/0.1 mL, i.v., Sagent Pharmaceuticals, Schaumburg, IL) for at least one week. Catheter patency was maintained by daily infusion of 0.1 mL heparinized saline (30 USP, Hospira, Lake Forest, IL).

Self-administration chambers. Cocaine self-administration was performed in standard operant chambers (28 x 26 x 20 cm, Med Associates Inc., St Albans, VT) that were placed in a ventilated, light- and sound-attenuating cubicle. The cocaine self-administration chambers were equipped with a swivel system allowing rats to move freely during self-administration sessions. Cocaine was delivered by a 15 r.p.m. syringe pump (Razel Scientific Instruments, Georgia, VT). The start of a session was signaled by the presentation of 2 retractable levers into the self-administration chamber. Pressing the right lever was programmed to deliver cocaine (volume: 0.1 mL in 4s) whereas pressing the left lever had no programmed consequences. During drug administration, a stimulus light above the active lever was illuminated and illumination lasted throughout a time-out period of 20 s, during which operant responding was not reinforced.

Cocaine self-administration training. One week after surgery, rats were trained to self-administer cocaine (0.5 mg/kg/infusion) under a fixed ratio 1 (FR1) schedule of reinforcement (one lever press resulted in one drug infusion, see: 2). Additional groups of cocaine-naive median raphe nucleus (MRN) and dorsal raphe nucleus (DRN) SERT KD and their control rats also underwent intravenous catheterization, were handled daily, and received daily infusion of heparinized saline, but were not exposed to the self-administration chambers (2).

Production of shRNA-encoding viral vectors. A web-based scoring algorithm (BLOCK-iT, Invitrogen) was used to generate a list of candidate shRNA sequences with a high SERT silencing probability. Adeno-associated viral serotype 2 (AAV2) vectors expressing one of two top-ranked sequences (shSERT1, target sequence: GCAACTGCACCAACTACTTCG and shSERT2, target sequence: GCCCTCTGTTTCTCCTGTTCA; loop sequence: CTTCCTGTCA) or a scrambled control sequence (shSCR, GTCTGTTTCCCTCGCTACTCT) were obtained from Applied Viromics (Fremont, CA). AAV capsid serotype 2 was used because this serotype has consistently been shown to yield small transduction volumes (3-5). A detailed description of the viral vector production and the purification procedures can be found on their website (www.appliedviromics.com). In short, complementary oligonucleotides encoding the sense and antisense strands separated by a 9-nucleotide loop were synthesized, annealed and cloned into a recombinant AAV2 backbone behind the mouse U6 promoter and flanked by inverted terminal repeats. This plasmid also contained the sequence of green fluorescent protein (GFP), which was used as a transduction reporter, under the control of the cytomegalovirus promoter. Viral particles were purified by anion-exchange column chromatography

(6). Viral vector stocks were delivered in phosphate buffered saline (PBS), pH 7.4, with 0.001% Pluronic F-68 (titers were 2.1-7.1 $x10^{11}$ GC/mL).

Intra-raphe nucleus infusion of viral vectors. At the end of cocaine self-administration training, rats were divided into 3 groups balanced by their number of infusions during the final training session. Rats were locally injected with either shSCR, shSERT1, or shSERT2. To this end, rats were anesthetized with 2-3% isoflurane and mounted in a stereotactic frame (David Kopf Instruments). A 30-gauge stainless steel needle pre-loaded with viral vector stock was lowered into the median (anterior-posterior: -8.0 mm from bregma, medio-lateral: 0.0 mm from midline, dorso-ventral: -8.6 mm from dura) or dorsal (anterior-posterior: -7.5 mm from bregma, medio-lateral: 0.0 mm from midline, dorso-ventral: -7.1 mm from dura) raphe nucleus (see Supplementary Figure S2). A volume of 2.0 µL was infused over a 20 min period using a Hamilton microsyringe controlled by a micro infusion pump. The combination of using this slow-infusion procedure and AAV serotype 2 typically resulted in transduction volumes not exceeding 1 mm^3 (see Supplementary Figure S3). The needle was left in place for an additional 5 min to minimize backflow up the needle track.

Short and long access cocaine self-administration. Two weeks after stereotactic surgery, one group of rats was allowed to self-administer 0.5 mg/kg/infusion of cocaine in daily 1 h sessions (limited or Short Access (ShA) group of rats) whereas another group of rats self-administered this dose of the psychostimulant in daily 6 h sessions (extended or Long Access (LgA) group of rats), for a total of 15 days (2).

Progressive ratio (PR) responding. In order to test the effects of reduced SERT expression on the motivation to work for cocaine, rats were allowed to self-administer cocaine under a PR schedule of reinforcement (see: 7). The test was performed one day following cocaine self-administration session 15. The number of lever presses required to obtain a single infusion of cocaine exponentially increased according to the following equation: number of responses per infusion = (5 x e $^{(\text{injection number} \times 0.2)}$) – 5 (for details: 8). When a rat failed to achieve this response requirement within a period of 60 min, the PR session was ended and the breakpoint was recorded. Twenty-four h after the PR test, the animals were subjected to an additional ShA or LgA cocaine self-administration session (see above).

Elevated plus-maze (EPM). The EPM apparatus consisted of two closed arms (50 x 10 x 50 cm) located perpendicular to two open arms (50 x 10 x 1 cm). The four arms were connected by a central arena (10 × 10 cm) and elevated 50 cm above the floor. One h before testing, animals were placed in a room adjacent to the EPM room. To start testing, rats were placed onto the central arena facing one of the open arms. Every rat was allowed to explore the EPM for 300 s and was tested only once. After each rat, the EPM was cleaned with 70% ethanol. Behavior was recorded and the number of entries into the closed and open arms as well as the total distance traveled was calculated using a video tracking system developed at the Radboud University Nijmegen Medical Centre (9). Twenty-four h after exposure to the EPM, rats were subjected to an additional ShA or LgA cocaine self-administration session (see above).

Perfusion. Twenty-four h following the last cocaine self-administration session, rats were deeply anesthetized using an overdose of 35% (v/v) chloral hydrate (injected volume: 3.5 mL, i.p., Sigma, St. Louis, Mo) and transcardially perfused with 200 mL saline followed by 400 mL ice-cold 4% paraformaldehyde (PFA) solution (Sigma). Brains were quickly removed, post-fixed in 4% PFA for 24 h, and placed in 30% sucrose solution at 4°C until sinking. Coronal 40 µm sections (200 µm interspaced) were made with a freezing microtome (Microm, Walldorf, Germany) and these sections were stored at 4°C in a cryoprotectant solution (50% 0.1 M PBS, 30% ethylene glycol, and 20% glycerol) until correct placement of the viral vector needle track was verified (see Supplementary Figure S2) and CRF immunohistochemistry was performed.

Corticotropin-releasing factor (CRF) immunohistochemistry. The CRF immunohistochemistry procedures are derived from Rouwette et al, 2008 (for details: 10). In short, brain slices were washed with 0.1 M PBS. To increase cell permeability, the slices were treated with 0.5% Triton X-100 (Sigma) for 30 min. After 3 additional washing steps with PBS, all slices were treated with 1% H_2O_2 for 60 min to inactivate endogenous peroxidase activity. Subsequently, the slices were washed with a solution of PBS and Bovine Serum Albumin (PerkinElmer, Boston, MA) and pre-incubated in 2% normal donkey serum (Jackson Immunoresearch Laboratories, West Grove, PA) for 1 h. Then, sections were incubated overnight in polyclonal (rabbit) CRF antiserum (1:2000, kindly provided by Dr. W.W. Vale, see: 11). The next day, after 3 washing steps in PBS, the sections were incubated with a secondary antibody (Biotin-SP-conjugated donkey anti rabbit IgG, 1:200, Jackson Immunoresearch Laboratories) for 60 min. After another 3 washing steps in PBS, the sections were

incubated in ABC reagent (Vector Labs, Burlingame, CA) for 1 h. The sections were washed in PBS twice and in 0.1 M Tris-HCl (pH7.4) buffer once. Immunostaining was visualized with 10 mg diaminobenzidine (DAB (D5637), Sigma) in 50 mL Tris buffer for 6 min after which 1% H_2O_2 was added for 6 min. The reaction was stopped by transferring the sections to distilled water for 2 min. The sections were then washed once in Tris buffer and twice in PBS. The sections were subsequently mounted on gelatin-coated glass slides and air-dried at 37°C overnight. After dehydration in escalating concentrations of alcohol followed by isopropanol and xylene, the sections were coverslipped using Entellan.

Immunodensity measurements. A Zeiss Axiophot microscope equipped with MBF Bioscience Stereo Investigator software was used to take 8-bit pictures (magnification: 100x) of the paraventricular nucleus (PVN) and the central nucleus of the amygdala (CeA). These brain regions were identified on the basis of the anatomical landmarks given by the stereotactic atlas of Paxinos and Watson (12). NIH ImageJ software (version 1.46r, available at www.rsb.info.nih.gov) was used to measure, for each brain region, the mean optical immunodensity (defined as the sum of the gray values of all the pixels in the brain region divided by the total number of pixels in this region), corrected for the optical immunodensity of the background (which was measured at 4 regions outside the brain region of interest). Per rat, quantification was performed in 3 sections of the PVN and 4 sections of the CeA. Measures were averaged over the different sections, providing for each brain region one value per animal.

***In situ* hybridization.** An independent group of cocaine-naive rats was used to determine local SERT KD over time. At 2, 4 or 8 weeks after the local infusion of AAV2 vectors encoding shSCR, shSERT1, or shSERT2 into the DRN, rats were sacrificed by decapitation and brains were harvested, snap-frozen in isopentane, and stored at −80°C. Six series of 20 µm coronal cryostat sections were collected on Superfrost Plus slides. A first series was processed for *in situ* hybridization with a GFP probe to assess the location and extent of viral transduction. A second series was processed for *in situ* hybridization with a SERT probe to evaluate SERT silencing efficiency within the transduced area. A plasmid containing the EGFP sequence subcloned into pGEM-T Easy was donated by Dr. Richard Rivera (The Scripps Research Institute, La Jolla, CA). A pBluescript II plasmid containing the rat SERT cDNA was obtained from Dr. Randy Blakely (Vanderbilt University, Nashville, TN, see: 13). Digoxigenin (DIG)-labeled riboprobes were synthesized using a kit (Roche, Indianapolis, IN). Sections were post-fixed in PFA 4%, and then acetylated in 0.1 M triethanolamine pH 8.0, acetic acid 0.2%. Following washes in salt sodium citrate (SSC) 2x, sections were dehydrated and defatted in a graded ethanol/chloroform series. Pre-hybridization and hybridization were performed at 70°C in a buffer containing 50% formamide, SSC 2x, Ficoll 0.1%, polyvinylpyrrolidone 0.1%, bovine serum albumin 0.1%, sheared salmon sperm DNA (0.5 mg/mL) and yeast RNA (0.25 mg/mL). Probes were diluted in the hybridization buffer (800 ng/mL) and incubated overnight on slides. Post-hybridization washes were performed in 50% formamide, SSC 2x and Tween-20 0.1%. Sections were then blocked for 1 h and incubated with anti-DIG antibody overnight at 4°C (Roche, 1:2000) in MABT buffer (0.1 M maleic acid pH 7.5, 0.15 M NaCl, Tween-20 0.1%) containing 10% normal goat serum. Following washes in MABT and incubation in

detection buffer (0.1 M Tris-HCl pH 9.5, 0.1 M NaCl, 0.05 M $MgCl_2$, Tween-20 0.1%), the reaction with NBT-BCIP was allowed to develop in the dark for 24 h at room temperature. Slides were rinsed, air dried and mounted in DPX (Sigma, St-Louis, MO). Sections were photographed using a Zeiss Axiophot microscope equipped with a QImaging Retiga 2000R color digital camera and QCapture software at 5x magnification. The ventricle was included in each picture as an anatomical landmark to enable overlay. Optical density of the hybridization signal was analyzed using NIH ImageJ software.

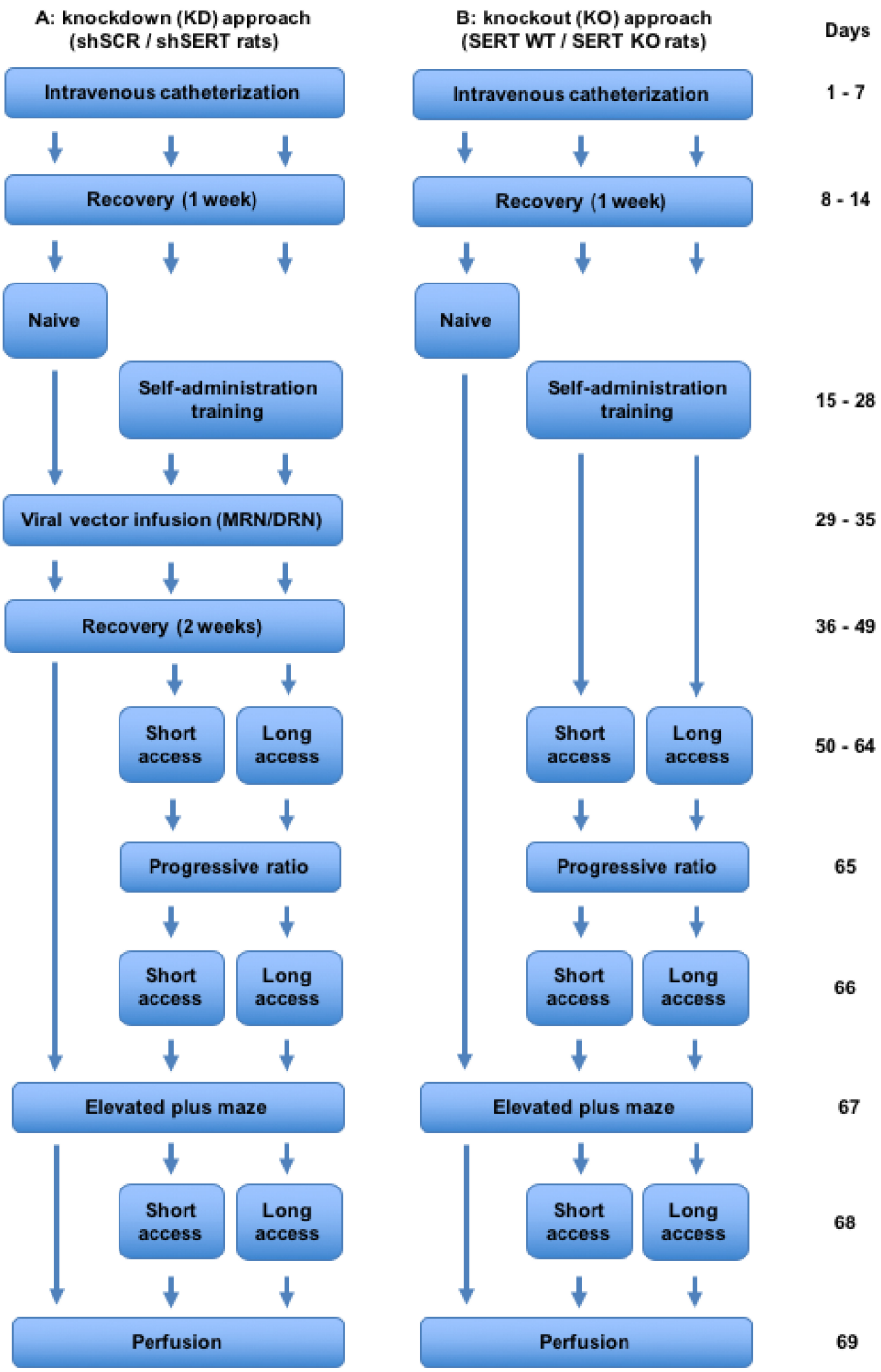
Approach B: constitutive SERT knockout (KO)

SERT KO rats (*SLC6A41*^{Hubr}) were generated by N-ethyl-N-nitrosurea (ENU) induced mutagenesis (14) and outcrossed with commercially available Wistar rats (Harlan, Ter Horst, the Netherlands) for at least ten generations (15). Male SERT KO rats and their wild-type (WT) counterparts, both weighing 250-300 g, were equipped with jugular vein catheters (1) and subjected to ShA and LgA cocaine self-administration (2), according to the procedures described above. In a pilot experiment (data not shown), 40% of the SERT KO animals showed signs of an overdose (seizures). The time-out period for the constitutive SERT KO approach was therefore increased from 20 s (see above) to 40 s. Additional groups of cocaine-naive SERT WT and SERT KO rats also underwent self-administration surgery, were handled daily, and received daily infusion of heparinized saline, but were not exposed to the self-administration chambers (2). Self-administration sessions were conducted until the cocaine intake of SERT KO rats reached a level similar to the cocaine intake of shSERT2 rats. CRF immunodensity measurements were not only

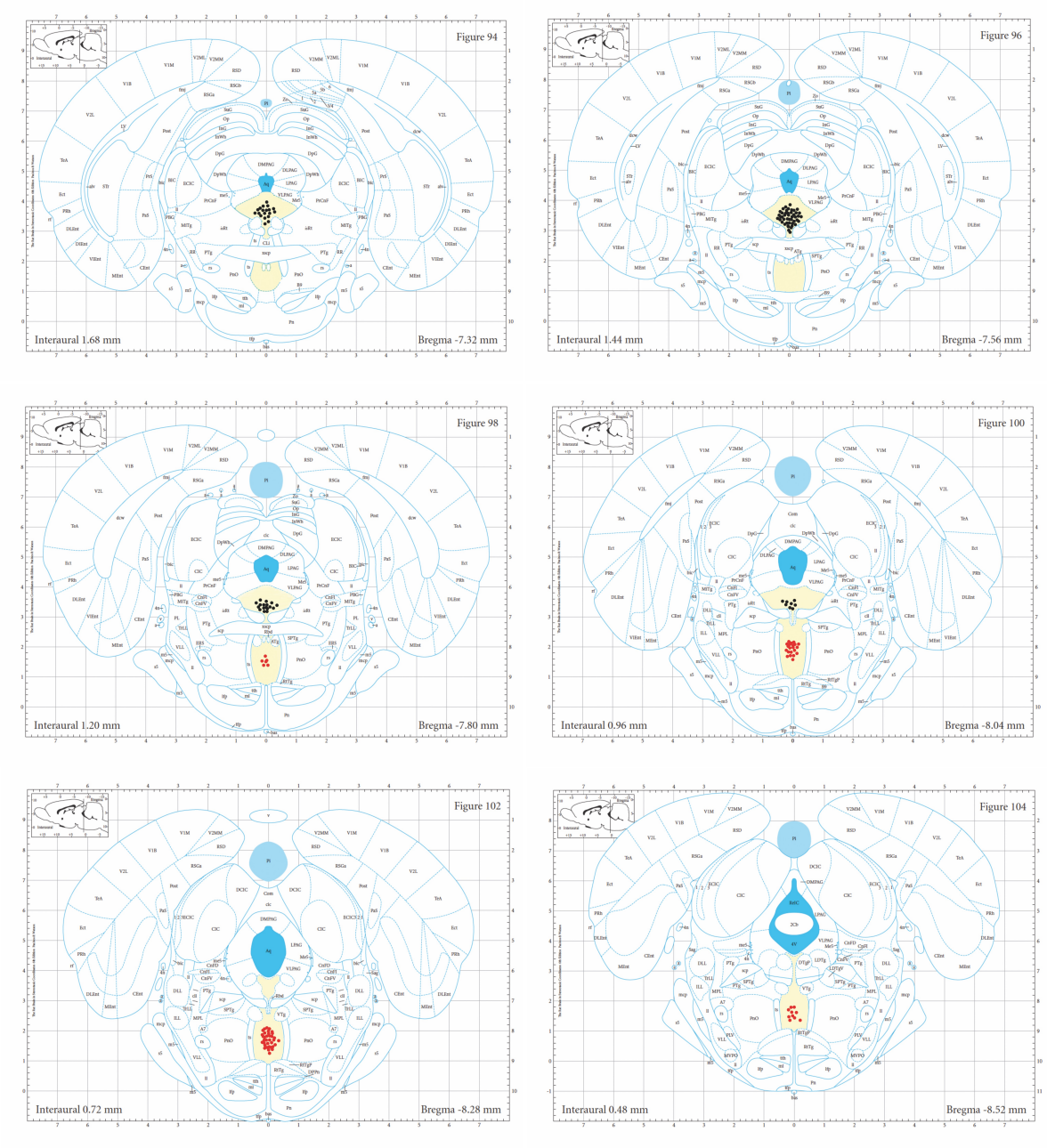
performed on sections taken from the PVN and CeA (see approach A above), but also on sections (n=4 / rat) taken from the bed nucleus of the stria terminalis (BNST).

Data analysis

Data are expressed as the mean ± SEM and analyzed using a two-way (repeated-measures) analysis of variance (ANOVA), followed by a one-way contrast ANOVA, post-hoc Fisher's Least Significant Difference (LSD) analysis or Student's t-test where appropriate (IBM SPSS Statistics version 23). Pearson product-moment coefficients were calculated to study putative correlations between two variables. A P-value smaller than 0.05 was considered statistically significant.



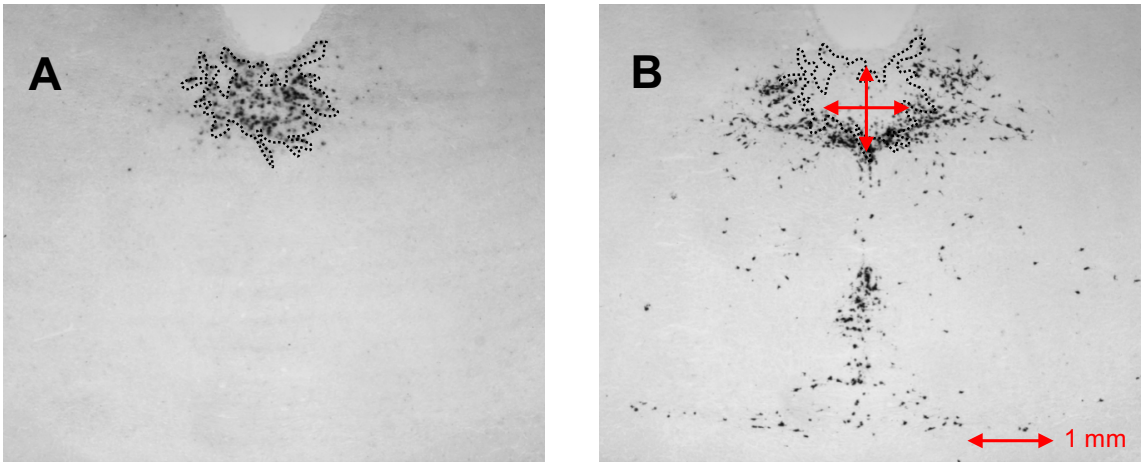
Supplementary Figure S1: Experimental timeline. The SERT knockout (KO) approach (B) was similar to the local SERT knockdown (KD) approach (A) except for the fact that there was no stereotactic surgery necessary in these animals and the time-out period during self-administration was increased from 20 to 40 s (see Supplementary Methods and Materials for details). Cocaine-naive animals were not exposed to the self-administration chambers during training and the various short and long access sessions.



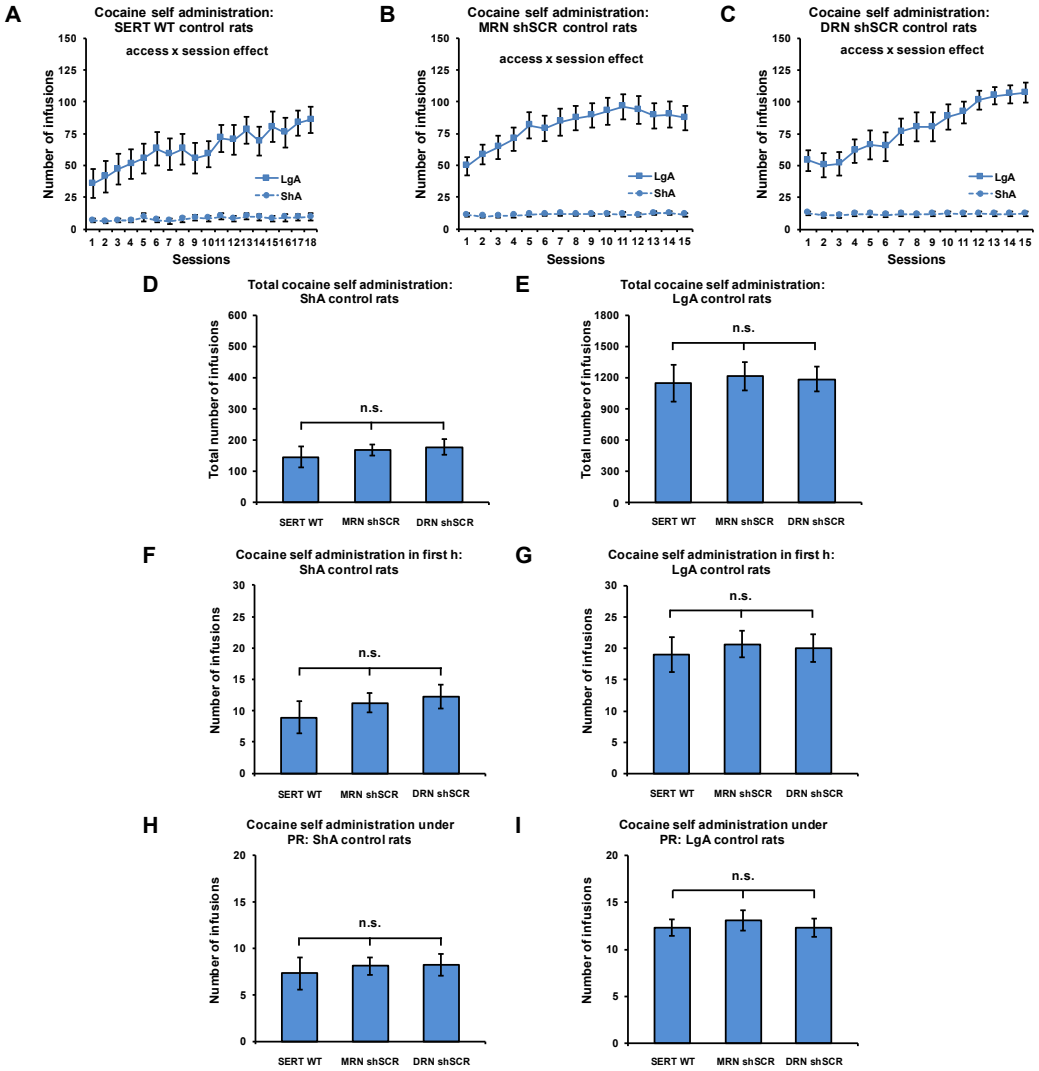
Supplementary Figure S2: Schematic representation of the tips of all stereotactically implanted and correctly placed needles that were used to knockdown SERT in DRN (black dots) and MRN (red dots) neurons. Coronal diagrams are taken from Paxinos and Watson, 2007 (1).

Supplementary Reference

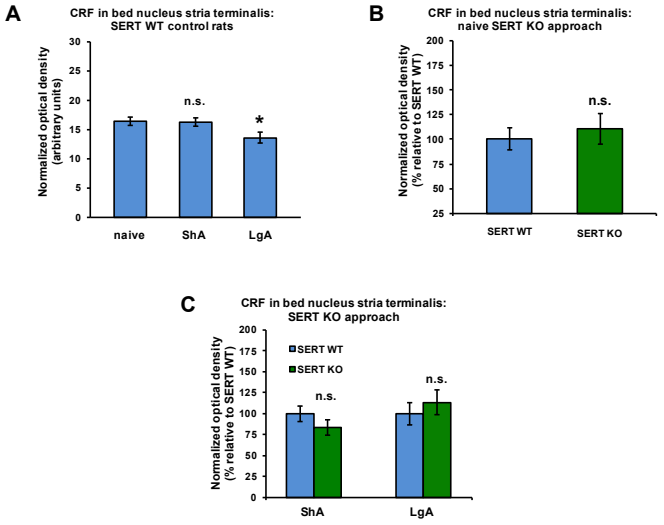
1. Paxinos G, Watson C (2007): *The rat brain in stereotaxic coordinates*. San Diego: Elsevier Academic Press.



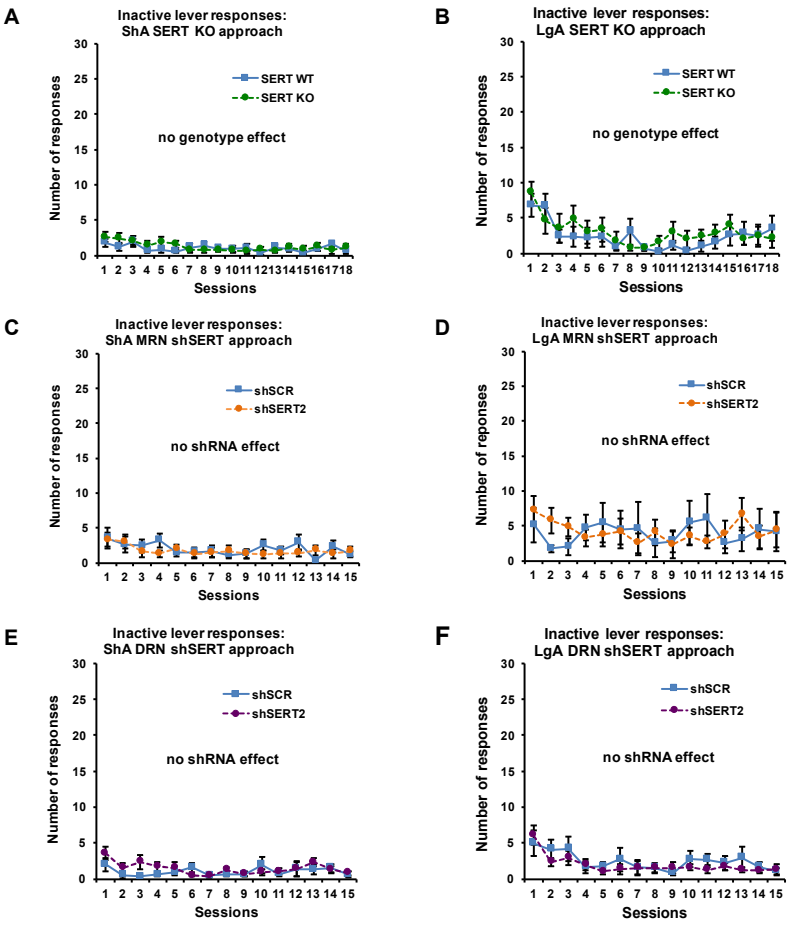
Supplementary Figure S3: Regional selectivity of virally-mediated SERT knockdown. Chromogenic *in situ* hybridization of GFP (**A**) and SERT (**B**) mRNA on adjacent serial sections from the brain of a rat injected in the DRN with shSERT2 and euthanized 4 weeks later. SERT expression is virtually ablated from the area transduced with the viral vector (dashed outline) while SERT expression outside of the transduced area (including the MRN) is intact.



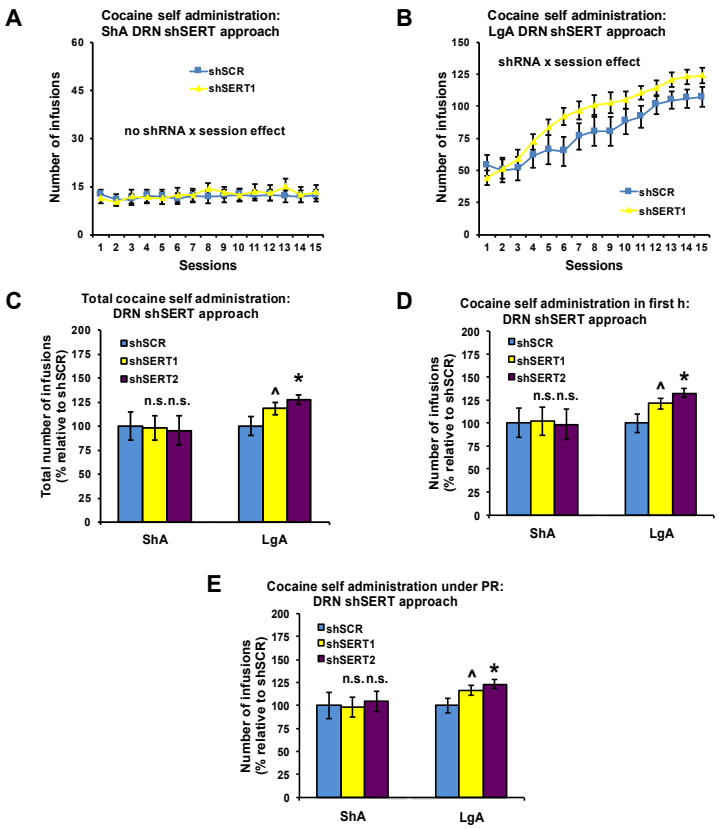
Supplementary Figure S4: LgA, but not ShA, to cocaine self-administration produced an escalation of the daily cocaine intake in the control rats of the SERT KO approach (A: access x session interaction: $F_{(17,374)}$=6.08, P<0.001; session effect LgA: P<0.001, session effect ShA: n.s.), the local SERT KD in the MRN (B: access x session interaction: $F_{(14,364)}$=12.42, P<0.001; session effect LgA: P<0.001, session effect ShA: n.s.), and the local SERT KD in the DRN (C: access x session interaction: $F_{(14,336)}$=18.29, P<0.001; session effect LgA: P<0.001, session effect ShA: n.s.). SERT WT rats required three more self-administration sessions than MRN and DRN shSCR animals to reach an equivalent total cocaine intake (D-E: approach effect: n.s.). The control groups did also not differ in their cocaine intake during the first h of the final self-administration session (F-G: approach effect: n.s.) and their intake under a progressive ratio (PR) schedule of reinforcement (H-I: approach effect: n.s.). Each graph shows mean ± S.E.M. of values obtained in SERT WT, MRN shSCR or DRN shSCR rats. n.s: no significant approach effect. ShA: SERT WT (blue): n=14, MRN shSCR (blue): n=13, DRN shSCR (blue): n=14; LgA: SERT WT (blue): n=10, MRN shSCR (blue): n=15, DRN shSCR (blue): n= 12.



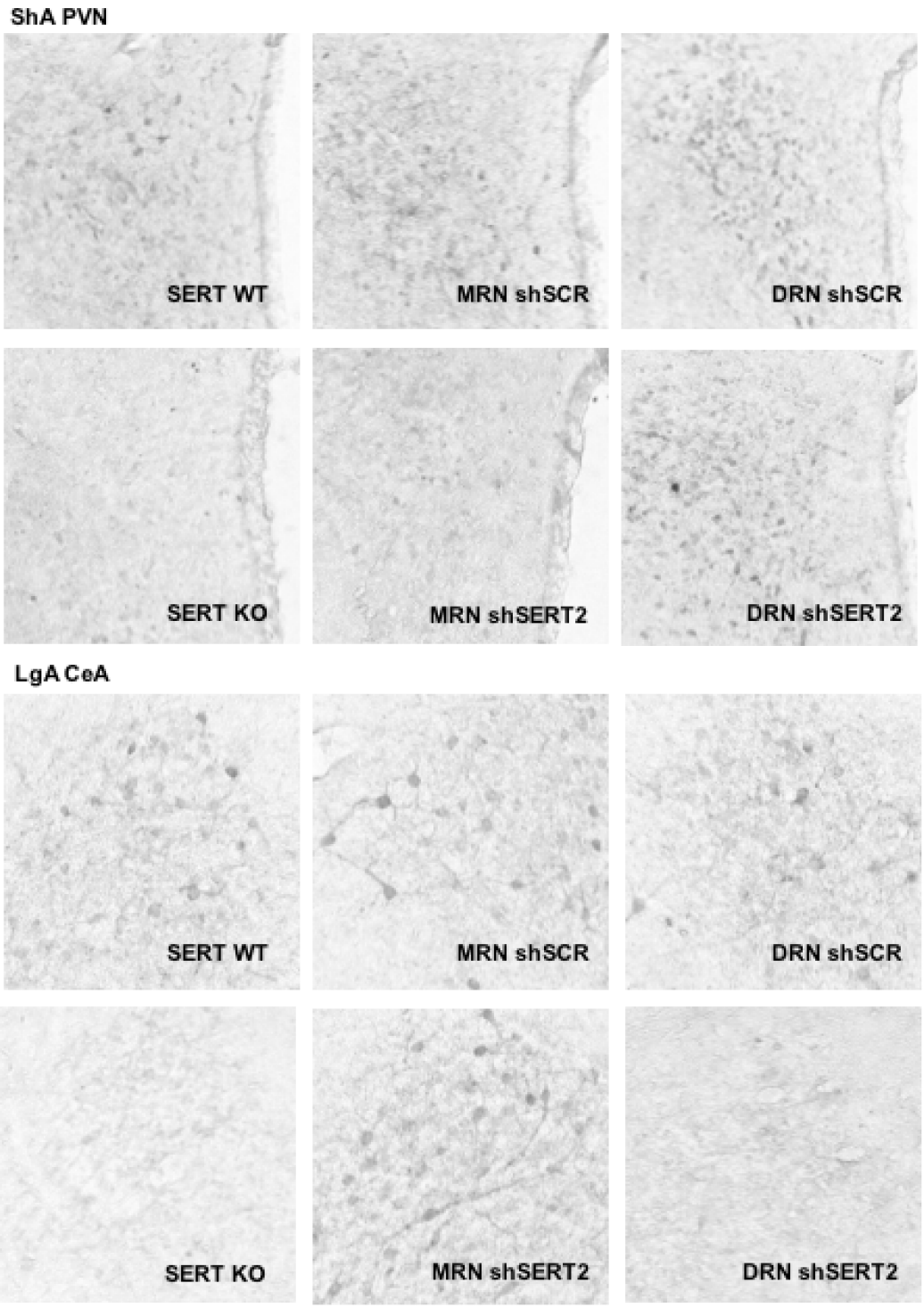
Supplementary Figure S5: LgA, but not ShA, to cocaine self-administration reduced CRF immunodensity levels in the BNST of SERT WT rats (A), and constitutive SERT KO had no effects on CRF levels in the BNST of animals that had either short (ShA), long (LgA) or no access (naive) to cocaine (B-C). *Significant decrease relative to cocaine naive, n.s: no difference relative to control. Data have been obtained from SERT KO rats, lacking SERT in neurons arising from both the MRN and DRN (see main Figure 2: naive: SERT WT (blue): n=13, SERT KO (green): n=10; and main Figure 3: ShA: SERT WT (blue): n=14, SERT KO (green): n=12; LgA: SERT WT (blue): n=10, SERT KO (green): n=11).



Supplementary Figure S6: No effects of constitutive SERT KO (A-B), MRN-specific SERT KD (C-D), or DRN-specific SERT KD (E-F) on the number of inactive lever presses during both ShA (left) and LgA (right) to cocaine. n.s: no significant change (no genotype/shRNA effect or no genotype/shRNA x session effect). The SERT-deletion-induced changes in active lever pressing can be found in main Figures 3-5. ShA: SERT WT (blue): n=14, SERT KO (green): n=12, MRN shSCR (blue): n=13, MRN shSERT2 (orange): n=13, DRN shSCR (blue): n=14, DRN shSERT2 (purple): n=16; LgA: SERT WT (blue): n=10, SERT KO (green): n=11, MRN shSCR (blue): n=15, MRN shSERT2 (orange): n=13, DRN shSCR (blue): n=12, DRN shSERT2 (purple): n=18.



Supplementary Figure S7: Intra-DRN infusion of shSERT1 increased the daily intake of cocaine in LgA, but not ShA, rats (A-B: shRNA x session interaction in LgA rats: $F_{(14,308)}$=1.76, P<0.05, shRNA x session interaction in ShA rats: n.s.). To allow direct comparison of cocaine self-administration in these rats with cocaine self-administration in rats marked by a lower or higher expression of SERT, the cocaine self-administration data of the rats infused with respectively shSCR or shSERT2 into the DRN have also been included (data reproduced from main Figure 5). When compared to the effect of shSERT2 (which reduced local SERT expression by ~75%), intra-DRN infusion of shSERT1 (which reduced local SERT expression by ~50%) resulted in an intermediate increase in cumulative cocaine intake (C: shRNA effect in LgA rats: $F_{(2,39)}$=4.09, P<0.05, shRNA effect in ShA rats: n.s.), intake during the first h of the final self-administration session (D: shRNA effect in LgA rats: $F_{(2,39)}$=3.67, P<0.05, shRNA effect in ShA rats: n.s.), and intake during a PR schedule of reinforcement (E: shRNA effect in LgA rats: $F_{(2,39)}$=3.83, P<0.05, shRNA effect in ShA rats: n.s.) in rats exposed to LgA, but not ShA, cocaine self-administration. In panels C-E, data are normalized to values obtained in shSCR counterparts. *Intake significantly higher than shSCR (LSD: P<0.05). ^Intake higher than shSCR and lower than shSERT2 (LSD: P<0.075), n.s: no significant change vs shSCR or shSERT2. ShA: shSCR (blue): n=14, shSERT1 (yellow): n=15, shSERT2 (purple): n=16; LgA: shSCR (blue): n=12, shSERT1 (yellow): n=12, shSERT2 (purple): n=18. The effects of shSERT1 and shSERT2 infusions on SERT expression in the DRN can be found in main Figure 2.



Supplementary Figure S8: Representative pictures illustrating the reported SERT reduction-induced changes in CRF immunodensity levels after ShA and LgA to cocaine self-administration (see also Main figures 3-5). The levels of CRF in the PVN (first and second rows) were reduced after ShA to cocaine in SERT KO (left column) and MRN (middle column), but not DRN (right column), SERT KD rats, whereas the levels of CRF in the CeA (third and fourth rows) were reduced after LgA to cocaine in SERT KO and DRN, but not MRN, SERT KD rats.

Correlation between SERT levels, CRF levels and anxiety

		SERT levels DRN	EPM open arm entries	CRF levels PVN	CRF levels CeA
MRN SERT KD approach	SERT levels MRN	n.s.	+ 0.621 p < 0.05	+ 0.552 p < 0.05	n.s.
	SERT levels DRN	X	n.s.	n.s.	n.s.
	EPM open arm entries		X	+ 0.812 p < 0.001	n.s.
	CRF levels PVN			X	n.s.
DRN SERT KD approach	SERT levels MRN	n.s.	n.s.	n.s.	n.s.
	SERT levels DRN	X	+ 0.780 p = 0.001	n.s.	+ 0.704 p < 0.01
	EPM open arm entries		X	n.s.	+ 0.574 p < 0.05
	CRF levels PVN			X	n.s.

Supplementary Table S1: Results of a Pearson's analysis to study the correlation between SERT levels, local levels of CRF and anxiety-related behavior in the cocaine-naïve animals of the SERT KD approach. A positive Pearson's correlation coefficient denotes a positive correlation. n.s: no significant correlation. MRN SERT KD approach: n=18 (see Main Figure 2, shSCR: n=9, shSERT2: n=9), DRN SERT KD approach: n=30 (see Main Figure 2: shSCR: n=15, shSERT2: n=15).

Correlation between cocaine intake and CRF levels

		CRF levels PVN	CRF levels CeA
ShA	SERT KO approach	- 0.429 P < 0.05	n.s.
	MRN SERT KD approach	- 0.469 P < 0.05	n.s.
LgA	SERT KO approach	n.s.	- 0.588 P < 0.01
	DRN SERT KD approach	n.s.	- 0.395 P < 0.05

Supplementary Table S2: Results of a Pearson's analysis to study the correlation between total cocaine intake and CRF levels. A negative Pearson's correlation coefficient denotes a negative correlation. n.s: no significant correlation. ShA (see Main Figures 3 and 4): SERT KO approach: n=26 (SERT WT: n=14, SERT KO: n=12), MRN SERT KD approach: n=26 (shSCR: n=13, shSERT2: n=13), LgA (see Main Figures 3 and 5): SERT KO approach: n=21 (SERT WT: n=10, SERT KO: n=11), DRN SERT KD approach: n=30 (shSCR: n=12, shSERT2: n=18).

Correlation between CRF levels, anxiety and the motivation to take cocaine

		CRF levels PVN	EPM open arm entries	PR intake
ShA	CRF levels CeA	n.s.	n.s.	n.s.
	CRF levels PVN	X	+ 0.255 p < 0.05	- 0.339 p < 0.05
	EPM open arm entries		X	- 0.341 p < 0.05
LgA	CRF levels CeA	n.s.	+ 0.211 p < 0.05	- 0.313 p < 0.05
	CRF levels PVN	X	n.s.	n.s
	EPM open arm entries		X	- 0.287 p < 0.05

Supplementary Table S3: Results of a Pearson's analysis to study the correlation between the observed SERT-deletion-induced local reductions of CRF and the increases in anxiety-related behavior (as illustrated by a decrease in open arm entries on the EPM) and the motivation to take cocaine (as illustrated by an increase in PR intake). A positive Pearson's correlation coefficient denotes a positive correlation, and a negative Pearson's correlation coefficient denotes a negative correlation. n.s: no significant correlation. The ShA correlation analysis was performed in ShA rats in which SERT reduction increased PR responding (main Figure 3 (SERT WT+SERT KO: n=14+12) and main Figure 4 (shSCR+shSERT2: n=13+13), main Figure 3 + main Figure 4: n=52). The LgA correlation analysis was performed in LgA rats in which SERT reduction increased PR responding (main Figure 3 (SERT WT+SERT KO: n=10+11) and main Figure 5 (shSCR+shSERT2: n=12+18), main Figure 3 + main Figure 5: n=51).

SERT expression levels

1. SERT KO approach	SERT WT	SERT KO	SERT WT vs SERT KO
SERT levels MRN (arbitrary units)	3.8 ± 0.7	0.2 ± 0.2	P < 0.001
SERT levels DRN (arbitrary units)	12.6 ± 2.0	0.3 ± 0.2	P < 0.001

2. MRN SERT KD approach	shSCR	shSERT2	shSCR vs shSERT2
SERT levels MRN (arbitrary units)	3.2 ± 0.4	1.0 ± 0.4	P < 0.01
SERT levels DRN (arbitrary units)	10.8 ± 0.9	12.0 ± 1.1	n.s.

3. DRN SERT KD approach	shSCR	shSERT2	shSCR vs shSERT2
SERT levels MRN (arbitrary units)	3.8 ± 0.4	3.9 ± 0.5	n.s.
SERT levels DRN (arbitrary units)	12.3 ± 0.9	2.5 ± 1.3	P < 0.001

Supplementary Table S4: Absolute values (± sem) of SERT mRNA expression in the MRN and DRN after constitutive SERT KO (measure 1) and raphe-specific SERT KD (measures 2 and 3). Data are obtained from the cocaine-naive rats depicted in Main Figure 2. Arbitrary units represent SERT optical density corrected for background density.

Absolute values of measures obtained in animals of constitutive SERT KO approach

1. cocaine SA total	SERT WT	SERT KO	WT vs KO
ShA	146 ± 35	239 ± 22	P < 0.05
LgA	1142 ± 176	1783 ± 56	P < 0.01
ShA vs LgA	P < 0.001	P < 0.001	

2. cocaine SA first h	SERT WT	SERT KO	WT vs KO
ShA	11 ± 2	20 ± 3	P < 0.05
LgA	19 ± 3	29 ± 2	P < 0.01
ShA vs LgA	P < 0.05	P < 0.05	

3. cocaine SA PR	SERT WT	SERT KO	WT vs KO
ShA	8 ± 2	14 ± 1	P < 0.01
LgA	12 ± 1	18 ± 1	P = 0.001
ShA vs LgA	P = 0.05	P < 0.05	

4. EPM open arm entries	SERT WT	SERT KO	WT vs KO
naive	14 ± 2	6 ± 2	P < 0.01
ShA	10 ± 2	4 ± 1	P < 0.05
LgA	9 ± 2	4 ± 1	P < 0.05
naive vs ShA	P < 0.05	n.s.	
naive vs LgA	P < 0.05	n.s.	
ShA vs LgA	n.s.	n.s.	

5. EPM total distance	SERT WT	SERT KO	WT vs KO
naive	1794 ± 82	1717 ± 94	n.s.
ShA	1755 ± 111	1778 ± 68	n.s.
LgA	1829 ± 97	1710 ± 98	n.s.
naive vs ShA	n.s.	n.s.	
naive vs LgA	n.s.	n.s.	
ShA vs LgA	n.s.	n.s.	

6. CRF in PVN	SERT WT	SERT KO	WT vs KO
naive	3.4 ± 0.4	2.3 ± 0.3	P < 0.05
ShA	2.1 ± 0.1	1.6 ± 0.2	P < 0.05
LgA	3.1 ± 0.4	2.8 ± 0.2	n.s.
naive vs ShA	P < 0.001	P < 0.05	
naive vs LgA	n.s.	n.s.	
ShA vs LgA	P < 0.01	P < 0.001	

7. CRF in CeA	SERT WT	SERT KO	WT vs KO
naive	9.2 ± 0.3	6.5 ± 0.8	P = 0.001
ShA	9.7 ± 0.6	8.7 ± 0.9	n.s.
LgA	7.9 ± 0.3	6.0 ± 0.6	P < 0.01
naive vs ShA	n.s.	n.s.	
naive vs LgA	P < 0.01	n.s.	
ShA vs LgA	P < 0.05	P < 0.05	

Supplementary Table S5: Absolute values (± sem) of the various measures of cocaine self-administration (SA), anxiety-related behavior on the elevated plus-maze (EPM) and CRF expression in the paraventricular nucleus (PVN) and central amygdala (CeA) depicted in main Figures 2 and 3, as well as results of pairwise comparisons between SERT WT and SERT KO rats and between the different types of access to cocaine (no access: naive, short access: ShA and long access: LgA).

Absolute values of measures obtained in animals of MRN-specific SERT KD approach

1. cocaine SA total	MRN shSCR	MRN shSERT2	shSCR vs shSERT2
ShA	168 ± 18	230 ± 16	P < 0.05
LgA	1212 ± 136	1191± 110	n.s.
ShA vs LgA	P < 0.001	P < 0.001	

2. cocaine SA first h	MRN shSCR	MRN shSERT2	shSCR vs shSERT2
ShA	11 ± 2	18 ± 2	P < 0.01
LgA	21 ± 2	20 ± 2	n.s.
ShA vs LgA	P < 0.01	n.s.	

3. cocaine SA PR	MRN shSCR	MRN shSERT2	shSCR vs shSERT2
ShA	8 ± 1	11 ± 1	P < 0.05
LgA	13 ± 1	13 ± 2	n.s.
ShA vs LgA	P < 0.01	n.s.	

4. EPM open arm entries	MRN shSCR	MRN shSERT2	shSCR vs shSERT2
naive	18 ± 5	5 ± 3	P < 0.05
ShA	10 ± 3	6 ± 3	P < 0.05
LgA	11 ± 3	11 ± 3	n.s.
naive vs ShA	P = 0.07	n.s.	
naive vs LgA	P = 0.07	n.s.	
ShA vs LgA	n.s.	n.s.	

5. EPM total distance	MRN shSCR	MRN shSERT2	shSCR vs shSERT2
naive	1445 ± 144	1269 ± 162	n.s.
ShA	1502 ± 113	1568 ± 122	n.s.
LgA	1588 ± 91	1579 ± 103	n.s.
naive vs ShA	n.s.	n.s.	
naive vs LgA	n.s.	n.s.	
ShA vs LgA	n.s.	n.s.	

6. CRF in PVN	MRN shSCR	MRN shSERT2	shSCR vs shSERT2
naive	3.0 ± 0.1	2.4 ± 0.1	P < 0.001
ShA	2.1 ± 0.1	1.8 ± 0.1	P < 0.01
LgA	2.5 ± 0.3	2.3 ± 0.2	n.s.
naive vs ShA	P < 0.001	P < 0.001	
naive vs LgA	n.s.	n.s.	
ShA vs LgA	n.s.	P < 0.05	

7. CRF in CeA	MRN shSCR	MRN shSERT2	shSCR vs shSERT2
naive	9.8 ± 0.3	9.9 ± 0.6	n.s.
ShA	8.9 ± 0.4	8.6 ± 0.4	n.s.
LgA	6.6 ± 0.4	6.7 ± 0.5	n.s.
naive vs ShA	n.s.	n.s.	
naive vs LgA	P < 0.001	P < 0.001	
ShA vs LgA	P < 0.001	P < 0.01	

Supplementary Table S6: Absolute values (± sem) of the various measures of cocaine self-administration (SA), anxiety-related behavior on the elevated plus-maze (EPM) and CRF expression in the paraventricular nucleus (PVN) and central amygdala (CeA) depicted in main Figures 2 and 4, as well as results of pairwise comparisons between MRN shSCR and MRN shSERT2 rats and between the different types of access to cocaine (no access: naive, short access: ShA and long access: LgA).

Absolute values of measures obtained in animals of DRN-specific SERT KD approach

1. cocaine SA total	DRN shSCR	DRN shSERT2	shSCR vs shSERT2
ShA	178 ± 26	170 ± 27	n.s.
LgA	1185 ± 121	1515 ± 60	P < 0.05
ShA vs LgA	P < 0.001	P < 0.001	

2. cocaine SA first h	DRN shSCR	DRN shSERT2	shSCR vs shSERT2
ShA	12 ± 2	13 ± 2	n.s.
LgA	20 ± 2	25 ± 1	P < 0.05
ShA vs LgA	P < 0.05	P < 0.001	

3. cocaine SA PR	DRN shSCR	DRN shSERT2	shSCR vs shSERT2
ShA	8 ± 1	9 ± 1	n.s.
LgA	12 ± 1	15 ± 1	P < 0.05
ShA vs LgA	P < 0.05	P < 0.001	

4. EPM open arm entries	DRN shSCR	DRN shSERT2	shSCR vs shSERT2
naive	14 ± 1	5 ± 1	P < 0.001
ShA	9 ± 2	9 ± 2	n.s.
LgA	9 ± 2	5 ± 1	P = 0.05
naive vs ShA	P = 0.06	n.s.	
naive vs LgA	P < 0.05	n.s.	
ShA vs LgA	n.s.	n.s.	

5. EPM total distance	DRN shSCR	DRN shSERT2	shSCR vs shSERT2
naive	1134 ± 77	1263 ± 140	n.s.
ShA	1227 ± 55	1317 ± 44	n.s.
LgA	1176 ± 113	1256 ± 69	n.s.
naive vs ShA	n.s.	n.s.	
naive vs LgA	n.s.	n.s.	
ShA vs LgA	n.s	n.s.	

6. CRF in PVN	DRN shSCR	DRN shSERT2	shSCR vs shSERT2
naive	3.6 ± 0.5	4.2 ± 0.7	n.s.
ShA	2.3 ± 0.5	2.6 ± 0.5	n.s.
LgA	4.3 ± 0.5	4.1 ± 0.4	n.s.
naive vs ShA	P = 0.07	P = 0.07	
naive vs LgA	n.s.	n.s.	
ShA vs LgA	P < 0.01	P < 0.05	

7. CRF in CeA	DRN shSCR	DRN shSERT2	shSCR vs shSERT2
naive	9.4 ± 0.4	7.2 ± 0.5	P < 0.01
ShA	8.8 ± 0.7	8.5 ± 0.5	n.s.
LgA	7.0 ± 0.6	5.0 ± 0.6	P < 0.05
naive vs ShA	n.s.	n.s.	
naive vs LgA	P < 0.01	P < 0.01	
ShA vs LgA	P = 0.06	P < 0.001	

Supplementary Table S7: Absolute values (± sem) of the various measures of cocaine self-administration (SA), anxiety-related behavior on the elevated plus-maze (EPM) and CRF expression in the paraventricular nucleus (PVN) and central amygdala (CeA) depicted in main Figures 2 and 5, as well as results of pairwise comparisons between DRN shSCR and DRN shSERT2 rats and between the different types of access to cocaine (no access: naive, short access: ShA and long access: LgA).

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