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Median and dorsal raphe serotonergic neurons control moderate versus compulsive cocaine intake

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Abstract

Background—Reduced expression of the serotonin transporter (SERT) promotes anxiety and cocaine intake in both humans and rats. We tested the hypothesis that median raphe nucleus (MRN) and dorsal raphe nucleus (DRN) serotonergic projections differentially mediate these phenotypes.

Methods—We used virally-mediated RNA interference to locally down-regulate SERT expression and compared the results to constitutive SERT knockout. Rats were allowed either short access (ShA, 1 h) or long access (LgA, 6 h) to cocaine self-administration to model moderate versus compulsive-like cocaine taking.

Results—SERT knockdown in the MRN increased cocaine intake selectively under ShA conditions and, like ShA cocaine self-administration, reduced CRF immunodensity in the paraventricular nucleus of the hypothalamus. In contrast, SERT knockdown in the DRN increased cocaine intake selectively under LgA conditions and, like LgA cocaine self-administration, reduced CRF immunodensity in the central nucleus of the amygdala. SERT knockdown in the

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MRN or DRN produced anxiety-like behavior, as did withdrawal from ShA or LgA cocaine self-administration. The phenotype of SERT knockout rats was a summation of the phenotypes generated by MRN- and DRN-specific SERT knockdown.

Conclusions—Our results highlight a differential role of serotonergic projections arising from the MRN and DRN in the regulation of cocaine intake. We propose that a cocaine-induced shift from MRN-driven serotonergic control of CRF levels in the hypothalamus to DRN-driven serotonergic control of CRF levels in the amygdala may contribute to the transition from moderate to compulsive intake of cocaine.

Keywords

serotonin transporter (SERT); knockout; knockdown and gene silencing; dorsal and median raphe nuclei; corticotropin-releasing factor (CRF); anxiety-related behavior; short and long access to cocaine self-administration

Introduction

In humans, reduced SERT expression and function (1–4) predicts an increased presentation of negative emotional states (1, 5) and increased intake of psychostimulants (6–8). These clinical findings are consistent with studies performed in SERT knockout (KO) rats showing that genetic inactivation of SERT increases not only anxiety-related behavior (5, 9), but also intravenous self-administration of ecstasy and cocaine (10, 11). In addition, cocaine increases locomotor activity and conditioned place preference more strongly in SERT KO than wild-type (WT) animals (10, 12).

Cocaine is known to inhibit the neuronal re-uptake of serotonin by blocking SERT. In the present study, we sought to determine the respective contribution of serotonergic projections from the median raphe nucleus (MRN) and the dorsal raphe nucleus (DRN) to cocaine intake behavior using virally-mediated local SERT knockdown (KD), and compare the results with constitutive SERT deletion in KO rats. Animals were exposed to either short or long access (ShA or LgA) cocaine self-administration (13) in order to evaluate the contribution of MRN and DRN serotonergic neurons to the moderate and compulsive intake of this drug (14). Extended access to cocaine self-administration recapitulates key elements of substance use disorders diagnosis, including gradual escalation of drug intake, enhanced motivation for the drug despite increased cost or negative consequences, and increased sensitivity to craving (for review: 15).

A second purpose of the present study was to determine how SERT deficiency may drive an increase in cocaine intake. It has been suggested that the increased psychostimulant intake observed in animals with a reduction of SERT is due to serotonin-induced changes in the synaptic levels of dopamine and/or noradrenaline (11). However, basal and cocaine-induced levels of these two catecholamines do not differ between SERT KO and WT animals (16, 17). We therefore hypothesize that the increased intake of cocaine in SERT-deficient subjects is due to serotonin-induced changes in non-catecholaminergic systems (16). Numerous studies have shown that corticotropin-releasing factor (CRF) signaling can produce anxiety-related behavior (18–22), which, in turn, may drive the escalation of cocaine intake and

stress-induced reinstatement of drug seeking behavior (for review: 23, 24–26). CRF is highly expressed in both the central nucleus of the amygdala (CeA) and the paraventricular nucleus of the hypothalamus (PVN) (27–30), and both regions are innervated by serotonergic neurons (31–33). Moreover, serotonin and various serotonergic modulators can alter the activity of CRF-expressing cells (34–39). These data suggest that CRF changes in the CeA and PVN may contribute to the increase in anxiety-related behavior and enhanced cocaine intake observed in humans expressing low levels of SERT. To further explore this possibility, we compared the effect of local and constitutive SERT down-regulation on baseline and cocaine withdrawal-induced CRF levels in the CeA and PVN, as well as on anxiety-like behavior in cocaine-naïve and cocaine-withdrawn rats.

Methods and Materials

A summary of the experimental design can be found in Supplementary Figure S1. Male Wistar rats (weighing 250–300 g) were first implanted with a catheter into the jugular vein (40) and were then trained to self-administer cocaine (0.5 mg/kg/infusion) under a fixed ratio 1 (FR1) schedule of reinforcement in daily 1-h sessions (41). An additional cocaine-naïve group of rats also underwent intravenous catheterization, but was not exposed to cocaine (41). For local knockdown experiments (approach A in Supplementary Figure S1), rats were infused with adeno-associated viral (AAV) vectors serotype 2, encoding either a short-hairpin RNA (shRNA) sequence designed to silence the expression of SERT (shSERT1 or shSERT2) or a scrambled control sequence (shSCR) into the MRN or DRN (Supplementary Figure S2). AAV vector serotype 2 infusion typically resulted in transduction volumes not exceeding 1 mm^3 (Supplementary Figure S3). Cocaine self-administration sessions were resumed 2 weeks after viral vector infusion in daily sessions of 1 h (ShA) or 6 h (LgA) (41). For constitutive KO experiments (approach B in Supplementary Figure S1), SERT KO rats (for details: 42, 43) and their WT counterparts were also trained to self-administer cocaine in 1-h sessions and were then split in ShA and LgA groups as well (41). For both approaches, once cocaine intake had successfully escalated under LgA conditions, rats were allowed to self-administer cocaine under a progressive ratio (PR) schedule of reinforcement in order to test the effects of reduced SERT expression on the motivation to work for cocaine (44, 45). On the following day, the rats were subjected to a regular ShA or LgA cocaine self-administration session, and another 24 h later, they were tested on the elevated plus-maze (EPM) to measure anxiety-related behavior (46). On the following day, rats were again subjected to a regular ShA or LgA cocaine self-administration session, and another 24 hours later, they were transcardially perfused with paraformaldehyde. Coronal brain sections containing the PVN, CeA, and bed nucleus of the stria terminalis (BNST) were then processed for CRF immunohistochemistry. Sections were incubated overnight in polyclonal rabbit CRF antiserum (1:2000, provided by Dr. Vale, 47), followed by a secondary antibody (Biotin-SP-conjugated donkey anti rabbit IgG, 1:200) for 60 min, and immunostaining was visualized using diaminobenzidine and H$_2$O$_2$ (for details: 48). Image-J (NIH) was used to measure the mean optical immunodensity in the brain regions of interest corrected for the optical immunodensity of the background. More details can be found in the Supplementary Information file.

Results

Effect of access duration on cocaine self-administration in control rats

The cocaine intake of control rats was equivalent across the 3 cohorts of rats that were used in this study (see Supplementary Figure S4). As expected, analysis of the pooled data from these three control subgroups showed that ShA cocaine self-administration remained stable over the testing days whereas LgA cocaine self-administration resulted in an escalation of the daily intake of cocaine (Figure 1A: access x session interaction: $F_{(14,1064)}=27.72$, $P<0.001$; session effect in LgA rats: $P<0.001$, session effect in ShA rats: n.s.). The cumulative cocaine intake was 7.5-fold larger in LgA than in ShA rats (Figure 1B: access effect: $F_{(1,76)}=170.89$, $P<0.001$). LgA sessions also increased cocaine intake during the first hour (h) of the final self-administration session by 2-fold compared to the 1-h intake of ShA sessions (Figure 1C: access effect: $F_{(1,76)}=26.10$, $P<0.001$), as well as the motivation to work for the drug under a progressive ratio (PR) schedule of reinforcement (Figure 1D: access effect: $F_{(1,76)}=26.88$, $P<0.001$).

Effect of cocaine self-administration on anxiety-like behavior and CRF levels in control rats

To analyze the withdrawal-induced changes in CRF and anxiety levels, a cocaine-naive control group was included (see Supplementary Methods and Materials). Twenty-four h after self-administration, open arm entries on the EPM were reduced after both ShA and LgA of cocaine (Figure 1E: access effect: $F_{(2,112)}=6.18$, $P<0.01$; naive vs ShA: $P<0.01$, naive vs LgA: $P<0.01$, LgA vs ShA: n.s.) while the total distance traveled on the maze was not affected (Figure 1F: access effect: n.s.). Withdrawal from ShA, but not LgA, to cocaine significantly reduced CRF immunodensity levels in the PVN (Figure 1G: access effect: $F_{(2,112)}=7.23$, $P=0.001$; naive vs ShA: $P<0.001$, naive vs LgA: n.s., LgA vs ShA: $P<0.01$) whereas withdrawal from LgA, but not ShA, of cocaine reduced CRF immunodensity levels in the CeA (Figure 1H: access effect: $F_{(2,112)}=4.08$, $P<0.05$; naive vs ShA: n.s., naive vs LgA: $P<0.01$; LgA vs ShA: $P<0.05$) and the BNST (Supplementary Figure S5: access effect: $F_{(2,34)}=3.88$, $P<0.05$; naive vs ShA: n.s., naive vs LgA: $P<0.05$; LgA vs ShA: $P<0.05$).

Validation of constitutive SERT KO and local SERT KD

No SERT mRNA was expressed in the raphe nuclei of constitutive SERT KO rats (Figures 2A–C), consistent with the complete abolition of SERT binding throughout the brain of these rats (43). Intra-raphe infusion of a viral vector expressing either of two shRNA sequences (shSERT1 or shSERT2) directed against the SERT transcript resulted in a reduction of SERT expression in transduced neurons (Figure 2D: shRNA effect: $F_{(2,42)}=32.21$, $P<0.001$) that reached statistical significance 4 weeks post-infusion for shSERT1 and 2 weeks post-infusion for shSERT2, compared to a non-targeting shRNA (shSCR). shSERT2 was more effective than shSERT1 in reducing local SERT expression (Figure 2D (LSD): shSCR vs shSERT1: $P<0.001$, shSCR vs shSERT2: $P<0.001$ and shSERT2 vs shSERT1: $P<0.001$). Eight weeks following intra-MRN injection of shSERT2, SERT expression levels in the MRN were reduced by ~75% (Figures 2E–F: shRNA effect: $F_{(1,16)}=13.07$, $P<0.01$), whereas SERT expression in the DRN was unaffected (Figure 2G: shRNA effect: n.s.). Eight weeks following intra-DRN injection of shSERT2, DRN SERT

expression levels were reduced to a similar extent (Figures 2H–I: shRNA effect: $F_{(1,28)}$=44.53, P<0.001), whereas SERT expression in the MRN was not affected (Figure 2J: shRNA effect: n.s.).

Effect of constitutive SERT KO and local SERT KD on anxiety-related behavior and CRF levels in cocaine-naïve rats

In the EPM, open arm entries were reduced in cocaine-naive SERT KO rats (Figure 2K: genotype effect: $F_{(1,21)}$=10.90, P<0.01) as well as in cocaine-naive rats infused with shSERT2 in either the MRN (Figure 2L: shRNA effect: $F_{(1,16)}$=6.12, P<0.05) or DRN (Figure 2M: shRNA effect: $F_{(1,28)}$=24.54, P<0.001). Neither constitutive nor local SERT downregulation affected the total distance traveled (Figure 2N, genotype effect: n.s. and Figures 2O–P, shRNA effect: n.s.). Constitutive SERT KO decreased CRF immunodensity in both the PVN (Figure 2Q: genotype effect: $F_{(1,21)}$=6.39, P<0.05) and CeA (Figure 2T: genotype effect: $F_{(1,21)}$=14.32, P<0.01). MRN-specific SERT KD reduced CRF immunodensity in the PVN (Figure 2R: shRNA effect: $F_{(1,16)}$=14.85, P=0.001), but not in the CeA (Figure 2U: shRNA effect: n.s.), whereas DRN-specific SERT KD reduced CRF immunodensity in the CeA (Figure 2V: shRNA effect: $F_{(1,28)}$=11.25, P<0.01), but not in the PVN (Figure 2S: shRNA effect: n.s.). CRF immunodensity in the BNST was not affected by constitutive SERT KO (Supplementary Figure S5) and was therefore not examined in SERT KD rats. In MRN SERT KD animals, both open arm entries and CRF levels in the PVN, but not CeA, were positively correlated with SERT levels in the MRN, but not DRN (Supplementary Table S1). In contrast, in the DRN SERT KD animals, open arm entries and CRF levels in the CeA, but not PVN, were positively correlated with SERT levels in the DRN, but not MRN (Supplementary Table S1).

Effect of constitutive SERT KO on cocaine intake

SERT constitutive KO increased the daily intake of cocaine in both ShA and LgA rats (Figures 3A–B: genotype effect: $F_{(1,43)}$=19.38, P<0.001; genotype x access interaction: n.s.). This daily increase resulted in a larger cumulative intake of cocaine under both access conditions in SERT KO vs SERT WT rats (Figure 3C: genotype effect: $F_{(1,43)}$=11.48, P<0.01, genotype x access interaction: n.s.). In addition, SERT constitutive KO increased the intake of cocaine during the first h of the final self-administration session (Figure 3D: genotype effect: $F_{(1,43)}$=9.43, P<0.01, genotype x access interaction: n.s.), as well as under a PR schedule of reinforcement (Figure 3E: genotype effect: $F_{(1,43)}$=18.13, P<0.001; genotype x access interaction: n.s.). Inactive lever responding was low and not affected by constitutive SERT KO (Supplementary Figure S6).

Effect of constitutive SERT KO on cocaine-induced anxiety-related behavior and CRF levels

Twenty-four h after ShA or LgA self-administration, open-arm entries, but not the total distance traveled, on the EPM were significantly reduced in SERT KO vs SERT WT rats (Figure 3F: genotype effect: $F_{(1,43)}$=11.19, P<0.01; genotype x access interaction: n.s; Figure 3G: genotype effect and genotype x access interaction: n.s.). Under ShA conditions, SERT constitutive KO decreased CRF immunodensity in the PVN (Figure 3H: genotype effect: $F_{(1,43)}$=5.38, P<0.05; genotype x access interaction: $F_{(1,43)}$=5.59, P<0.05; genotype

effect in ShA rats: $F_{(1,24)}$=6.95, P<0.05; genotype effect in LgA rats: n.s.), but not CeA (Figure 3I). In contrast, under LgA conditions, SERT constitutive KO decreased CRF immunodensity in the CeA (Figure 3I: genotype effect: $F_{(1,43)}$=5.52, P<0.05; genotype x access interaction: $F_{(1,43)}$=5.74, P<0.05; genotype effect in LgA rats: $F_{(1,19)}$=8.97, P<0.01; genotype effect in ShA rats: n.s.), but not PVN (Figure 3H). The cumulative cocaine intake of SERT WT and KO rats was negatively correlated with both CRF immunodensity in the PVN under ShA conditions and CRF immunodensity in the CeA under LgA conditions (Supplementary Table S2). SERT constitutive KO did not affect CRF immunodensity in the BNST under either ShA or LgA conditions (Supplementary Figure S5).

Effect of MRN-specific SERT KD on cocaine intake

MRN-specific SERT KD increased the daily intake of cocaine in ShA, but not in LgA, rats (Figures 4A–B: shRNA x access interaction: $F_{(1,50)}$=4.87, P<0.05; shRNA effect in ShA rats: $F_{(1,24)}$=7.19, P<0.05; shRNA effect in LgA rats: n.s.). This increased daily self-administration resulted in a larger cumulative intake of cocaine in shSERT2 vs shSCR rats under ShA, but not LgA, conditions (Figure 4C: shRNA x access interaction: $F_{(1,50)}$=9.93, P<0.01; shRNA effect in ShA rats: $F_{(1,24)}$=7.18, P<0.05; shRNA effect in LgA rats: n.s.). MRN-specific SERT KD increased the first-h cocaine intake of ShA, but not LgA, rats (Figure 4D: shRNA x access interaction: $F_{(1,50)}$=17.15, P<0.001; shRNA effect in ShA rats: $F_{(1,24)}$=9.48, P<0.01; shRNA effect in LgA rats: n.s.). MRN-specific SERT KD also increased PR responding under ShA, but not LgA, conditions (Figure 4E: shRNA x access interaction: $F_{(1,50)}$=10.57, P<0.01; shRNA effect in ShA rats: $F_{(1,24)}$=6.42, P<0.05; shRNA effect in LgA rats: n.s.). Inactive lever responding was low and not affected by MRN-specific SERT KD (Supplementary Figure S6).

Effect of MRN-specific SERT KD on cocaine-induced anxiety-related behavior and CRF levels

MRN-specific SERT KD reduced open arm exploration on the EPM in ShA, but not LgA, rats (Figure 4F: shRNA x access interaction: $F_{(1,50)}$=7.06, P<0.05; shRNA effect in ShA rats: $F_{(1,24)}$=5.56, P<0.05; shRNA effect in LgA rats: n.s.), without affecting the total distance traveled (Figure 4G: shRNA effect and shRNA x access interaction: n.s.). MRN-specific SERT KD decreased CRF immunodensity in the PVN in ShA, but not LgA, rats (Figure 4H: shRNA x access interaction: $F_{(1,50)}$=11.92, P<0.01; shRNA effect in ShA rats: $F_{(1,24)}$=8.49, P<0.01; shRNA effect in LgA rats: n.s.). There was no effect of MRN-specific SERT KD on CRF immunodensity in the CeA (Figure 4I: shRNA effect and shRNA x access interaction: n.s.). The cumulative cocaine intake of MRN shSCR and shSERT2 rats was negatively correlated with CRF immunodensity in the PVN under ShA conditions (Supplementary Table S2).

Effect of DRN-specific SERT KD on cocaine intake

DRN-specific SERT KD increased the daily intake of cocaine in LgA, but not in ShA, rats (Figures 5A–B: shRNA x access interaction: $F_{(1,56)}$=4.13, P<0.05; shRNA effect in LgA rats: $F_{(1,28)}$=7.29, P<0.05; shRNA effect in ShA rats: n.s.). This increased daily self-administration resulted in a larger cumulative intake of cocaine in shSERT2 vs shSCR rats under LgA, but not ShA, conditions (Figure 5C: shRNA x access interaction: $F_{(1,56)}$=5.23,

P<0.05; shRNA effect in LgA rats: $F_{(1,28)}$=7.20, P<0.05; shRNA effect in ShA rats: n.s.). DRN-specific SERT KD increased the first-h cocaine intake of LgA, but not ShA, rats (Figure 5D: shRNA x access interaction: $F_{(1,56)}$=3.82, P=0.05; shRNA effect in LgA rats: $F_{(1,28)}$=6.79, P<0.05; shRNA effect in ShA rats: n.s.). DRN-specific SERT KD also increased PR responding under LgA, but not ShA, conditions (Figure 5E: shRNA x access interaction: $F_{(1,56)}$=4.09, P<0.05; shRNA effect in LgA rats: $F_{(1,28)}$=7.25, P<0.05; shRNA effect in ShA rats: n.s.). Inactive lever responding was low and not affected by DRN-specific SERT KD (Supplementary Figure S6).

Effect of DRN-specific SERT KD on cocaine-induced anxiety-related behavior and CRF levels

DRN-specific SERT KD reduced open arm exploration on the EPM in LgA, but not ShA, rats (Figure 5F: shRNA x access interaction: $F_{(1,56)}$=8.00, P<0.01; shRNA effect in LgA rats: $F_{(1,28)}$=6.79, P<0.05; shRNA effect in ShA rats: n.s.), without affecting the total distance traveled (Figure 5G: shRNA effect and shRNA x access interaction: n.s.). There was no effect of DRN-specific SERT KD on CRF immunodensity in the PVN (Figure 5H: shRNA effect and shRNA x access interaction: n.s.). However, DRN-specific SERT KD decreased CRF immunodensity in the CeA in LgA, but not ShA, rats (Figure 5I: shRNA x access interaction: $F_{(1,56)}$=13.73, P<0.001; shRNA effect in LgA rats: $F_{(1,28)}$=7.34, P<0.05; shRNA effect in ShA rats: n.s.). The cumulative cocaine intake of DRN shSCR and shSERT2 rats was negatively correlated with CRF immunodensity in the CeA under LgA conditions (Supplementary Table S2).

The quantitative relationship between DRN-specific SERT KD and cocaine intake was evaluated using the shSERT1 sequence, which induces a less severe down-regulation of SERT transcript expression compared to shSERT2 (see Figure 2D). A gene-dosage effect was observed, whereby the phenotype generated by shSERT1 was intermediate between the phenotypes generated by shSCR and shSERT2 (Supplementary Figures S7C–E). Despite being milder than with shSERT2, the increase in cocaine self-administration elicited by shSERT1 was statistically significant (Supplementary Figures S7A–B).

Correlations between CRF levels, anxiety-related behavior and motivation for cocaine self-administration

A Pearson analysis revealed moderate, but significant, correlations between CRF levels, open arm exploration on the EPM, and motivation to take cocaine, depending on the duration of access to cocaine self-administration. CRF immunodensity in the PVN, but not the CeA, significantly correlated with anxiety-like behavior under ShA conditions. Changes in anxiety-like behavior and PVN CRF levels also correlated with an increased motivation to press for cocaine (i.e., increased PR responding) under ShA, but not LgA, conditions (Supplementary Table S3). In contrast, CRF immunodensity in the CeA, but not in the PVN, significantly correlated with anxiety-like behavior under LgA conditions. Changes in anxiety-like behavior and CeA CRF levels also correlated with an increased motivation to press for cocaine (i.e., increased PR responding) under LgA, but not ShA, conditions (Supplementary Table S3).

Discussion

This study shows that central reduction of SERT has profound effects on both the daily intake of cocaine and the motivation to take the drug. The phenotype of SERT KO rats, in which SERT expression is abolished in neurons arising from both the MRN and DRN, could be dissociated in two complementary and non-overlapping components by local KD of SERT expression in either the MRN or the DRN. MRN-specific KD recapitulated the increased cocaine intake of SERT KO rats under ShA conditions, while DRN-specific KD recapitulated the increased cocaine intake of SERT KO rats under LgA conditions. A first implication of these findings is that the observed phenotype of SERT KO rats does not result from neurodevelopmental alterations, since SERT KD was initiated in adulthood. Furthermore, considering that ShA self-administration models moderate cocaine use whereas LgA self-administration models compulsive cocaine use (for review: 14, 49), we propose that the transition from moderate to compulsive cocaine intake is accompanied by a shift from MRN- to DRN-driven serotonergic control over behavior. The effect of SERT downregulation on cocaine intake may be hypothesized to result from local desensitization of postsynaptic $5\text{-}HT_{1A}$ receptors in projection areas of MRN and DRN serotonergic neurons, which was previously described in SERT KO rats, and proposed as a potential mechanism for the facilitation of psychostimulant self-administration (10, 50). Unknown is whether the cocaine intake increase elicited by SERT reduction results from increased extracellular baseline levels of serotonin (16, 17, 43) or reduced cocaine-induced serotonin release (16, 17).

Based on our finding that SERT deletion does not alter basal or cocaine-stimulated dopamine and noradrenaline release, we previously proposed that serotonin-mediated changes in non-catecholaminergic systems may underlie the increased cocaine intake of SERT KO rats (16). The current data obtained in both SERT KO and SERT KD rats show that 24 h after ShA and LgA cocaine self-administration, CRF immunodensity levels are lower in the PVN and CeA, respectively. These brain region-specific reductions in CRF immunodensity levels correlated with cumulative cocaine intake, suggesting that the exacerbation of CRF effects by SERT downregulation may result from increased cocaine exposure. *In vivo* microdialysis previously demonstrated that extracellular levels of CRF in the amygdala strongly increase during withdrawal from cocaine (51). Accordingly, a decrease in CRF immunoreactivity may reflect an increased release of CRF from intracellular vesicles into the extracellular space. This interpretation has been proposed in earlier studies to explain decreases in CRF immunoreactivity associated with pharmacological evidence of increased CRF signaling (52, 53).

In addition to various studies showing that CRF transmission alters the activity of serotonergic neurons (for review: 54), the results of the present study indicate that serotonin also regulates the levels of CRF. This finding is consistent with previous reports showing that serotonin or serotonergic agents lead to changes in the CRF system (34–39). The observed changes in CRF immunodensity levels in the present study may result from serotonergic afferents controlling the release of CRF by local CRF-containing neurons located in the PVN and CeA (55, 56), or by extrinsic CRF afferents (57, 58). Changes in CRF levels were accompanied by, and correlated with, a decrease in open-arm exploration

on the EPM. In turn, this increase in anxiety-related behavior was accompanied by, and correlated with, an increase in the motivation to take cocaine. Together these data support various studies indicating that CRF-induced changes in emotional behavior can promote cocaine intake and seeking (24, 25, 53, 59–61).

The present study reveals the neuroanatomical specificity of the relationship that ties central serotonergic projections, local CRF levels, anxiety-related behavior, and the motivation for cocaine taking. ShA cocaine intake changed CRF levels in the PVN, but not CeA, whereas LgA cocaine intake changed CRF levels in the CeA, but not PVN. Serotonergic signaling in the BNST activates local CRF-expressing neurons (22) and mediates the anxiety-like state associated with withdrawal from chronic intermittent ethanol exposure (62, 63). We found that LgA cocaine self-administration reduced CRF levels in the BNST, consistent with the contribution of CRF signaling in the BNST to cocaine seeking (64, 65). However, BNST CRF levels were not further affected by SERT KO, indicating that CRF signaling in the BNST is likely not involved in the behavioral effects of SERT reduction reported in the present study.

Our local KD approach suggests that cocaine-induced changes in the levels of CRF in the PVN and CeA, and the concomitant anxiogenic-like effects, are produced by cocaine-induced changes in serotonergic neurons of the MRN and DRN, respectively. This hypothesis is supported by various anatomical studies (31–33) showing that neurons arising from the MRN project to brain regions that are located along the brain midline (e.g., PVN) whereas neurons arising from the DRN project to laterally located brain regions (e.g., CeA, see also: 57, 66, 67, 68).

Conclusions

Altogether, the present study supports the novel hypothesis that a cocaine-induced shift from MRN-driven serotonergic control of CRF levels in the hypothalamus to DRN-driven serotonergic control of CRF levels in the amygdala plays an important role in the transition from moderate to compulsive intake of cocaine in dependent individuals. Moderate cocaine intake is primarily driven by the positive incentive properties of cocaine, while compulsive cocaine intake is, at least in part, driven by the negative emotional states associated with withdrawal (69). The dual role of serotonergic neurons from the MRN and DRN in the regulation of cocaine self-administration under ShA versus LgA conditions provides a neurobiological basis for the contribution of serotonin signaling to both positive and negative reinforcement associated with the development of compulsive cocaine seeking (70).

Implications

The finding that CRF levels in the CeA and PVN as well as open-arm entries on the EPM were reduced in cocaine-naive SERT KO and SERT KD rats indicates that individuals carrying an allelic variant of the serotonin transporter polymorphic region decreasing SERT gene transcription are not only vulnerable to the transition from moderate cocaine intake to drug dependence, but may also be at risk for the initiation of cocaine use. In addition, serotonin-induced changes in CRF and anxiety may contribute to the previously reported

increased intake of non-psychostimulant drugs of abuse (e.g., alcohol (71–73), opiates (8, 74, 75) and nicotine (76–78)) in these individuals. Finally, our study suggests that restoring the balance between MRN and DRN serotonergic activity may reverse the dysregulation of brain emotional systems that promote cocaine use, as was previously proposed for mood disorders (79, 80). We propose that during the early stages of cocaine dependence, MRN-targeted treatment may be beneficial, whereas at later stages DRN-targeted treatment may be more effective. In this respect, it is worth mentioning that the DRN and MRN display unique neurochemical features (e.g., differential serotonin receptor subtypes and densities), which could be leveraged for selective manipulation via systemic pharmacological interventions (for review: 80).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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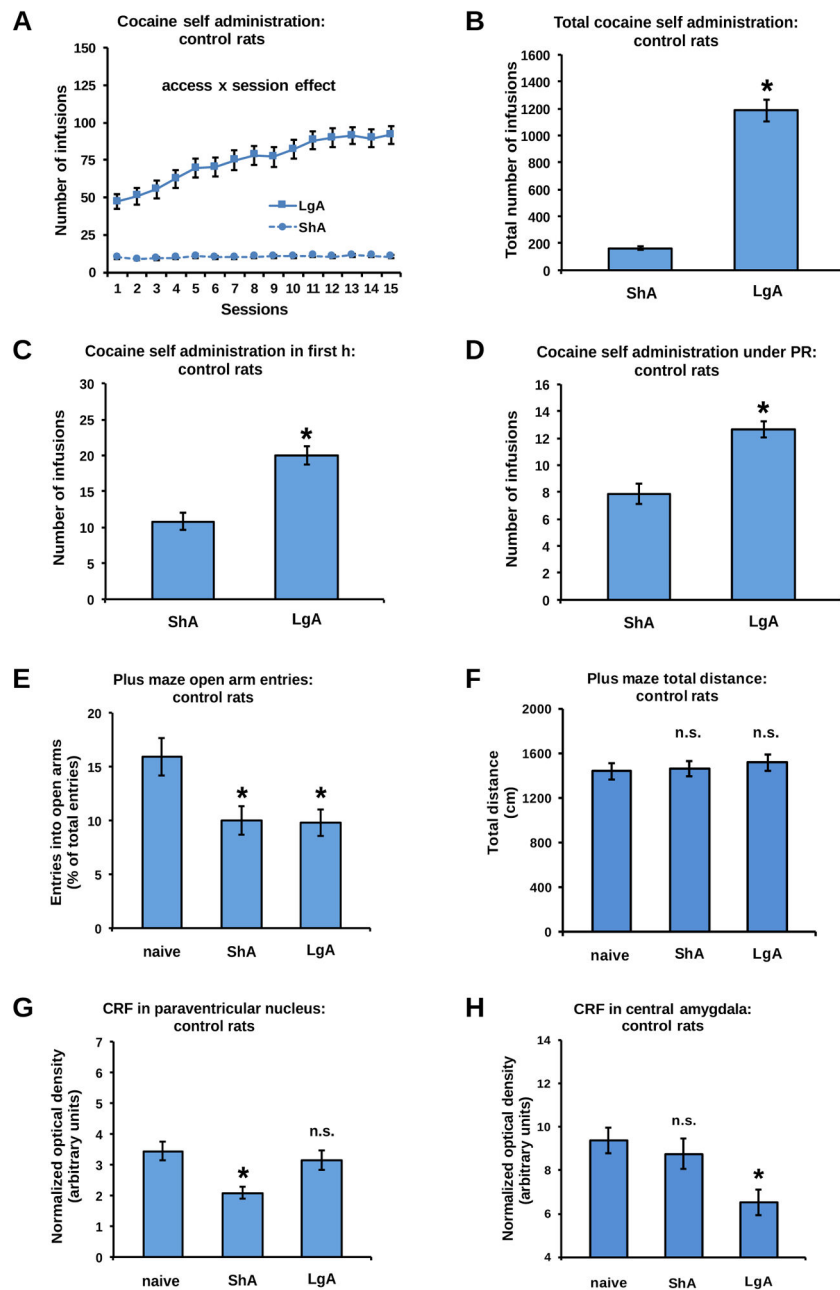
Figure 1.

In control rats, daily LgA to cocaine self-administration produced a gradual escalation of cocaine intake (A), reflected by a higher cumulative intake (B), a higher intake during the first h of the final self-administration session (C), and a higher intake during a PR schedule of reinforcement (D), when compared to daily ShA self-administration. Withdrawal from both ShA and LgA to cocaine reduced the number of open arm entries on the EPM (E), without affecting locomotor activity (F). ShA cocaine self-administration reduced CRF immunodensity levels in the PVN (G), but not in the CeA (H). In contrast, LgA cocaine self-administration reduced CRF in the CeA (H), but not the PVN (G). *Significant change

(P<0.05) vs ShA or naive, n.s: no significant change. Given their similar total intake of cocaine (see supplementary Figure S4), data of SERT WT and shSCR rats were pooled and each graph shows the mean ± S.E.M. of this combined group of animals. Naive (blue): n=37 (SERT WT: n=13, MRN shSCR: n=9, DRN shSCR: n=15), ShA (blue): n=41 (SERT WT: n=14, MRN shSCR: n=13, DRN shSCR: n=14), LgA (blue): n=37 (SERT WT: n=10, MRN shSCR: n=15, DRN shSCR: n=12).
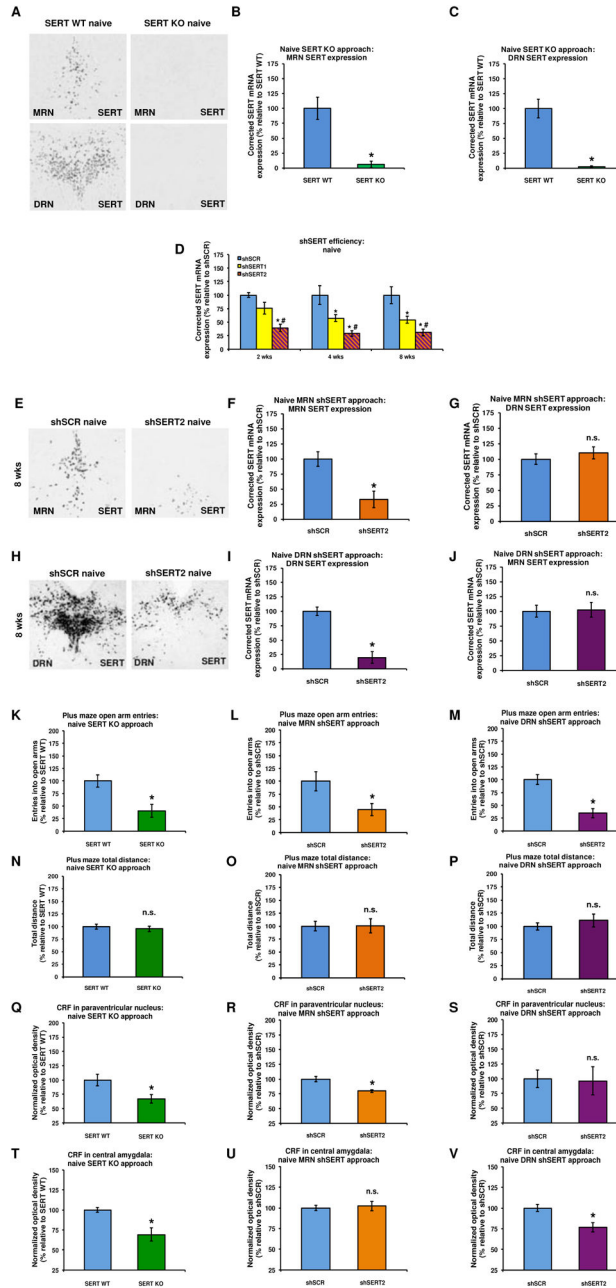
Figure 2.

In situ hybridization revealed no SERT mRNA expression in the MRN (A and B) and DRN (A and C) of SERT KO rats. Intra-raphe infusion of a viral vector encoding either of two shRNA sequences targeting SERT mRNA (shSERT1 or shSERT2) reduced the local expression of SERT mRNA (D). shSERT2 was more effective than shSERT1 in reducing local SERT expression. Eight weeks following intra-MRN injection of shSERT2, SERT expression levels in the MRN, but not DRN, were reduced (E, F and G). Eight weeks following intra-DRN injection of shSERT2, DRN, but not MRN, SERT expression levels were reduced to a similar extent (H, I and J). Both constitutive SERT KO and local SERT

KD reduced the number of open arm entries on the EPM (K, L and M) without affecting locomotor activity (N, O and P) in cocaine-naïve rats. The increase in anxiety-related behavior in cocaine-naïve SERT KO rats (K) was accompanied by a decrease in CRF immunodensity in both the PVN (Q) and CeA (T). In contrast, the increase in anxiety-related behavior in cocaine naïve MRN shSERT rats (L) was accompanied by a decrease in CRF immunodensity in the PVN (R), but not the CeA (U), whereas the increase in anxiety-related behavior in cocaine-naïve DRN shSERT rats (M) was accompanied by a decrease in CRF immunodensity in the CeA (V), but not the PVN (S). All data are normalized to values obtained in SERT WT or shSCR control rats (see Supplementary Table S4 for absolute values of SERT expression and Supplementary Tables S5–S7 for absolute values of cocaine intake, EPM behavior and CRF levels). *Significant decrease (P<0.05) relative to SERT WT or shSCR, #significant difference (P<0.05) between shSERT2 and shSERT1, n.s: no difference relative to control. SERT WT (blue): n=13, SERT KO (green): n=10; MRN shSCR (blue): n=9, MRN shSERT2 (orange): n=9; DRN shSCR (blue): n=15, DRN shSERT2 (purple): n=15.
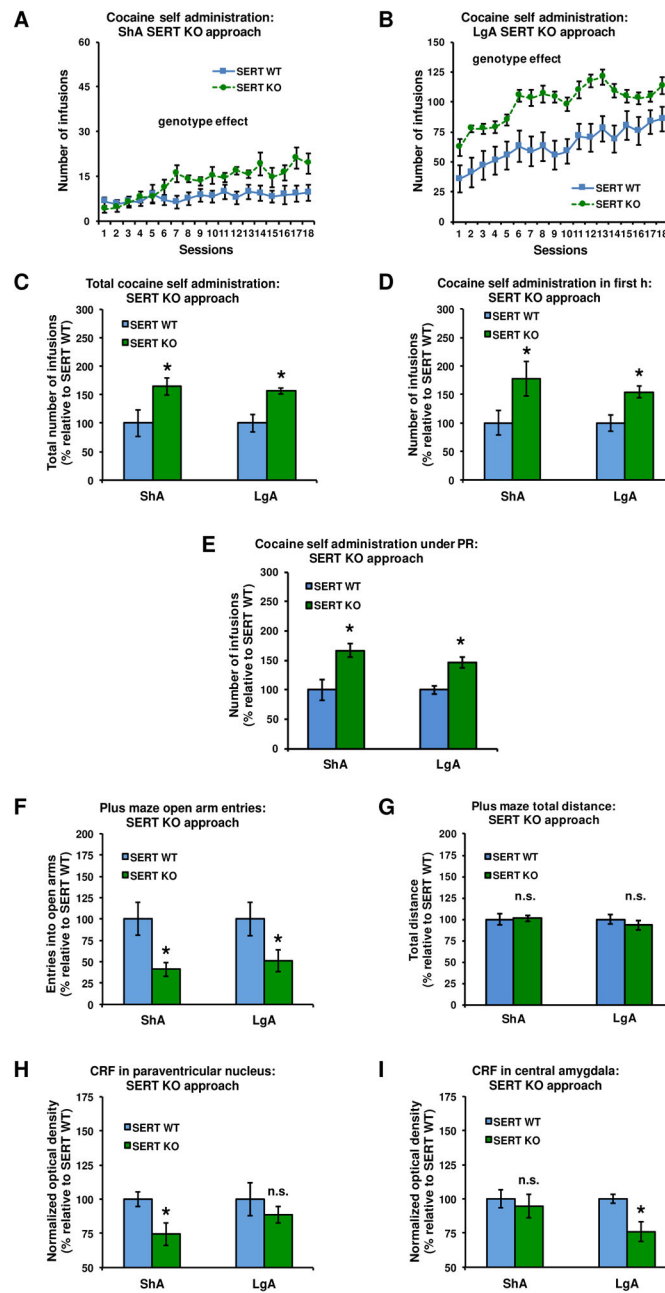
Figure 3.
Constitutive SERT KO increased daily cocaine intake (A–B), cumulative cocaine intake (C), intake during the first h of the final self-administration session (D), and intake during a PR schedule of reinforcement (E) in rats exposed to either ShA or LgA cocaine self-administration. SERT KO also reduced open arm entries, but not locomotor activity, on the EPM after both durations of cocaine access (F and G). The SERT KO-induced increase in ShA cocaine intake (A) was accompanied by a reduction of CRF immunodensity levels in the PVN (H), but not CeA (I). In contrast, the SERT KO-induced increase in LgA intake of cocaine (B) was accompanied by a reduction of CRF in the CeA (I), but not PVN (H) (see

Supplementary Figure S8 for representative pictures). In panels C–I, data are normalized to values obtained in SERT WT counterparts (see Supplementary Table S5 for absolute values). *Significant change (P<0.05) vs SERT WT, n.s: no significant change. ShA: SERT WT (blue): n=14, SERT KO (green): n=12; LgA: SERT WT (blue): n=10, SERT KO (green): n=11.
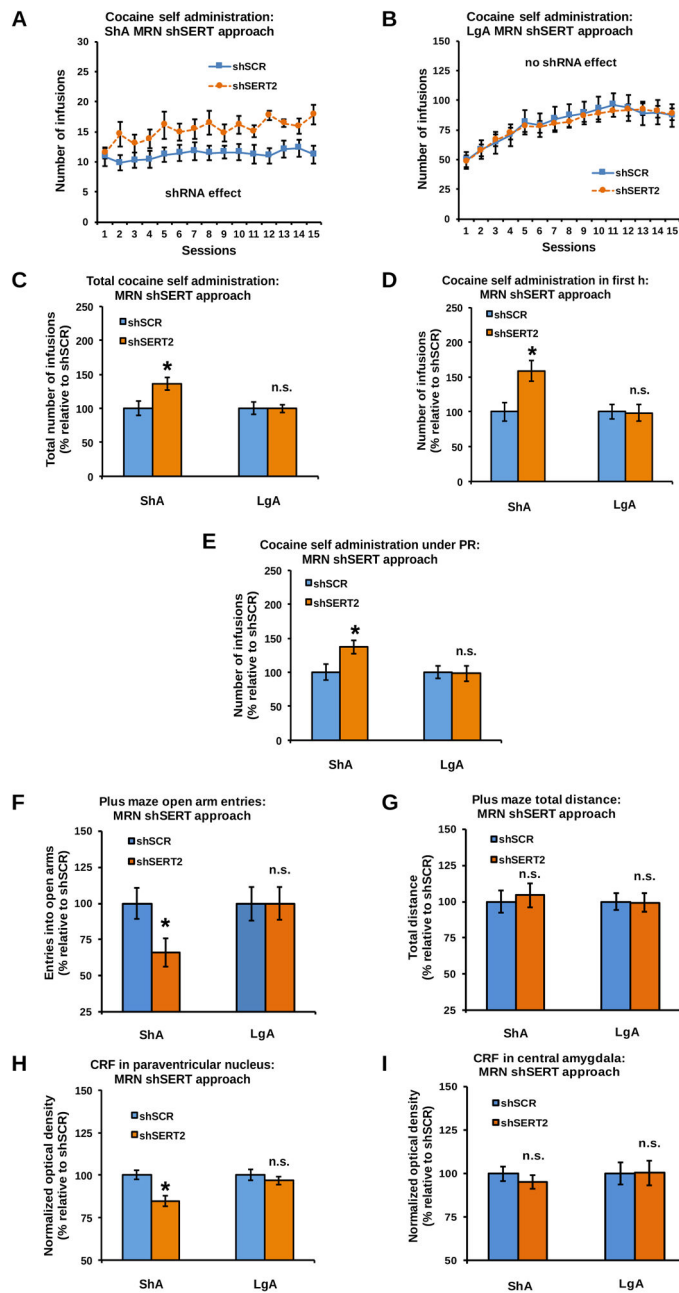
Figure 4.
SERT KD in the MRN increased daily cocaine intake (A–B), cumulative cocaine intake (C), intake during the first h of the final self-administration session (D), and intake during a PR schedule of reinforcement (E) in rats exposed to ShA, but not LgA, cocaine self-administration. MRN-specific SERT KD also reduced open arm entries, but not locomotor activity, on the EPM after ShA, but not LgA, to cocaine (F and G). The lack of shSERT2 effects on LgA cocaine intake (B) is accompanied by a lack of effect on CRF immunodensity in the PVN (H) and CeA (I) of LgA rats. In contrast, the shSERT2-induced increase in ShA cocaine intake (A) was accompanied by a reduction of CRF immunodensity

in the PVN (H), but not CeA (I), of ShA rats (see Supplementary Figure S8 for representative pictures). In panels C–I, data are normalized to values obtained in shSCR counterparts (see Supplementary Table S6 for absolute values). *Significant change (P<0.05) vs shSCR, n.s: no significant change. ShA: shSCR (blue): n=13, shSERT2 (orange): n=13; LgA: shSCR (blue): n=15, shSERT2 (orange): n=13.
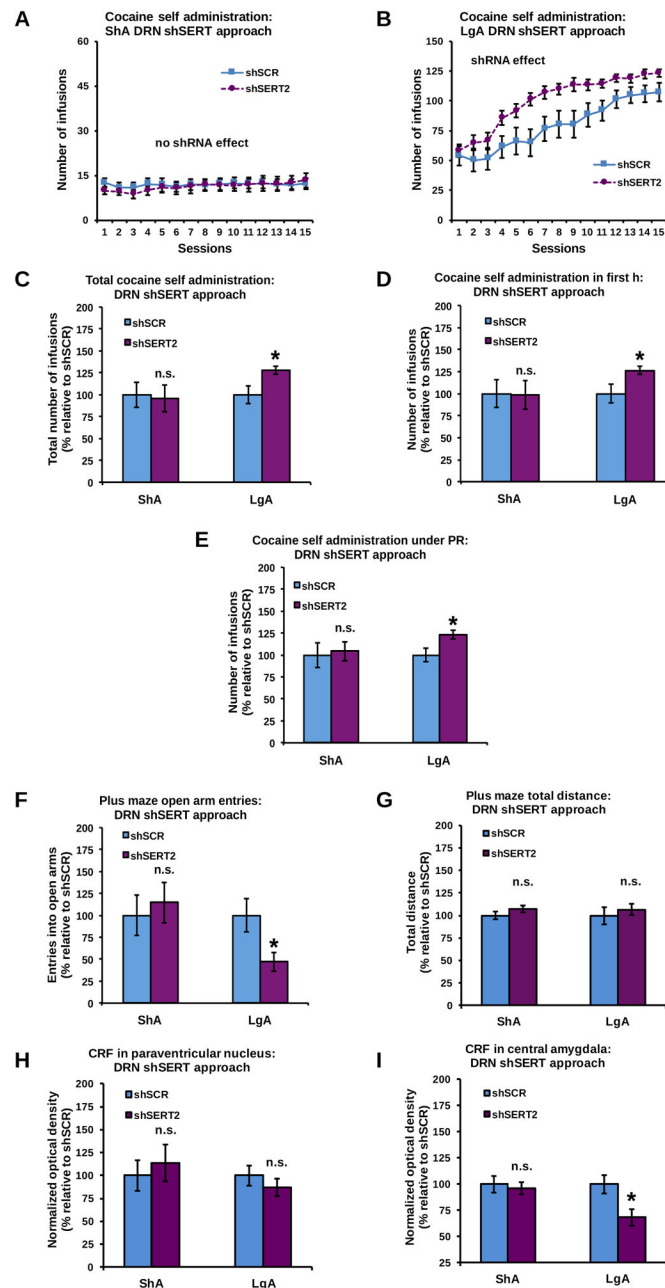
Figure 5.
SERT KD in the DRN increased daily cocaine intake (A–B), cumulative cocaine intake (C), intake during the first h of the final self-administration session (D), and intake during a PR schedule of reinforcement (E) in rats exposed to LgA, but not ShA, cocaine self-administration. DRN-specific SERT KD also reduced open arm entries, but not locomotor activity, on the EPM after LgA, but not ShA, to the cocaine (F and G). The lack of shSERT2 effects on ShA cocaine intake (A) is accompanied by a lack of effect on CRF immunodensity in the PVN (H) and CeA (I) of ShA rats. In contrast, the shSERT2-induced increase in LgA cocaine intake (B) was accompanied by a reduction of CRF immunodensity

in the CeA (I), but not PVN (H), of LgA rats (see Supplementary Figure S8 for representative pictures). In panels C–I, data are normalized to values obtained in shSCR counterparts (see Supplementary Table S7 for absolute values). *Significant change (P<0.05) vs shSCR, n.s: no significant change. ShA: shSCR (blue): n=14, shSERT2 (purple): n=16; LgA: shSCR (blue): n=12, shSERT2 (purple): n=18. Changes in cocaine self-administration following intra-DRN infusion of shSERT1 are presented in Supplementary Figure S7.