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Dopamine, norepinephrine and serotonin transporter gene deletions differentially alter cocaine-induced taste aversion

Jermaine D. Jones [a,*], F. Scott Hall [b], George R. Uhl [b], Anthony L. Riley [a]

[a] Psychopharmacology Laboratory, Department of Psychology, American University, Washington, D.C, United States
[b] Molecular Neurobiology Branch, National Institute on Drug Abuse, Intramural Research Program, Baltimore, MD, United States

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ABSTRACT

Although cocaine is primarily known for its powerful hedonic effects, there is evidence that its affective experience has a notable aversive component that is less well understood. A variety of pharmacological and molecular approaches have implicated enhanced monoamine (MA) neurotransmission in the aversive effects of cocaine. Although numerous studies have yielded data supportive of the role of the monoamines (indirectly and directly), the specific system suggested to be involved differs across studies and paradigms (Freeman et al., 2005b; Grupp, 1997; Roberts and Fibiger, 1997). Monoamine transporter knockout mice have been useful in the study of many different aspects of cocaine effects relevant to human drug use and addiction, yet an assessment of the effects of deletion of the genes for the dopamine, norepinephrine and serotonin transporters (DAT, NET, and SERT, respectively) on cocaine's aversive properties has yet to be performed (Uhl et al., 2002). In the current investigation, the strength of cocaine-induced aversions was compared among three groups of transgenic mice with deletions of the genes responsible for the production of one of the monoamine transporters. When compared to their respective WT controls, dopamine transporter deletion slightly attenuated cocaine-induced aversion while deletion of SERT or NET resulted in a more significant delay in the onset and strength of cocaine-induced taste aversions. The data lead us to conclude that the action of cocaine to inhibit NET contributes most substantially to its aversive effects, with some involvement of SERT and minimal contribution of DAT.

1. Introduction

The powerful reinforcing effects of cocaine are believed to underlie its abuse liability (Kuhar et al., 1991). Although cocaine's potent hedonic effects dominate its affective experience, reports indicate that cocaine has aversive effects as well (Anthony et al., 1989; Blanchard and Blanchard, 1999; Estelles et al., 2004; McDougle et al., 1994; Rosen and Kosten, 1992; Yang et al., 1992). It has been suggested that these aversive effects impact the rewarding effects of cocaine to the extent that they may significantly influence its initial use and subsequent abuse potential (Lynch and Carroll, 2001; Riley and Simpson, 2001; Stolerman and D'Mello, 1981). A number of experimental procedures have been used to specifically assay these aversive effects (Ettenberg and Geist, 1991; Koob et al., 1997; Riley and Simpson, 2001). One such procedure is conditioned taste aversion (CTA) learning, a type of Pavlovian conditioning in which a novel taste is paired with the aversive effects of a compound (Garcia et al., 1955). Following repeated exposure to the taste-drug pairing, the subjects will typically avoid consumption of the novel solution (i.e., display a taste aversion), the degree of which is considered to be directly related to the potency of the aversive effects of the drug (Riley and Tuck, 1985; see also http://www.CTALearning.com).

Manipulation of the aversive affective experience produced by cocaine administration may provide a means of abuse intervention and, accordingly, there has been increased interest in determining the physiological basis of cocaine's aversive properties. Cocaine non-selectively inhibits the reuptake of monoamine neurotransmitters through its blockade of their neuronal plasma membrane transporters, thereby acting as a potent indirect agonist of the norepinephrine (NE), dopamine (DA) and serotonin (5-HT) systems (Taylor and Ho, 1978). Although cocaine has multiple sites of action, its effects upon dopaminergic neurotransmission have long been thought to mediate most of its behavioral effects, most notably, locomotor stimulation and reward (Kuhar et al., 1991).

Interestingly, research from a variety of pharmacological and molecular approaches has implicated actions upon these systems as the basis of the aversive effects of cocaine as well (Freeman et al., 2005a; Goudie and Thornton, 1975; Grupp, 1997; Roberts and Fibiger, 1975; Serafine and Riley, 2009). Direct comparisons of the taste aversions produced by selective monoamine transporter inhibitors to

* Corresponding author. Substance Use Research Center, Columbia College of Physicians and Surgeons, New York State Psychiatric Institute, 1051 Riverside Drive, New York, NY 10032, United States. Tel.: +1 212 543 5151.
E-mail address: JermaineDJones@gmail.com (J.D. Jones).

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those produced by cocaine have repeatedly observed that only NET inhibitors are capable of matching the aversive potency of cocaine (Freeman et al., 2005b; Jones et al., 2009), although results from other approaches have yielded conflicting results (Freeman et al., 2008; Serafine and Riley, 2009). The majority of previous research assessing the specific neurochemistry of cocaine-induced aversions has been pharmacological in nature (either by mimicking or antagonizing cocaine-induced effects) and with this approach comes unique methodological concerns (i.e., selectivity of the pharmacological agent employed). For that matter, one of the fundamental questions regarding many of the effects of cocaine is whether multiple sites of action contribute, or can contribute, to its effects.

Transgenic techniques present an alternative approach to help elucidate the specific roles of DA, 5-HT and NE transmission in cocaine-induced CTA. These techniques, particularly gene knockouts (KO), have proven useful in investigations of the biochemical basis underlying the behavioral effects of many commonly abused drugs (Cordero-Erausquin et al., 2000; Giros et al., 1996; Hall et al., 2001, 2009; Miner et al., 1995; Pan et al., 2009; Sora et al., 1998; Thomsen et al., 2009; Tzavara et al., 2009). To date, research utilizing transgenic mice lacking the monoamine transporters (DAT, NET and/or SERT) has primarily been used to assess their roles in the ability of cocaine to modulate reward and locomotor behavior (Chen et al., 2006; Giros et al., 1996; Hall et al., 2002; Hall et al., 2009; Rocha, 2003; Sora et al., 1998, 2001; Thomsen et al., 2009; Xu et al., 2000a). However, this work has indirectly implicated the contributions of the MA systems in the aversive effects of cocaine as well. Specifically, KO mice lacking either NET or SERT display enhanced cocaine-induced place preferences (CPP), an effect that is even further enhanced in NET/SERT double KO mice (Hall et al., 2002; Sora et al., 1998; Xu et al., 2000a; see discussion in Hall et al., 2004). This suggests that in wild-type subjects with intact transporters, cocaine-induced elevations in NE and/or 5-HT may be aversive and, thus, counteract the normally rewarding effects of cocaine mediated elsewhere (Uhl et al., 2002). Given that such work only indirectly addresses the role of these neurotransmitters in cocaine-induced aversions, a direct assessment of the ability of subjects with DAT, NET or SERT gene deletions to acquire aversions is needed. Accordingly, in the following investigation cocaine-induced conditioned taste aversions were assessed in groups of KO mice with lifelong deletions of the genes responsible for the production of either the DA, NE or 5-HT transporters using homozygous (−/−) monoamine transporter KO mice (DAT KO, SERT KO, and NET KO) compared to wild-type littermate controls.

2. Methods and materials

2.1. Subjects

The DAT, SERT and NET knockout mice and wild-type littermate controls used in these experiments were bred from DAT/SERT and NET/SERT double knockout lines that have been previously described (Hall et al., 2002; Sora et al., 2001). These double knockout lines had been derived by crosses of the original single knockout lines (Bengel et al., 1998; Sora et al., 1998; Xu et al., 2000b). Thus, these gene knockouts were expressed on a mixed C57BL/6J-129 background (ES cells were from R1, J1, and AK7 cell lines from different 129 substrains; see Jackson Labs for details: http://www.jaxmice.jax.org/jaxnotes/archive/481e.html). All of the original single gene knockout lines were produced in the typical fashion (e.g. some type of 129-derived ES cells and subsequent backcrossing for one generation to C57BL/6). Because the mice from the double knockout lines have been bred for more than 10 generations by heterozygous crosses within a small breeding population (e.g. <100 breeding pairs) it is likely that fixation of alleles has occurred at most loci. However, because the fixation process is random, which particular loci become fixed to the C57 or 129 alleles is likely to differ between lines, giving them more "129-like" or "C57-like" characteristics. This would depend on the phenotype being considered and likely accounts for the differences between WT controls discussed below, which is a factor that makes the littermate control design used here essential. Although the lines used to produce these mice were double knockout lines, only single gene knockouts were used in these experiments. DAT KO and SERT KO mice were from the DAT/SERT double knockout line, and NET KO mice were from the NET/SERT double knockout line. Therefore, separate WT controls were needed for the NET KO mice (designated NET WT), but the same WT controls were used for both DAT KO and SERT KO mice (designated DAT/SERT WT). All subjects were bred from heterozygote crosses producing −/− and +/+ littermates. Mice were genotyped using polymerase chain reaction (PCR) amplification using two internal primers, one targeted at the knockout insertion sequence and one targeted at the WT gene, and one external primer, which generated two products identifying the WT and KO genes. The DAT and SERT transgenic knockout insertion sequences contained a neomycin gene (NEO), while the NET KO contained a green fluorescent protein gene insert (GFP). PCR using TSG DNA polymerase (Bio Basic, Inc., Canada) was performed on DNA that was eluted from tail tip fragments after digestion overnight in Protease K. For DAT genotyping the external primer (AGT GTG TGC AGG GCA TGG TGT A) and the WT primer (TAG GCA CTG CTG ACG ATG ACT G) produced a 500 bp band, while the external primer and the NEO primer (CTC GTC GTG ACC CAT GGC GAT) produced a 600 bp band. For SERT genotyping the external primer (GCT CTC AGT CTT GTC TCC ATA AC) and the WT primer (TGC TGA CTG GAG TAC AGG CTA G) produced a 620 bp band, while the external primer and the NEO primer (CTC GTC GTG ACC CAT GGC GAT) produced an 800 bp band. For NET genotyping the external primer (GCT CTG TCC CTG TGC TTC ACG) and the WT primer (TGA GGC CTA AGC TGG AGC TCG) produced a 600 bp band, while the external primer and the GFP primer (CGG TGA ACA GCT CCT CGC CC) produced a 470 bp band. A total of 204 subjects were utilized, and as is common for most transgenic studies male and female mice of each genotype were employed. Since no sex differences were observed the data presented here has been collapsed across gender (see Statistical analysis section for more detail). At the time of testing all subjects were between the ages of 12 and 20 weeks. Each genotype/dose group consisted of between 8 and 12 mice with subjects only being used in one condition (*N*'s: D/S WT = 50, DAT KO = 32, SERT KO = 42, NET WT = 33, and NET KO = 37).

2.2. Apparatus/housing

All subjects were individually housed in Plexiglas bins (44.5 × 23 × 20 cm) fitted with wire-grated tops. Subjects were maintained under a 12:12 LD cycle (lights on at 0800 h) and at an ambient temperature of 23 °C. Harland Rat and Mouse Laboratory Diet was available *ad libitum* throughout the experiment. Water was available *ad libitum* until the experimental procedures were initiated (see below). All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council, 2003) and were approved by the American University Institutional Animal Care and Use Committee.

2.3. Drugs and solutions

Cocaine hydrochloride (HCl) (generously supplied by the National Institute on Drug Abuse) was dissolved in saline and subcutaneously (sc) administered in a concentration of 10 mg/ml (cocaine doses expressed as the salt).

2.4. Drug conditioning

On Day 1 of this phase, subjects were allowed 1 h access to a novel 0.1% sodium saccharin solution during their scheduled fluid-access period. Based on their level of saccharin consumption, a computer program was used to assign subjects of a particular genotype to one of the four drug conditions. For example, following saccharin presentation at Trial 1, the level of saccharin consumption for all SERT KO subjects was calculated. Based upon the amount of saccharin they consumed, SERT KO subjects were assigned to one of the 4 drug conditions in a manner which ensured roughly equivalent group means. This stratified method of assignment ensures roughly equivalent levels of consumption between drug conditions, within each genotype. The accuracy of this procedure was always confirmed by an ANOVA showing that among the four drug conditions of a particular genotype, there was no significant difference at Trial 1. Within 10–15 min following saccharin access, subjects were injected sc with one of four doses of cocaine (0, 18, 32 and 50 mg/kg). This 1/4 log function has been shown to represent a wide range of cocaine's aversive effects within this preparation, producing significant dose-dependent aversions in both rats and mice (Busse et al., 2005; Freeman et al., 2005a; Jones et al., 2009). The 0 mg/kg dose consisted of a vehicle injection of saline which was isovolumetric with the highest dose of drug. The different drug doses and genotypes resulted in the following groups: DAT/SERT WT (0, 18, 32, and 50 mg/kg); NET WT (0, 18, 32, and 50 mg/kg); DAT KO (0, 18, 32, and 50 mg/kg); SERT KO (0, 18, 32, and 50 mg/kg); and NET KO (0, 18, 32, and 50 mg/kg). On the 3 days following each conditioning day, all subjects were allowed 1 h access to water without drug administration. This alternating procedure of conditioning/water recovery was repeated for a total of four complete cycles.

2.5. Statistical analysis

Analysis of variance (ANOVA) was first employed to compare absolute saccharin consumption between the two WT conditions following conditioning with all 4 drug doses. Subsequently comparisons were made among all 5 genotypes conditioned with saline alone in order to examine baseline differences in saccharin preference. Although not mentioned in the Results section, comparisons between male and female mice of both WT conditions found no significant sex difference (all p's ≥ than 0.6). Subsequent analyses were performed without respect to this variable (collapsed across sex). In order to control for the significant baseline differences observed among the 5 genotypes (addressed in more detail in the results section), the data were transformed into percent shift from baseline scores [(TRIAL {2, 3, 4} × 100) / TRIAL 1 − 100 = % Shift].

For subsequent analyses, in order to take into account potential baseline differences in consumption and because there were differences between the two WT conditions, comparisons of the degree of aversions (as measured by % shift score) were made using Repeated-Measure Analysis of Variance (ANOVA). The between-subject factors for each of these analyses were DOSE (0, 18, 32, and 50) and/or GENOTYPE (D/S WT, DAT KO, SERT KO, NET WT, and NET KO) and the within-subjects factor was TRIAL (2,3,4). To determine the basis of significant effects in the overall ANOVA, separate analyses compared the two wild-type strains, as well as comparing the knockouts to their respective wild-type littermates. When significant effects were observed in these ANOVAs, follow-up ANOVAs were used to compare saccharin consumption at each trial and for each dose of drug. When necessary, Tukey's HSD post-hoc tests were utilized to specify between genotype and/or trial differences.

3. Results

3.1. Comparisons absolute saccharin consumption

Between the two WT conditions, ANOVA revealed significant main effects of Trial F (3, 225) = 67.92, p<0.001, DOSE F (3, 75) = 26.97, p<0.001 and GENOTYPE F (1, 75) = 5.91, p<0.05, along with significant Trial × DOSE F (9, 225) = 10.71, p<0.01 and Trial × GENOTYPE interactions F (3, 225) = 5.55, p<0.01. Differences in consumption following saline-only administration (Fig. 1) highlight differences in saccharin preference or neophobia among all five genotypes, further validating the necessity of the percent shift transformation [Genotype main effect: F (4, 42) = 8.32, p<0.001]. Subsequent comparisons were made utilizing a % shift score in order to control for background strain differences observed between the two WT conditions in these analyses.

3.2. Percent shift comparisons among all five genotypes

An initial analysis using Repeated-Measures ANOVA compared % shift scores across Trials, Doses and Genotypes. This analysis found significant main effects for TRIAL: F (2, 348) = 20.42, p<0.001, DOSE: F (3, 174) = 28.66, p<0.001 and GENOTYPE: F (4, 174) = 3.92, p<0.005, along with significant TRIAL × DOSE: F (6, 348) = 8.79, p<0.001 and TRIAL × GENOTYPE: F (8, 348) = 1.98, p<0.05 interactions. Subsequent analyses revealed the nature of the overall effect of GENOTYPE and the GENOTYPE × TRIAL interaction shown in this initial analysis, part of which was due to background strain differences. These differences necessitated separate comparisons of DAT KO, SERT KO and NET KO mice to their respective WT controls.

3.3. Percent shift comparison between D/S WT and NET WT

ANOVA comparing % shift scores between the D/S WT and NET WT groups was performed in order to assess potential background strain differences between the two lines (Fig. 2). This analysis revealed significant main effects of GENOTYPE [F (1, 75) = 3.99, p<0.05], TRIAL [F (2, 15) = 18.17, p<0.001] and DOSE [F (3, 75) = 28.59, p<0.001]. Significant interactions were also observed for GENOTYPE × DOSE [F (3, 75) = 2.82, p<0.05] and TRIAL × DOSE [F (6, 150) = 5.38, p<0.001], and the GENOTYPE × TRIAL interaction approached significance [F (2, 150) = 2.98, p = 0.057]. Differences between these two groups further demonstrate the potential confounding effects of comparing genotypes which do not share the same genetic background. Planned comparisons between the two genotypes at each trial found no significant differences between the two groups for Trial 2 with any of the drug doses tested. Yet, at Trial 3 the degree of suppression in saccharin consumption was smaller in the NET WT group than the D/S WT group following conditioning with saline (p<0.001), 18 (p<0.001) and 32 (p<0.05) mg/kg of drug. For the final trial (Trial 4), the % decrease was smaller in the NET WT group following conditioning with saline (p<0.001) and 18 (p<0.05) mg/kg of cocaine. Thus, in order to control for the confounding influence of background strain, the analyses of the effects of NET, SERT and DAT knockout reported below were only made between genotypes with a common genetic background (i.e., D/S background = D/S WT, DAT KO and SERT KO; and NET background = NET WT and NET KO).

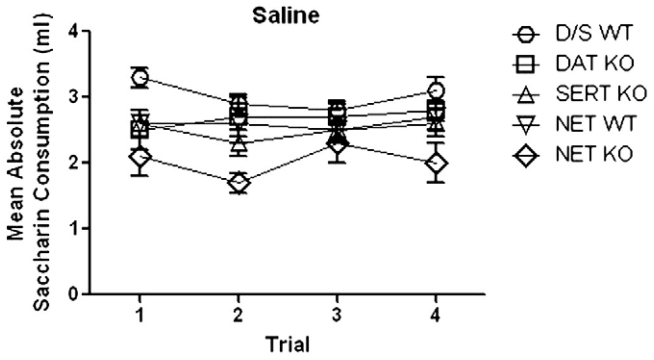

Fig. 1. Illustrates the mean (±SEM) absolute saccharin consumption for all subjects conditioned with saline (0 mg/kg) on conditioning Trials 1–4.

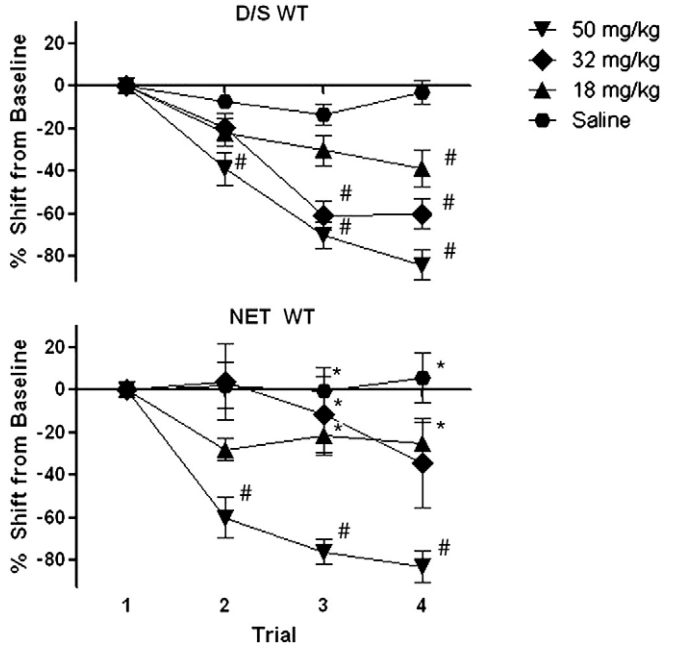

Fig. 2. Illustrates the mean (±SEM) percent decrease in saccharin consumption between Trial 1 (baseline) and Trials 2–4 for D/S WT and NET WT subjects after conditioning with 0, 18, 32 or 50 mg/kg doses of cocaine. *Denotes significant difference between the two WT groups when conditioned with the same dose. #Denotes significant difference from the saline-conditioned control group of the same genotype.

3.4. Percent shift comparisons among D/S WT, DAT KO and SERT KO mice

Omnibus comparison of the % shift in saccharin consumption across TRIAL, DOSE and GENOTYPE found significant main effects of GENOTYPE $F(2, 112) = 4.97$, $p<0.01$, DOSE $F(3, 112) = 21.68$ $p<0.001$, and TRIAL $F(2, 112) = 21.71$ $p<0.001$, as well as a significant TRIAL × DOSE interaction $F(6, 224) = 8.66$, $p<0.001$. As such, planned analyses sought to determine whether there were differences in the onset and strength of the aversions among the D/S background groups (Fig. 3). These analyses compared consumption among the four dose conditions, at each trial, as well as across trials for each dose.

In order to examine differences in the rate of aversion development among the three genotypes, the first analysis compared changes in saccharin consumption following conditioning with each active DOSE of cocaine (18, 32, and 50 mg/kg) versus saline (0 mg/kg) at each TRIAL (separately for each genotype). On Trial 2 (% shift for Trial 1 was 0 for all groups), a significant suppression in saccharin consumption was observed in D/S WT subjects conditioned with the highest dose versus saline-treated subjects (50 mg/kg; $p<0.05$). On this trial, DAT KO and SERT KO groups conditioned with 18, 32 and 50 mg/kg of cocaine did not differ significantly from subjects of the same genotype conditioned with saline. On Trial 3 conditioning with 32 mg/kg cocaine significantly reduced saccharin consumption in D/S WT mice ($p<0.001$) and DAT KO mice ($p<0.05$), but not SERT KO mice (versus saline-treated controls). Similarly, for Trial 3 the 50 mg/kg dose significantly reduced saccharin in these two genotypes (D/S WT: $p<0.001$, DAT KO $p<0.05$). The effects of the 32 and 50 mg/kg doses in SERT KO subjects were not significant for Trial 3. On Trial 4, the lowest dose of cocaine (18 mg/kg) significantly reduced saccharin consumption in the D/S WT group ($p<0.01$), but not in either of the KO groups. On Trial 4, the 32 mg/kg dose produced a significant suppression of consumption in all three genotypes (D/S WT: $p<0.001$, DAT KO: $p<0.005$, and SERT KO: $p<0.05$), as did the highest dose of cocaine (D/S WT: $p<0.001$, DAT KO: $p<0.01$, SERT KO: $p<0.01$).

In order to compare differences in the strength (degree of suppression) of cocaine-induced aversions, further analysis compared

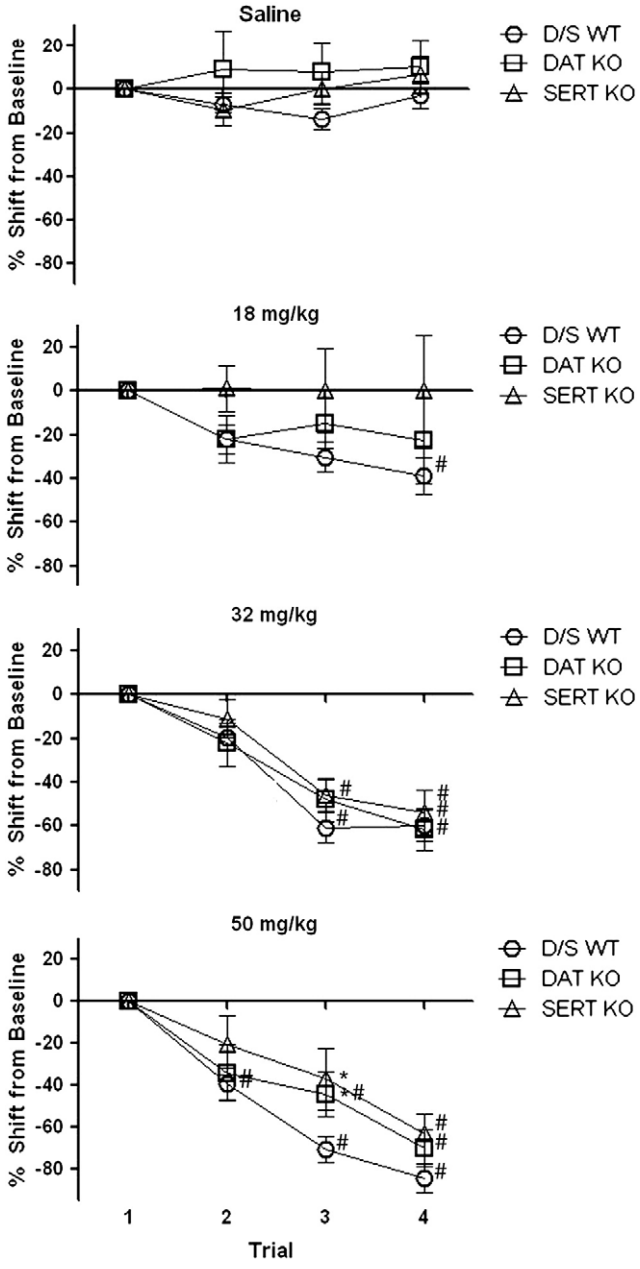

Fig. 3. Illustrates the mean (±SEM) percent decrease in saccharin consumption between Trial 1 (baseline) and Trials 2–4 for D/S WT, DAT KO and SERT KO subjects after conditioning with 0, 18, 32 or 50 mg/kg doses of cocaine. *Denotes significant difference from the WT group conditioned with the same dose. #Denotes significant difference from the saline-conditioned control group of the same genotype.

the degree of suppression in each of the three GENOTYPES (D/S WT, DAT KO, and SERT KO) at each TRIAL (separately for each DOSE). This analysis found that significant differences among the three groups were only observed in response to conditioning with 50 mg/kg of cocaine on Trial 3. On this trial, the percent decrease from baseline for both SERT KO ($p<0.05$) and DAT KO ($p<0.05$) mice was significantly smaller than for D/S WT mice (Fig. 4). No other dose comparisons were significant. When conditioned with the same dose of drug, the DAT KO and SERT KO groups never differed significantly from each other at any trial.

3.5. Percent shift comparisons between NET WT and NET KO mice

Again, based upon the results of the omnibus ANOVA [GENOTYPE $F(1, 62) = 2.97$, $p<0.05$; DOSE $F(3, 62) = 10.06$ $p<0.001$; TRIAL $F(2,$

124) = 4.58 $p<0.05$; TRIAL × DOSE; $F(6, 124) = 2.63$, $p<0.05$] planned analyses sought to determine if there were differences in the onset and strength of the aversions between the NET WT and NET KO groups (Fig. 4). In order to assess differences in the onset of the aversions between the two genotypes, we first compared changes in saccharin consumption following conditioning with each active DOSE of cocaine (18, 32, and 50 mg/kg) versus saline (0 mg/kg) at each TRIAL (separately for each genotype). On Trial 2, the largest dose of cocaine (50 mg/kg) significantly suppressed saccharin consumption compared to saline treatment in NET WT mice ($p<0.01$). None of the cocaine doses had significant effects in NET KO subjects on Trial 2. On Trial 3, only the 50 mg/kg dose significantly suppressed saccharin consumption in NET WT mice ($p<0.01$), while again cocaine was ineffective in NET KO subjects at all doses. Similarly on Trial 4, significant suppression of saccharin consumption was only observed in response to conditioning with the largest cocaine dose, but this was observed in both NET WT ($p<0.001$) and KO mice ($p<0.05$).

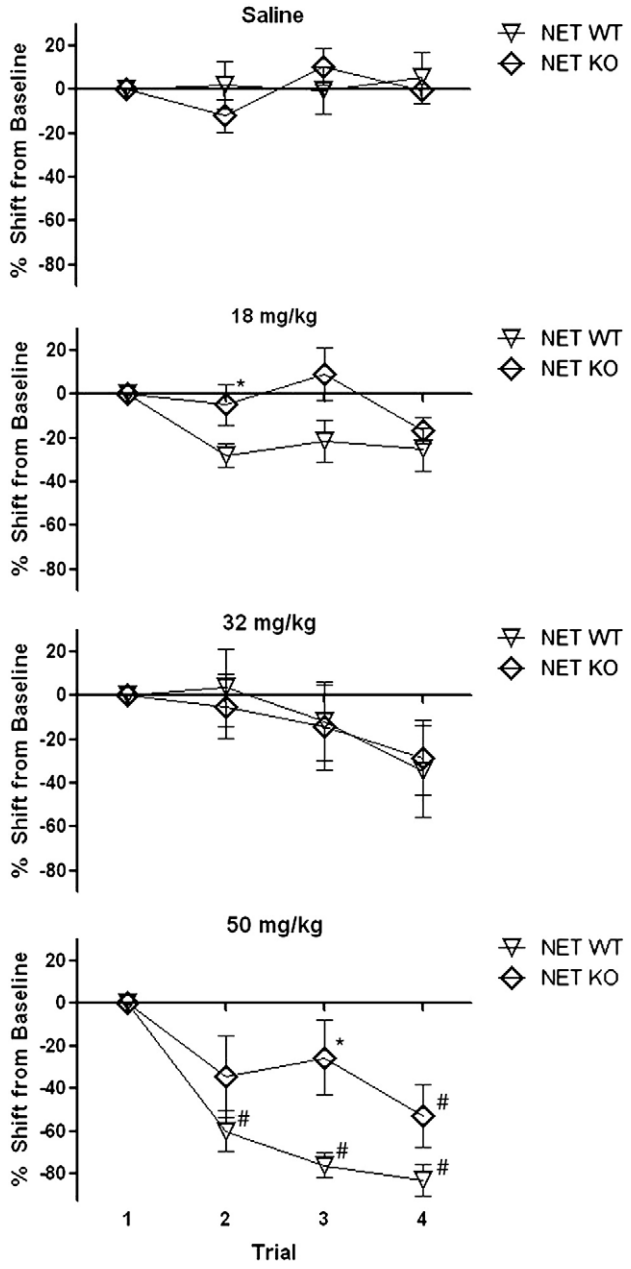

Fig. 4. Illustrates the mean (±SEM) percent decrease in saccharin consumption between Trial 1 (baseline) and Trials 2–4 for NET WT, and NET KO subjects after conditioning with 0, 18, 32 or 50 mg/kg doses of cocaine. *Denotes significant difference from the WT group conditioned with the same dose. #Denotes significant difference from the saline-conditioned control group of the same genotype.

In order to examine differences in the strength of cocaine-induced taste aversion, planned comparisons assessed the degree of suppression produced between the two GENOTYPES (NET WT and NET KO) on each TRIAL (separately for each DOSE). This analysis revealed significant GENOTYPE differences in saccharin consumption following conditioning with 18 mg/kg (Trial 2: $p<0.05$) and 50 mg/kg (Trial 3: $p<0.05$) of cocaine (Fig. 4). For both of these trials, the percent decrease in suppression produced by cocaine was significantly smaller in NET KO mice.

4. Discussion

Transgenic manipulations of the monoamine transporters, as well as other monoamine system related genes, have proven to be an effective tool in investigating the biological basis of many cocaine-mediated effects (for a review, see Hall et al., 2004). Not surprisingly, previous studies have concentrated upon the rewarding or reinforcing effects of cocaine. The results reported here are the first to examine directly the aversive effects of cocaine in monoamine transporter knockout mice, although the potential importance of cocaine aversion for explaining the effects of SERT KO and NET KO on cocaine CPP has been hypothesized previously (Uhl et al., 2002). In the current study, individual groups of monoamine transporter KO mice were conditioned with various doses of cocaine in order to assess the effects of DA, NE and 5-HT transporter deletions on the acquisition of cocaine-induced CTA.

Since the knockout mice used here were from two separate lines, it was important to consider the genetic background of the control mice and to compare the knockouts to littermate controls. Reduced cocaine CTA was observed overall for NET WT mice compared to D/S WT mice. For the D/S WT group, all three doses of cocaine-induced significant aversions in a dose-dependent manner were compared to saline-treated controls. DAT deletion slightly reduced the strength of cocaine-induced CTA. Unlike their WT controls, conditioning with 18 mg/kg of cocaine did not produce a significant suppression of saccharin consumption in the DAT KO group and aversions were only observed in response to conditioning with the two highest doses. SERT transporter deletion appeared to produce a somewhat greater reduction in the strength of cocaine-induced CTA. In SERT KO mice, only the highest dose of cocaine (50 mg/kg) was able to significantly reduce saccharin consumption. Although direct dose and trial comparisons between the DAT and SERT KO mice did not identify any differences in the strength of the cocaine-induced aversions, there was a delay in the development of cocaine-induced aversions in SERT KO mice compared to both D/S WT mice and DAT KO mice, suggesting that they were less sensitive to the aversive properties of cocaine than either of these groups. Although the highest conditioning dose induced significant aversions in all three genotypes, the rate of acquisition of these aversions differed significantly between genotypes. The aversion developed most rapidly in the D/S WT mice (Trial 2), compared to DAT KO (Trial 3) and SERT KO mice (Trial 4). Additionally the strength of the aversion produced by this dose of cocaine was reduced in both knockout groups, but only for Trial 3.

As mentioned above, when compared to D/S WT mice, decreased sensitivity to the aversive properties of cocaine was observed in NET WT mice. Specifically, NET WT mice exhibited delayed rate of acquisition and reduced strength of their aversions. These differences between strains of wild-type mice are not surprising. Previous research has shown that the behavioral effects of cocaine can differ significantly as a function of mouse strain (Rocha et al., 1998a,b). More relevant to the current investigation, other studies have also observed differences in cocaine CTA between C57 and 129 mouse strains (de Bruin et al., 2006; Randall-Thompson, 2005). In NET WT and NET KO mice, suppression of saccharin consumption compared to

saline-treated mice was only observed following conditioning with 50 mg/kg cocaine. For the WT group, suppression began at an earlier point during conditioning (Trial 2) and persisted throughout all subsequent trials, while in NET KO mice decreased consumption was only observed for final trial. Additionally, the strength of the suppression in saccharin consumption produced by cocaine was reduced in NET KO mice compared to WT mice. On multiple trials, saccharin consumption was more suppressed by pairing with cocaine in WT mice. Although it may be argued that the lack of a significant cocaine dose-response effect within the NET WT group limits the conclusions which can be drawn from these results, rarely in the rodent taste aversion literature are higher doses employed due to animal health and safety concerns. Nevertheless, in contrast to the relatively small effects observed for DAT KO or SERT KO, NET KO produced relatively large reductions in the ability of cocaine to induce a taste aversion.

Therefore, these experiments demonstrate that DAT, SERT and NET gene deletion all altered the sensitivity to the aversive effects of cocaine, although on far too different degrees. Only a minimal effect of DAT KO was found, a somewhat greater effect of SERT KO and the largest effect produced by NET KO.

Based on these findings, it appears that inhibition of the norepinephrine transporter contributes most substantially to the aversive effects of cocaine in the CTA preparation, with some involvement of SERT inhibition and the least contribution by DAT inhibition. Research characterizing the behavioral phenotypes of NET KO and SERT KO mice provides additional support for this hypothesis. Microdialysis studies have correlated increased NE transmission with depression (Mokrani et al., 1997). Forced-swim and tail suspension tests also reveal changes in depressant-like behavior in both NET KO and SERT KO mice; although these effects are dependent on strain and other considerations (Dziedzicka-Wasylewska et al., 2006; Holmes et al., 2002; Perona et al., 2008; Xu et al., 2000a). SERT KO mice also have impaired stress coping and fear conditioning (Wellman et al., 2007), and Holmes et al. (2002, 2003a,b) reported that SERT KO mice exhibit exaggerated neuroendocrine and adreno medullary responses to stress and potentiated anxiety-like behavior in response to novelty as well as in the elevated plus maze, the light–dark box and the open field. Researchers often attribute the elevated anxiety-like behavioral phenotypes found in these models to elevated extracellular 5-HT and/or NE (Kalueff et al., 2006), although some studies have concluded that antidepressant-like effects result from increased 5-HT and NE transmission (Gainetdinov and Caron, 2003; Perona et al., 2008).

Other findings in transgenic mouse models support these conclusions. 5-HT_{1B} and 5-HT_{2C} receptor KO mice have both been shown to self-administer cocaine at higher levels in comparison to wild-type controls (Pattij et al., 2003, Rocha et al., 2002). One interpretation of these results may be that this removes an aversive component of cocaine's hedonic effects mediated by these receptors.

Although it would appear that increases in 5-HT neurotransmission may be involved in the aversive effects of cocaine, there is also evidence that enhanced 5-HT neurotransmission is an essential component of cocaine's rewarding effects. Elimination of the dopamine transporter has not been shown to abolish the rewarding effects of cocaine in most studies (Giros et al., 1996; Medvedev et al., 2005; Rocha et al., 1998a,b; Sora et al., 1998), but deletion of both DAT and SERT do abolish cocaine conditioned place preference (Sora et al., 2001). This finding strongly suggests that enhanced 5-HT neurotransmission contributes to cocaine reward (Sora et al., 2001), at least under some circumstances. In other circumstances it appears that much of the rewarding effects of cocaine is mediated by DAT. A transgenic modification of DAT that prevents cocaine binding, without substantially affecting uptake and the consequent compensatory changes, was sufficient to eliminate cocaine conditioned place preference (Chen et al., 2006). Finally, a more detailed study of cocaine self-administration demonstrated that it was largely eliminated in the same line that had previously been shown to have normal cocaine CPP (Thomsen et al., 2009). Thus, although deletion of DAT has substantial effects on the rewarding and reinforcing effects of cocaine, it would appear that 5-HT neurotransmission also contributes to these rewarding effects, as well as the aversive affective components of cocaine.

In a previous study from this laboratory, DAT was implicated in the aversive effects of cocaine. Randall-Thompson (2005) observed attenuated cocaine-induced taste aversion, but intact cocaine-induced place preference, in DAT knockout mice. In the current study, a slight attenuation in the magnitude and onset of cocaine CTA was also found in DAT KO mice. However, the observation by Randall-Thompson and colleagues is difficult to compare to the current, more extensive examination of cocaine CTA, due to a number of methodological differences from the present investigation. That study examined only one dose of cocaine and also used a combined CPP/CTA design. The authors acknowledged that the interaction of the aversion and place preference procedures had a detrimental impact on the overall results.

Returning to a consideration of the present findings, although single transporter deletions were effective at attenuating the aversive effects of cocaine, cocaine CTA was not eliminated with any single gene deletion. It may be that the actions of cocaine upon multiple transporters contribute to cocaine-induced taste aversions; an implication supported by research on the rewarding aspects of cocaine, discussed above. If the actions of cocaine at multiple transporters constitute the basis of its aversive effects, then it might be expected that cocaine-induced taste aversions could only be attenuated (and not eliminated) by single transporter deletions. Our research, and that of others, suggests that deletion of both the NET and SERT genes would have the most influence upon the aversive properties of cocaine and their combined deletion may more substantially diminish cocaine CTA.

Another possibility why single transporter deletions may not have completely eliminated the acquisition of cocaine-induced taste aversions may be the adaptations in these systems that have been shown to occur in response to transporter deletion (Benoit-Marand et al., 2000; Fabre et al., 2000; Jones et al., 1999; Kim et al., 2005). Considerable biochemical changes have been observed in all three monoamine transporter knockouts, including altered receptor expression, changes in neurotransmitter regulation and decreased autoreceptor function (for summary, see Gainetdinov and Caron, 2003). Such adaptations have been suggested to be responsible for the maintenance of cocaine-mediated behaviors including CPP and self-administration in DAT KO mice (Rocha, 2003; Sora et al., 1998). Because of these potential compensatory alterations in the normal mechanisms underlying reward and aversion in DAT, NET and SERT KO mice the effects observed in these mice may not necessarily reflect the mechanisms controlling the aversive effects of cocaine in WT mice; although it raises the possibility that there may be individual differences in the basis of cocaine between individuals. Evidence that such profound alterations occur includes evidence that selective serotonin (fluoxetine) and norepinephrine (nisoxetine) inhibitors gain reinforcing properties in DAT KO mice that are not found in WT mice (Hall et al., 2002). However, a notable consideration is that the findings obtained here using gene knockouts are in agreement with most pharmacological research utilizing CTA (Jones et al., 2009; Serafine and Riley, 2009; though see Freeman et al., 2008 for conflicting results).

From consideration of the present results, it is clear that the ability of cocaine to induce CTA is primarily mediated by its effects on NET, with some contribution by SERT, and perhaps a minor contribution by DAT. Future studies may confirm this multi-transporter hypothesis using multiple transporter knockouts, as was done for cocaine CPP (Sora et al., 2001). However, in order to better define the relative contributions of each system, other investigations may benefit from

targeting specific monoamine receptors. Transporter deletion affects all of the neurons receiving those monoamingeric inputs that express that transporter, whereas receptor knockouts would only affect a subset expressing that particular receptor, likely to be expressed in specific brain areas, and involved in a subset of the actions of cocaine. There is compelling evidence suggesting that there are substantial and conflicting ways in which different receptors for the same neurotransmitter can contribute to the affective profile of a compound (Carey et al., 2005; Di Chiara et al., 2004; Parsons et al., 1998; Pruitt et al., 1995; Rocha et al., 2002; Shippenberg et al., 1993; Woolverton and Kleven, 1988). Collectively, these data, along with studies of the mechanisms underlying the rewarding effects of cocaine, indicate that varying the proportion of the monoaminergic effects at each of the three monoamine transporters is an important and critical determinant of the relative rewarding and aversive consequences of monoamine transporter blockers that could lead to significantly different affective profiles for different drugs, or between individuals that differ in the functioning of these transporters.

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