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Response of Limbic Neurotensin Systems to Methamphetamine Self-Administration

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Abstract

Methamphetamine (METH) abuse is personally and socially devastating. Although effects of METH on dopamine (DA) systems likely contribute to its highly addictive nature, no medications are approved to treat METH dependence. Thus, we and others have studied the METH-induced responses of neurotensin (NT) systems. Neurotensin is associated with inhibitory feedback action on DA projections and NT levels are elevated in both the nucleus accumbens and dorsal striatum after non-contingent treatment with high doses of METH. In the present study we employed a METH self-administration (SA) model (linked to lever pressing) to demonstrate that substitution of a NT agonist for METH, while not significantly affecting motor activity, dramatically reduced lever pressing but was not self-administered per se. We also found that nucleus accumbens NT levels were elevated via a D1 mechanism after 5 sessions in rats self-administering METH (SAM), with a lesser effect in corresponding yoked rats. Extended (15 daily sessions) exposure to METH SA manifested similar NT responses; however, more detailed analyses revealed: (i) 15 d of METH SA significantly elevated NT levels in the nucleus accumbens shell and dorsal striatum, but not the nucleus accumbens core, with a lesser effect in the corresponding yoked METH rats; (ii) the elevation of NT in both the nucleus accumbens shell and dorsal striatum significantly correlated with the total amount of METH received in the self-administering, but not the corresponding yoked, METH rats; and (iii) a NT agonist blocked, but a NT antagonist did not alter, lever-pressing behavior on day 15 in SAM rats. After 5 days in SAM animals, NT levels were also elevated in the ventral tegmental area, but not frontal cortex of rats self-administering METH.

Keywords

neurotensin; methamphetamine; dopamine; nucleus accumbens; self-administration; dorsal striatum

The personal and social devastation caused by methamphetamine (METH) abuse/addiction is severe and likely due to its very addictive nature, a refractory dependence, and an abuse pattern that can cause psychosis, violence and criminal behavior (Meredith et al., 2005). Even though it has been demonstrated that profound actions on dopamine (DA) systems mediate many of METH's harmful effects (Fleckenstein et al., 2007), no

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pharmacotherapeutics are currently approved for METH dependence (Elkashelf et al., 2008). Consequently, it is important to study DA-linked systems to identify effective novel strategies to treat METH addiction. To this end, we have investigated the role of the neuropeptide, neurotensin (NT) in METH abuse models.

Neurotensin is particularly interesting in this regard due to its neuromodulatory connection with limbic DA systems (Ferraro et al. 2007; Merchant et al. 1988). Neurotensin associated with limbic systems is synthesized in the nucleus accumbens and frontal cortex and interacts with mesolimbic and mesocortical DA systems, respectively (Azzi et al. 1998). Like the DA pathways, these limbic NT projections likely originate for the most part in the ventral tegmental area (VTA) and help regulate the limbic DA pathways through mechanisms initiated by either D1 or D2 receptor activation. Overall, activation of limbic NT systems or receptors antagonizes DA activity (Merchant et al. 1988; Feifel et al., 2008), with an apparent physiological role to counteract overactive DA pathways (Wagstaff et al., 1994), leading to the conclusion that NT is a natural neuroleptic; thus, it has been suggested that NT agonists would be effective treatment for hyper-DA limbic psychoses such as schizophrenia (Feifel et al., 2008; Hadden et al., 2005). Study of the role of DA receptors in regulating NT systems has revealed that stimulation of D1 or D2 receptors have opposite effects, either increasing or decreasing the NT tissue levels in the nucleus accumbens, respectively (Merchant et al., 1989). In this regard, others and we found that the NT increases in nucleus accumbens content caused by D1 receptor activation are likely due to an elevated expression of NT precursor mRNA. This suggests that increased NT content in these structures is an accumulation of NT from activated synthesis (Adams et al. 2001; Castel et al., 1994a; Merchant et al., 1994).

Much like D1 receptor activation, non-contingent high doses of METH (~10 mg/kg) also increase both NT tissue levels and associated mRNA expression in the nucleus accumbens. These effects are blocked by a D1, but not a D2, antagonist implying that high doses of METH exert a D1 influence on NT limbic systems (Merchant et al. 1988; Hanson et al. 1992). Interestingly, high-dose METH does not alter NT release in the nucleus accumbens (Wagstaff et al., 1996), suggesting that consequent NT tissue level changes are not due to changes in release per se, but likely due to elevated synthesis and accumulation; thus, there probably is little alteration in NT-mediated feedback inhibition under high-dose METH conditions in limbic structures permitting dramatic increases in DA release caused by METH actions on monoamine transporters (Fleckenstein et al., 2007). Although these types of non-contingent studies clearly implicate NT limbic systems in the immediate pharmacological effects of high doses of METH and its neurobiology, their relationship to human abuse is unclear. Thus, the relevance of these NT responses to critical drug dependence issues such as drug wanting, anticipation, contingent administration and extinction is unknown.

In order to mimic more accurately the real-life situation of human psychostimulant abuse, the present study determined the response of NT systems to METH SA (contingent on appropriate lever pressing) paradigms. In these studies the contingent NT responses in rats trained to lever press for METH i.v. infusions, were determined by comparing NT tissue levels in the nucleus accumbens and related structures (e.g., VTA and frontal cortex: Azzi et al. 1998) during stable patterns of 5–15 d METH SA to those of yoked-METH (YM) and yoked-saline (YS) animals.

The treatment paradigm was based on a modification of the operant-training method described by Fuchs et al. (2005) and See et al. (2007). Using this contingent model we confirmed the inhibitory action of NT systems on METH effects because replacement of METH with a NT agonist 1 hr into the 6th SA session stopped lever pressing: this finding

suggests that systemic administration of a NT agonist does not substitute for METH and is not self-administered per se. We also observed that NT tissue levels in the nucleus accumbens shell, but not the core, were increased by METH SA through a D1, but not a D2, mechanism. Further scrutiny revealed that: (i) the increases in NT levels were ~ 2-fold greater in the self-administering METH (SAM) vs. yoked-METH (YM) rats; and (ii) after 15 d the changes in NT levels in both the nucleus accumbens shell and the dorsal striatum significantly correlated with the total METH exposure in the SAM, but not in the corresponding YM animals. Further examination revealed that METH SA also elevated NT in the ventral tegmental area (VTA), but not frontal cortex.

2. Experimental procedures

2.1. Animals

Male Sprague-Dawley rats (300–350 gm; Charles River Laboratories, Raleigh, NC) were allowed to acclimate to home cages, the procedure room and an ambient temperature of 25 degrees C for at least 1 week prior to experimentation. All groups used for SA (contingent) studies were initially group-housed except when placed individually into operant chambers for food training (Fuchs et al., 2005). After catheter implantation, these rats were kept individually housed for the remainder of the experiments. These rats were killed 6 h after the final 5th or 15th METH SA daily session when NT tissue levels were determined. This time point was previously shown to cause a significant striatal NT response after METH SA (Frankel et al. 2011). For the non-contingent studies represented in Fig. 2, the rats received a single investigator-administered dose of 10 mg/kg METH, s.c. and were killed 12 h later: this time point was selected because it has been shown in other studies to cause a maximum NT response to non-contingent psychostimulant treatment (Alburges et al. 2011).

2.2. Food Training

Because we have observed that in our treatment paradigm, food training predicted an animal's ability to acquire drug SA, all rats used were required to pass food training as described previously (Fuchs et al., 2005; See et al., 2007; Frankel et al. 2011). Briefly, rats were restricted to approximately 85% of their free-feeding food weight, and then placed in Coulbourn operant chambers connected to a PC computer running Graphic State software. Each chamber was equipped with two retractable levers, a food-pellet dispenser between the levers, and a house light on the wall opposite the levers. One lever was "active" resulting in the delivery of a food pellet while the other lever had no programmed consequences. Training consisted of a schedule of food reinforcement (45 mg Rodent Grain food pellets; Bio-Serv Delivering Solutions) FR 1 with only the stimulus-appropriate (drug-lever) eliciting the reward. If, during the overnight (15 h) food-training session a rat received 50 pellets, the FR was increased to 2. If the rat obtained another 50 food pellets, the FR increased to 3 for the balance of the session. Rats remained in the food-training phase for either 4 days or until they achieved 2 consecutive sessions on the FR 3 schedule after which they received a jugular vein catheter implant (~90% of the rats successfully completed food training).

2.3. Drugs and Chemicals

Methamphetamine hydrochloride was furnished by the National Institute on Drug Abuse, National Institute of Health (Bethesda, MD) and infusion quantities were calculated as the free base.

In a study to determine the effect of activating NT receptors on METH SA, a selective agonist (Lys(CH₂NH)lys-Pro, Trp-*tert*-Leu-Leu-Oet [PD149163; PD] (Petrie et al. 2004), and stable NT fragment [NT (8–13)], was obtained from the National Institutes of Health,

National Institute of Mental Health). This PD compound stimulates central nervous system NT1 receptors when delivered systemically (Feifel et al., 2008) and was used to replace METH after 1 h into the 6th SA session (Fig. 1). During this period, each lever press by the rat infused 0.1 mg/infusion i.v. of PD. Because there is no report of administering this PD compound i.v. in a SA format, we selected a dose that when administered 1–3 times by lever pressing would deliver a dose similar to the 0.25–1.0 mg/kg used by others for investigator-administered s.c. treatment (Feifel et al. 2008; Frankel et al. 2011). It is noteworthy that the 0.1 mg i.v. dose of PD per se had no observable effects on locomotion or motor function of the rats. Lever pressing during the 7th session also caused a similar dose of PD infusion at an FR1 rate. METH SA was restored to that used for the 5th session conditions for the 8th – 11th sessions.

In a separate study, the effect of stimulating, or blocking, the NT1 receptors on METH SA was examined in SAM rats on the 15th day of SA by injecting the NT agonist PD149163 (0.5 mg/kg, s.c.; Feifel et al. 2008) or a NT antagonist (SR48692 [purchased from Tocris Bioscience; 0.3 mg/kg; Antonelli et al. 2007; Wagstaff et al. 1994], respectively, 15 minutes prior to the session. In the experiments to study the DA receptor mechanisms underlying the NT responses to contingent METH treatments, yoked-METH rats were pretreated by i.p. injections of saline, 0.5 mg/kg of either the D1 (SCH23390 from Research Biochemicals Inc.), or D2 (eticlopride from Research Biochemicals Inc.) receptor antagonists. These drugs were administered 15 min prior to each of the 5 sessions (see Fig. 3).

2.4. METH Self-administration Training

Operant training was based on procedures as previously described (Fuchs et al., 2005; See et al., 2007; Frankel et al. 2011). Self-administration sessions were conducted during the light cycle; however, animals were exposed to a 14/10 h light/dark environment while in their home cages. Each self-administering METH (SAM) rat underwent 4-h sessions in Coulbourn operant chambers for 5 to 15 consecutive days and were exposed to the presentation of an identical right and left lever. One of the levers was selected (it did not matter which) as active such that appropriate pressing resulted in a primary stimulus of METH infusion (0.06 mg/infusion) followed by lever retraction for a 20-second time-out period until subsequent presentation of the levers. The SAM rats selectively pressed the active lever >90% of the time. Data collection and reinforcer delivery were controlled by a PC computer using Graphic State Notation (Coulbourn Instruments). Prior to initiation of SA training, each SAM rat was randomly paired with two yoked rats. The yoked animals were prepared and treated identically as the SAM animals except that both levers in the operant chamber had no programmed consequences. Furthermore, these yoked animals received either METH (0.06 mg/infusion; YM) or saline (equal volume; YS) at times determined by the behavior of the linked SAM rats. After each SA session, all rats were returned to their home cages and given access to 6 gms of Purina rat chow. After 1 day of METH SA at an FR 1, the FR was increased within the session to an FR 2 after 25 presses, an FR 3 after 30 presses and finally an FR 5 after 45 presses (rats typically reached the FR 5 stage toward the conclusion of the 2nd, or beginning of the 3rd, session of acquisition and remained throughout the study).

The YS rats averaged ~19 lever presses on what had been the active lever for food training, during their first session of being yoked to SAM animals. The lever-pressing behavior dropped off to 1–3 presses/session by the 4th and 5th sessions prior to sacrificing as previously described (Frankel et al. 2011). For SAM animals lever pressing became relatively stable for the 3–15 sessions. It should also be noted that because of the yoking arrangement, the pattern of METH infusions for the YM rats was identical to that of the SAM animals. For these studies SAM rats were considered to have reached criterion if they maintained relatively steady lever pressing by the 3rd day of SA (with ~ 0.6 mg/kg/day

METH infusions and self-administered a total of at least 3 mg of i.v. METH by the 5th day; this was typical of 80% of our SAM animals). Six hours after the 5th (Figs. 2, 3, 6) or 15th (Figs. 4, 5) SA session, the rats were killed, brains removed and frozen on dry ice.

2.5. Catheter implantation

After food training, rats were anesthetized with Equithesin (i.p.) and indwelling catheters consisting of a screw-type connector, silastic tubing (10 cm i.d., 0.64 mm o.d., 1.19 mm) Prolite polypropylene monofilament mesh and cranioplastic cement were implanted beneath the skin of the back (at the shoulder-blades). The outlet of the catheter ran subcutaneously around the underside of the animal with the end inserted into the right jugular vein. The catheter was secured to the surrounding tissue with sutures. A 0.1-ml antibiotic solution containing Cefazolin (10.0 mg/ml) dissolved in heparinized saline (70 U/ml; Sigma, St Louis, MO) was flushed through the catheter for 3 days after surgery to extend catheter patency. Thereafter, catheters were flushed with 0.1 ml of heparinized saline before and after each SA session to prevent clotting. Stylets were inserted into the catheters when rats were not connected to infusion pumps. All experiments were approved by the University of Utah Institutional Animal Care and Use Committee and adhered to the National Academy of Sciences 'Guide for the Care and Use of Laboratory Animals'.

2.6. Analysis of Neuropeptide Levels in Tissue

Levels of NT were determined by specific and sensitive RIAs previously described (Frankel et al., 2007; Alburges and Hanson 1999). The whole nucleus accumbens (Fig. 2, 3), or the nucleus accumbens shell and core (Fig 4,5), VTA and frontal cortex (Fig. 7) were dissected (Paxinos and Watson, 1982) and frozen at -80°C until assayed for the appropriate neuropeptide. Mean peptide levels (pg/mg protein) for NT in YS control animals were: 509 for nucleus accumbens shell; 456 for nucleus accumbens core; 661 for VTA; and 36 for frontal cortex. To facilitate comparisons, data were normalized by dividing with respective control mean values.

Statistical Analysis

Three or more groups were compared by two-way analysis of variance followed by a Newman-Keuls post hoc test or Student's t-test. All results, were considered significant when $p < 0.05$. The significance of correlation between NT tissue levels and amount of METH administered (Fig. 5) was determined by calculating a Pearson correlation coefficient.

3. Results

Because activation of limbic NT systems or receptors has been reported to antagonize DA activity (Merchant et al. 1988; Wagstaff et al. 1994; Feifel et al. 2008; Torregrossa and Kalivas 2008), we determined if substitution of the NT1 receptor agonist, PD149163, for METH would influence lever-pressing behavior after a METH self-administration pattern had been established. Thus, 1 h into the 6th METH SA session a switch was made without disturbing the rats, such that each lever press caused an iv infusion of 0.1 mg of PD rather than 0.06 mg of METH. As shown in Fig. 1, the lever-pressing behavior dramatically decreased from $16 \pm 7/1$ h (4-h pressing rate of 64 ± 28 ; open column on day 6) to $1.8 \pm 0.8/2^{\text{nd}}-4^{\text{th}}$ hours (4-h pressing rate of 2.4 ± 1.1 ; black column on day 6). The PD-treated rats behaved normally while in the operant chamber and ate normally overnight after returned to their home cage. During the 7th session, lever pressing continued to be associated with PD SA resulting in a total lever-pressing activity of 2.9 ± 0.7 . After the METH infusions were restored for the 8th session, there was a significant restoration of lever pressing;

however the combination of lever pressing during sessions 9, 10 and 11 only reached $52 \pm 11\%$ of the lever pressing combination of the same rats for the 3rd, 4th, and 5th sessions.

In order to assess the METH-induced responses of NT systems associated with the nucleus accumbens, the tissue levels of this neuropeptide were measured in this limbic structure of rats that non-contingently received 1 s.c. injection of METH (10 mg/kg/administration). As previously reported (Geisler and Zahm 2006; Merchant et al. 1988), investigator-administered (noncontingent) METH increased the nucleus accumbens level of NT (Fig. 2: $t(10)=2.3$, $p<0.05$). To compare this METH effect with that in rats which self-administered (contingent) this stimulant, NT levels of the nucleus accumbens were measured in animals that received i.v. METH infusions in response to appropriate lever pressing (labeled SAM in Fig. 2). As with the non-contingent drug treatment, NT tissue content in these animals was significantly elevated vs. YS (control) animals ($F(2,26)=24.17$, $p<0.05$). A similar, but significantly less increase in striatal NT levels was also observed in the corresponding YM group.

We examined the mechanism underlying the effect of METH SA on nucleus accumbens NT systems. Initially, this was attempted by pretreating SAM rats with either a D1 (SCH23390) or D2 (eticlopride) antagonist prior to placing these animals in their operant chamber for the first METH SA session. However, because both of these antagonists substantially interfered with lever pressing it was not possible to ascertain if the METH-induced NT changes in SAM animals were mediated by DA receptors. Consequently, we employed an indirect approach to study the mechanisms in this NT response by pretreating the YM rats prior to each of the 5 sessions of METH infusions which were determined by the lever-pressing behavior of linked SAM animals (see Fig. 3). The YM+sal effect compared to the YS+sal group was blocked by a SCH23390 pretreatment (see YM+SCH vs. YM+sal and YS+sal: $F(6,89)=12.58$, $p<0.05$). In contrast, the eticlopride pretreatment had no effect of its own, nor did it block the increase in NT that occurred in the YM animals (see YM+etic vs. YM+sal and YS+etic). These findings suggest that D1, but not D2, receptors are involved with these contingent METH effects on the nucleus accumbens NT systems.

Because the SA protocols we used for both Figs. 2 and 3 were for 5 d, we next determined if this length of time was sufficient to produce a stable pattern of operant responses and if the NT responses to METH SA were similar with longer protocols. In addition, we also wanted to examine how the core and shell of the nucleus accumbens contributed to the NT responses. These objectives were achieved by employing a 15-d period for METH self-administration (Fig. 4). Using this extended protocol we observed that: (i) under the conditions described in the Experimental procedures section there was stable METH infusion/session from the 3rd (0.59 ± 0.1 mg) to the 15th (0.69 ± 0.16 mg) day with a mean of 0.66 ± 0.2 mg/session ($N=8-16$); (ii) dorsal striatal NT levels were significantly ($p<0.02$) elevated in both SAM and YM vs YS rats 6 h after the 15th SA session ($F(2,31)=11.45$, $p<0.05$), with the SAM effect significantly greater than the YM effect; (iii) we examined a similar pattern in nucleus accumbens shell to that in the dorsal striatum and the effects shown in Fig. 2 after 5 d of SA ($F(2,28)=40.24$, $p<0.05$); and (iv) in contrast, we did not observe significant changes in the nucleus accumbens core in either SAM or YM vs. corresponding YS rats ($F(2,27)=2.77$, $p=0.08$). We next determined if there was a correlation between the changes in the dorsal striatal (DS) and nucleus accumbens shell (NAs) NT levels and the total amount of METH self-administered (Fig. 5). We found a significant correlation in the dorsal striatal ($r^2=0.40$; $p=0.04$) and nucleus accumbens shell ($r^2=0.54$; $p<0.01$) regions of SAM. In contrast, despite receiving identical patterns of i.v. infusions of METH, there was no significant correlations found in the dorsal striatal ($p=0.83$) or nucleus accumbens shell ($p=0.78$) regions of the corresponding YM rats.

To confirm that activation of NT1 receptors had an inhibitory effect on SA behavior after 15 d in SAM rats, we pretreated these animals with the NT1 agonist, PD19163 prior to their last session. Similar to our findings in Fig. 1, stimulation of NT1 receptors blocked SA activity in the SAM rats after 14 d of SAM activity. In contrast, we observed no alterations in lever-pressing behavior in similar SAM rats after a pretreatment with the NT1 antagonist, SR48692 (Fig. 6)

Because the findings in Figs. 2, 3, 4 and 5 suggested that the accumbens NT system (particularly in the shell region) responds to METH SA, it is possible that NT projections from the ventral tegmental area (VTA; Geisler and Zahm 2006) contributed to this response. To test this possibility, we also examined NT levels in the VTA in SAM and YM rats. As with the accumbens NT response, we found that NT levels in the VTA were also increased in the SAM vs. the YS animals ($F(2,60)=6.13$, $p<0.05$); however, there was no significant change in the NT levels in corresponding YM animals. In order to determine if another NT projection from VTA responded in a manner similar to that associated with the nucleus accumbens, we measured NT levels in the frontal cortex of SAM rats. This structure was examined because in non-contingent studies the NT responses in the frontal cortex were opposite of those in the nucleus accumbens (Merchant et al. 1988) and we wanted to determine if there also existed a similar difference after METH SA. In contrast to the nucleus accumbens and VTA responses, NT in the frontal cortex, was not significantly altered in either the SAM or associated YM rats (see Fig. 7).

4. Discussion

Neurotensin is closely associated with central DA systems, such as those found in the nucleus accumbens, suggesting that NT systems play an important role in mediating behavior under regulation of ventral striatal DA projections. Despite overwhelming evidence of linkage between NT and DA pathways, there is some controversy as to the precise function of NT systems as it relates to DA regulation; thus, its influence on DA activity is somewhat dependent on site (e.g., cell body vs. terminals), and means (e.g., directly into brain regions vs. systemically) of NT agonist/antagonist administration (Ramos-Ortolaza et al. 2009; Robledo et al. 1993). However, relative to limbic regions, studies suggest that NT indirectly regulates pre-junctional dopaminergic transmission in the nucleus accumbens by increasing glutamate release followed by increases in GABA release, which in turn exerts an inhibitory action on DA transmission (Ferraro et al. 2007; Ferraro et al. 1998). Due to its apparent antagonistic outcome on limbic DA activity, NT has been referred to as a natural neuroleptic (Feifel et al. 2008; Hadden et al. 2005) and has been suggested to be useful in the management of excessive DA activity (Feifel et al. 2008; Chartoff et al., 2004). This possibility is supported by the observations of Wagstaff et al. (1994) that a NT-selective antiserum (to complex and inactivate extracellular NT) administered into the ventricles and a systemically administered NT antagonist substantially enhance both METH-induced release of DA in the nucleus accumbens and locomotor activity.

While the role of NT in regulating the DA function of the ventral striatum requires further elucidation, more is known about how changes in DA activity influence the limbic NT systems associated with the nucleus accumbens. These studies have identified D-1-mediated increases in accumbens levels of NT in response to activation of DA receptors either directly by selective agonists (Singh et al. 1992) or indirectly by psychostimulants such as METH (Merchant et al. 1988). In contrast, it also has been reported that activation of the D-2 receptor with a selective agonist actually decreases NT content in the ventral striatum (Merchant et al., 1989).

Dopamine-related changes in the NT systems of the nucleus accumbens have also been monitored by assessing the synthesis of this neuropeptide through measurements of the mRNA for its NT/neuromedin N precursor. Such studies demonstrated that changes in DA receptor activity caused by either selective direct D1-receptor agonists or indirect agonists such as METH, significantly increase the levels of NT mRNA in the nucleus accumbens (Adams et al. 2001) probably associated with VTA projections (Geisler and Zahm 2006) and the elevated synthesis likely contributes to the increases in the associated NT tissue content (Castel et al. 1994b).

Taken together, these studies suggest that activation of D1 receptors either by a selective D1 agonist or a high dose of METH, influences NT signaling and synthesis, increasing tissue accumulation of this peptide in the nucleus accumbens. In contrast, D2 receptor activation appears to have an opposite effect (i.e., it decreases striatal NT content). To appreciate better the interaction between limbic NT and DA systems especially after METH administration, it is necessary to identify how the actual release of NT is affected by METH treatment. To this end, Wagstaff et al. (1996) reported that NT release is increased in nucleus accumbens after a low, but not after the high METH dose that is required to elevate striatal NT level, in fact, exposure of striatal tissue slices to a high concentration of METH is reported to actually reduce NT release (Hanson et al. 1992). Taken together these findings suggest that investigator-controlled administration of METH high doses either does not change, or reduces, NT release patterns per se, while increasing synthesis and levels of NT in the mesolimbic pathway.

As previously mentioned, until the present report, all studies of METH-induced NT changes in the limbic system have been based on non-contingent models. Others and we have elucidated many of the elements of this drug-neuropeptide interaction; however, while these findings have been extremely helpful in our attempt to better understand the pharmacology of this potent stimulant, their link to the critical issues related to human abuse is unclear. Thus, the relevance of these NT responses to important drug dependence elements such as drug wanting, anticipation, contingent administration and extinction are unknown. The design of the present METH SA study was intended to help address these questions.

Employing the operant training described in the Experimental procedures we confirmed other reports that systemic stimulation of NT receptors inhibit DA activation caused by stimulants such as METH (Feifel et al. 2008; Hadden et al. 2005; Wagstaff et al. 1994). The study represented in Fig. 1 was intended to test the possibility that NT agonists block METH SA because they stimulate DA release themselves and consequently substitute for METH in a SA paradigm. This possibility was eliminated as we observed that substitution of the PD compound for METH 1 hr into the 6th session dramatically reduced lever pressing while allowing normal motor activity. The reduced lever-pressing effect of PD continued into day 7, while lever-pressing continued to be associated with i.v. injections of the NT agonist. However, on days 8–11 lever-pressing activity rebounded after METH was returned to the syringes. These findings suggest that systemic activation of the NT receptors inhibited the operant behavior associated with METH SA, while not substituting for the stimulant and apparently the PD compound does not possess rewarding properties itself. The lever-pressing on days 9–11 vs. 3–5 suggests a significant long-term suppression of the lever-pressing even after the PD compound was likely no longer present suggesting a continual persistence of the inhibitory effect of activating the NT receptors even after direct effects of the agonist was gone. This finding is consistent with the report of Boules et al. (2011) that NT69L, another NT agonist, also blocks lever pressing associated with nicotine SA suggesting that activation of NT1 receptors suppresses operant behavior linked with stimulant SA in general.

As described in Experimental procedures, the YS rats previously food-trained behaved as expected and the manifested lever-pressing associated with food extinguished very quickly once the rats were placed in the chambers and learned that their operant behavior no longer was associated with a reward nor any consequence. It was also observed that those SAM rats which received METH infusions contingent to appropriate operant responding, typically lever pressed stably to receive a very consistent dose of METH/session after the 2nd to 3rd session for either a 5-d or 15-d treatment. This suggests that under these conditions a steady lever-pressing behavior for METH was established early and continued for many days.

The findings presented in Figs. 2 and 3 demonstrate that similar nucleus accumbens NT changes occurred in both non-contingent and contingent METH administration, suggesting that under both types of conditions, there exists the presence of a direct pharmacology of METH that is independent of contingency-related elements. However, the observation that NT changes in the nucleus accumbens were significantly enhanced in the SAM vs. YM rats may be because in the SAM rats there existed factors such as cue response, motivation, anticipation and task persistence or drug-seeking that like the METH pharmacology also enhanced DA activity and increased NT levels. These METH-induced changes in NT levels support the belief that the nucleus accumbens networks have a role in mediating the rewarding effects of drugs and integrates inputs from limbic and cortical regions, linking motivation with action (Carlezon and Thomas 2009).

The fact that METH SA elevated NT levels in the nucleus accumbens suggested that the mechanism for this NT response was activation of D1 receptors due to METH-induced release of DA associated with the mesolimbic pathway. This possibility was tested by pretreating rats prior to METH session with either a D1 (SCH23390) or a D2 (eticlopride) antagonist. Interestingly, when either drug was given to the SAM rats prior to a SA session they would not lever press in contrast to the robust operant behavior that normally occurred (see description in the Experimental procedures). This finding could mean that activation of both of these DA receptor types contribute to operant behavior associated with reward. However, a lack of lever pressing (and of course a lack of METH infusion) by the SAM animals made it impossible to determine if these DA receptors were important to the NT responses to METH in these animals. To address this problem, we instead administered the antagonists prior to the sessions for the YM group. The results in Fig. 3 supported the hypothesis that these nucleus accumbens NT effects were mediated by the D1, and not the D2, receptor. Because previous non-contingent studies demonstrated that such an elevation in NT tissue content is due to activation of NT synthesis and apparently not associated with release of NT per se (Adams et al. 2001; Wagstaff et al. 1996), we speculate that such is also true under these contingent conditions. The apparent lack of a D2 role in the increases of NT levels in the nucleus accumbens in SAM rats is consistent with the observations that activation of this DA receptor causes a decrease in accumbens NT levels (Merchant et al. 1989). However, it is worth mentioning that in contrast to METH SA, we have observed that extinction of this behavior causes a reduction in NT levels in the nucleus accumbens and that this effect is blocked by D2, and not D1, antagonist, suggesting that the D2 receptor plays an important role in the extinction of operant behaviors (data unpublished).

Another important conclusion that also is implied by the present findings is that the NT system primarily responsible for these effects of METH SA is likely specifically related to the VTA-associated mesolimbic pathway (Geisler and Zahm 2006). This was supported by the finding that VTA NT levels were similarly elevated in SAM rats (Fig. 7). However, it is noteworthy that we did not observe changes in NT levels in the frontal cortex, another terminal region for NT projections originating in the VTA. This suggests that the NT systems associated with the mesocortical pathway responds to METH SA differently than the mesolimbic-related NT pathway. This is consistent with previous reports that non-

contingent METH treatments cause opposite NT effects in the nucleus accumbens and the frontal cortex (Merchant et al. 1989).

It should be mentioned that while this is the first demonstration that SA of METH causes limbic NT responses that resemble those caused by non-contingent high doses of METH, a recent report by Ramos-Ortolaza (2009) looked at the effect of cocaine SA on NT levels in the nucleus accumbens after five 3-h SA sessions. Despite some paradigm differences, this group reported that like our findings with METH, cocaine SA also increased NT levels in the nucleus accumbens. However, this study did not include yoked cocaine groups for comparisons, so it was not possible to determine which DA receptor mediated the NT effect (i.e., D1 and D2 antagonists cannot be given directly to the rats lever pressing for drug because these antagonists block lever pressing activity).

Because others have observed that non-contingent cocaine treatments, like D1 agonists, elevate NT tissue levels in nucleus accumbens, it is probable that like with our METH studies that the NT responses caused by cocaine SA, were also due to D1 activation (Hanson et al. 1995).

We also wanted to determine if longer periods of METH SA would have similar effects on NT systems. It was important to establish the fact that our shorter contingent protocol (~5 d) represented a stable model of METH SA as reported by other investigators (Schwendt et al. 2009). To accomplish this we allowed rats to lever press for METH for 15 d as described in the Experimental procedures. We observed even with the longer protocol that lever-pressing activity remained stable after the initial 2–3 days. In this study we measured NT levels separately in the nucleus accumbens shell and core (Fig. 4). We observed that the NT levels were increased in the shell of both SAM and YM rats after 15 days of SA, much like that observed in the whole nucleus accumbens after 5 d (Figs. 2 and 3) with the SAM effect being significantly greater than the YM group. However, we did not find significant changes in the core suggesting that the limbic NT responses under these conditions were more prominent in the shell. We also examined the NT effects in the dorsal striatum under these extended conditions and observed a pattern of increased NT tissues level similar to that in the shell and like that previously reported (Frankel et al. 2011).

We next examined if the increases in NT levels were directly linked with the operant behavior. This was accomplished by testing the correlation between NT tissue levels in both the nucleus accumbens shell and the dorsal striatum and the total amount of METH received by either the SAM or YM rats during the 15 d. For the SAM, but not the YM, animals, the total amount of METH infused was directly related to lever-pressing activity and a measure of operant behavior. We found a significant correlation between METH infusion and NT levels in the SAM, but not the YM, rats in both the nucleus accumbens shell and the dorsal striatum. Under these conditions, the findings demonstrate that the changes in the NT system did not correlate significantly with exposure to METH alone (e.g., YM rats), but the correlation became significant when operant responses were also considered (e.g., SAM rats). This observation supports the conclusion that the NT systems in the nucleus accumbens shell and dorsal striatum contribute to elements related to contingent behavior such as anticipation, motivation, etc.

We also examined the effects of activating and blocking NT1 receptors on the pattern of lever-pressing after the longer METH SA paradigm. As with the shorter SAM protocol (i.e., 5 d), treatment with the agonist PD compound blocked the operant behavior associated with METH SA. In contrast, blockade of the NT1 receptor with the SR compound did not significantly alter lever pressing under similar conditions. These findings further suggest that the role of NT receptors in SA activity is similar with either the short or long SA

protocols in that NT1 activation reduces lever pressing. However, the endogenous NT systems do not appear to play an active role in this SA behavior regardless the length of the training.

In summary, the present findings strongly suggest that the limbic NT system is involved in mediating responses to METH SA and may well contribute to elements that are critical to METH dependence. In addition, these somewhat selective NT effects appear to be specifically associated with increases in D1 receptor activity related to mesolimbic projections and confirm the relevance of much of the previous non-contingent METH studies on limbic neuropeptide responses to issues of dependence and addiction to this potent stimulant.

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Abbreviations

SA	self-administration
METH	methamphetamine
SAM	self-administering methamphetamine
YM	yoked-methamphetamine
YS	yoked-saline
NT	neurotensin
DA	dopamine
VTA	ventral tegmental area
PD	PD149163

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Highlights

- NT agonist reduced self-administration of, but did not substitute for, METH
- 5–15 days of METH self-administration raised NT levels in nucleus accumbens shell
- Differences in NT responses occurred in self-administering METH vs. yolk METH rats
- NT changes mediated by METH self-administration were D1-mediated
- Changes in accumbens NT correlated with lever presses in SAM but not YM rats

Fig. 1.

Effect of substituting the NT1 receptor agonist, PD149163, for METH in response to lever pressing in rats trained to self-administer this stimulant. METH was replaced with the NT agonist 1 h into the 6th session. The PD compound remained in the syringe for the remainder of the self-administration period as well as throughout the session on the 7th d. The METH was restored to the syringe for the 8–11 sessions. See Experimental procedures for more details. The open columns represent lever pressing for METH and the dark columns represent lever-pressing for PD149163 for 4 h (note the columns for METH and PD149163 on day 6 represent values were adjusted to a 4 h rate). (N=8–9).

Fig. 2.

Elevation of NT levels in the whole nucleus accumbens after non-contingent (12 hr after a single 10 mg/kg, sc METH injection) and contingent (6 h after 5 daily sessions of METH self-administration as described in Experimental procedure) METH exposures. Values represent mean percent $\pm$ S.E.M. of respective saline (Sal) or yoked saline (YS) controls (N=9–10). * and **P<0.05 vs other corresponding groups.

Fig. 3. METH-induced increases in nucleus accumbens NT content of self-administering METH (SAM+sal), and to a lesser degree, yoked METH (YM+sal) vs. Yoked-Saline (YS+sal) rats. All rats were pretreated prior to each session with either SCH23390 (SCH), eticlopride (etic.) or saline (sal) and sacrificed 6 h after the final (5th) session. The increase in nucleus accumbens NT levels of YM rats was blocked by pretreatment with the D1 antagonist SCH23990 (YM+SCH), but not the D2 antagonist eticlopride (YM+etic) (N=7–27). Values represent mean percents of YS+sal $\pm$ S.E.M. (N=7–27) * P<0.05 vs. all other groups. **P<0.05 vs. all other groups but not each other.

Fig. 4.

Effects of 15 days of METH self-administration on NT levels in nucleus accumbens shell and core and the dorsal striatum. The patterns of elevation of NT content in the dorsal striatum, nucleus accumbens shell, but not the core, were similar to that observed in the whole nucleus accumbens after 5 days of METH self-administration as shown in Fig. 2. Values represent mean percents of corresponding YS groups (N=11–12). * and ** $P < 0.05$ vs. all other corresponding groups.

Fig. 5.

Using data from the rats represented in Fig. 4, individual NT levels in the nucleus accumbens shell (NAs) and dorsal striatum (DS) were correlated with the corresponding total amounts of METH infused i.v./animal for the 15 days of the experiment in both SAM and YM groups. Significant correlations were observed in the SAM, but not the YM groups from both the nucleus accumbens shell and the dorsal striatum. The Pearson correlation coefficients and p values are expressed for each group in the corresponding figures.

Fig. 6. Effects of pretreatment with PD149163 (PD) or SR48692 (SR) on lever pressing in SAM rats on the 15th d of SA. The NT1 agonist (PD) blocked lever pressing but the NT1 antagonist (SR) did not significantly alter the operant behavior. (N=7–15) *P<0.05 vs. the other group.

Fig. 7. METH self-administration induced increases in NT levels of the VTA, but not frontal cortex, of both SAM and YM vs. Yoked-Saline (YS) rats. Values represent mean percents of YS $\pm$ S.E.M (N=9–10). *P<0.05 vs. YS, but not each other.