Published in final edited form as:

Neurotox Res. 2016 May ; 29(4): 569–582. doi:10.1007/s12640-016-9605-9.

Methamphetamine, d-amphetamine and p-chloroamphetamine induced neurotoxicity differentially effect impulsive responding on the stop-signal task in rats

Teri M. Furlong[†], Lee S. Leavitt, Kristen A. Keefe, and Jong-Hyun Son

Department of Pharmacology and Toxicology, 30 S. 2000 E., University of Utah, Salt Lake City, UT 84112, USA

Abstract

Abused amphetamines, such as d-amphetamine (AMPH) and methamphetamine (METH), are highly addictive and destructive to health and productive lifestyles. The abuse of these drugs is associated with impulsive behavior, which is likely to contribute to addiction. The amphetamines also differentially damage dopamine (DA) and serotonin (5-HT) systems, which regulate impulsive behavior; therefore, exposure to these drugs may differentially alter impulsive behavior to effect the progression of addiction. We examined the impact of neurotoxicity induced by three amphetamines on impulsive action using a stop-signal task in rats. Animals were rewarded with a food pellet after lever pressing (*i.e.* a go trial), unless an auditory cue was presented and withholding lever press gained reward (*i.e.* a stop trial). Animals were trained on the task and then exposed to a neurotoxic regimen of either AMPH, p-chloroamphetamine (PCA), or METH. These regimens preferentially reduced DA transporter levels in striatum, 5-HT transporter levels in prefrontal cortex, or both, respectively. Assessment of performance on the stop-signal task beginning one week after the treatment revealed that AMPH produced a deficit in go-trial performance, whereas PCA did not alter performance on either trial type. In contrast, METH produced a deficit in stop-trial performance (*i.e.* impulsive action) but not go-trial performance. These findings suggest that the different neurotoxic consequences of substituted amphetamines are associated with different effects on inhibitory control over behavior. Thus, the course of addiction and maladaptive behavior resulting from exposure to these substances is likely to differ.

Keywords

Amphetamine; stop-signal task; behavioral inhibition; impulsive action; dopamine; serotonin

[†]Author of correspondence: Dr. Teri M. Furlong, Dept. Pharmacology and Toxicology, 30 S. 2000 E., Rm 201, Salt Lake City, UT 84112, Tel: (801) 585-1253, Fax: (801) 585-5111, teri.furlong@utah.edu.

Ethical approval

All applicable national and institutional guidelines for the care and use of animals were followed. All procedures were approved by the research ethics committee at the University of Utah, where the studies were conducted.

Conflict of interest

The authors declare that they have no conflict of interest

Introduction

Drug addiction is a chronic relapsing disorder characterized by a loss of control over drug use. Understanding how this compulsive pursuit of drugs develops, and why abstinence is so difficult after prolonged abuse will be important for the treatment of addiction. Both clinical and preclinical studies suggest that impulsive behavior is not only involved in the initiation of drug-taking, but may also affect the maintenance of drug-seeking, compulsive drug-use, and reinstatement following abstinence (Perry and Carroll 2008; Jentsch et al. 2014). Impulsive behavior occurs following quick decision-making, when there is an inability to stop or withhold a response despite negative consequences (Dalley et al. 2011; Jentsch et al. 2014). This kind of poor inhibitory control over behavior is key feature of drug addiction, where maladaptive drug-use continues seemingly without consideration of negative consequences to health and livelihood (Perry and Carroll 2008; Jentsch et al. 2014).

While impulsivity may be a pre-existing trait that pre-disposes an individual to initiate drug use, it is also likely to be consequence of drug exposure (Perry and Carroll 2008; Dalley et al. 2011). It is well recognized that extensive drug use causes changes to the brain that alter normal-decision making processes and perpetuate the development of addiction (Scott et al. 2007; Perry and Carroll 2008; Dean et al. 2013). Of particular concern are amphetamine (AMPH), and its analog methamphetamine (METH), because of the high incidence of addiction and abuse, cost to society, and devastating impact on health and productive lifestyles (Scott et al. 2007; Berman et al. 2009; Dobkin and Nicosia 2009). Further, the amphetamines have direct toxic effects on the brain, causing long-term damage to dopamine (DA), serotonin (5-HT) or both systems (Belcher et al. 2005; McCann et al. 1998; Sekine et al. 2001; Volkow et al. 2001; Sekine et al. 2006; Berman et al. 2009). These systems are known to be involved in regulating impulsivity (Dalley and Roiser 2012; Jentsch et al. 2014); thus, damage to DA and/or 5-HT systems resulting from exposure to amphetamines may contribute to the increased incidence of impulsivity seen in individuals who abuse these drugs. For example, METH abuse is associated with impairments in the ability to withhold responding (i.e. impulsive action), as well as other measures of poor behavioral control (Monterosso et al. 2005; Hoffman et al. 2006; Simons et al. 2008; Henry et al. 2011; Tolliver et al. 2012). However, the degree to which these behaviors are pre-existing versus a consequence of drug-exposure is difficult to determine in human drug abusers (Dalley et al. 2011).

In animals, impulsive action is commonly assessed using stop-signal or go/no-go tasks, where animals are required to inhibit an action in the presence of a cue in order to gain a favorable outcome (Winstanley et al. 2006; Eagle et al. 2008; Perry and Carroll 2008; Eagle and Baunez 2010; Dalley et al. 2011; Jentsch et al. 2014). Several lines of evidence suggest that DA and 5-HT systems modulate performance on such tasks (Eagle et al. 2008). There is also further evidence that the interaction between these systems is important for impulse control (Harrison et al. 1997; Koskinen and Sirvio 2001). Examining the effects of prior exposure to amphetamines on such tasks in rodents will, therefore, provide insight into whether deficits in impulsive action can arise as a consequence of damage to DA and/or 5-HT systems induced by exposure to amphetamines.

The neurotoxic effect of d-AMPH is relatively selective for DA over 5-HT, whereas METH affects both DA and 5-HT systems (Belcher et al. 2005). In contrast, another amphetamine analog, the designer drug p-chloroamphetamine (PCA), is relatively selective for 5-HT over DA (Belcher et al. 2005; Masaki et al. 2006). Given the different neurotoxic potential of the amphetamines, it is likely that their impact on impulsive behavior also differs, but this has not yet been established. Understanding the functional consequences of neurotoxicity induced by the amphetamines will not only be informative regarding the potential damage to humans following long-term drug abuse, but will also provide further insight into the roles of DA and 5-HT in impulsive behavior. Such insight is important as alterations to these systems are part of the pathology of a number of psychiatric illness associated with failure of behavioral inhibition, including attention-deficit and hyperactivity disorder, Parkinson's disease, obsessive-compulsive disorder, and schizophrenia (Eagle et al. 2008, 2009; Eagle and Baunez 2010).

In the current study, we examined the impact of prior exposure to neurotoxic regimens of amphetamines on impulsive action in rats using the stop-signal task. Rats were first trained on the task, during which a lever press was rewarded with a positive food outcome, except when an auditory tone stop-signal was presented, in which case animals were required to inhibit the response in order to gain the reward. Rats were then treated with a neurotoxic dosing regimen of an amphetamine, and then continued on the task so that pre-performance could be compared to post-performance. Hence, animals acquired the task before neurotoxicity was induced in order to establish whether impulsive action developed following drug exposure. In the first experiment, rats were exposed to d-AMPH or PCA, which predominately affect DA and 5-HT respectively, or vehicle. In the second experiment, rats were exposed to METH under hyperthermic conditions that induce neurotoxicity to both DA and 5-HT, or under conditions that protect from neurotoxicity (i.e. cool conditions that prevent hyperthermia)(Ali et al. 1994; Bowyer et al. 1994). Neurotoxicity to DA and 5-HT systems was then confirmed in prefrontal cortex (PFC) and striatum using radioligand binding of dopamine and serotonin transporters (DAT and SERT, respectively). Thus, we sought to determine whether exposure to a range of amphetamines alter performance to a similar extent on the stop-signal task despite differential impact on DA and 5-HT systems.

Materials and methods

Animals

Male Sprague-Dawley rats (250–300 g; Charles River, Wilmington, MA) were individually housed in hanging wire cages in a colony room controlled for temperature and lighting (12:14 hr, lights on at 6 AM). Animals had free access to standard chow and water except during behavioral procedures, during which food was restricted to maintain body weight at approximately 85% of free-feeding weight. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Utah, and were in accordance with the *Guide for the Care and Use of Laboratory Animals* (8th Ed., National Research Council).

Drug treatment

Animals were treated with AMPH, PCA or METH. The AMPH (d-amphetamine hemisulfate salt) and PCA (DL-p-chloroamphetamine hydrochloride) were purchased from Sigma-Aldrich. The METH [(±)-methamphetamine hydrochloride] was kindly provided by the National Institute on Drug Abuse (Rockville, MD). All doses were calculated as the free-base and administered in a volume of 1ml/kg, s.c..

In experiment 1, there were 3 treatment groups: Control (n=5), AMPH (n=5), or PCA (n=4) groups, and in experiment 2, there were 2 treatment groups: normothermic-METH (n=4), or neurotoxic-METH (n=3) groups. All animals were housed in plastic tub-cages (33 × 28 × 17 cm) in groups of 3–5, from 12 hr prior to treatment to 12 hr following treatment. During treatment, rats received four injections at 2-hr intervals and body temperatures were monitored every hour via a rectal probe. In experiment 1, these injections were 4 × 0.9% saline (Control group), 4 × 5 mg/kg AMPH (AMPH group) or 4 × 5 mg/kg PCA (PCA group). In experiment 2, both groups were treated with 4 × 10 mg/kg of METH. Rats in the neurotoxic-METH group were treated under ambient conditions and experienced METH-induced hyperthermia, while rats in the normothermic-METH group were kept under a fan to prevent the METH-induced increase in body temperature. The maximum temperature of all rats was controlled by placing animals in a cage over wet ice if their rectal temperature reading exceeded 40 °C. For rats in the normothermic group, the maximum temperature was set at 37.5°C, and animals were placed on ice if their recorded rectal temperature exceeded that, in order to maintain these rats in the same body temperature range as the Control group in experiment 1.

We chose a normothermic control group for the second experiment, because of the established effects of hyperthermia on the neurotoxic potential of METH (Ali et al. 1994; Bowyer et al. 1994). Thus, we could then determine whether hyperthermic compared to normothermic conditions also differentially impact cognition and behavior (Belcher et al. 2008). By conducting the experiment in this way, we are limited in our ability to quantify the extent of monoamine loss in rats in the normothermic group, but not limited regarding their behavioral performance given the within subjects design comparing pre- versus post- task performance (see below).

Stop-signal task apparatus and procedure

The stop-signal task took place in standard operant chambers housed within sound- and light-attenuating boxes (Coulbourn Instruments, Whitehall, PA). Each chamber had two retractable levers on either side of a recessed trough where a pellet dispenser delivered a 45-mg sucrose pellet (Research Diets, New Brunswick, NJ) when appropriate. A light was located inside the trough, and a series of 3 small LED lights were located above each of the levers. The chamber was illuminated by a 3-W, 24-V house light located on the wall opposite the levers, unless otherwise stated, and also contained a speaker that was connected to a tone generator (1 KHz tone; Coulbourn Instruments, Whitehall, PA). Head entry into the trough was detected via an infrared photobeam. A computer equipped with Graphic State 3.0 software (Coulbourn Instruments, Whitehall, PA) controlled event timing and recorded lever presses.

During the stop-signal task, animals were rewarded with a pellet for lever pressing on ‘go’ trials, and rewarded for not lever pressing on ‘stop’ trials within a single session (fig 1) (Eagle and Robbins 2003). Animals were initially trained for go responses only, in 20 min daily sessions, where lever-press earned pellet reinforcement. Each session began with the free delivery of a pellet and illumination of the trough light. Head entry into the trough to retrieve the pellet then initiated the next trial, in which the trough light was extinguished, the left lever extended and the left LED lights illuminated. Responding on the left lever resulted in the delivery of a pellet, illumination of the trough light, and retraction of the lever. If the left lever was not pressed within 60 sec, it was retracted (*i.e.* an omitted trial). There was a maximum of 200 trials in each session, and 8.6 ± 0.78 sessions were required before animals reached criterion, which was defined as $>70\%$ correct responding on the go trials on two consecutive days. Animals were then trained to press the right lever for pellet reinforcement. The daily sessions were similar, except that left lever responses resulted in entry of the right lever and illumination of the right LED lights rather than pellet delivery. A pellet was then delivered if the right lever was pressed. Failure to press the right lever resulted in a 5-sec time-out period, during which the house light was extinguished (*i.e.* a failed go trial). In order to ensure a rapid response, the right lever was presented for a limited period. Initially, this period was 30 sec, but it was reduced each session so that it was approx. 0.5 sec greater than the reaction time of the individual rat on its last session. Reaction time was calculated for each rat as the average time between right lever entry and right lever press on the successful trials within a session. By the final session, the right lever was available for 1–2.5 sec depending on the individual rat’s ability to maintain criteria of pressing the right lever on more than 70% of trials on three consecutive sessions (8.5 ± 0.9 sessions required to reach criteria).

Stop trials were then introduced randomly within a session (20 % of total trials) (fig 1). During a stop trial, an auditory tone was presented while the right lever was present, and the right-lever press did not result in pellet delivery. Instead, withholding the response for the length of the stop trial (*i.e.* until the right lever retracted), resulted in pellet delivery, so that the tone served as a stop-signal. Initially, the tone was presented for the length of time that the right lever was presented, but the length of the tone presentation was gradually reduced across sessions to 100 msec in duration. The tone was presented randomly within the first 50% of the time period that the right lever was available so that it could not be anticipated, and so that approximately 80% of trials could be performed correctly (Eagle et al. 2007). Pressing the right lever during the stop-signal resulted in a timeout period of 5 sec, during which the house-light extinguished and the right lever retracted (*i.e.* a failed stop trial). The initial period that the right lever was available was 1 sec during a stop trial, and 1–2.5 sec during a go trial (as above). These periods were adjusted for each session, for each individual rat, until they were the same for both trial types with at least 70% of responses correct on both trials for three consecutive sessions (between 1 and 2.3 sec depending on the rat, which required 19.2 ± 1.3 sessions to reach this criteria on average).

Animals then underwent treatment with substituted amphetamines (as described above). The stop-signal task resumed one week later, where the daily 20-min sessions continued for a further 5 days. The period that the right lever was available was the same as it was for last

three sessions prior to treatment for each rat (i.e. between 1 and 2.3 sec depending on the rat).

Trial accuracy (*i.e.* the percentage of successful trials) for both the go and stop trials was calculated for the last 3 pre-treatment sessions, as well as the 5 post-treatment sessions, for each group. This was done by dividing the number of successful trials (*i.e.* pressing the right lever on go trials and not pressing the right lever on stop trials) by the total number of initiated trials for each animal (*i.e.* the left lever was pressed; omitted trials were not included). Mean reaction time (mRT) during the go trials was calculated as the time between right lever entry and right lever press for each trial, and the percentage of omitted trials was calculated as the number of failures to press the left lever to initiate a trial divided by the total number of times the left lever was presented into the chamber. These values were each averaged across the three pre-treatment and five post-treatment sessions to yield one measure for each animal for the pre-treatment and post-treatment sessions.

Dopamine transporter (DAT) and serotonin transporter (SERT) autoradiography

Dopamine transporter (DAT) binding in striatum and serotonin transporter (SERT) binding in PFC were used to confirm neurotoxicity for each group. Immediately following the last session on the Stop-Go task, rats were sacrificed by exposure to CO₂ for 1 min. Brains were removed rapidly and frozen in 2-methylbutane (Mallinckrodt Baker, Phillipsburg, NJ), chilled on dry ice and stored at -80 °C. The prefrontal cortex and striatum were then sectioned at 12-μm on a freezing cryostat (Cryocut 1800, Cambridge Instruments, Bayreuth, Germany), collected onto glass slides (SuperFrost Plus), and stored at -20°C until use.

DAT and SERT were determined by [¹²⁵I] RTI-55 binding (PerkinElmer, Waltham, MA; see (Belcher et al. 2008; Son et al. 2013). Sections were pre-incubated in assay buffer (10 mM NaPO₄, 120 mM NaCl, 100 mM sucrose) for 5 min to remove endogenous ligands, and then incubated in assay buffer containing 25 pM [¹²⁵I] RTI-55 for 2 hr at room temperature. To examine DAT in striatum, 100 nM fluoxetine was also added to these incubations to block radioligand binding to SERT, given that [¹²⁵I] RTI-55 binds with both DAT and SERT (Boja et al. 1992; Belin et al. 2008). PFC sections incubated in fluoxetine showed no binding (data not shown). The sections were then rinsed in assay buffer (~4°C), then distilled water (~4°C), and rapidly dried under a stream of low-heated air. Finally, the slides were apposed to Biomax film (Eastman Kodak, Rochester, NY) for 24 hr, and the films then developed.

Image analysis

Autoradiographic images were captured and analyzed using the image analysis program ImageJ (<http://rsbweb.nih.gov/ij/>), by an experimenter blinded to treatment group (Son et al. 2013). Densitometric analysis was used to yield an average density (arbitrary gray) value for the dorsomedial (DMS), dorsolateral (DLS), ventromedial (VMS), and ventrolateral (VLS) striatum, as well as the orbitofrontal (OFC), infralimbic (IL), prelimbic (PrL), and anterior cingulate (ACC) cortex (see figs 2B and 3B). The average density of white matter in the sections was subtracted from these values to correct for background labeling. DAT was analyzed across 4 sections of the striatum (between +1.8 and +0.8 mm from Bregma), and SERT was analyzed across four sections of the prefrontal cortex (between +2.7 and +3.7 mm

from Bregma) for each animal, and then averaged for each region in each group. Data are presented as the average density for each group, as well as a percentage of the appropriate control for each experiment.

Statistical analysis

Data are presented as mean $\pm$ SEM, and were analyzed using planned orthogonal contrasts comparing within and between subjects factors. For experiment 1, the between subjects factors were the Control group compared to the two drug-treated groups (AMPH and PCA), and the two drug groups compared to each other. For experiment 2, the neurotoxic-METH group was compared to the normothermic-METH group. The within subjects factor for body temperature was time (0–7 hours following treatment), and for the stop-go task, trial type (either go or stop trials) and session (either pre-treatment or post-treatment sessions). Simple main effects were used to determine the source of any interaction. Analysis of DAT and SERT binding was conducted using separate one-way ANOVAs for each brain region examined, followed by post-hoc analysis using a Bonferroni correction factor. Differences were considered significant when $p < 0.05$ in all cases.

Results

Body temperature

Body temperature was monitored each hour during drug-treatment, and is shown for each group in fig 2A for experiment 1, and fig 3A for experiment 2. It can be seen in fig 2A that body temperature was elevated for the AMPH and PCA groups compared to the Control group, and in fig 3A that the neurotoxic-METH group had elevated body temperature compared to the normothermic-METH group, reaching hyperthermic levels (Bowyer et al. 1994). For experiment 1, there was a significant main effect of AMPH and PCA treatment compared to control treatment ($F_{(1,11)}=164.7$, $p < 0.001$), but no difference between the AMPH and PCA treatment groups ($F_{(1,11)}=0.01$, $p=0.922$). There was also a significant main effect of time ($F_{(1,11)}=5.73$, $p=0.036$), but no significant interaction between time and treatment ($F_{(1,11)}=1.90$, $p=0.195$). Analysis at each time point showed that there was no difference in body temperature between the drug-treated and control groups prior to the first injection ($F_{(1,11)}=0.51$, $p=0.490$), but there were significant differences at every time point post-injection ($F_{(1,17)} > 6.73$, $p < 0.025$, for all analyses), except for the final time point ($F_{(1,11)}=3.71$, $p=0.080$). For experiment 2, there was a significant main effect of treatment group ($F_{(1,5)}=506.0$, $p < 0.001$), a significant main effect of time ($F_{(1,5)}=237.5$, $p < 0.001$), and a significant interaction ($F_{(1,5)}=84.0$, $p < 0.001$). Analysis at each time point showed that there was no difference between the neurotoxic and normothermic- METH groups prior to the first injection ($F_{(1,5)}=0.12$, $p=0.74$), but there were differences at all time-points post-injection ($F_{(1,5)} > 7.5$, $p < 0.02$, for all analyses).

DA and 5-HT depletions following AMPH and PCA

The results of the [125 I] RTI-55 autoradiography for experiment 1 are shown in figure 2B–D. Large reductions in DAT binding were seen in all striatal regions examined following AMPH treatment compared to control (81–89 % reduction from control for all regions), whereas only small reductions were seen following PCA treatment (2–10 % reduction from

control for all regions). In contrast, large reductions in SERT binding were seen in all PFC regions examined after PCA treatment (79–92 % reduction from control for all regions), while smaller reductions were seen following AMPH treatment (23–33 % reduction from control for all regions). One-way ANOVA of the mean grey values revealed that there were differences between groups in DAT binding in DMS ($F_{(2,13)} = 1072.7$, $p < 0.001$), DLS ($F_{(2,13)} = 406.6$, $p < 0.001$), VMS ($F_{(2,13)} = 614.7$, $p < 0.001$), and VLS ($F_{(2,13)} = 699.5$, $p < 0.001$), and in SERT binding in the OFC ($F_{(2,13)} = 360.2$, $p < 0.001$), IL ($F_{(2,13)} = 290.6$, $p < 0.001$), PrL ($F_{(2,13)} = 331.5$, $p < 0.001$), and ACC ($F_{(2,13)} = 247.3$, $p < 0.001$). Post-hoc analysis of DAT binding revealed differences between the control group and the AMPH group in all striatal regions ($p < 0.001$ for all comparisons), and differences between the control group and the PCA group in the VMS and VLS ($p < 0.012$ for both comparisons), but not DMS or DLS ($p > 0.9$ for both comparisons). The AMPH group also differed from the PCA group for all striatal regions examined ($p < 0.001$), indicating that DAT loss was greater for the AMPH group than the PCA group. Post-hoc analysis of the SERT binding in PFC confirmed that the Control group was significantly different from the AMPH group and the PCA group in all regions examined ($p < 0.001$ for AMPH and PCA, for all analyses). The PCA group also differed to the AMPH group for all PFC regions examined ($p < 0.001$), indicating that SERT loss was greater for the PCA group compared to the AMPH group. These findings support prior work that AMPH has greater neurotoxic effects on DA than 5-HT systems, whereas PCA has greater neurotoxic effects on 5-HT than DA systems (Belcher et al. 2005).

DA and 5-HT depletions following METH

The results of the [125 I] RTI-55 autoradiography for experiment 2 are shown in figure 3B–D. Large reductions in both DAT and SERT binding were seen in all striatal and PFC regions examined in the neurotoxic METH group as compared to the normothermic METH group (72–88 % reduction in DAT and 58–80 % reduction in SERT, for all regions). One-way ANOVA of the mean grey values confirmed that there were differences between the two groups in DAT binding in DMS ($F_{(1,6)} = 484.8$, $p < 0.001$), DLS ($F_{(1,6)} = 300.2$, $p < 0.001$), VMS ($F_{(1,6)} = 345.7$, $p < 0.001$), and VLS ($F_{(1,6)} = 324.2$, $p < 0.001$), and in SERT binding in the OFC ($F_{(1,6)} = 441.4$, $p < 0.001$), IL ($F_{(1,6)} = 101.4$, $p < 0.001$), PrL ($F_{(1,6)} = 352.6$, $p < 0.001$), and ACC ($F_{(1,6)} = 3492$, $p < 0.001$). This finding supports prior work that has established that the partial loss of DA and 5-HT that is indicative of neurotoxicity following METH exposure largely depends on METH-induced hyperthermia (Ali et al. 1994; Bowyer et al. 1994).

Effect of amphetamines on the stop-signal task

We evaluated the effect of neurotoxicity induced by different amphetamines on behavioral inhibition by comparing performance on the stop-signal task before and after treatment across 2 experiments. At the end of the pretreatment phase, groups were matched for performance so that the time period that the levers were presented during the stop and go trials was the same for all groups (tables 1A and 1B, analyses not shown). The total number of go and stop trials also did not differ between groups before or after treatment, nor did the total number of omitted trials, which were low overall (tables 1A and 1B). For the analysis of total trials for experiment 1, there was no main effect of drug treatment (AMPH and PCA)

versus control treatment ($F_{(1,11)}=0.01$, $p=0.92$) and no difference between the two drug-treated groups ($F_{(1,11)}=0.01$, $p=0.94$). There was a main effect of trial type, given that the go trials made up 80% of the total trials and stop trials the remaining 20% of trials each session ($F_{(1,11)}=152.0$, $p<0.001$). Importantly, there was no effect of pre- versus post- treatment session ($F_{(1,11)}=1.78$, $p=0.21$), and no significant interactions between these factors ($F_{(1,11)}<0.51$, $p>0.49$ for all analyses). For the analysis of total trials for experiment 2, there was also no main effect of neurotoxic METH versus normothermic METH treatment ($F_{(1,5)}=1.16$, $p=0.33$), an effect of trial type ($F_{(1,5)}=69.4$, $p<0.001$), no effect of pre- versus post- treatment sessions ($F_{(1,5)}=0.00$, $p=0.98$), and no significant interactions ($F_{(1,5)}<1.07$, $p>0.35$, for all analyses).

For the number of omitted trials in experiment 1, there were no differences between the control and drug-treated groups or between the AMPH and PCA groups ($F_{(1,11)}<0.11$, $p>0.75$, for both analyses), but there was an effect of session ($F_{(1,11)}=8.1$, $p=0.02$), and no interaction ($F_{(1,11)}=0.38$, $p=0.55$). From inspection of table 1, these analyses indicate that the number of omitted trials was less in the post-treatment indicating improved performance over time for all groups. For experiment 2, there was no effect of treatment ($F_{(1,5)}=1.7$, $p=0.25$), no effect of session ($F_{(1,5)}=0.34$, $p=0.59$), and no interaction ($F_{(1,5)}=0.07$, $p=0.80$). Overall, these analyses indicate that the total number of omitted trials was not adversely impacted by treatment for any of the groups.

Finally, the AMPH, PCA and vehicle groups showed similar mRTs in both the pre- and post-treatment sessions (table 1A). Statistical analysis comparing mRTs of the AMPH and PCA groups to the control group confirmed there were no differences between groups ($F_{(1,11)}=0.92$, $p=0.36$), no differences across pre- and post- treatment sessions ($F_{(1,11)}=1.71$, $p=0.22$), and no interaction ($F_{(1,11)}=0.001$, $p=0.98$). The neurotoxic- and normothermic METH groups also showed similar mRTs to each other during the pre- and post-treatment sessions (Table 1B). There were no significant differences between groups ($F_{(1,5)}=2.4$, $p=0.18$), no differences across sessions ($F_{(1,5)}=0.33$, $p=0.59$), and no interaction ($F_{(1,5)}=1.0$, $p=0.36$). Hence, neurotoxicity induced by any of these substituted amphetamines did not affect mRTs on go trials.

Effects of AMPH and PCA on trial accuracy

Figure 4 shows performance accuracy on the stop and go trials in experiment 1 for the pre- and post-treatment sessions. It can be seen that the percentage of successful trials for the Control group following treatment was similar to what it was prior to treatment. However, trial accuracy decreased post-treatment for the drug-treated groups, particularly for the AMPH group, where successful go trials were reduced. Statistical analysis revealed that overall performance on the go versus stop trials did not differ ($F_{(1,11)}=0.15$, $p=0.71$), but there was an overall difference in performance between the pre- and post-treatment sessions ($F_{(1,11)}=24.5$, $p<0.001$). Importantly, there was a significant interaction between session and treatment group for the control vs. drug groups ($F_{(1,11)}=6.97$, $p=0.023$), as well as for AMPH group vs. PCA group ($F_{(1,11)}=6.37$, $p=0.028$), but not between session and trial-type ($F_{(1,11)}=0.53$, $p=0.48$). Analysis of session for each treatment group revealed a significant effect for the AMPH group ($F_{(1,11)}=35.6$, $p<0.001$), but not the control group ($F_{(1,11)}=0.61$,

$p=0.451$) or PCA group ($F_{(1,11)}=3.81$, $p=0.077$). When session was considered for the AMPH group, a difference between pre and post treatment was found for the go trials ($F_{(1,11)}=8.67$, $p=0.013$), while the difference between sessions for stop trials was not significant ($F_{(1,11)}=3.1$, $p=0.11$). These analyses indicate that performance of the AMPH group was reduced post-treatment for go-trials, unlike the performance of the Control and PCA groups.

Effects of METH on trial accuracy

Figure 5 shows trial accuracy for animals with and without METH-induced neurotoxicity. It can be seen that the percentage of successful trials was decreased for the neurotoxic-METH group post-treatment for the stop trials, but not the go trials. Statistical analysis revealed significant differences in overall performance on the go vs. stop trials ($F_{(1,5)}=7.0$, $p=0.046$) and for pre- versus post- sessions ($F_{(1,5)}=20.0$, $p=0.007$). There was also a significant interaction between session and treatment group ($F_{(1,5)}=13.7$, $p=0.014$), session and trial type ($F_{(1,5)}=23.9$, $p=0.005$), but not trial type and treatment group ($F_{(1,5)}=0.77$, $p=0.42$). Most importantly, the 3-way interaction between group, session and trial type was significant ($F_{(1,5)}=10.8$, $p=0.022$). Analysis of the session by trial type interaction for each group revealed a significant effect for the neurotoxic-METH group ($F_{(1,5)}=39.0$, $p=0.002$), but not the normothermic-METH group ($F_{(1,5)}=1.12$, $p=0.338$). Comparison of each trial-type for the neurotoxic-METH group confirmed a difference across sessions for the stop trials ($F_{(1,5)}=48.60$, $p<0.001$), but not the go trials ($F_{(1,5)}=5.50$, $p=0.066$). There were no differences across sessions for either trial type for the normothermic-METH group ($F_{(1,5)}<0.665$, $p>0.665$). These findings suggest that the neurotoxic-METH group demonstrated a reduction in successful stop trials post-treatment, unlike the normothermic-METH group.

Discussion

The present study confirmed prior work showing that exposure to different substituted amphetamines has varied neurotoxic effects on DA and 5-HT systems, and extended those findings by showing that animals with neurotoxicity induced by these agents show varied behavioral effects as assessed by performance on the stop-signal task. Specifically, AMPH and the neurotoxic-METH exposure paradigms caused significant depletions of striatal DA, as well as damage to PFC 5-HT systems, whereas exposure to PCA was associated with relatively selective damage to 5-HT systems, consistent with previous findings (Brunswick et al. 1992; Ali et al. 1994; Ruotsalainen et al. 2000; Belcher et al. 2005; Masaki et al. 2006; Son et al. 2013). In the present work, we found that AMPH-induced neurotoxicity was associated with reduced go trial accuracy, whereas METH-induced neurotoxicity was associated with a selective reduction in stop-trial accuracy. PCA-induced neurotoxicity was not associated with significant alterations of either trial type. This study, therefore, demonstrates that the different monoamine neurotoxicity profiles induced by different substituted amphetamines are associated with different behavioral profiles on the stop-signal task. This finding has important implications for understanding the behavioral mechanisms associated with the development of addiction to these drugs, as well as for possible courses of treatment.

The effect of AMPH-induced neurotoxicity on go-trial accuracy

AMPH-induced neurotoxicity was associated with impaired performance on go trials, in which animals did not press (the right) lever in order to gain reward. This finding was not due to an inability to lever press, as the number of omitted trials and number of trials initiated by left-lever press did not differ from those of the other groups. Further, the right lever was sometimes pressed on stop trials, and when the left lever was pressed on go trials, mRT was not reduced. Non-specific effects on arousal and locomotion, that have been shown to reduce go-trial accuracy, can therefore be ruled out (Koskinen and Sirvio 2001; Perry and Carroll 2008; Bari and Robbins 2013). Prior work has suggested that a reduction in go trial accuracy is associated with altered behavioral activation, rather than altered behavioral inhibition, and is attributed to decreased attentional capacity, reduced stimulus detection and indiscriminate responding (Bari and Robbins 2013). Therefore, our data suggest that AMPH-induced neurotoxicity may be associated with impairment in attentional processes.

Although our study is the first to examine impulsive behavior following AMPH-induced neurotoxicity, other studies provide evidence that an impairment in attentional processes resulting from AMPH exposure may underlie the decrease in go trial accuracy observed in the present work. For example, a relatively large dose of AMPH ($6 \times 4\text{--}5$ mg/kg) repeated across days (rather than within one day, as in the current study) decreased accuracy on the 5-choice-serial-reaction-time task (5-CSRTT) (Fletcher et al. 2007). This task shares a cue-signaled 'go' response component to the stop-signal task, and is used to examine sustained and divided attention (Robbins 2002). The deficit on the 5-CSRTT, as well as deficits seen in an attention-shift task, were attributed to reduced attentional capacity resulting from the AMPH exposure (Fletcher et al. 2005; Fletcher et al. 2007). Further, heavy, long-term AMPH abuse in humans, including d-AMPH abuse, is also associated with deficits in attention, concentration, learning and working memory (McKetin and Mattick 1997; McKetin and Mattick 1998; Ornstein et al. 2000; Ersche et al. 2006; Reske et al. 2010). Past exposure to AMPH may, therefore, promote further drug use to combat these adverse effects on attention, especially given that acute AMPH exposure increases attention and concentration, and improves go-trial performance (Eagle and Robbins 2003).

AMPH exposure in the current study was associated with a loss of DA terminals in striatum, and while not examined, DA terminals in cortex and hippocampus were also likely to have been effected (Belcher et al. 2008). A number of studies have implicated DA systems in go-, rather than stop-, trial performance. For example, widespread blocking of D1/D2 receptors prolongs go, but not stop, reaction times (Eagle et al. 2007), and blocking D2, D3 or D4 receptors reduces go, but not stop, trial accuracy (Bari and Robbins 2013). Deficits in go performance seen in the 5-CSRTT following AMPH exposure are also rescued by D1 receptor activation in the PrL (Fletcher et al. 2007). Conversely, antagonism of D2 receptors in DMS decreases go trial performance in the stop-signal task (Eagle et al. 2011), suggesting that AMPH-induced loss of DA terminals in these regions may underlie the reduced accuracy on go trials observed in the present work. Further studies using localized targeting of DA depletions will be required to determine the precise regions affected by AMPH that are important for go trial performance. For example, inactivation of IL or OFC effect go- and

not stop- trial performance (Bari et al. 2011), so DA in these regions may also be of importance.

Lack of effect of PCA-induced neurotoxicity on the stop-signal task

PCA-induced neurotoxicity to 5-HT systems did not significantly impact performance on the stop-signal task, despite inducing large depletions, as evidenced by decreased SERT binding throughout the PFC. This lack of an effect is in agreement with a previous demonstration that widespread depletion of 5-HT by administration of the 5-HT-selective neurotoxin, 5,7-di-hydroxytryptamine, does not impact performance on this task (Eagle et al. 2009). Depletion of 5-HT terminals also fails to reduce go accuracy on the 1- or 5-CSRTT (Harrison et al. 1997; Ruotsalainen et al. 2000; Winstanley et al. 2004). Therefore, despite the well-recognized role that 5-HT plays in impulsivity, 5-HT is not involved in all aspects of behavioral inhibition, and this is likely to include impulsive action, as measured by performance on the stop-signal task (Eagle et al. 2008; Eagle and Baunez 2010; Dalley and Roiser 2012).

Prior studies have shown, however, that behavioral deficits do emerge following 5-HT loss when the demands of the behavioral tasks are increased. For example, when waiting time is increased (*i.e.* the time between the go-cue and required response) (Harrison et al. 1997; Ruotsalainen et al. 2000; Eagle et al. 2009), when the intensity of the go-cue is reduced (Harrison et al. 1997), or when lesions are induced prior to any training (Masaki et al. 2006). Thus, while several lines of evidence, including the findings reported herein, suggest that 5-HT may not be necessary for ‘action inhibition’, we cannot exclude a role of 5-HT in other aspects of impulsivity in the stop-signal task not examined in the current study; *e.g.* waiting for reinforcement (*i.e.* ‘action restraint’) (Harrison et al. 1997; Ruotsalainen et al. 2000; Winstanley et al. 2004; Eagle et al. 2008; Eagle et al. 2009; Jentsch et al. 2014). Whether PCA exposure influences impulsivity in humans is not known given the lack of reports of such abuse. However, it has been demonstrated that humans with a polymorphism of the 5-HT transporter, that reduces its expression and efficiency, do not display deficits in the stop-signal task, in line with the current findings (Clark et al. 2005).

The effect of METH-induced neurotoxicity on stop-trial accuracy

In contrast to the reduction in go-trial accuracy and the lack of effects associated with the relatively selective DA and 5-HT loss induced by AMPH- and PCA, respectively, combined DA and 5-HT loss induced by METH was associated with a decrease in stop-trial accuracy. Reduced stop-trial accuracy is thought to be reflective of impulsive action, defined as the inability to inhibit an inappropriate action, and is indicative of reduced inhibitory control over behavior (Winstanley et al. 2006; Eagle et al. 2008; Perry and Carroll 2008; Eagle and Baunez 2010; Dalley et al. 2011; Jentsch et al. 2014). METH abuse in humans is also associated with deficits in stop-trial, but not go-trial performance (Monterosso et al. 2005), as well as deficits in other types of behavioral inhibition (Hoffman et al. 2006; Simons et al. 2008; Henry et al. 2011; Tolliver et al. 2012).

It is difficult to determine whether impaired inhibitory control is a pre-existing trait predisposing an individual to abuse or a consequence of METH abuse in humans (Dalley et

al. 2011). The present findings suggest that impulsive action can arise as a consequence of METH-induced neurotoxicity. Further, this deficit is not simply due to METH exposure given that normothermic-METH group did not demonstrate stop-trial impairment. In accordance, we have previously demonstrated that increases in preservative responding for reward, another indication of poor inhibitory control over behavior, are a consequence METH-induced neurotoxicity (Son et al. 2013). Our findings are of particular importance, given that impulsive action is predictive of escalated and persistent drug-taking in animals (Dalley et al. 2007; Belin et al. 2008; Diergaarde et al. 2008; Economidou et al. 2009), thus providing a possible mechanism by which METH induced neurotoxicity could contribute to the chronic relapsing nature of METH addiction.

We observed reductions in DAT binding in striatum and SERT binding in PFC that could underlie the deficits seen in impulsive action. Importantly, decreases in DAT and SERT binding are found in these same regions in humans with a history of METH abuse (McCann et al. 1998; Sekine et al. 2001; Volkow et al. 2001; Sekine et al. 2006). A number of subregions within the striatum and PFC have been shown to be important for stop-trial performance. This includes the ACC and PrL, where pharmacological inactivation reduces stop-, but not go-, trial accuracy (Bari et al. 2011), and the DMS where blockade of DA receptors alters stop-trial performance (Eagle et al. 2011). In contrast, inactivation of IL and OFC, have no effect on stop-trial performance (Eagle and Robbins 2003; Bari et al. 2011; Eagle et al. 2011), suggesting these regions may not be important loci of the presently observed behavioral impairment, despite METH-induced monoamine toxicity in these brain regions. It is also worth considering that the neurotoxic effects of METH on norepinephrine (NE) may also be important, given NE has been implicated in stop-, but not go-, trial performance (Eagle and Robbins 2003; Robinson et al. 2008; Bari et al. 2011; Eagle et al. 2011). Although METH is considered selective for DAT and SERT, it has been shown previously to significantly reduce NE in lateral amygdala and dorsomedial hypothalamus (Brunswick et al. 1992). Thus, damage to NE in these regions may also be important for deficits in stop-trial performance in rats with METH-induced neurotoxicity.

AMPH and PCA, which each share aspects of the neurotoxic profile of METH, did not induce the same behavioral deficit as METH. Prior work has similarly shown that deficits in novel object recognition associated with METH-induced monoamine neurotoxicity are also not seen following either AMPH or PCA-induced toxicity to DA and 5-HT systems (Belcher et al. 2005; Belcher et al. 2008). It may therefore be the case that interactions between damage to DA and 5-HT systems or damage to another system (e.g. NE) by METH are ultimately important for the behavioral outcome. DA and 5-HT are known to interact to regulate impulsive behavior (Winstanley et al. 2005). Therefore, loss of both these systems may interact to prevent the go-trial deficit that was seen following AMPH treatment. Indeed, prior work has shown that impairments on the 5-CSRTT that are seen following lesions of central 5-HT or the PrL are prevented by antagonism of DA receptors (Harrison et al. 1997; Koskinen and Sirvio 2001; Passetti et al. 2003; Pezze et al. 2009). These findings suggest that behavioral deficits resulting from loss of one of these systems can be rescued by reduced function in the other system. Alternatively, the go-trial impairment following AMPH may arise because of competition between an impaired system (DA) and a relatively intact system (5-HT). Such an idea has been proposed, for example, as a basis for why

smaller rather than larger brain lesions sometimes have a greater impact on cognitive performance (Baxter and Murray 2001; Belcher et al. 2005). Clearly, understanding the intricacies of the interplay between the functions of different neurotransmitter systems within the same brain structure, as well as across different brain structures, will require region-selective manipulations of those systems.

Finally, it is worth noting that while the acute dosing paradigm used in the current study does not model the typical pattern of gradually escalating doses seen in human abuse, it produces the neurotoxic DA and 5HT profiles that are seen with long-term drug abuse in humans more readily than do escalating dose paradigms (Krasnova et al. 2010; Marshall and O'Dell 2012). For example, multiple dosing of METH across a longer time period produces less severe or shorter lasting monoamine damage, sometimes failing to produce effects on these systems, or failing to produce other indications of brain damage such as neuroinflammation that are evident in the human condition (Krasnova et al. 2010; McFadden et al. 2013; Marshall and O'Dell 2012; Schwendt et al. 2009). Further, although self-administration paradigms model drug-seeking and motivational and learning aspects of the addiction process (Marshall and O'Dell 2012; Schwendt et al. 2009), such a paradigm would introduce confounds to the current study. For example, if pre-existing impulsivity determined the degree to which an animal self-administered the stimulants (Belin et al. 2008; Diergaarde et al. 2008), and in turn the degree of resulting neurotoxicity, then performance on the stop-signal task would be confounded by this pre-determined variable. Thus, given that the acute, binge-regimen dosing paradigm produces persistent neurochemical changes similar to those seen following extensive self-administration in animals and long-term abuse in humans (Krasnova et al. 2010; Marshall and O'Dell 2012), we think that it is the best model to understand the impact of monoamine damage induced by substituted amphetamines on inhibitory control over behavior.

Conclusions and implications

The most important finding of the current study was that AMPH- and METH-induced neurotoxicity differentially impact performance on the stop-signal task. Specifically, AMPH-induced neurotoxicity was associated with impaired go-trial accuracy, whereas METH-induced neurotoxicity was associated with impaired stop-trial accuracy. As noted above, these findings suggest the possibility that AMPH-induced DA loss is associated with impairment of attentional processes, whereas METH-induced monoamine loss may be associated with development of impulsive actions. The stop-signal task used in the present study is thought to have translational relevance to human conditions characterized by poor control over impulsivity (Eagle et al. 2008; Eagle and Baunez 2010). Of particular interest is that responsivity to pharmacotherapy may be determined by individual differences in behavioral control (Eagle et al. 2008). Given that deficits in inhibitory control over actions and deficits in attention have been reported in human METH and AMPH abusers, respectively, the present findings have the potential to improve the development of effective therapeutic approaches to manage METH and AMPH dependence. For example, drugs that specifically improve stop-trial accuracy (e.g. modafinil or atomoxetine) may be more beneficial to METH abusers (Eagle et al. 2007; Robinson et al. 2008), and drugs that improve go processes (e.g. methylphenidate, propranolol or prazosin) may be more

beneficial to AMPH abusers (Eagle et al. 2007; Bari and Robbins 2013). Future studies examining the effects of these drugs in animal models of stimulant-induced neurotoxicity will be necessary to confirm this potential.

Acknowledgments

Funding

This study was funded by National Institute on Drug Abuse grant DA024036.

References

- Ali SF, Newport GD, Holson RR, Slikker W Jr, Bowyer JF. Low environmental temperatures or pharmacologic agents that produce hypothermia decrease methamphetamine neurotoxicity in mice. *Brain Res.* 1994; 658(1–2):33–38. [PubMed: 7530580]
- Bari A, Mar AC, Theobald DE, Elands SA, Oganya KC, Eagle DM, Robbins TW. Prefrontal and monoaminergic contributions to stop-signal task performance in rats. *J Neurosci.* 2011; 31(25): 9254–9263. [PubMed: 21697375]
- Bari A, Robbins TW. Noradrenergic versus dopaminergic modulation of impulsivity, attention and monitoring behaviour in rats performing the stop-signal task: possible relevance to ADHD. *Psychopharmacology (Berl).* 2013; 230(1):89–111. [PubMed: 23681165]
- Baxter MG, Murray EA. Opposite relationship of hippocampal and rhinal cortex damage to delayed nonmatching-to-sample deficits in monkeys. *Hippocampus.* 2001; 11(1):61–71. [PubMed: 11261774]
- Belcher AM, Feinstein EM, O'Dell SJ, Marshall JF. Methamphetamine influences on recognition memory: comparison of escalating and single-day dosing regimens. *Neuropsychopharmacology.* 2008; 33(6):1453–1463. [PubMed: 17637607]
- Belcher AM, O'Dell SJ, Marshall JF. Impaired object recognition memory following methamphetamine, but not p-chloroamphetamine- or d-amphetamine-induced neurotoxicity. *Neuropsychopharmacology.* 2005; 30(11):2026–2034. [PubMed: 15900317]
- Belin D, Mar AC, Dalley JW, Robbins TW, Everitt BJ. High impulsivity predicts the switch to compulsive cocaine-taking. *Science.* 2008; 320(5881):1352–1355. [PubMed: 18535246]
- Berman SM, Kuczenski R, McCracken JT, London ED. Potential adverse effects of amphetamine treatment on brain and behavior: a review. *Mol Psychiatry.* 2009; 14(2):123–142. [PubMed: 18698321]
- Boja JW, Mitchell WM, Patel A, Kopajtic TA, Carroll FI, Lewin AH, Abraham P, Kuhar MJ. High-affinity binding of [125I]RTI-55 to dopamine and serotonin transporters in rat brain. *Synapse.* 1992; 12(1):27–36. [PubMed: 1411961]
- Bowyer JF, Davies DL, Schmued L, Broening HW, Newport GD, Slikker W Jr, Holson RR. Further studies of the role of hyperthermia in methamphetamine neurotoxicity. *J Pharmacol Exp Ther.* 1994; 268(3):1571–1580. [PubMed: 8138969]
- Brunswick DJ, Benmansour S, Tejani-Butt SM, Hauptmann M. Effects of high-dose methamphetamine on monoamine uptake sites in rat brain measured by quantitative autoradiography. *Synapse.* 1992; 11(4):287–293. [PubMed: 1502685]
- Clark L, Roiser JP, Cools R, Rubinsztein DC, Sahakian BJ, Robbins TW. Stop signal response inhibition is not modulated by tryptophan depletion or the serotonin transporter polymorphism in healthy volunteers: implications for the 5-HT theory of impulsivity. *Psychopharmacology (Berl).* 2005; 182(4):570–578. [PubMed: 16163530]
- Dalley JW, Everitt BJ, Robbins TW. Impulsivity, compulsivity, and top-down cognitive control. *Neuron.* 2011; 69(4):680–694. [PubMed: 21338879]
- Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW. Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science.* 2007; 315(5816):1267–1270. [PubMed: 17332411]

- Dalley JW, Roiser JP. Dopamine, serotonin and impulsivity. *Neuroscience*. 2012; 215:42–58. [PubMed: 22542672]
- Dean AC, Groman SM, Morales AM, London ED. An evaluation of the evidence that methamphetamine abuse causes cognitive decline in humans. *Neuropsychopharmacology*. 2013; 38(2):259–274. [PubMed: 22948978]
- Diergaarde L, Pattij T, Poortvliet I, Hogenboom F, de Vries W, Schoffelmeer AN, De Vries TJ. Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. *Biol Psychiatry*. 2008; 63(3):301–308. [PubMed: 17884016]
- Dobkin C, Nicosia N. The War on Drugs: Methamphetamine, Public Health, and Crime. *Am Econ Rev*. 2009; 99(1):324–349. [PubMed: 20543969]
- Eagle DM, Bari A, Robbins TW. The neuropsychopharmacology of action inhibition: cross-species translation of the stop-signal and go/no-go tasks. *Psychopharmacology (Berl)*. 2008; 199(3):439–456. [PubMed: 18542931]
- Eagle DM, Baunez C. Is there an inhibitory-response-control system in the rat? Evidence from anatomical and pharmacological studies of behavioral inhibition. *Neurosci Biobehav Rev*. 2010; 34(1):50–72. [PubMed: 19615404]
- Eagle DM, Lehmann O, Theobald DE, Pena Y, Zakaria R, Ghosh R, Dalley JW, Robbins TW. Serotonin depletion impairs waiting but not stop-signal reaction time in rats: implications for theories of the role of 5-HT in behavioral inhibition. *Neuropsychopharmacology*. 2009; 34(5):1311–1321. [PubMed: 19005464]
- Eagle DM, Robbins TW. Inhibitory control in rats performing a stop-signal reaction-time task: effects of lesions of the medial striatum and d-amphetamine. *Behav Neurosci*. 2003; 117(6):1302–1317. [PubMed: 14674849]
- Eagle DM, Tufft MR, Goodchild HL, Robbins TW. Differential effects of modafinil and methylphenidate on stop-signal reaction time task performance in the rat, and interactions with the dopamine receptor antagonist cis-flupenthixol. *Psychopharmacology (Berl)*. 2007; 192(2):193–206. [PubMed: 17277934]
- Eagle DM, Wong JC, Allan ME, Mar AC, Theobald DE, Robbins TW. Contrasting roles for dopamine D1 and D2 receptor subtypes in the dorsomedial striatum but not the nucleus accumbens core during behavioral inhibition in the stop-signal task in rats. *J Neurosci*. 2011; 31(20):7349–7356. [PubMed: 21593319]
- Economidou D, Pelloux Y, Robbins TW, Dalley JW, Everitt BJ. High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence. *Biol Psychiatry*. 2009; 65(10):851–856. [PubMed: 19181308]
- Ersche KD, Clark L, London M, Robbins TW, Sahakian BJ. Profile of executive and memory function associated with amphetamine and opiate dependence. *Neuropsychopharmacology*. 2006; 31(5):1036–1047. [PubMed: 16160707]
- Fletcher PJ, Tenn CC, Rizos Z, Lovic V, Kapur S. Sensitization to amphetamine, but not PCP, impairs attentional set shifting: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. *Psychopharmacology (Berl)*. 2005; 183(2):190–200. [PubMed: 16220338]
- Fletcher PJ, Tenn CC, Sinyard J, Rizos Z, Kapur S. A sensitizing regimen of amphetamine impairs visual attention in the 5-choice serial reaction time test: reversal by a D1 receptor agonist injected into the medial prefrontal cortex. *Neuropsychopharmacology*. 2007; 32(5):1122–1132. [PubMed: 17047670]
- Harrison AA, Everitt BJ, Robbins TW. Central 5-HT depletion enhances impulsive responding without affecting the accuracy of attentional performance: interactions with dopaminergic mechanisms. *Psychopharmacology (Berl)*. 1997; 133(4):329–342. [PubMed: 9372531]
- Henry BL, Minassian A, van Rhenen M, Young JW, Geyer MA, Perry W. Translational Methamphetamine ARCG. Effect of methamphetamine dependence on inhibitory deficits in a novel human open-field paradigm. *Psychopharmacology (Berl)*. 2011; 215(4):697–707. [PubMed: 21279333]
- Hoffman WF, Moore M, Templin R, McFarland B, Hitzemann RJ, Mitchell SH. Neuropsychological function and delay discounting in methamphetamine-dependent individuals. *Psychopharmacology (Berl)*. 2006; 188(2):162–170. [PubMed: 16915378]

- Jentsch JD, Ashenhurst JR, Cervantes MC, Groman SM, James AS, Pennington ZT. Dissecting impulsivity and its relationships to drug addictions. *Ann N Y Acad Sci.* 2014; 1327:1–26. [PubMed: 24654857]
- Koskinen T, Sirvio J. Studies on the involvement of the dopaminergic system in the 5-HT₂ agonist (DOI)-induced premature responding in a five-choice serial reaction time task. *Brain Res Bull.* 2001; 54(1):65–75. [PubMed: 11226715]
- Krasnova IN, Justinova Z, Ladenheim B, Jayanthi S, McCoy MT, Barnes C, Warner JE, Goldberg SR, Cadet JL. Methamphetamine self-administration is associated with persistent biochemical alterations in striatal and cortical dopaminergic terminals in the rat. *PLoS One.* 2010; 5(1):e8790. [PubMed: 20098750]
- Marshall JF, O'Dell SJ. Methamphetamine influences on brain and behavior: unsafe at any speed? *Trends Neurosci.* 2012; 35(9):536–545. [PubMed: 22709631]
- Masaki D, Yokoyama C, Kinoshita S, Tsuchida H, Nakatomi Y, Yoshimoto K, Fukui K. Relationship between limbic and cortical 5-HT neurotransmission and acquisition and reversal learning in a go/no-go task in rats. *Psychopharmacology (Berl).* 2006; 189(2):249–258. [PubMed: 17016708]
- McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA. Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [¹¹C]WIN-35,428. *J Neurosci.* 1998; 18(20):8417–8422. [PubMed: 9763484]
- McFadden LM, Hadlock GC, Allen SC, Vieira-Brock PL, Stout KA, Ellis JD, Hoonakker AJ, Andrenyak DM, Nielsen SM, Wilkins DG, Hanson GR, Fleckenstein AE. Methamphetamine self-administration causes persistent striatal dopaminergic alterations and mitigates the deficits caused by a subsequent methamphetamine exposure. *J Pharmacol Exp Ther.* 2012; 340(2):295–303. [PubMed: 22034657]
- McKetin R, Mattick RP. Attention and memory in illicit amphetamine users. *Drug Alcohol Depend.* 1997; 48(3):235–242. [PubMed: 9449023]
- McKetin R, Mattick RP. Attention and memory in illicit amphetamine users: comparison with non-drug-using controls. *Drug Alcohol Depend.* 1998; 50(2):181–184. [PubMed: 9649970]
- Monterosso JR, Aron AR, Cordova X, Xu J, London ED. Deficits in response inhibition associated with chronic methamphetamine abuse. *Drug Alcohol Depend.* 2005; 79(2):273–277. [PubMed: 15967595]
- Ornstein TJ, Iddon JL, Baldacchino AM, Sahakian BJ, London M, Everitt BJ, Robbins TW. Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers. *Neuropsychopharmacology.* 2000; 23(2):113–126. [PubMed: 10882838]
- Passetti F, Levita L, Robbins TW. Sulpiride alleviates the attentional impairments of rats with medial prefrontal cortex lesions. *Behav Brain Res.* 2003; 138(1):59–69. [PubMed: 12493630]
- Perry JL, Carroll ME. The role of impulsive behavior in drug abuse. *Psychopharmacology (Berl).* 2008; 200(1):1–26. [PubMed: 18600315]
- Pezze MA, Dalley JW, Robbins TW. Remediation of attentional dysfunction in rats with lesions of the medial prefrontal cortex by intra-accumbens administration of the dopamine D(2/3) receptor antagonist sulpiride. *Psychopharmacology (Berl).* 2009; 202(1–3):307–313. [PubMed: 18985321]
- Reske M, Eidt CA, Delis DC, Paulus MP. Nondependent stimulant users of cocaine and prescription amphetamines show verbal learning and memory deficits. *Biol Psychiatry.* 2010; 68(8):762–769. [PubMed: 20605137]
- Robbins TW. The 5-choice serial reaction time task: behavioural pharmacology and functional neurochemistry. *Psychopharmacology (Berl).* 2002; 163(3–4):362–380. [PubMed: 12373437]
- Robinson ES, Eagle DM, Mar AC, Bari A, Banerjee G, Jiang X, Dalley JW, Robbins TW. Similar effects of the selective noradrenaline reuptake inhibitor atomoxetine on three distinct forms of impulsivity in the rat. *Neuropsychopharmacology.* 2008; 33(5):1028–1037. [PubMed: 17637611]
- Ruotsalainen S, Miettinen R, MacDonald E, Koivisto E, Sirvio J. Blockade of muscarinic, rather than nicotinic, receptors impairs attention, but does not interact with serotonin depletion. *Psychopharmacology (Berl).* 2000; 148(2):111–123. [PubMed: 10663425]
- Schwendt M, Rocha A, See RE, Pacchioni AM, McGinty JF, Kalivas PW. Extended methamphetamine self-administration in rats results in a selective reduction of dopamine transporter levels in the

- prefrontal cortex and dorsal striatum not accompanied by marked monoaminergic depletion. *J Pharmacol Exp Ther.* 2009; 331(2):555–562. [PubMed: 19648469]
- Scott JC, Woods SP, Matt GE, Meyer RA, Heaton RK, Atkinson JH, Grant I. Neurocognitive effects of methamphetamine: a critical review and meta-analysis. *Neuropsychol Rev.* 2007; 17(3):275–297. [PubMed: 17694436]
- Sekine Y, Iyo M, Ouchi Y, Matsunaga T, Tsukada H, Okada H, Yoshikawa E, Futatsubashi M, Takei N, Mori N. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. *Am J Psychiatry.* 2001; 158(8):1206–1214. [PubMed: 11481152]
- Sekine Y, Ouchi Y, Takei N, Yoshikawa E, Nakamura K, Futatsubashi M, Okada H, Minabe Y, Suzuki K, Iwata Y, Tsuchiya KJ, Tsukada H, Iyo M, Mori N. Brain serotonin transporter density and aggression in abstinent methamphetamine abusers. *Arch Gen Psychiatry.* 2006; 63(1):90–100. [PubMed: 16389202]
- Simons JS, Dvorak RD, Batien BD. Methamphetamine use in a rural college population: associations with marijuana use, sensitivity to punishment, and sensitivity to reward. *Psychol Addict Behav.* 2008; 22(3):444–449. [PubMed: 18778139]
- Son JH, Kuhn J, Keefe KA. Perseverative behavior in rats with methamphetamine-induced neurotoxicity. *Neuropharmacology.* 2013; 67:95–103. [PubMed: 23159331]
- Tolliver BK, Price KL, Baker NL, LaRowe SD, Simpson AN, McRae-Clark AL, Saladin ME, DeSantis SM, Chapman E, Garrett M, Brady KT. Impaired cognitive performance in subjects with methamphetamine dependence during exposure to neutral versus methamphetamine-related cues. *Am J Drug Alcohol Abuse.* 2012; 38(3):251–259. [PubMed: 22257306]
- Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, Logan J, Wong C, Miller EN. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am J Psychiatry.* 2001; 158(3):377–382. [PubMed: 11229977]
- Winstanley CA, Dalley JW, Theobald DE, Robbins TW. Fractionating impulsivity: contrasting effects of central 5-HT depletion on different measures of impulsive behavior. *Neuropsychopharmacology.* 2004; 29(7):1331–1343. [PubMed: 15054475]
- Winstanley CA, Eagle DM, Robbins TW. Behavioral models of impulsivity in relation to ADHD: translation between clinical and preclinical studies. *Clin Psychol Rev.* 2006; 26(4):379–395. [PubMed: 16504359]
- Winstanley CA, Theobald DE, Dalley JW, Robbins TW. Interactions between serotonin and dopamine in the control of impulsive choice in rats: therapeutic implications for impulse control disorders. *Neuropsychopharmacology.* 2005; 30(4):669–682. [PubMed: 15688093]

Fig. 1.

Schematic representation of a single trial in the stop-signal task. (1). Each session began with head entry to the trough, following illumination of the trough light. (2). The left lever was then extended, the left LED lights illuminated and the trough light extinguished. If the left lever was not pressed within 1–2.5 sec, it was retracted and the trial was recorded as an omission. (3 and 4). Pressing the left lever led to extension of the right lever and illumination of the right LED lights. The trial then progressed randomly as either a go or stop trial, 80% and 20% of the time, respectively. The insert shows that the right lever was available for a limited time period depending on the rat (1–2.5 sec, see text for details). Within the first 50% of this period, a 100-msec tone was randomly presented (stop trial) or not presented (go trial). (3). On Go trials, pressing the right lever resulted in delivery of a pellet reward, and the trial was recorded as a successful trial. Failure to press the right lever was recorded as a failed trial and initiated a 5 sec time-out period, wherein the house-light was extinguished for 5 sec with the levers retracted. (4). On stop trials, withholding lever press following the tone stimulus resulted in pellet reward. Pressing the right lever was recorded as a failed trial and resulted in a time-out period. New trials in all cases were initiated by head entry into the trough (1).

Fig. 2.

Temperature and DA and 5-HT depletions following AMPH and PCA treatment (for experiment 1). (A). Rectal temperature was elevated at each hour following drug-treatment compared to control treatment. (B). Representative photomicrographs of [¹²⁵I] RTI-55 autoradiography for DAT and SERT for each group from striatum and PFC. (C). Quantification of mean grey values of the autoradiography for each group. Larger reductions in DAT binding were seen following AMPH treatment compared to control and PCA treatments in the striatum. (D). Larger reductions in SERT binding were seen following PCA treatment compared to control and d-AMPH treatments in the PFC. Mean $\pm$ SEM. Asterisk indicate significant differences between drug- and control treatments ($p < 0.05$). Striatum: DMS: dorsomedial, DLS: dorsolateral, VMS: ventromedial, VLS: ventrolateral. Cortex: OFC: orbitofrontal, IL: infralimbic, PrL: prelimbic, ACC: anterior cingulate

Fig. 3.

Temperature and DA and 5-HT depletions following METH treatment (for experiment 2).

(A). Rectal temperature was elevated at each hour for the neurotoxic-METH-group

compared to normothermic-METH group where animals were cooled to maintain

temperature below 37.5°C. (B). Representative photomicrographs of [¹²⁵I] RTI-55

autoradiography for DAT and SERT for both groups from striatum and PFC. (C).

Quantification of mean grey values of the autoradiography for each group. Large reductions

in DAT binding were seen following neurotoxic-METH treatment compared to

normothermic-METH treatment in the striatum. (D). Large reductions were also seen in

SERT binding in the PFC for neurotoxic versus normothermic-METH group. Mean ± SEM.

Asterisk indicate significant differences between drug- and control treatments (p<0.05).

Striatum: DMS: dorsomedial, DLS: dorsolateral, VMS: ventromedial, VLS: ventrolateral.

Cortex: OFC: orbitofrontal, IL: infralimbic, PrL: prelimbic, ACC: anterior cingulate

Fig. 4.

Stop and go trial accuracy for experiment 1. The percentage of successful trials for the control group did not differ before and after treatment for either trial type (first panel). However, go trial accuracy was significantly reduced pre- versus post- treatment for the AMPH group, whereas stop trial accuracy was not significantly affected (middle panel). For PCA, neither trial was affected (last panel). Asterisk indicate significant differences between pre- and post- trial ($p < 0.05$). Mean $\pm$ SEM

Fig. 5.

Stop and go trial accuracy for experiment 2. The percentage of successful trials for the normothermic-METH group did not differ before and after treatment for either trial type (first panel). Go trial accuracy was also not significantly affected for the neurotoxic METH group, but stop trial accuracy was significantly reduced pre- versus post- treatment (last panel). Asterisk indicate significant differences between pre- and post- trial ($p < 0.05$). Mean $\pm$ SEM

Table 1A and 1B Tables showing Mean $\pm$ SEM for each variable measured on the stop-signal task before (pre) and after treatment (post) for each group (in experiment 1 and 2, respectively). None of the variables differed pre- versus post-treatment, or between the treatment groups. Therefore, non-specific effects on task performance can be ruled out. Lever present shows the average time (in seconds) that the right lever was available during the stop and go trials. Total trials show the average number of go and stop trials for each group (*i.e.* the left lever was presented. Note that stop trials make up 20% of total trials), and the omitted trials show the average number of trials that were not initiated (*i.e.* the left lever was not pressed). mRT on go trials shows the time (in seconds) between entry of the right lever and press of the right lever.

Table 1

	A. Exp 1			B. Exp 2		
		pre	post		pre	post
Lever present (sec)	Control	1.8 $\pm$ 0.2	1.8 $\pm$ 0.2	norm-METH	1.7 $\pm$ 0.1	1.7 $\pm$ 0.2
	AMPH	1.6 $\pm$ 0.2	1.6 $\pm$ 0.2	toxic-METH	1.8 $\pm$ 0.2	1.8 $\pm$ 0.3
	PCA	1.5 $\pm$ 0.2	1.5 $\pm$ 0.2			
Total go trials (#trials)	Control	99.6 $\pm$ 5.3	95.3 $\pm$ 14.1	norm-METH	89.2 $\pm$ 23.4	87.2 $\pm$ 24.6
	AMPH	99.1 $\pm$ 21.5	88.5 $\pm$ 21.8	toxic-METH	111.7 $\pm$ 13.1	116.8 $\pm$ 16.0
	PCA	110.8 $\pm$ 11.0	82.4 $\pm$ 4.9			
Total stop trials (#trials)	Control	24.6 $\pm$ 2.1	23.8 $\pm$ 4.2	norm-METH	23.2 $\pm$ 6.0	20.1 $\pm$ 6.1
	AMPH	26.7 $\pm$ 5.1	23.4 $\pm$ 5.6	toxic-METH	28.8 $\pm$ 3.8	28.7 $\pm$ 4.8
	PCA	26.9 $\pm$ 3.1	21.1 $\pm$ 0.9			
Omitted trials (#trials)	Control	1.0 $\pm$ 0.5	0.3 $\pm$ 0.2	norm-METH	1.6 $\pm$ 1.0	1.0 $\pm$ 0.3
	AMPH	1.1 $\pm$ 0.6	0.4 $\pm$ 0.3	toxic-METH	0.7 $\pm$ 0.6	0.3 $\pm$ 0.2
	PCA	0.7 $\pm$ 0.4	0.5 $\pm$ 0.3			
mRT go trials (sec)	Control	1.4 $\pm$ 0.1	1.5 $\pm$ 0.2	norm-METH	1.2 $\pm$ 0.1	1.1 $\pm$ 0.1
	AMPH	1.3 $\pm$ 0.1	1.5 $\pm$ 0.2	toxic-METH	1.3 $\pm$ 0.1	1.4 $\pm$ 0.1
	PCA	1.2 $\pm$ 0.1	1.3 $\pm$ 0.1			