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Development of stereotyped behaviors during prolonged escalation of methamphetamine self-administration in rats

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Abstract

Rationale—Experimental animal studies have shown that repeated administration of psychostimulants, such as methamphetamine (METH), results in an altered behavioral response profile, which includes the sensitization of both locomotor and stereotyped behaviors. Although sensitization of these behaviors has been characterized in detail during bolus, investigator-administered drug administration, little is known about the development or expression of stereotypies during psychostimulant self-administration.

Objective/Methods—The present study investigated in rats the expression of focused stereotyped behaviors during an extended access, escalation procedure of METH self-administration. Over several weeks during stepwise extended daily access to METH (3-, 6- and 12-h) followed by exposure to 24-h ‘binges’, rats gradually increased daily drug intake.

Results—During the escalation procedure, the rats’ behavioral response evolved from locomotor activation to progressively more focused stereotypies, culminating in continuous oral behaviors (licking, gnawing and chewing), interrupted only by episodic lever presses. Sensitization of stereotyped behaviors was evident, particularly with regard to oral behaviors that exhibited a more rapid onset and intensification in the apparent absence of greater drug intake.

Conclusions—Our data demonstrate that stepwise extended daily access to METH (3-, 6-, 12- and 24-h) self-administration in rats closely approximates motivational, pharmacokinetic, as well as behavioral patterns of human METH abuse. The accompanied appearance of sensitization of intense focused stereotyped behaviors, which is probably a consequence of escalation of drug intake, resembles stereotypies associated with investigator-initiated METH administration and may parallel the development of stimulant-induced psychosis seen in human abusers.

Keywords

Methamphetamine; self-administration; escalation; binge; stereotypy; sensitization

Introduction

Methamphetamine (METH) is a highly addictive psychomotor stimulant drug, and its use may lead to deleterious psychiatric symptoms, including cognitive impairments and

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schizophrenia-like psychosis, particularly with long-term, high-dose abuse patterns such as 'bingeing' (Nordahl et al. 2003; Meredith et al. 2005; Rogers et al. 2008). Although the factors contributing to these impairments have not been fully characterized, evidence indicates that duration and pattern of exposure to stimulant drugs play important roles (Simon et al. 2002; Davidson et al. 2001). Thus, efforts to utilize preclinical models to understand potential underlying mechanisms should simulate the duration and pattern of human drug exposure. Attempts to accurately model the pattern of human METH abuse in experimental animals have been made, using both contingent and non-contingent drug administration procedures (Hemby et al. 1997; Segal et al. 2003; Kitamura et al. 2006; Belcher et al. 2008; Rogers et al. 2008; Kuczenski et al. 2009). For example, observations indicate that a progressive escalation in dosage typically precedes high dose maintenance patterns (Simon et al. 2002) or 'binge' regimens in humans. Thus, we have argued that a similar administration regimen is an important component of drug delivery that should be used in animal models (Segal and Kuczenski 1997; 1999). This approach has proven useful in identifying long-term consequences of METH intake, such as tolerance (O'Neil et al. 2006) and histopathological damage (Kuczenski et al. 2007).

Nevertheless, typical non-contingent METH administration procedures do not provide the opportunity to investigate potential relationships between drug-induced dysfunctions and motivational aspects of drug taking (Rogers et al. 2008), whereas rodent intravenous self-administration (IVSA) procedures introduce that possibility. For example, rats with extended access to 6-h of METH IVSA have shown escalated drug intake (Kitamura et al. 2006) and increased drug seeking (Dalley et al. 2007; Rogers et al. 2008) compared to animals with shorter access. In addition, those authors hypothesized that extending daily drug access to 6-h leads to cognitive impairments, similar to those seen in human METH addicts who engaged in repeated 'binge-runs' (Rogers et al. 2008). Nevertheless, in general, contingent drug administration procedures have not included substantial dose escalation, and the typical 6-h drug exposure in extended access IVSA procedures does not adequately simulate the day-long exposure associated with 'maintenance' or 'binge' patterns of METH use in humans (Cho et al. 2001). Taken together, contingent and non-contingent METH administration procedures address and simulate different aspects of stimulant abuse, and accordingly differences in their neurobiological effects have been reported (see, for example, Stefanski et al. 1999; Jacobs et al. 2003; but see Winsauer et al. 2003; Kiyatkin and Brown 2004; Stuber et al. 2005). Importantly, experimental animal models that include motivational aspects, as well as exposure patterns similar to those seen in human METH abusers, have not been extensively explored in METH research.

Stereotypy is a cardinal feature of a broad range of neuropsychiatric disorders, and is also a fundamental feature of the behavioral syndrome induced by psychomotor stimulant drugs (Rylander 1969; 1972; Schiörring 1977; Davis and Schlemmer 1980; Ridley 1994; Randrup and Munkvad 1972). Behaviors of patients that typify stereotypy in clinical disorders range from repetitions of single or multiple movements (motor stereotypies) to repetitive, inflexible patterns of attention, emotion, planning and cognition (Canales and Graybiel 2000). Furthermore, with repeated administration, the responsiveness to stimulants is progressively enhanced (sensitization), and some evidence suggests that the susceptibility to psychotic symptoms associated with stimulant abuse may also be progressively augmented (Brady et al. 1991; Satel et al. 1991; Angrist 1994; Gawin and Khalsa 1996; Post and Kopanda 1976). Considerable efforts have been made to understand the mechanisms underlying the altered behavioral response profile, which includes the increased expression of both locomotor and stereotyped behaviors (Segal et al. 1981; Robinson and Becker 1986; Kalivas et al. 1993; Segal and Kuczenski 1994). With repeated stimulant administration, stereotypies exhibit a more rapid onset, become more intense and may appear at a lower acute dose than would typically produce stereotypy (Robinson and Becker 1986; Segal and

Kuczenski 1994). Although characterization of stimulant-induced behavioral changes has been achieved in detail during bolus, investigator-administered non-contingent drug administration procedures (e.g., Segal et al. 2003; Kuczenski et al. 2009), little is known about the development or the expression of stereotypies during chronic, high dose stimulant self-administration.

The present study investigated METH self-administration in a drug exposure pattern which mimics the progressive increase in drug consumption that typically precedes chronic, high-dose or 'binge' patterns of human METH abuse. We furthermore evaluated onset and expression of focused, repetitive behaviors in relation to METH exposure times and actual drug intake during progressive drug self-escalation.

Methods and Materials

Subjects

Adult male Sprague Dawley rats (350–450 g) purchased from Harlan (Harlan Labs, Gilroy, CA) were single housed with *ad libitum* access to food and tap water. The vivarium was temperature (20°C) and humidity (55 ± 5%) controlled and maintained on a reversed 12-h dark (8:00 a.m. to 8:00 p.m.), 12-h light cycle to allow the experiments to be performed during the normal active phase of the rats' awake/sleep cycle. All animal facilities, as well as surgical and experimental procedures, were in accordance with National Institutes of Health and Association for the Assessment and Accreditation of Laboratory Animal Care guidelines and were approved by the Institutional Animal Care and Use Committee.

Surgery

After one week of acclimation, animals were anaesthetized under an isoflurane/oxygen mixture (1–3% isoflurane) and chronic indwelling catheters were implanted into the right jugular vein as described previously (Hadamitzky et al. 2011).

Methamphetamine self-administration

After a 5-day recovery period from surgery, IVSA of METH was initiated in custom-made experimental chambers (30 × 30 × 38 cm) placed within ventilated sound-attenuating boxes. Each chamber was maintained at constant temperature (20°C) and humidity (55 ± 5%). One wall of the chamber was equipped with two retractable levers (active and inactive, 6 cm apart) located 6 cm above the metal grid floor of the chamber. Active lever presses resulted in an infusion of METH at a dose of 0.05 mg/kg in a volume of 0.05 ml over a period of 1.5 s (fixed-ratio; FR1). Responses on the inactive lever were recorded but had no consequences. The delivery of METH was paired with the activation of the cue light located above the levers, which remained illuminated throughout a 20 s timeout (TO) period, while the lever was inactive. Levers retracted immediately at the end of the session. Rats' performance was considered stable when the subject pressed the active lever at least 10 times over three consecutive days.

After acquisition of METH IVSA and six additional days of 1-h daily exposure sessions, one group of rats remained in this restricted self-administration mode (limited access; LMT, n=8), while the other group was tested on a self-administration escalating drug intake protocol (extended access; EXT, n=8) as described previously with modifications (see Hadamitzky et al. 2011). Briefly, drug access time was progressively extended over successive six-day periods, progressing from 3-, 6- and 12-h daily sessions, with two drug free days in between changes in the duration of self-administration sessions. Subsequently, animals were given free access to METH for 24-h 'binge-runs', while each 'binge' was followed by two drug-free days. Once rats of the EXT group were given prolonged access to

the drug for 12- and 24-h, they remained in the experimental chambers and were no longer returned to the vivarium. From this time point onwards, each morning the chambers were serviced and animals were weighed. Food and water were available *ad libitum* within the chambers. To maintain the light and dark cycles during extended access, the chambers were equipped with white (8:00 p.m. to 8:00 a.m.) and red lights (8:00 a.m. to 8:00 p.m.). Data collection and test session functions were controlled by computers and MED-PC IV software (MED Associates, St. Albans, VT).

Throughout the experiment, the weights of the animals remained relatively stable. However, during 24-h access the animals' bodyweight declined by approximately 17%, but the lost weight was regained by the animals during the drug free days (data not shown).

Behavioral analysis

The rats' behavior was continuously monitored and recorded digitally during the sessions using micropinhole cameras equipped with wide-angle lenses mounted on the doors of the chambers. Video images were collected via GV-800 BNC capture cards and Geovision software, and stored on DVD media for subsequent evaluation. Raters unaware of the specific experimental conditions evaluated the recordings on the basis of behavioral ethograms and rating procedures established previously with modifications (Segal and Kuczenski 1987). Briefly, specific behaviors, such as focused sniffing (restricted to approximately a 3-cm diameter area of floor or wall), repetitive head and limb movements, and oral movements (chewing, licking, and biting) were investigated. In the EXT group, behavior was always rated during the first hour of exposure on the last day of each self-administration access period (1-, 3-, 6-, 12- and 24-h). For comparison, the LMT group was analyzed at three corresponding time points, that is, at the beginning (*early*), in the middle (*middle*), and at the end (*late*) of the overall period of limited 1-h access. Stereotypy behaviors were rated as percentage of time engaged in intervals of 10 min length.

Plasma methamphetamine and amphetamine

After finishing the experiments, all animals were euthanized. The EXT group was euthanized during a 'binge' after six hours of exposure to METH, and the LMT group immediately after finishing its last 1-h session. For pharmacokinetic analyses, trunk blood was collected in ethylenediaminetetraacetic acid tubes after decapitation, and plasma was isolated and immediately frozen on dry ice. Plasma concentrations of METH and AMPH, its primary active metabolite, were determined by NMS Laboratories (Willow Grove, PA).

Drugs

Methamphetamine hydrochloride (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline. The dose represents the free base.

Data analyses

The descriptive statistics are based on means, and variance as indicated by the standard error of the mean (SEM). Statistical analyses were conducted using SigmaStat version 2.0 software (SPSS, Chicago, IL). Data (active lever presses/drug intake in mg/kg) obtained during the escalation course as well as during the first hour were subjected to a two-way analysis of variance (ANOVA) with *Group* (LMT vs. EXT) as one factor and *Time* (days or sessions) as a within-subjects factor. Changes in the pattern of active lever presses or drug intake in the first hour during extended access were also investigated. Using a two-way repeated measures ANOVA, drug intake over 10 min intervals was analyzed within the EXT group with *Session* (first vs. last 6-h session) as one factor and *Interval* (10 min) as the second factor. Post hoc comparisons were performed using the Student–Newman–Keuls

test. Stereotypy data from the LMT and the EXT group were analyzed using one-way ANOVA, with Bonferroni's corrections for post hoc comparisons after statistically significant effects in the ANOVA. Differences in blood plasma levels of METH and AMPH were evaluated with t-tests.

Results

METH self-administration

Fig. 1 depicts the course of active lever presses and total METH intake. LMT animals exhibited a stable pattern of drug intake and ANOVA revealed no significant changes over the course of drug access ($F(26,182)=1.078$, $p=.371$). In contrast, EXT rats progressively increased their intake over stepwise extended access periods. An ANOVA revealed a main effect of *Group* ($F(1,364)=138.52$, $p<.001$), a main effect of *Time* ($F(26,364)=28.24$, $p<.001$), and a *Group* $\times$ *Time* interaction ($F(26,364)=27.69$, $p<.001$). Post hoc comparisons indicated that EXT rats increased METH intake above the level observed during the first escalation session (Day 7), with the increase reaching significance beginning with the 8th escalation session onward (Day 14; $p<.05$).

To further analyze the nature of escalation, drug intake during the first hour of each session was compared between LMT and EXT groups (Fig. 1b). While drug intake remained low and stable in LMT rats, the EXT group progressively increased METH intake over stepwise extended access periods. An ANOVA showed a main effect of *Group* ($F(1,364)=42.1$, $p<.001$), a main effect of *Time* ($F(26,364)=5.55$, $p<.001$), and a *Group* $\times$ *Time* interaction ($F(26,364)=3.49$, $p<.001$). Post hoc analysis revealed that on corresponding days, METH intake of EXT rats during the first hour of each session was significantly elevated above the level of LMT rats, starting from the ninth session onward ($p<.05$).

Development of stereotyped behaviors

Over the course of extended access METH self-administration, stereotyped behaviors expressed by the animals progressively changed from predominantly focused sniffing during short access to intense oral behaviors during moderate and extended access. The temporal profiles of focused stereotypies observed during the first hour of exposure on the last day of each extended access period are summarized in Fig. 2 and 3.

EXT animals exhibited a progressive change in the qualitative nature of stereotyped behaviors over the METH escalation course. During 1-h access, EXT animals exhibited typical low-moderate stimulant dose locomotor activation (data not shown) along with frequent and extensive focused sniffing, generally directed at the floor and walls of the experimental chamber (Fig 2a). During 3-h access, repetitive head movements, nearly absent during 1-h access, substantially contributed to the stereotypy profile during the first hour of drug exposure (Fig. 2b). As access was further extended, oral stereotypies consisting of biting and licking at the walls and floor of the chamber, progressively predominated the expressed behavioral response (Figs. 2c, Fig 3). Specifically, the extended access course led to a significant increase in the percentage of time engaged in focused oral stereotypies in the first hour ($F(2,19)=3.953$; $p<.0037$). Post hoc comparisons revealed that at the 12-h access oral stereotypies were significantly higher compared to the 6-h access (Fig. 4; $p<.05$). Examination of video recordings revealed that behaviors which were expressed during the initial hour persisted till the end of the session, only interrupted by brief episodes of apparent sleep during the 24-h access. Notably, the animals exhibited few episodes of exploratory locomotion and typically remained in a single location in the chamber, leaving that location only to move to the active lever for drug self-administration.

As would be expected, LMT animals, characterized at three time points within the limited 1-h access course (*early, middle, late*), exhibited a behavioral response profile which was virtually identical to the one seen and described in EXT animals during their 1-h sessions, that is, predominantly focused sniffing with few or no episodes of repetitive movements or oral behaviors (see Fig. 2a for EXT animals).

Sensitization of the stereotypy response

Over the entire course of exposure, the qualitative features of stereotyped behaviors in LMT animals did not change from predominantly focused sniffing. However, ANOVA revealed a significant increase in the percentage of time engaged in focused sniffing (0–60 min, $F(2,7)=105.3$; $p<.0001$). Post hoc comparisons indicated that both the *middle* and *late* days were significantly different from the *early* day ($p<.001$). In addition, the onset of these behaviors was more rapid during the later sessions (ANOVA; 0 – 10 min, $F(2,7)=31.2$; $p<.0001$), and post hoc comparisons revealed that all three time points were significantly different from each other ($p<.01$; Fig. 4a). Comparisons of overall drug intake in LMT animals over the three time points revealed no significant differences (ANOVA; $F(2,14)=3.22$; $p=.071$). Both the increase in time engaged in focused sniffing and the more rapid onset of this behavior in the absence of changes in drug intake are indicative of sensitization of perseveration with successive exposures to the drug.

As seen in the LMT group, examination of EXT animals revealed a variety of evidence that sensitization of the stereotypy response was occurring during repeated exposure to METH (Fig. 4b). For example, whereas animals were engaged primarily in focused sniffing and repetitive head movements during the first 6-h session, by the last 6-h session substantial oral stereotypies appeared within the initial 20-min interval and predominated throughout the remainder of the hour-long rating period (Fig. 5a, b). In contrast to the dramatic change in behaviors, drug consumption exhibited few changes (Fig. 5c). In addition, the onset of these behaviors was different, as well. Post hoc comparisons showed that at the 12-h access the onset of the behavioral response was more rapid (0 – 10 min, $F(2,19)=7.998$; $p<.0003$) compared to the 6-h access ($p<.05$).

Plasma methamphetamine and amphetamine

During the hour before euthanasia, rats' drug intake was approximately 0.35 mg/kg in the EXT group and 0.15 mg/kg in the LMT group. Hence, METH plasma levels achieved at extended access were significantly increased compared to the limited access group (LMT group = 61.4 ± 9.7 ng/ml; EXT group = 99.3 ± 11.4 ng/ml; $t = 2.2$, $p<.05$). Regarding AMPH, the primary metabolite of METH, plasma levels were also different between the groups (LMT group = 11.9 ± 2.5 ; EXT group = 31 ± 6.6 ng/ml; $t = 2.1$, $p<.05$).

Discussion

The present study introduced in rats a simulation of a chronic high-dose or 'binge' pattern of human METH abuse by allowing rats to self-administer the drug for progressively longer periods of time up to 24-h. This simulation includes a number of components that we suggest are critical for accurate representation in experimental animals of human METH exposure. In particular, the simulation sought to promote a METH exposure profile that might reproduce the neurochemical adaptations that are associated with stimulant-induced dysfunctions and may be useful in studies with translational relevance.

First, as noted in the Introduction, data indicate that the neurobiological consequences of contingent and non-contingent drug administration procedures are different (Hemby et al. 1997). Although the potential significance of these differences remains controversial

(Stefanski et al. 1999; Jacobs et al. 2003; but see Winsauer et al. 2003; Kiyatkin and Brown 2004; Stuber et al. 2005), self-administration models in rodents clearly exhibit greater etiological and face validity with respect to the human disease from the perspective of drug taking and seeking.

Second, substantial evidence suggests that METH abuse most likely starts with a period of relatively gradual dose escalation. With gradual dose escalation, tolerance develops to the sympathomimetic effects of the stimulants, and abusers are able to survive higher doses (Fischman and Schuster 1974, 1977; Schuster and Fischman 1975; Schmidt et al. 1985b; Angrist 1994b). As a consequence, both the dose and frequency of stimulant administration can be increased, presumably to achieve and maintain high levels of the euphoria produced by these drugs (Angrist 1987; Angrist 1994b; Gawin and Khalsa 1996). We have therefore consistently argued that in animal models of chronic, high-dose METH exposure and 'bingeing', escalation in dosage is a critical component. In the present self-administration escalating drug intake procedure, rats (EXT group) increased their total daily amount of drug from approximately 0.5 mg/kg/day with limited 1-h access to approximately 14.5 mg/kg/day during unlimited 24-h access (Fig. 1a). These latter amounts maintained METH plasma levels in our rats during a 24-h session ($99.3 \text{ ng/ml} = 0.66 \text{ }\mu\text{M}$) within the range of typical values reported in human METH abusers (near $1 \text{ }\mu\text{M}$) (Melega et al. 2007). Because the dopamine transporters in humans and rats, the primary site of action for METH, exhibit similar sensitivity to the drug (Owens et al. 1997; Eshelman et al. 2001), and because the plasma level of METH is a good predictor for extracellular brain concentrations of the drug, we would suggest that both rats and humans "seek" comparable effective plasma, and thus, brain concentrations of the drug to engage the dopamine transporter. As a consequence, we further suggest that, at least from a motivational perspective, extended access METH self-administration in rats provides the most appropriate model to achieve a translationally relevant METH dosing regimen.

As previously shown in rodents, extended access to METH for 6-h leads to escalation of drug intake, evident by increased intake during the first hour of exposure (Kitamura et al. 2006; Hadamitzky et al. 2011). Our present results are also consistent with those observations, given that the EXT group increased its METH intake in the first hour from approximately 0.5 mg/kg during the 1-h access to approximately 1.4 mg/kg during the 6-h access. Further extension of access to 12- and 24-h only resulted in a modest further escalation of intake during the first hour of exposure (Fig. 1b; It should be noted, however, that because animals exposed to 12- and 24-h access to METH remained within the experimental chamber with levers withdrawn and also had free access to food and water throughout the drug exposure, the change in cues involved with transfer of animals from the vivarium to the experimental chamber and food and water availability during short access periods might have affected subsequent responding during the first hour). In contrast, METH intake in rats with limited access (LMT group) remained low and stable throughout the overall time course of 1-h daily exposure, similar to the level observed during 1-h access of the EXT group (ca. 0.5 mg/kg).

Species differences in METH pharmacokinetics between rats (half-life: $< 1 \text{ h}$) and humans (half-life: $10\text{--}12 \text{ h}$; Cho et al. 2001) offer a significant challenge to simulate the temporal parameters of typical human METH exposure patterns in rats (Segal and Kuczenski 2006). In this regard, the final critical component of the present self-administration escalating drug intake procedure is provided by the extended access sessions. Specifically, the 12- and 24-h sessions allow a better approximation to the day-long exposure characteristics of maintenance patterns of human METH abusers (Simon et al. 2002). Importantly, since stimulant-induced psychotic states most likely develop during sustained intake of relatively high doses of the drug over an extended period of time (Segal and Schuckit 1983), the 24-h

sessions offer an appropriate condition to investigate potential mechanisms underlying these behaviors within 'binge-runs'.

Similar to the results reported by Fowler and colleagues (2007), which were obtained during cocaine self-administration, the present data indicate that self-administered METH, during sufficiently prolonged exposure sessions, can support perseverative movements and intense stereotyped behaviors. LMT animals only exhibited focused sniffing, which predominated throughout the course of multiple 1-h self-administration sessions. In EXT animals, however, the qualitative nature of the stereotypies progressed from focused sniffing, through repetitive head and body movements to intense oral behaviors as the duration of the self-administration sessions and the magnitude of lever presses and drug intake gradually increased. This pattern of qualitative change in the nature of stereotyped behaviors is identical to the profile we typically observed under our experimental conditions after bolus injection of increasing doses of AMPH-like stimulants (see, for example, Segal and Kuczenski 1987; Kuczenski and Segal 1989). Continuous, intense oral stereotypies were also the predominant behavior seen during a 72-h 'binge' using human METH pharmacokinetics simulated in the rat (Kuczenski et al. 2009). It is unlikely that the availability of higher unit doses of METH to LMT animals would have resulted in similar, more intense focused stereotypies because rats titrate their drug intake based on the dose, and thus would not achieve levels of METH sufficiently high to support these behaviors within 1 hour sessions.

In spite of the development of tolerance to the sympathomimetic effects of the stimulants, sensitization of the locomotor and stereotypy effects of these drugs are a prominent feature after repeated administration (Segal and Mandell 1974). Furthermore, tolerance to the sympathomimetic effects does not appear to contribute to sensitization of locomotion or stereotypy (Kuczenski et al. 1982). As has been observed during investigator-administered stimulant exposure, sensitization of the stereotypy responses was evident in both LMT and EXT animals during METH self-administration. In the former group, repeated daily exposure resulted in a more rapid onset and increased magnitude of focused sniffing, and ultimately led to a stable, unchanging, sensitized profile (Fig. 4a). With extended access to METH, rats exhibited a more rapid onset and increased magnitude of the stereotyped behavior associated with each extended access period, including repetitive head movements expressed during intermediate length sessions (Fig. 2b, c), as well as the oral stereotypies expressed during the longer sessions (Fig. 3a, b). The development of the sensitized behavioral profile appeared to occur across daily sessions (Fig. 5a, b), whereas there was little evidence of sensitization developing during drug-free days [e.g., compare the last day of the 3-h sessions (Fig. 2b) with the first day of the 6-h sessions (Fig. 5a).

Behavioral perseveration is a prominent feature of the stimulant response in both experimental animals and humans, and motor stereotypies and perseverative thought processes are behaviors that are expressed in stimulant-induced psychosis (Kramer et al. 1967; Randrup and Munkvad 1967; Snyder 1973; Sudilovsky 1975). Perseveration of both motor and cognitive patterns may be integral to the pathophysiology of the psychotic state (Segal and Janowsky 1978). Evidence indicates that the psychotogenic response, that is, the development of stimulant psychosis and psychotic symptoms, are progressively enhanced. The present results indicate that extended access METH self-administration in rats produces a pattern of behavioral augmentation characterized by a progressively more perseverative and restricted response. Ellinwood and colleagues (1973) postulated that motor stereotypies, restricted perceptions and repetitious thinking might be subserved by common mechanisms (see also Sudilovsky 1975). Thus, although it is not entirely clear that perseverative tendencies are significantly implicated in the development of stimulant psychosis (Angrist

and Gershon 1970; Bell 1973), an understanding of the factors contributing to the augmented stereotypy response may provide insight into the etiology of stimulant psychosis.

Converging evidence indicates that chronic METH exposure results in long-term functional changes of the dopaminergic and serotonergic brain systems (Volz et al. 2007; Chang et al. 2007), and dopamine transmission in the dorsal part of the corpus striatum (caudate-putamen) is particularly affected (Davidson et al. 2001; Kita et al. 2003; Cadet et al. 2003; McCann and Ricaurte 2004). These changes in dopamine transmission have been suggested to lead to dysregulation of the striatal modulatory action on corticostriatal/ thalamocortical circuits, and therefore influence numerous behavioral and cognitive functions (Steiner and Kitai 2000; Tekin and Cummings 2002; Kalivas 2002; Martinez et al. 2003; Dalley et al. 2004). Hence, Schwendt and colleagues (2009) showed that long term extended access to 6-h METH self-administration in rats leads to a persistent decrease in dopamine transporter protein (DAT) levels in the prefrontal cortex and dorsal striatum. Similar, though less intense adaptations in striatum also occurred in our animals (Melega, Hadamitzky, Kuczenski unpublished observations). It is unlikely that these decreases in DAT represent the “neurotoxicity” typically associated with acute high dose METH administration. First, the decreases in DAT reported by Schwendt and colleagues (2009) were not accompanied by decrements in other protein markers typically associated with neurotoxicity. Second, in spite of the more intense exposure to METH which we utilized, the decrement in DAT which we observed was markedly less than those authors reported, likely reflecting the more gradual increase in access times which we used. Third, plasma levels of METH in our animals were substantially less than typically associated with neurotoxicity (O’Neil et al. 2006). Rather the self-administered escalating drug intake procedure was probably associated with compensatory neuro-adaptations in certain brain areas, contributing to the changes of the behavioral response profile and its onset. Such neuroadaptations are also suggested to be responsible for the development of idiopathic psychosis (Druhan et al. 1998), while it is still unclear whether they reflect compensatory alterations or neuronal damage, or whether there is a possible exhibition of functional recovery over time (Segal et al. 2003).

Taken together, contingent and non-contingent METH administration procedures can incorporate different aspects of human stimulant abuse. We here present a promising methodology that simulates the motivational, pharmacokinetic, and behavioral patterns of METH self-administration in humans. Furthermore, we showed that this procedure led to qualitative and quantitative changes in the animals’ behavioral response profile, while the onset of this altered response profile may be attributed to escalated drug intake. Clinical findings revealed that a progressive enhancement in responsiveness to stimulants (sensitization) plays an important role in the appearance of stimulant-induced paranoid psychosis (Brady et al. 1991; Satel et al. 1991; Angrist 1994; Gawin and Khalsa 1996; Post and Kopanda 1976). Even though, it is not clear that perseverative tendencies are significantly implicated in the development of stimulant psychosis (Angrist and Gershon, 1970; Bell 1973), an understanding of the factors contributing to the augmented stereotypy response may provide insight into the etiology of stimulant psychosis, and thus allow for further behavioral and neurochemical investigations in animals.

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Fig. 1.

Active lever presses and drug intake during limited access (LMT; $n = 8$) and extended access (EXT; $n = 8$) METH self-administration during the total session (a); and during the initial hour of each session (b). Significant difference within the EXT group is indicated by an asterisk ($p < .05$ vs. day 7), difference between the EXT and LMT group by a circle ($p < .05$). Activity during the first hour of the first two 12-h sessions was disrupted for some animals due to short term swivel malfunction. All data are expressed as means $\pm$ SEM.

Fig. 2.

Onset and intensity of the behavioral response after METH availability at short (**a**) and moderate (**b**, **c**) conditions of the EXT group. Stereotyped behaviors were rated as the percentage of time engaged in a particular behavior during observation intervals of 10 min length during the first hour of the last session of the distinct access periods (fsn= focused sniffing; rhm= repetitive head movements; oral= oral behaviors). Data are expressed as means $\pm$ SEM. Note that due to recording malfunction only six animals were evaluated during short access.

Fig. 3.

Onset and intensity of the behavioral response after METH availability at extended access conditions of the EXT group. Stereotyped behaviors were rated as the percentage of time engaged in a particular behavior during observation intervals of 10 min length during the first hour of the last session of the distinct access periods (fsn= focused sniffing; rhm= repetitive head movements; oral= oral behaviors). Data are expressed as means $\pm$ SEM.

Fig. 4.

Onset and intensity of the behavioral response predominantly expressed during limited (a; $n=8$) and extended METH availability (b; $n=8$). Behaviors were rated as percentage of time engaged during observation times of the first 10 min (left hand series; white bars) and during the whole first hour (right hand series; dark bars). At limited access focused sniffing was compared at three specific days of the course (*early*, *middle*, and *late*); significant differences vs. *early* are indicated by asterisks ($p < .05$), difference between *late* and *middle* is indicated by a cross ($p < .05$). At extended access, oral stereotypes were compared between 6-, 12- and 24-h; significant differences vs. 6-h are indicated by asterisks ($p < .05$). Data are expressed as means $\pm$ SEM.

Fig. 5.

Change of the stereotypy response profile and occurrence of sensitization during repeated exposure to METH at moderate access. Data represent percentage of time engaged in stereotyped behaviors during the first hour of the first (a) and the last (b) 6-h session (fsn= focused sniffing; rhm= repetitive head movements; oral= oral behaviors; n=8). The drug intake during these two sessions is presented in (c). Significant difference is indicated by an asterisk ($p < .05$). Data are expressed as means $\pm$ SEM.