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Behavioral flexibility predicts increased ability to resist excessive methamphetamine self-administration

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ABSTRACT

Drug addiction is often associated with cognitive deficits and behavioral inflexibility that may contribute to the development and maintenance of addictive behaviors by reducing addicts' ability to control their behavior toward the drug. In this study, we investigated the relationships between pre-drug levels of behavioral flexibility and the risk to develop uncontrolled methamphetamine (METH) self-administration. First, we measured individual performance in an inter-dimensional set-shifting procedure in which animals have to switch between an external visual rule and an internal side rule in order to obtain food pellets. Then we allowed rats to self-administer methamphetamine for twenty long 14h sessions and we investigated the relationships between behavioral flexibility and measures of control over drug intake. Rats rapidly acquired to self-administer high levels of METH which resulted in moderate weight loss. After several sessions of self-administration, whereas some rats progressively increased their METH intake, other rats showed very long voluntary pauses between drug injections and showed no escalation in METH self-administration. Interestingly, we found that behavioral flexibility is correlated with METH self-administration and that more flexible rats take less METH and do not escalate drug taking. These results suggest that traits of behavioral flexibility may protect against the development of excessive and dysregulated drug taking. Conversely, the inability to adapt behavioral responses as a function of the environmental contingencies may contribute to the risks to develop addiction to methamphetamine.

INTRODUCTION

Psychostimulant addiction is often associated with deficits in decision-making and other executive functions (Stalnaker et al., 2009; Verdejo-Garcia and Bechara, 2009). These deficits may not only affect the daily cognitive performance and behavior of addicts but also contribute to the difficulties of addicts to refrain from drug use and therefore they could be seen as integral part of the addiction pathology (Goldstein and Volkow, 2002). Among psychostimulants, methamphetamine (METH) is considered a large concern because it is among the most abused illicit drug in the world ((UNODC), 2014) and because of its neurotoxicity (Cadet and Krasnova, 2009). METH users show a wide variety of cognitive deficits ranging from impaired attention, memory, processing speed to executive functions (Dean et al., 2013; Scott et al., 2007). However, in humans it is difficult to determine the contribution of pre-existing cognitive impairments that could increase the vulnerability to develop addiction (Grant and Chamberlain, 2014). The use of animal models can provide important information about the contribution of different factors in the development of addiction. For example, previous studies in rodents have shown that exposure to drugs such as cocaine and amphetamines produce cognitive deficits (Stalnaker et al., 2009; Winstanley et al., 2010). However, concerning the role of pre-existing traits, most studies have focused on trait impulsivity and have shown that high impulsivity predicts the development of addiction-like behavior, maintenance and relapse (Dalley et al., 2011; Pattij and De Vries, 2013). Surprisingly, the role of other cognitive traits on the vulnerability to addiction has been mostly neglected.

Behavioral flexibility can be defined as the ability to change his/her own behavior to react to changing external conditions and maximize gain or minimize loss (Darrah et al., 2008; Stalnaker et al., 2009). In the context of addiction, behavioral flexibility may be involved in the transition from recreational to compulsive use of drugs. In fact, whereas drugs produce strong pleasurable effects that drive initial taking, at one point, these pleasurable effects are counterweighted and overwhelmed by the negative consequences of addiction (Koob and Volkow, 2010). Therefore,

flexible individuals might be expected to quit drug use when negative effects overcome the positive effects. Importantly, behavioral flexibility procedures could allow measuring aspects of compulsivity traits in humans and animals (Fineberg et al., 2010).

In preclinical models, behavioral inflexibility can be operationally defined as the inability of animals to adapt their behavior when external or internal contingencies change. In inter-dimensional set-shifting tasks (Darrah et al., 2008), for example, rats learn a visual-cue discrimination strategy (i.e. respond on the one of two levers that is signaled by light) and then, shift to using an egocentric spatial response strategy (press on the left or right lever only) to obtain reward (Darrah et al., 2008; Eagle and Baunez, 2010; Floresco et al., 2009). This task is cognitively demanding because, in order to succeed, rats have to 1) be attentive to external cues and the consequences of their action; 2) inhibit a behavior that was previously reinforced while ignoring confounding cues and 3) implement an alternative strategy. Rats that are inflexible have difficulties in changing their behaviors according to the new experimental contingencies and make more errors (Darrah et al., 2008; Stalnaker et al., 2009).

Here, we investigated whether it would be possible to predict METH-taking behaviors from trait flexibility. For this, first we trained rats in an operant set-shifting procedure and then we allowed them to self-administer METH for 20 long (14h) sessions, which are known to lead to escalation and dysregulation of drug-taking behavior (Kitamura et al., 2006; Krasnova et al., 2013; Krasnova et al., 2010; Parsegian et al., 2011; Parsegian and See, 2014). Finally, we investigated the relationships between basal levels of behavioral flexibility and measures of excessive METH-taking behavior.

MATERIALS AND METHODS

For detailed description of the methods see supplementary material.

Subjects

Twenty-eight adult (11-12 weeks of age), male Sprague-Dawley rats (Janvier, France), experimentally naive at the start of the study, were housed in a temperature- and humidity-controlled room and maintained on a 12-hour light/dark cycle (light on at 7.00 AM). Animals were housed three per cages during habituation to the animal facility and one per cage for the entire experimental period. All experiments were conducted during the light phase in accordance with European Union directives (2010/63/EU) for the care of laboratory animals.

Set-Shifting Task

Starting one week before the beginning of the experiments, food was restricted to approximately 15 – 18g per day, which maintained rats at 85-90% of their weight. Feeding occurred in their home cages 1h after the experimental session. Rats had unlimited access to water.

Behavioral flexibility experiments were performed in Coulbourn experimental chambers controlled by GraphState software (Coulbourn Instruments, Allentown, PA, USA; www.coulbourn.com). The procedure was similar to that designed by Darrah and colleagues (Darrah et al., 2008) with the difference that levers instead of nose-pokes were used as operanda. In brief, after shaping of operant responses, rats underwent sixteen training sessions to assess trait behavioral flexibility. In these sessions the operant cages were equipped with two identical levers, each surmounted by a diode green light that could be switched on or off according to the experimental phase of the procedure. The food tray was located on the opposite wall in order to force animals to reinitialize behavior and limit side biases. In each trial, in order to obtain food pellets, rats had to choose between the two levers based on two rules in different sensory dimensions: a visual dimension or an egocentric spatial dimension.

For the visual rule dimension, animals had to follow the position of a light above one of the two levers, which indicated which lever was active in a given trial. For the egocentric rule dimension, animals had to keep responding on the same side (right or left) and to ignore the position of the light. In each session, if a rat produced ten consecutive correct trials the rule was changed from one dimension to the other for a maximum of four different sets so that each dimension was presented twice per session. If animals did not reach this criterion, the trials continued with the same rule until the end of the experimental session. In each session there were four possible sets: i) rule light (L), ii) rule side right (SR), iii) rule light (L) and iv) rule side left (SL). The session ended when a rat completed the four different sets or after 45 min whichever occurred first. Four configurations were possible: 1) L > SR > L > SL; 2) SR > L > SL > L; 3) L > SL > L > SR and 4) SL > L > SR > L. The starting set was counterbalanced among rats and changed daily for each rat so the four different configurations were presented every four sessions in ascending order. The number of total, correct and incorrect trials was measured. Basal levels of flexibility were calculated as individual average percentage of errors/trial across the last four sessions, which allowed evaluating behavioral performance in each of the four configurations. Flexibility was measured by the ability to adapt from one rule to the other and therefore rats that showed lower percentage of errors were considered more flexible.

METH self-administration apparatus and procedure

Starting at the end of the last set-shifting session and until the end of the experiment, rats were fed *ad libitum*. One day after the end of flexibility training, rats were implanted with intra-jugular catheters and had one week of recovery before self-administration sessions started. METH self-administration experiments were performed in Imetronic experimental chambers equipped with nose-pokes as operanda (Imetronic, Pessac, France; www.**imetronic**.com). Twenty 14h METH self-administration sessions were conducted using a Fixed-Ratio 1 (FR1)

schedule of reinforcement, which included the rats' dark phase (6pm-8am). METH was available at a dose of 0.06 mg per injection. METH intake was calculated based on rats' weight. Based on pilot experiments, in order to minimize drug-induced health deterioration and to maximize animal survival, we imposed pauses of 1-2 days every 5-6 self-administration sessions and we limited the total number of sessions to twenty. Escalation of METH taking was measured as the percentage of increase of METH intake compared to the average intake in the first three sessions.

Statistical analysis.

Differences in behavioral flexibility and drug-taking behavior were assessed by one- or two-way ANOVA for repeated measures. Results showing significant overall changes were subjected to Dunnets or Student-Newman-Keuls post-hoc test. For correlation analysis, we calculated Z-test coefficients using Statview software. To divide animals in high- and low-flexibility groups we used a median split. Differences were considered significant when $p < 0.05$.

RESULTS

Behavioral Flexibility Performance

The first four sessions allowed rats to learn the procedure, which explains the increase in the number of trials completed and in the number of errors committed as they were increasingly faced to multiple rule changes (Fig. 1A). Rats learned relatively rapidly to switch from one rule to another to obtain food pellets, and within 4-5 sessions most rats were able to complete the four sets of the set-shifting task. While in the first sessions the percentage of error for each set was around 40% (a value close to the 50% chance level), within 5-6 sessions it reached a value of 30%, and after 10 sessions rats' behavioral performance stabilized at a group average error percentage value of 23-24% (Fig. 1A-B-C). Statistical analysis by one-way ANOVA for repeated measures revealed a significant effect of session for trials ($F(27, 405)= 8.61$, $p < 0.0001$), for errors ($F(27, 405)= 8.90$, $p < 0.0001$) and for % of errors ($F(27, 405)= 11.78$, $p < 0.0001$). Basal level of flexibility, calculated as individual average percentage of errors across the last 4 sessions, showed a Gaussian distribution (Fig. 1D) with individual flexibility ranging from 19 to 29 % of errors/trial (25%-75% percentiles = 22.36-24.91; mean = 23.68; median = 23.67).

Self-administration of METH

Eight days after the end of set-shifting task, self-administration of METH started. All rats learned to self-administer METH within the first 14h session showing high levels of preference for the active lever (Fig. 2A) and taking about 100 injections of METH/session (Fig. 2B). Initially, the average intake of METH was 12-13 mg/kg and then increased to 17-18 mg/kg with peaks of ~20 mg/kg (Fig 2C). This increase was in part related to a significant loss of weight in METH rats. In fact, within a few sessions of METH self-administration, rats' weight decreased from 450 grams to about 380 grams and then stabilized at this level (Fig. S1). By the tenth day of self-administration, rats started to show a significant escalation of

METH-taking behaviors with intakes 50 % higher than baseline levels (Fig. 2D). Statistical analysis by one-way ANOVA for repeated measures revealed a significant effect of active/inactive nose poke ($F(1, 27)= 49.60$, $p < 0.0001$), of session for the number of injections ($F(27, 513)= 2.57$, $p < 0.01$), METH intake ($F(21, 513)= 3.74$, $p < 0.0001$) and for escalation ($F(21, 513)= 3.69$, $p < 0.0001$).

Whereas the group average showed relatively low levels of variability, we observed that some rats, after the first 2-3 sessions, started taking "pauses" in their self-administration, reducing their intake to levels lower than the first day. In several instances, this reduction was associated with long pauses (up to several hours) in METH-taking behavior, which often occurred at the beginning of the session before the first METH injection (for an example of this self-administration pattern, see Fig. S2). These pauses were followed by a normal pattern of self-administration with frequent and well-spaced injections of METH. These sessions of voluntary reduction of METH intake were scattered between sessions in which METH self-administration was regular and frequent. Some rats showed many days of this voluntary reduction in METH intake, some showed only a few days of reduction and only six rats did not show any day of reduction.

Relationships between basal levels of flexibility and METH self-administration

We then investigated whether basal levels of flexibility were correlated with measures of METH self-administration after short and long exposure to METH. After 10 sessions of self-administration neither intake nor escalation were correlated with levels of flexibility (Fig. 3A and 3C). In contrast, intake in the later 10 sessions of METH self-administration was significantly and positively correlated with basal levels of behavioral flexibility (Correlation = 0.48, Z-Value = 2.63, $p < 0.01$) (Fig. 3B). In fact, individuals that made more errors in the set-shifting procedure were more likely to self-administer more METH. Similarly, levels of escalation of METH taking during the last 10 sessions of self-administration were also

positively correlated with basal levels of flexibility (Correlation = 0.50, Z-Value = 2.76, $p < 0.01$) (Fig. 3D).

Finally, we divided rats in two groups based on their behavioral flexibility (high and low) based on a median split and re-analyzed their self-administration behavior. We found that highly flexible rats showed significantly lower METH intake and lower escalation compared to low-flexibility animals (Fig. 4A-B). Statistical analysis by two-way ANOVA for repeated measure of METH intake and escalation revealed a significant effect of flexibility (intake, $F(1, 26) = 7.01$, $p < 0.05$; escalation: $F(1, 26) = 10.53$, $p < 0.01$) and of session (intake: $F(19, 494) = 3.76$, $p < 0.0001$; escalation $F(19, 494) = 3.80$, $p < 0.0001$) and a significant flexibility X session interaction for escalation ($F(19, 494) = 1.79$, $p < 0.05$).

DISCUSSION

In this study, we demonstrate that pre-existing traits of behavioral flexibility predict the propensity to develop excessive METH taking behavior after long, but not short, periods of self-administration. In fact, rats that perform better in set-shifting tasks self-administer less METH and show less escalation of METH intake but this difference becomes significant only after more than ten sessions of METH self-administration. These results suggest that flexibility phenotypes might influence the risk to develop addiction to methamphetamine.

In the set-shifting procedure used in this study, adapted from a previous publication (Darrah et al., 2008), rats have to perform repeatedly extra-dimensional shifts from a visual strategy to an egocentric side strategy in order to obtain food pellets. Rats have to learn two types of responses and switch between them while ignoring cues that are not relevant to the specific tasks. Therefore, this procedure measures executive functions that allow individuals to be attentive to external and internal cues, to process the consequences of their actions and to inhibit inappropriate behavior. Based on all these factors, individuals are then able to select the most appropriate behavioral response. After an initial learning phase, rats are tested repeatedly on the same set of rules that are alternated within and between sessions and therefore they have to choose among strategies that they have already learned. In this respect, the procedure is different from other set-shifting procedures in which flexibility is measured in a single session or two (Dalton et al., 2011; Ding et al., 2014; Haluk and Floresco, 2009). It is possible that these procedures measure different aspects of behavioral flexibility; the former (Darrah et al. 2009 and present work) models perseverative behavior and strategy selection and the latter (Dalton et al., 2011; Ding et al., 2014; Haluk and Floresco, 2009) models the ability to rapidly adapt to new contingencies and to implement a novel behavioral strategy. The fact that these procedures may measure different aspects of flexibility is confirmed by the analysis of our data showing that behavioral flexibility on the first day, the first two or the

first four days does not correlate with behavioral flexibility on the last 4 days when behavior has stabilized (data not shown). A possible limitation of our procedure is that, with training, rats may learn to compensate initial behavioral deficits. However, this limitation appears to be outweighed by the fact that because of repetitive testing, this procedure is less likely to be influenced by confounding factors such as reactivity to stress and novelty, general learning deficits and appears to reveal a stable phenotype.

In this series of experiments, rats were allowed to self-administer METH in very long 14h self-administration sessions. Rats learned rapidly to self-administer METH and showed very high intake of METH from the first day. They started off with an average of 12.5 mg/kg and reached up to 20 mg/kg. Previous studies investigating METH self-administration in 15h sessions, found that METH intake starts at a low 3 mg/kg and gets at 14-15 mg/kg in the last sessions (Krasnova et al., 2013; Krasnova et al., 2010). In our study, rats had a steeper learning curve, reached similar levels of self-administration after 7-8 sessions and had twelve additional sessions (20 vs 8) to further increase their daily and total intake. Under these conditions, rats lost weight from about 450 to 380 g (> 15% weight), which is consistent with the known anorexigenic effects of METH (Krasnova et al., 2013; Krasnova et al., 2010; Saito et al., 1995). In addition, several rats alternated days of escalation in METH intake with days of voluntary reduction in METH intake, characterized by very long pauses in self-administration behavior, which resulted in considerable reduction in METH intake. This behavior is similar to that previously reported under condition of continuous access (24h/day) to amphetamine and methamphetamine which was also characterized by periods of active self-administration and pauses that can last several hours (Pickens and Harris, 1968; Yokel and Pickens, 1973). Under those conditions, METH self-administration was associated with high levels of toxicity and deaths of the rats occurred within a few weeks (Pickens and Harris, 1968; Yokel and Pickens, 1973). Similarly, in pilot experiments, we found that this level of

self-administration is close to the physical limits of rats and lethal METH toxicity. Therefore, these elements suggest that METH self-administration under the present conditions is associated with adverse effects and that the escalation of METH found in this study truly reveals a loss of control over METH use.

The main aim of this paper was to investigate whether traits of behavioral flexibility could predict the development of excessive METH self-administration. Our results demonstrate that rats that makes less errors and are more flexible are less prone to self-administer METH excessively and less prone to show escalation of METH taking. Importantly, these differences were not evident after short periods of self-administration but were unmasked only after longer periods of self-administration. Therefore, these results suggest that levels of flexibility influence the ability to limit METH consumption when adverse effects start occurring and that the facility in adapting behaviors to a changing environment may represent a protective factor against the development of METH addiction. Whereas in humans METH users show deficits in executive functions including behavioral flexibility (Scott et al., 2007) compared to healthy controls, in those studies it is difficult to determine whether these deficits represent a pre-existing trait that predispose to METH abuse, whether they are the consequences of some forms of METH toxicity (Dean et al., 2013; Grant and Chamberlain, 2014) or both. Our study suggests that behavioral inflexibility can render individuals more vulnerable to develop excessive METH-taking behavior. In turn, because escalation of METH self-administration has been shown to induce deficits in behavioral flexibility (Parsegian et al., 2011), this could lead to an aggravation of initial deficits which finally would results in even bigger difficulties in limiting or controlling his/her own drug intake. This reciprocal correlation resembles the one previously described between addiction and impulsivity traits. In fact, on the one hand, impulsivity traits predict the risks to develop dysregulated drug use and on the other hand, drug taking can induce impulsivity (Dalley et al., 2011; Pattij and De Vries, 2013).

Importantly, work from Ersche and colleagues suggest that deficits in executive functions appear to be endophenotypes that predispose individuals to the development of drug addiction (Ersche et al., 2012a; Ersche et al., 2012b; Ersche et al., 2010).

Our results clearly demonstrate that animals that are more cognitively flexible are protected from developing excessive use of METH. Behavioral inflexibility appears an important feature of drug addiction because individuals continue to take drugs even when the positive effects of drugs are clearly overwhelmed by the negative consequences of drug taking. It is therefore possible to hypothesize that the same behavioral and neurobiological mechanisms are involved in the set-shifting task and in refraining from excessive METH self-administration. A key region for such executive control would be the prefrontal cortex that has been indeed shown to be involved in set-shifting procedure (Darrah et al., 2008; Eagle and Baunez, 2010; Floresco et al., 2009) and in uncontrolled drug taking (Chen et al., 2013; Kasanetz et al., 2013). However, other mechanisms independent from behavioral flexibility may participate in these effects and it is possible that animals selected for behavioral flexibility share characteristics other than cognitive control that may lead them to reduce their intake. Future studies making use of more direct measure of efficacy of reinforcement, such as progressive ratio schedule, and of resistance to punishment are needed to draw more definitive conclusions about the behavioral mechanisms underlying the relationships between behavioral flexibility and METH self-administration.

In conclusion, this study demonstrates that behavioral flexibility in a set-shifting task predicts the propensity to develop excessive METH self-administration. Therefore, capacities in adapting behavior in response to external contingencies may represent a cognitive trait that would modulate individual ability to refrain from drug use when negative consequences ensue. Because excessive drug use can in turn further worsen cognitive flexibility, inflexible

individuals would be at great risk to develop addiction as they become more and more unable to change their behavior.

AUTHOR CONTRIBUTIONS

MI, NT and MS conceived and designed the experiments. MI and MS performed experiments and analyzed data. All authors contributed to the writing of the manuscript.

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FIGURE LEGENDS

Fig. 1. Behavioral flexibility performance in the set-shifting procedure. A) Number of trials completed, B) number of errors committed and C) % errors/trials during the 16 training sessions of set-shifting training. Flexibility was measured by lower percentages of errors/trials, which reflects the ability to adapt behavior as rapidly and efficiently as possible when rules changed during the sessions. D) Distribution of basal levels of flexibility measured as individual average of % errors/trial in the last 4 set-shifting sessions. The vertical dotted line in panel D indicates the median flexibility value (23.67% errors/trial). Data are expressed as means ± SEM from 28 rats. Note that for panel A the number of trials is significantly higher in all sessions compared to session 1 but symbols are not shown for reasons of clarity. For panels A-C, one-way ANOVA for repeated measure followed by Dunnett's post-doc test. **, $p < 0.05$ and $p < 0.01$ compared to session 1.

Fig. 2. Self-administration of METH. A) Number active and inactive responses, B) number of injections, C) METH intake and D) escalation of METH intake compared to the first three days of self-administration during the 20 self-administration sessions. Data are expressed as means ± SEM from 28 rats. Note that for panel A active responses are significantly higher than inactive responses for all sessions other than session 1 but symbols are not shown for reasons of clarity. For panels B-D, one-way ANOVA for repeated measure followed by Dunnett's post-doc test * and **, $p < 0.05$ and $p< 0.01$ compared to baseline.

Fig. 3. Correlation between behavioral flexibility and self-administration of METH after short and long periods of access to METH . Correlation between basal levels of flexibility in the set-shifting procedure and average daily METH intake during A) the first and B) the last 10 sessions of self-administration. Correlation between basal levels of flexibility in the

set-shifting procedure and average escalation of METH taking behavior during C) the first and D) the last 10 sessions of self-administration. Basal flexibility was calculated as the average % of errors/trial during the last 4 set-shifting sessions. Linear regressions and 95% intervals were calculated and Z scores were obtained. R^2 for intake were 0.08 ($p = 0.15$) for the first 10 sessions and 0.23 ($p < 0.01$) for second 10 sessions. R^2 for escalation were 0.01 ($p = 0.68$) for the first 10 sessions and 0.25 ($p < 0.01$) or second 10 sessions.

Fig. 4. Influences of high and low levels of flexibility on self-administration of METH. A) METH intake and B) escalation of METH intake compared to the first three days of self-administration during the 20 self-administration sessions in high and low flexible rats. Rats were classified as high or low flexible based on a median split (median value = 23.67 % errors/trial). Data are expressed as means ± SEM of 14 rats. Two-way ANOVA for repeated measure followed by Dunnett's post-doc test. * and **, $p < 0.05$ and $p< 0.01$ compared to baseline levels; $, $p < 0.05$ compared to high flexible rats.

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Fig. 1

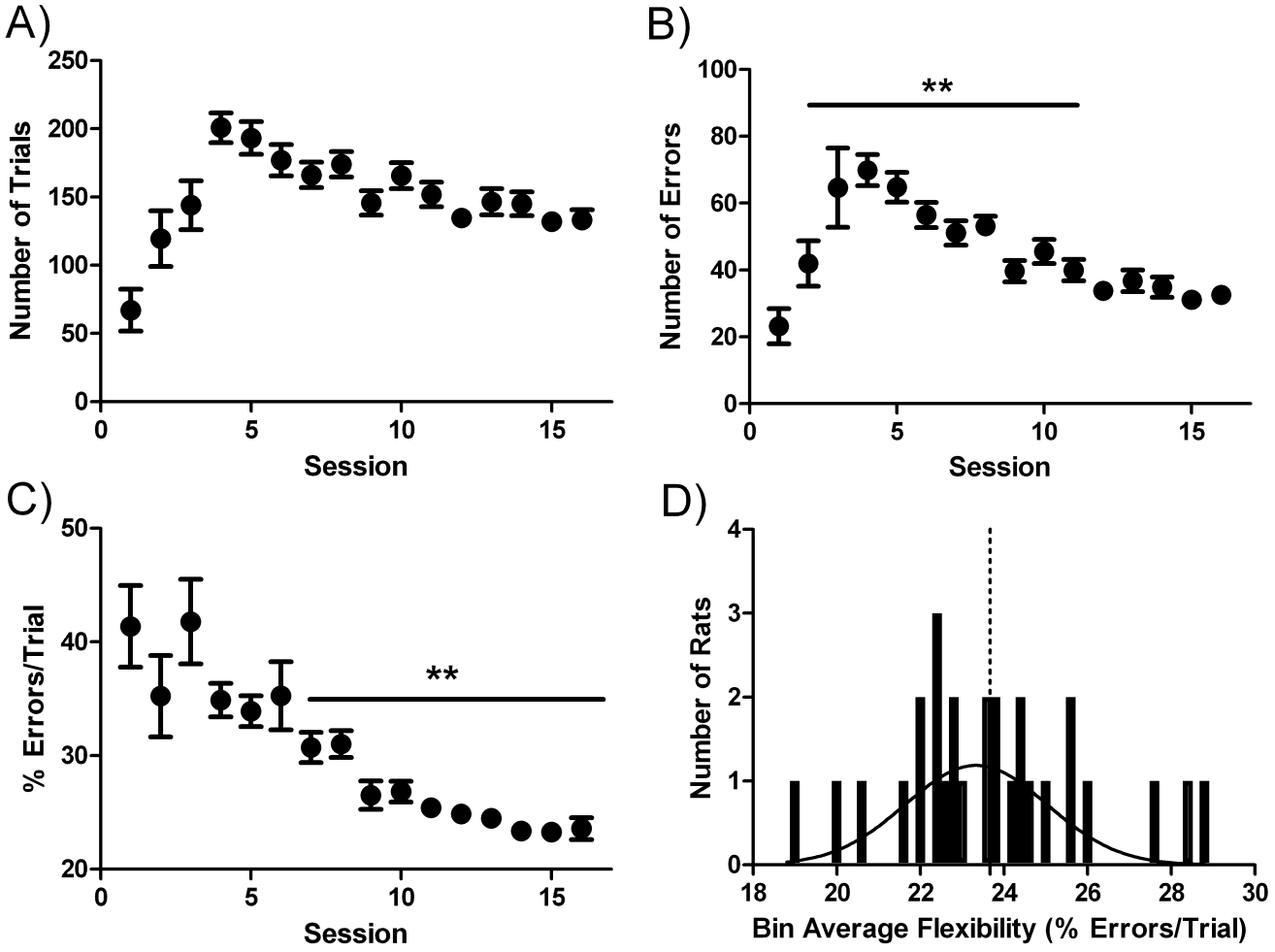

A)
Number of Trials
250
200
150
100
50
0
0
5
10
15
Session
B)
Number of Errors
100
80
60
40
20
0
**
0
5
10
15
Session
C)
% Errors/Trial
50
40
30
20
**
0
5
10
15
Session
D)
Number of Rats
4
3
2
1
0
18
20
22
24
26
28
30
Bin Average Flexibility (% Errors/Trial)

Fig. 2

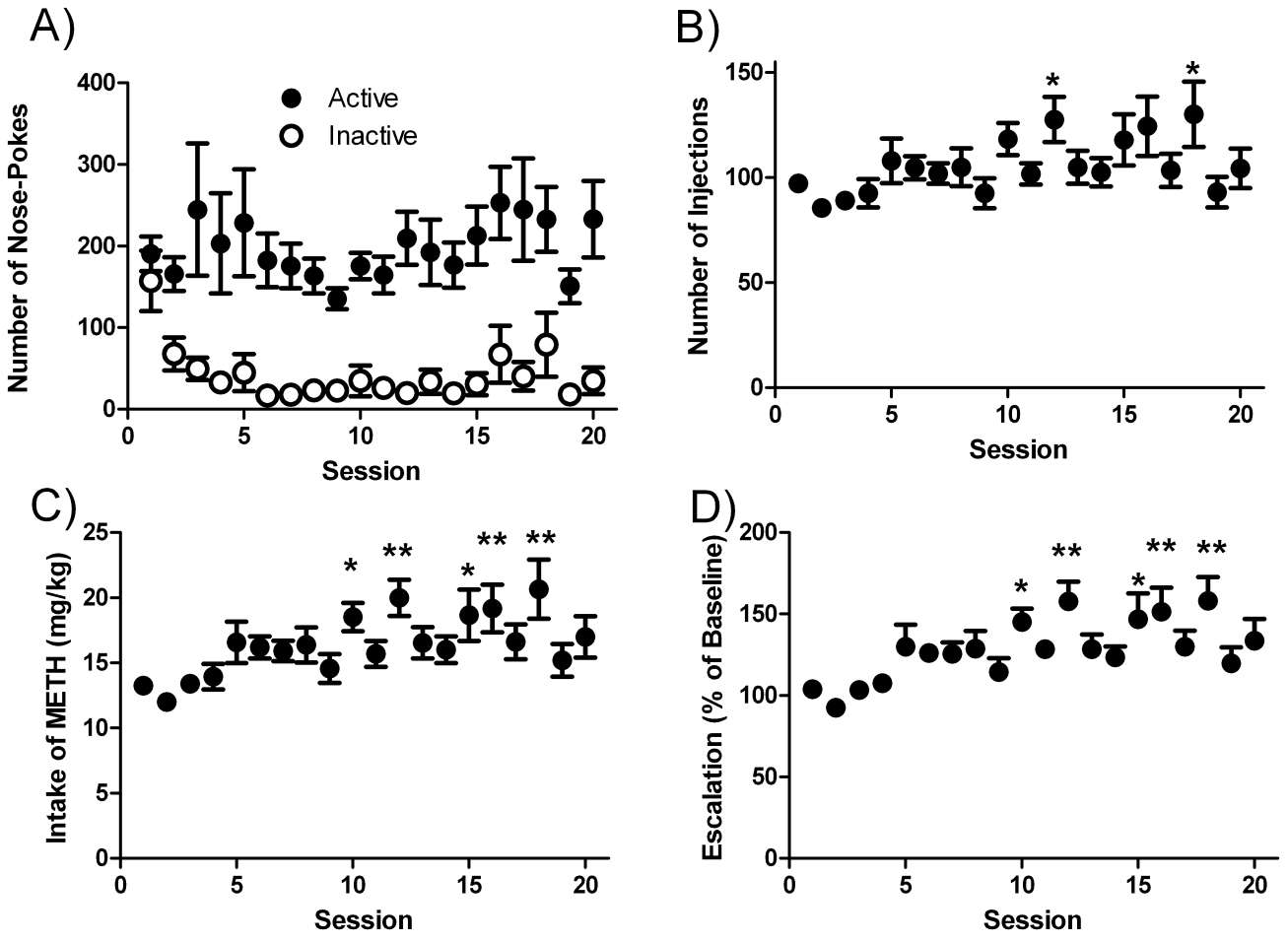

A)
Number of Nose-Pokes
Active
Inactive
Session
B)
Number of Injections
Session
C)
Intake of METH (mg/kg)
Session
D)
Escalation (% of Baseline)
Session

Fig. 3

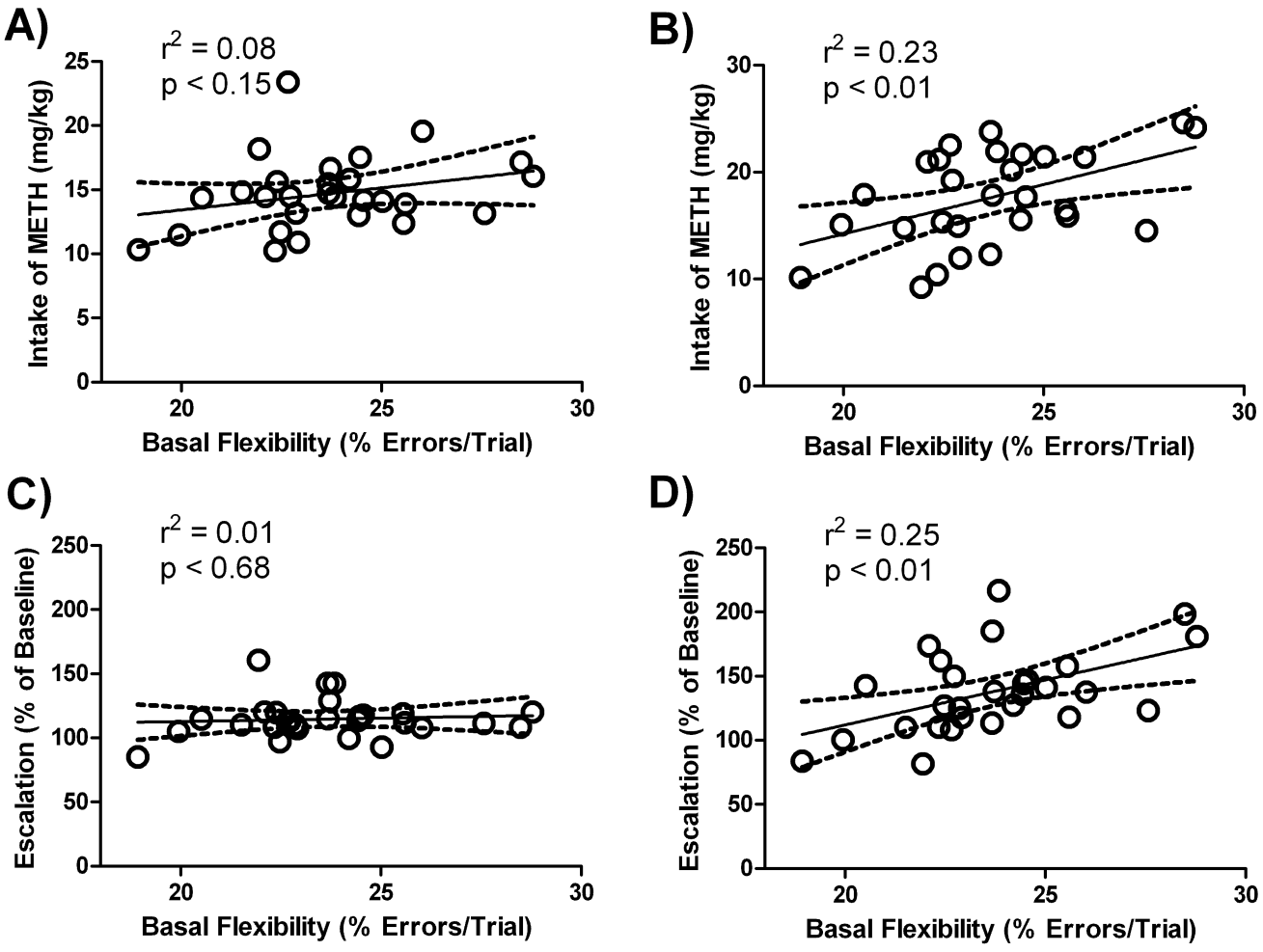
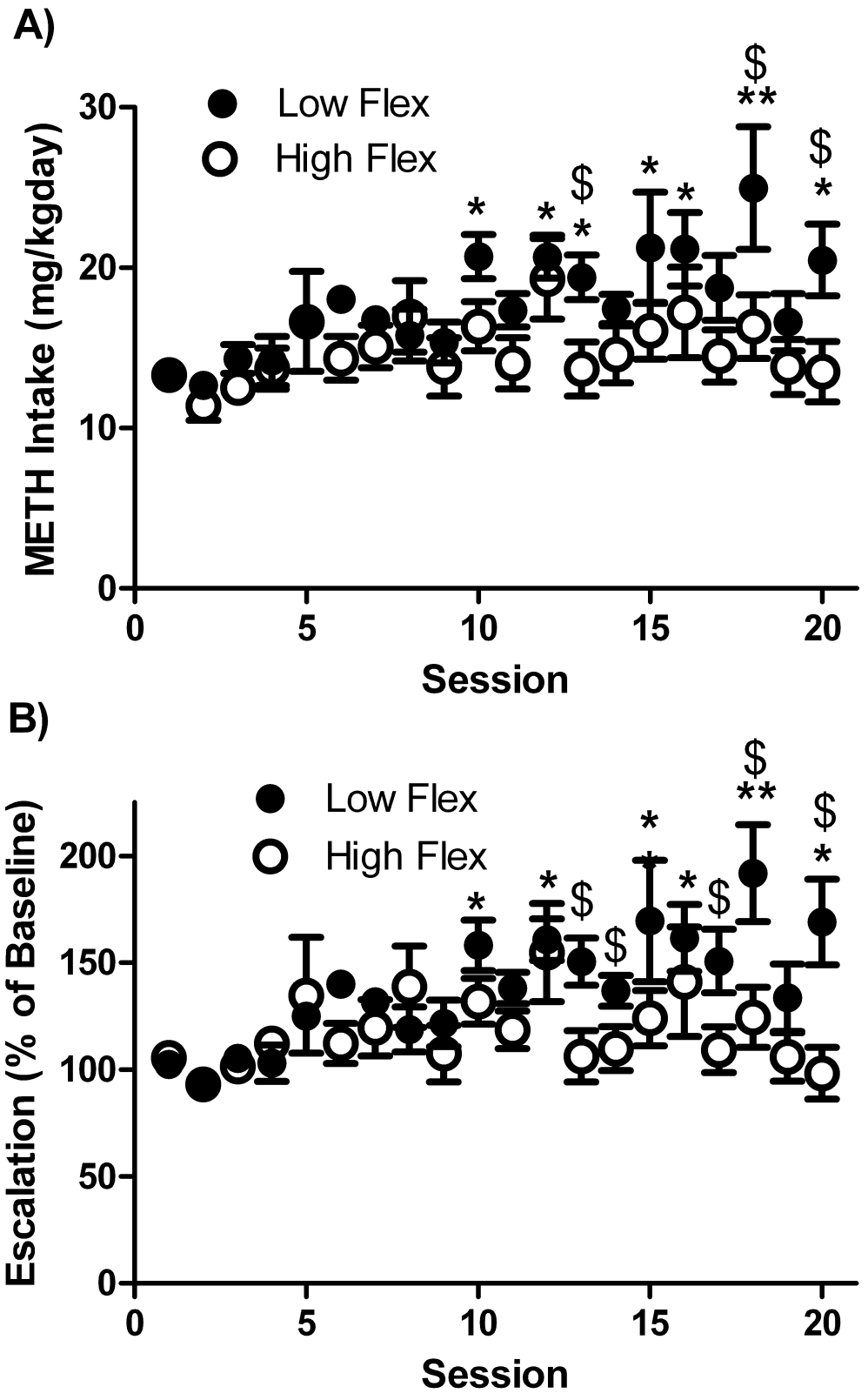

A)
r2 = 0.08
p < 0.15
Intake of METH (mg/kg)
Basal Flexibility (% Errors/Trial)
B)
r2 = 0.23
p < 0.01
Intake of METH (mg/kg)
Basal Flexibility (% Errors/Trial)
C)
r2 = 0.01
p < 0.68
Escalation (% of Baseline)
Basal Flexibility (% Errors/Trial)
D)
r2 = 0.25
p < 0.01
Escalation (% of Baseline)
Basal Flexibility (% Errors/Trial)

Fig. 4


A)
Low Flex
High Flex
METH Intake (mg/kgday)
30
20
10
0
0
5
10
15
20
Session
B)
Low Flex
High Flex
Escalation (% of Baseline)
200
150
100
50
0
0
5
10
15
20
Session