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ORIGINAL INVESTIGATION

Reduction of impulsivity with amphetamine in an appetitive fixed consecutive number schedule with cue for optimal performance in rats

Marion Rivalan · Stéphanie Grégoire · Françoise Dellu-Hagedorn

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Abstract
Rationale Impulsivity is a key feature of many psychopathologies such as mania, personality disorders or attention deficit–hyperactivity disorder (ADHD). Most experimental paradigms assessing impulsive behaviour also require non-specific capacities such as time estimation. This may interact with the measures and mask the beneficial effects of psychostimulants—the most commonly used treatment for ADHD—on impulsivity, given that these drugs speed up the internal clock.
Objectives The present experiment investigated the effects of suppressing behaviours non-specific to impulsivity in a fixed consecutive number (FCN) schedule and examined whether amphetamine, previously shown to increase impulsive responses in this task, could have beneficial effects when impulsive responses are promoted.
Materials and methods Food-deprived rats were trained to press one lever of a two-lever operant chamber eight times before pressing the other lever to obtain food. Premature ending of responses resulted in absence of food delivery and reset the counter. A cue light indicating the required number of presses was present ($FCN8_{cue}$) and removed after training (FCN8). Rats were then trained under an $FCN16_{cue}$ schedule to be challenged with *d*-amphetamine (0.125, 0.25 and 0.5 mg/kg).
Results The cue improved performances, and similar scores were obtained under $FCN16_{cue}$ compared to FCN8. Premature responses under these two conditions were unrelated. Amphetamine reduced impulsive responses in $FCN16_{cue}$ at the lower dose.
Conclusions Suppression of capacities non-specific to impulsivity in the FCN schedule, associated with conditions that permit the expression of inhibitory deficits, allows the beneficial effects of psychostimulants observed clinically to be evidenced experimentally.

Keywords Behavioural inhibition · *d*-Amphetamine · Appetitive reinforcement · Operant behaviour · Rat · Time estimation · Discriminative stimulus

M. Rivalan · S. Grégoire · F. Dellu-Hagedorn (✉)
Université Bordeaux 2; Université Bordeaux 1; CNRS; UMR 5227,
146 rue Léo Saignat - BP31,
33076 Bordeaux Cedex, France
e-mail: francoise.dellu@lnpb.u-bordeaux2.fr

Introduction

Impulsivity is a complex behavioural trait that is prominent in several psychiatric disorders such as mania, personality and conduct disorders, attention deficit hyperactivity disorder (ADHD) as well as substance abuse. Research on this personality trait and related disorders would benefit from better agreement on its definition and measures. Efforts to establish operational definitions of impulsivity have been developed recently in animal models aimed to clarify several aspects of this non-unitary trait (Dellu-Hagedorn 2006; Evenden 1999). However, one conceptual obstacle in behavioural neuroscience is the issue of whether a given behavioural procedure unequivocally assesses the psychological processes that the task purports to measure (construct validity). All of the potential procedural contingencies that mediate performance may not always be taken into consideration by the experimenter. The discrepancy between the effects of psychostimulants—the most commonly administered treatment for ADHD (Fone and Nutt 2005)—on impulsivity in laboratory tasks may well be related to this problem.

One important parameter that could have a major influence on the measures of impulsivity and their modulation by

psychostimulants is time estimation, a process that may not be directly related to impulsivity but may contribute to impulsive behaviour (Evenden 1999). Dopaminergic manipulations are believed to effect a change in interval timing: Amphetamine and related compounds cause an immediate leftward shift in the timing function relative to baseline trials at low doses, speeding up the passage of time (for review, see McDonald and Meck 2004). In comparison with tasks in which timing is alleviated, performances in impulsive tasks based on time estimation should be impaired by psychostimulants. Given that psychostimulants are expected to reduce at least some aspects of impulsive behaviour as it is observed clinically, opposite effects on time estimation and impulsivity could mask the beneficial effects of these drugs on impulsivity. This hypothesis could explain, partially at least, the detrimental effects of amphetamine on various impulsive responses in which time estimation is required (Charrier and Thiebot 1996; Cook and Kelleher 1962; Dews 1958; Evenden and Meyerson 1999; Evenden 1998a,b; Evenden and Ryan 1996): A predominant effect on time estimation could lead to enhanced impulsive-like responses.

The fixed consecutive number (FCN) schedule first designed by Mechner and Latranyi (1963) aims to measure behavioural inhibition capacities through the rat's ability to terminate an action to reach a goal (Evenden and Ko 2005; Evenden and Meyerson 1999; Evenden 1998a–c). Hungry rats are required to complete a sequence of an FCN of responses on one of the levers (FCN lever) before a response on the second lever (reinforcement lever) results in food delivery. An impulsive response is assumed when the response requirement is not completed before pressing the reinforcement lever, resulting in the absence of food delivery and resetting of the counter. Organisation of this sequential behaviour may be internally generated by retrieving an action pattern that fits the task requirement. These internally generated actions require, at least in part, estimation of the time that has elapsed during lever pressing given that no indication is given of the number of responses they execute. Estimation of the performance by the animals may also be related to a "pseudocounting strategy" related to the response rate or the effort that the animal has made, and it is possible that the consistency of the response rate may account for performances in this task.

Under this schedule, amphetamine consistently reduces the proportion of rewarded chains by inducing premature switches to the reinforcement lever (Evenden and Meyerson 1999; Evenden 1998a,b). One hypothesis could be that the detrimental effects of amphetamine on time estimation may have induced premature responses and thus masking a possible positive effect on inhibitory processes. Whatever the strategies involved to perform this task that may have biased the measure of impulsivity, the prediction is that amphetamine would have reduced impulsive behaviour in an FCN schedule in which these biases are reduced and that mainly requires inhibition of premature responses.

To reduce the involvement of these non-specific components in the task, one possibility is to provide an external discriminative stimulus that signals the completion of the response requirement. Previous data have shown that adding an external discriminative stimulus to the FCN task drastically modifies the pattern of responding and reduces the detrimental effects of amphetamine on impulsivity (Laties 1972; Laties et al. 1981). Similar changes have been obtained in two other experimental paradigms: (1) in a differential-reinforcement of low rates (DRL) schedule that requires that an animal wait for a specified length of time before a response is reinforced (Wiley et al. 2000) and (2) in the fixed-interval (FI) schedule of reinforcement, in which the first operant response after a fixed period of time is reinforced (Laties and Weiss 1966). However, in the presence of an external cue, the tasks were much easier and drastically reduced impulsive responses, thus precluding any pharmacological effect on impulsivity.

The present study aimed to obtain a purer measure of behavioural inhibition deficit in a cued version of the FCN task *that favours impulsive behaviour* and to show that under these conditions, according to clinical data, behavioural inhibition can be enhanced by amphetamine at low doses.

For this purpose, it was demonstrated that performances in the FCN schedule that requires the control of internally generated behaviours by the subject are independent of performances in a cued version of this task that favours impulsive behaviour. Performances of the same group of rats were compared in variations of the task with different signalling conditions. Under the signalled condition, a stimulus light was on during lever presses on the FCN lever until the optimal number of presses was reached. The end of the signal marked the availability of a food pellet when pressing the reinforcement lever, thus suppressing internally controlled behaviours. Under the unsignalled condition, no signal was ever presented. By enhancing the difficulty of the task (increased number of presses required to obtain food, FCN16), promoting impulsive responses, it was possible to compare performances in signalled vs unsignalled conditions at the same level of difficulty. With these comparisons, it was possible to distinguish between individuals only impaired in the uncued version of the task (individuals with difficulties in controlling internally generated behaviours, i.e. timing) and individuals whose scores worsened in the cued version of the task that was more demanding in inhibitory control (thus mainly presenting inhibitory deficits).

The effects of amphetamine on performance under the signalled condition were tested. Given that amphetamine is expected to reduce behavioural inhibitory deficits (Santosh and Taylor 2000), one would expect to reveal a beneficial effect of amphetamine on inhibition in this modified task

that enhances impulsive responses and reduces non-specific parameters related to internally generated behaviour.

Materials and methods

Subjects

Forty eight male Sprague–Dawley rats (Charles River, Lyon, France) were received at 6 weeks of age. They were housed in groups of four in a temperature- (23°C) and humidity-controlled room (60%) on an inverted 12-h light–dark (8:00–20:00) schedule. Animals were under dietary restriction. Food rationing was adjusted to maintain their weight around 85% of their expected weight at the same age. A week before the beginning of the experiments, animals were handled for a few minutes every day.

Apparatus

The apparatus consisted of eight sound-insulated light-tight outer chambers each containing a two-lever conditioning box (Imetronic, Pessac, France), as previously described (Dellu-Hagedorn et al. 2004). The boxes (32×32×22 cm) were constructed from white plastic panels with a Plexiglas door. They were equipped with a fan providing a background noise. Each box was permanently illuminated with a diffuse 17-lux light source located in the middle of the ceiling. An additional light (2 lux) was sited between the two levers, 16 cm above the floor. The floor consisted of 5-mm-diameter stainless steel bars spaced 1.5 cm apart. Two stainless steel levers protruded horizontally 1 cm from the wall situated to the left of the door, 16 cm apart and 6 cm above the grid floor. A tray was situated centrally on the opposite wall. Food pellets (45 mg, Bioserv, USA) were delivered into the tray by a food dispenser. Data collection was automated by a control software (Imetronic, Pessac) running on a computer outside the testing room.

Procedure

This task (adapted from Evenden 1998a) measures that ability of rats to carry out a chain of sequential acts to achieve a goal. The schedule requires a fixed minimum number of responses on one of the levers, before a response on the second lever results in food delivery. A reduction in the average chain length is considered to be a loss of behavioural inhibition.

Fixed consecutive number schedule 8

Training Thirty minutes before a session, rats were placed in their home cage, in the light-attenuated experimental room. On the first day, the right lever was inserted into the box, and every press resulted in the delivery of a food pellet in the tray after collecting the pellet. On the following day, the left lever was inserted and the same schedule of reinforcement was employed. This alternation procedure was continued until the rats had obtained at least 60 pellets within 45 min with each lever.

FCN training was then started. During training, the two levers were available. At the beginning of the test, the light between the two levers was on (cue light). Each training session lasted 45 min. On the first day, the rats were required to press the left lever first (FCN lever) and then the right lever (reinforced lever) to obtain food (FCN1). When 60 pellets were obtained within a session, the FCN requirement was increased to 2 and, according to the same criterion, to 3, 4, 5 and 8 (test condition). During this training period, the cue light was switched off when the rats had completed the number of consecutive presses required on the FCN lever to obtain food. This cue light was on again when rats visited the tray. If the chain was shorter than the number required, the rat had to start a new chain. If the chain was longer, it had no consequence, and the pellet was delivered when the rat pressed the reinforced lever.

Testing (1) *FCN8 with cue light* ($FCN8_{cue}$): Rats that failed to reach the criterion to be tested under a FCN8 schedule after 20 training sessions were excluded. The other rats were tested under the FCN8 schedule until they reached stable performances. Test sessions lasted a maximum of 45 min and ended when 100 pellets had been obtained. The mean scores of each animal obtained after stabilisation over the last four sessions were recorded.

(2) *FCN8 without cue light (FCN8)*: The effect of the cue light on the performances in the FCN task was investigated. After completing the FCN8 test with the cue light, rats were tested for three consecutive days under the same experimental conditions except that the cue light remained off during the whole test. The mean scores of each animal obtained from these three sessions were recorded.

Fixed consecutive number schedule 16 with cue light

Training After completing the FCN8 test without cue light, rats were trained again under a FCN8 with the presence of the cue light indicating that an optimal number of lever presses was reached. To increase the difficulty of the task, rats were then trained under a $FCN12_{cue}$ schedule during two consecutive 30-min sessions and then under a

$FCN16_{cue}$ schedule until they reached a stable level of performance.

Testing Test sessions lasted a maximum of 30 min and ended when 100 pellets were obtained. The mean scores of each animal obtained from the last three sessions were recorded.

Amphetamine

This experiment was performed 12 days after completion of the $FCN16_{cue}$ task. A dose–effect of amphetamine (*d*-amphetamine sulfate, Cooperative Pharmaceutique Française, Melun) on scores obtained in the $FCN16_{cue}$ task was tested. All rats were tested with three doses (0.5, 0.25 and 0.125 mg/kg) injected subcutaneously in sterile 0.9% saline vehicle at a volume of 1 ml/kg. Amphetamine was administered 30 min before testing under $FCN16_{cue}$ for 15 min. The amphetamine injections were administered 1 week apart (in decreasing concentrations). The effect of amphetamine was compared to scores obtained after subcutaneous saline injection the day before, according to the same experimental protocol.

Data measures and analysis

The following parameters were recorded:

Response efficiency This is defined as the number of presses on the FCN lever divided by the total number of food deliveries. This shows the average number of lever responses required to obtain a food pellet (minimum value=8 for FCN8, 16 for FCN16).

Chain length This is defined as the mean length of the chain of responses made on the FCN lever before switching to the reinforced lever. According to Evenden (1998a), this parameter reflects impulsivity; a shortening of this value indicates a reduction in the average chain length and an increase in impulsivity.

Percentage of rewarded chains This is defined as rewarded chains as a proportion of the total number of chains made on the FCN lever before a response on the reinforced lever was made. Among rewarded chains, some are just as long as necessary and reflect high response efficiency, whereas some others exaggeratedly exceed the number of presses required and reflect low response efficiency. These rewarded inefficient chains might be considered as a perseverative behaviour: a tendency to pursue a goal-directed behaviour that is no longer appropriate. They obviously influence the response efficiency score but also the mean length of chain. The percentage of rewarded chains would be more appropriate than the mean chain length to reflect impulsive behaviour given that the rewarded inefficient chains have the same weight as the rewarded efficient chains in this calculation. The percentage of rewarded chains is calculated independently of their length (between the optimal and the maximum ones), contrarily to the mean chain length. A decrease in the percentage of rewarded chains reflects an increase in impulsivity.

Response rate This is defined as the mean total number of responses on both levers per second (the data obtained were almost identical to the response rate calculated with mean total responses on the FCN lever per second).

Latency in collecting food pellets This is defined as the mean latency in visiting the food magazine after a rewarded chain of presses has been terminated by a press on the reinforced lever.

Learning of the procedure before reaching test phase This is the number of sessions needed to reach the test phase (learning score).

Duration of optimal chains This is the mean duration of eight consecutive responses on the FCN lever during FCN8 and $FCN8_{cue}$.

Distribution of chain length Two complementary representations of chain length distribution were made based on (1) the percentage of chains achieving at least *n* number of responses and (2) number of chains with a length of *n* consecutive presses as a percentage of the total number of chains.

Proportions of very long (at least six responses above the number requested) and short chains of responses compared to optimal length were also considered. Response efficiency scores were used to attest to the stability of performance (variation of response efficiency below 2 over three consecutive sessions). The change in proportion of rewarded chains in FCN8 compared to $FCN16_{cue}$ was calculated.

Comparisons of the scores of the whole sample in two experimental conditions were carried out using a paired *t* test. Correlations between scores were performed using Bravais–Pearson's correlation test. Comparisons of scores between subgroups of impulsive (IMP), intermediate (INT) and non-impulsive (NIMP) individuals according to their scores in the $FCN16_{cue}$ task were calculated using analysis of variance (ANOVA), followed by simple main effects (SME) or post-hoc comparisons (Newman–Keuls [NK] test). Student's *t* test was used to compare group scores or to assess variation in proportion of rewarded chains. Vehicle values were combined with the drug treatment data in a one-way ANOVA. Differences between drug treatments and the control were tested using Dunnett's *t* test. All comparisons were made at the 5% level. The normality of the variable distribution was

verified using Shapiro–Wilk's test. A logarithmic transformation was performed when necessary to normalise variables.

Results

Effect of indicating optimal performance (cue light) in the FCN task

Four rats were excluded from this analysis because they could not reach the criterion for the test phases or were inactive during the tests. The remaining rats (n=44) needed 5.2±0.2 training sessions to reach the test conditions.

Comparison of the scores in the FCN8 task with and without cue light

As expected, introducing a cue light indicating the optimal number of lever presses improved the performance accuracy of the rats compared to the unsignalled protocol. After training with a cue light leading to 88% of rewarded chains, the removal of the cue induced a decrease in performance (74%) for all the rats except for four subjects that had the best scores under both schedules. Addition of the cue increased the percentage of rewarded chains (t=7.65; df=43; p<0.001) and enhanced response efficiency (t=8.14, p<0.001) without changing the mean chain length. This was associated with a significant decrease in response rate (t=4.66; p<0.001), consistent with an increase in the mean duration of optimal chains (15±0.5 vs 13.6±0.5 s; t=5.53, df=42, p<0.001; Table 1).

These results can be better explained by analysis of chain length distribution (Fig. 1). The cue light changed the general shape of the chain length distribution curves. With no cue light, chains were symmetrically distributed on a Gaussian-like curve, with a maximum obtained for chains of nine responses. In the presence of a cue light, a 50% reduction in unrewarded chains was observed (26.2 vs 12%) and a marked peak of chains of eight responses (21.1%), which corresponds to the optimal response to obtain food. Subsequently, the percentage of efficient presses progressively decreased, and no difference in the curve shape was observed for chains with more than ten responses, compared to condition with no cue.

There was no correlation between the percentages of rewarded chains in either experimental condition (r=0.15; df=43; ns), whereas the percentages of very long chains were correlated (r=0.61; df=43; p<0.001). No correlation

Table 1 Performances under an appetitive FCN schedule: effects of a cue light indicating optimal performance

Conditions	Response rate (responses/s)	Mean chain length (responses)	Response efficiency (responses on FCN lever/ food delivery)	Rewarded chains (%)
FCN8 without cue	0.60±0.02*	10.1±0.2	14.1±0.2*	73.8±1.8*
FCN8 with cue	0.55±0.02	10.3±0.2	11.9±0.2	88.5±0.9
FCN16 with cue	0.67±0.04*	17.1±0.5*	27.8±3.2*	74.2±3.5*

The data shown are the means±SEMs.
Statistically significant differences with FCN8 with cue experimental condition (paired t test).
*p<0.001

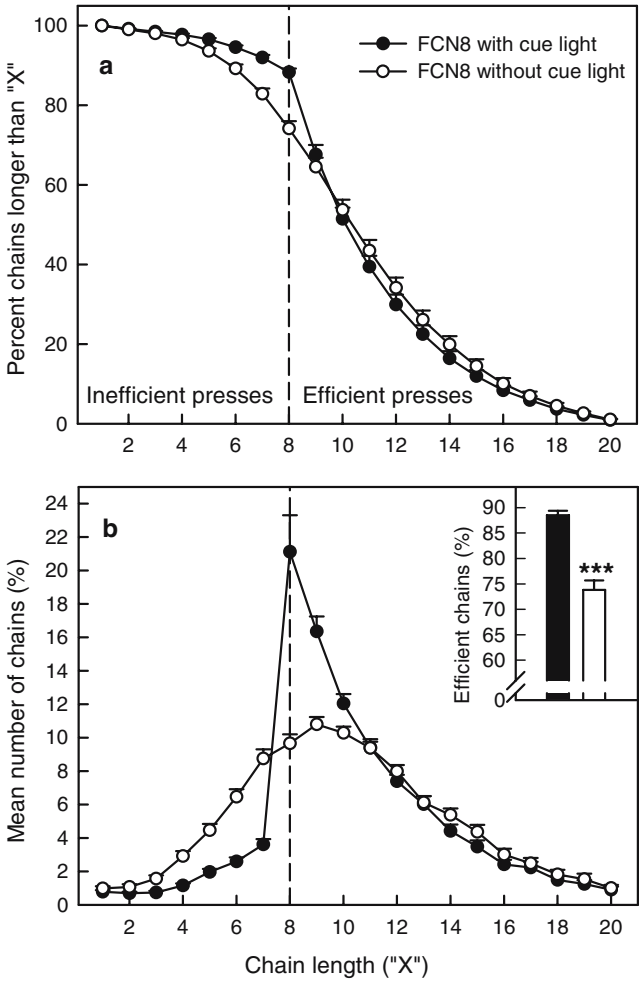

Fig. 1 Effects of cue light signalling when optimal number of presses has been reached to obtain food on the chain length distribution of rats responding under a FCN8 schedule of reinforcement. The *horizontal axis* shows the chain length, and the *vertical axis* shows **a** the percentage of chains achieving at least that number of responses or **b** the number of chains of each length as a percentage of the total number of chains. Optimal performance is indicated by the *vertical dotted line*. *Inset*: percentages of rewarded chains in the two experimental conditions. The presence of a cue increased the percentage of rewarded chains and decreased the number of unrewarded ones. It drastically changed the shape of the chain length distribution curve. Statistical comparison (paired t test): ***p<0.001

between learning score and proportion of rewarded chains in the cue and the no cue conditions could be shown (r= 0.12 and 0.17, respectively; df=43; ns).

Enhanced difficulty of the FCN_{cue} task

The presence of the cue light markedly reduced the number of premature chains but did not completely eliminate them: 11.5% of chains remained unrewarded, suggesting that this protocol can still allow expression of premature responses. To recruit more impulsive-like behaviour and evidence inhibitory deficit, the difficulty of the task was enhanced by increasing the number of responses required to obtain food ($FCN16_{cue}$). The rats' scores in $FCN16_{cue}$ were compared to those obtained in $FCN8_{cue}$ (Table 1). Under this new experimental condition, rats readily increased their number of responses and reached a stable level of scores after three to four sessions. The increased number of responses required to obtain food was associated with a higher response rate (t= 4.34; df=43; p<0.001) and a higher mean chain length (t= 15.62; df=43; p<0.001). The average chain length was about 108% of the target chain compared to 129% for $FCN8_{cue}$. However, rats were less efficient in performing the $FCN16_{cue}$ task compared to $FCN8_{cue}$ as shown by a marked decrease in response efficiency and proportion of rewarded chains (t= 5.09 and 4.80, respectively; df=43, p<0.001). About 74% of chains resulted in 14% less food than in $FCN8_{cue}$. Percentages of rewarded chains in $FCN16_{cue}$ and $FCN8_{cue}$ were positively correlated (r=0.65; df=42; p<0.001). A positive correlation was also found between the proportion of rewarded chains and response rate (r=0.57, df=43, p<0.001).

Although increasing the number of responses required to obtain food had no obvious effect on the general shape of the chain length distribution curve, two major differences were observed: a more pronounced number of unrewarded chains that increased progressively with the number of responses and an expected shift of the distribution to the right with a similar peak for chains of 16 responses instead of eight (Fig. 2). A quarter of the rats achieved 70% or less rewarded chains in $FCN16_{cue}$, whereas all the rats achieved more than 70% of rewarded chains in $FCN8_{cue}$. The proportion of very long chains of responses did not differ significantly between $FCN8_{cue}$ (11.7%±1.5) and $FCN16_{cue}$ (13.6%±2.3; t=1.16; df=43; ns).

Independence of the scores obtained in the FCN schedules with and without cue light

The proportion of rewarded chains did not significantly differ under FCN8 and $FCN16_{cue}$ (Table 1). However, no significant correlation between the proportions of rewarded chains obtained in these two experimental conditions could be demonstrated. To compare impulsive individuals in both

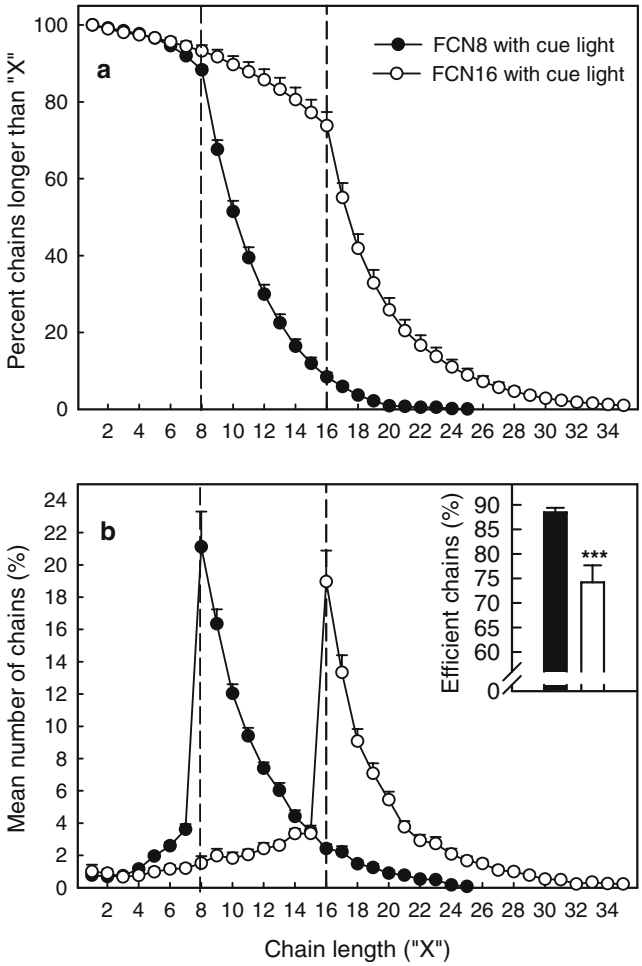

Fig. 2 Effects of doubling the number of presses required to obtain food on the chain length distribution of rats responding under an FCN schedule of reinforcement with a cue light indicating that the optimal number of presses has been reached. The *horizontal axis* shows the chain length, and the *vertical axis* shows **a** the percentage of chains achieving at least that number of responses or **b** the number of chains of each length as a percentage of the total number of chains. Optimal performance in both conditions is indicated by the *vertical dotted lines*. *Inset*: percentages of rewarded chains in the two experimental conditions. The shift of the distribution to the right induced by the higher number of presses required was associated with a higher percentage of unrewarded chains. Statistical comparison (paired t test): ***p<0.001

conditions, subgroups of impulsive (IMP), intermediate (INT) and non-impulsive (NIMP) individuals according to their proportion of rewarded chains in $FCN16_{cue}$ were created. The IMP group (n=11) included all the rats with a score below 70%, the NIMP group (n=24) included rats with a score above 80% and the INT group included the remainder (n=9). The proportion of rewarded chains was significantly higher in IMP compared to both INT and NIMP ($F_{2, 41}$=61.26; p<0.001; NK, p<0.001) in $FCN16_{cue}$ (Fig. 3a) but did not differ significantly between the three groups in FCN8 with no cue (Fig. 3b). Variations in the proportion of rewarded chains between FCN8 vs $FCN16_{cue}$ conditions were calculated to compare the gain vs loss in

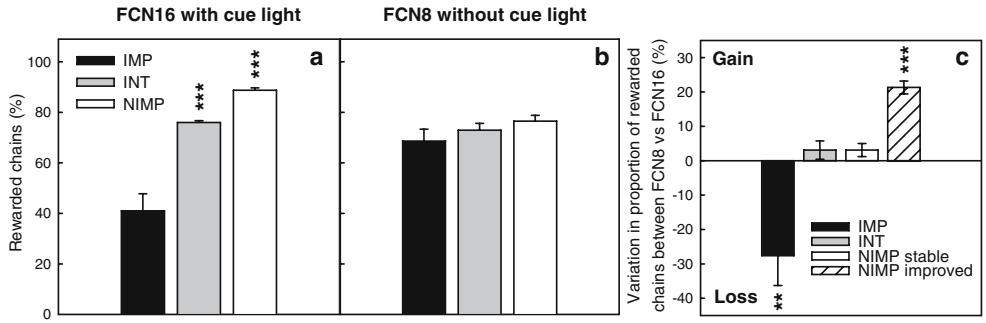

Fig. 3 Relationships between premature responses of rats responding under FCN schedules of reinforcement with and without a cue light indicating that the optimal number of presses has been reached. **a** Percentages of rewarded chains in $FCN16_{cue}$ of three groups of rats selected according to their scores in this task: non-impulsive rats (NIMP, proportion of rewarded chains above 80%, n=24), impulsive rats (IMP, scores below 70%, n=11) and the remainder (INT, n=9). **b** Percentages of rewarded chains of the three groups in the FCN8 schedule. No difference between groups is observed. **c** Comparisons of the groups according to the gain vs loss of rewarded chains induced by the change in experimental conditions (from FCN8 to $FCN16_{cue}$). Within NIMP, two subgroups could be distinguished: rats that had stable scores and rats that improved their scores between the two experimental conditions. Statistical comparisons (NK): ***p<0.001; test for significant variation of the scores (t test): ***p<0.001; **p<0.01

rewarded chains because of the changes in experimental conditions. This analysis revealed that IMP made fewer rewarded chains than in FCN8 (t=3.17, df=10; p<0.01), whereas INT had similar scores (t=1.15, df=8, ns). NIMP globally increased rewarded chains in $FCN16_{cue}$ compared to FCN8 conditions (t=5.3, df=23, p<0.001), but marked inter-individual differences were observed. Within NIMP, two kinds of performance could be distinguished: rats (n=12) that improved their performance in the presence of the cue by at least 15% (mean variation in rewarded chains: 21.3±1.9%; t=11.2, df=11; p<0.001) and rats (n=12) with no significant variation in scores with and without cue (mean variation in rewarded chains: 3.1±1.9%; t=1.63; df=11, ns) (Fig. 3c).

Amphetamine

Amphetamine (0.125, 0.25 and 0.5 mg/kg) or saline vehicle was injected subcutaneously 30 min before testing, and the $FCN16_{cue}$ task was shortened to 15 min to ensure optimal effect of the drug during behavioural measure. It is noteworthy that the proportion of rewarded chains increased with time over a 30-min session and that scores obtained during the first 15 min were significantly lower than in a 30-min task (Table 1), better allowing a possible improvement of the performances by the drug.

Among the 44 rats tested, six were too inactive to be tested after amphetamine injection (at 0.25 and 0.5 mg/kg) and were eliminated from this experiment. Mean number chains of presses of these rats was 6.8±0.8 compared to 35.8±2.3 for the others. Three of them had more than 70% rewarded chains during a previous experiment under $FCN16_{cue}$. Moreover, response efficiency could not be calculated for some rats after amphetamine injections (0.25 and 0.5 mg/kg) because they could not obtain any food reinforcement in spite of a fairly high level of activity (n=6).

The effects of amphetamine on parameters measured in the $FCN16_{cue}$ schedule are summarised in Table 2. Amphetamine markedly increased the response rate ($F_{3,\ 111}$=7.92; p<0.001). This was only statistically significant at the lower dose (0.125 mg/kg). Amphetamine also markedly changed the chain length ($F_{3,\ 111}$=18.95; p<0.001): It was increased at the lower dose, had no effect at the intermediate dose and was decreased at the higher dose.

Table 2 Effects of amphetamine on performances under an appetitive FCN schedule

	Response rate (responses/s)	Mean chain length (responses)	Response efficiency (responses on FCN lever/food delivery)	Rewarded chains (%)
Amphetamine (FCN16 with cue)				
Vehicle	0.64±0.05	13.8±0.6	37.0±5.1	55.1±4.2
0.125 mg/kg	0.76±0.05*	16.2±0.6*	25.5±2.3**	74.1±4.6*
0.25 mg/kg	0.70±0.06	14.5±0.7	28.9±3.1	61.2±5.0
0.5 mg/kg	0.66±0.05	12.5±0.7***	37.8±5.5	43.6±3.8**

The data shown are the means±SEMs.
Statistically significant differences between the amphetamine treatment and vehicle (Dunnett's t test).
*p<0.001; **p<0.01; ***p<0.05

Similarly, amphetamine markedly changed the proportion of rewarded chains ($F_{3,\ 111}$=24.96, p<0.001): It increased the percentage of rewarded chains at the lower dose, had no effect at the intermediate dose and decreased the percentage of rewarded chains at the higher dose. The increased chain length and proportion of rewarded chains at 0.125 mg/kg were associated with enhanced efficiency in responding (t=2.93, df=35, p<0.01). These effects are best illustrated by analysis of the chain length distributions (Fig. 4). Amphetamine had a significant effect on the proportion of unrewarded chains: both on very short chains (below nine responses) and longer ones (from 9 to 15 responses; $F_{3,\ 111}$=14.58 and 6.57, respectively, p< 0.001). Amphetamine also had significant effects on the proportion of rewarded chains; the more significant effects being observed within the range of 16 (optimal) to 22 responses ($F_{3,\ 111}$=21.67; p<0.001). The proportion of very long chains (above 22 responses) was also modified by amphetamine ($F_{3,\ 111}$=3.19; p<0.05). Amphetamine had no effect on chain length distribution at 0.25 mg/kg and had opposite effects at 0.125 mg/kg compared to 0.5 mg/kg. At the lower dose, amphetamine decreased the proportion of unrewarded chains by half, with an effect on very short chains as well as longer ones (Dunnett's t test, p<0.01). Inversely, this dose increased the proportion of fairly long rewarded chains (from 16 to 22 responses) more than the proportion of very long inefficient (above 22) rewarded chains (p<0.001 and p<0.01, respectively). At the higher dose, amphetamine increased the proportion of very short chains (p<0.01) and decreased the proportion of fairly long ones (p<0.001).

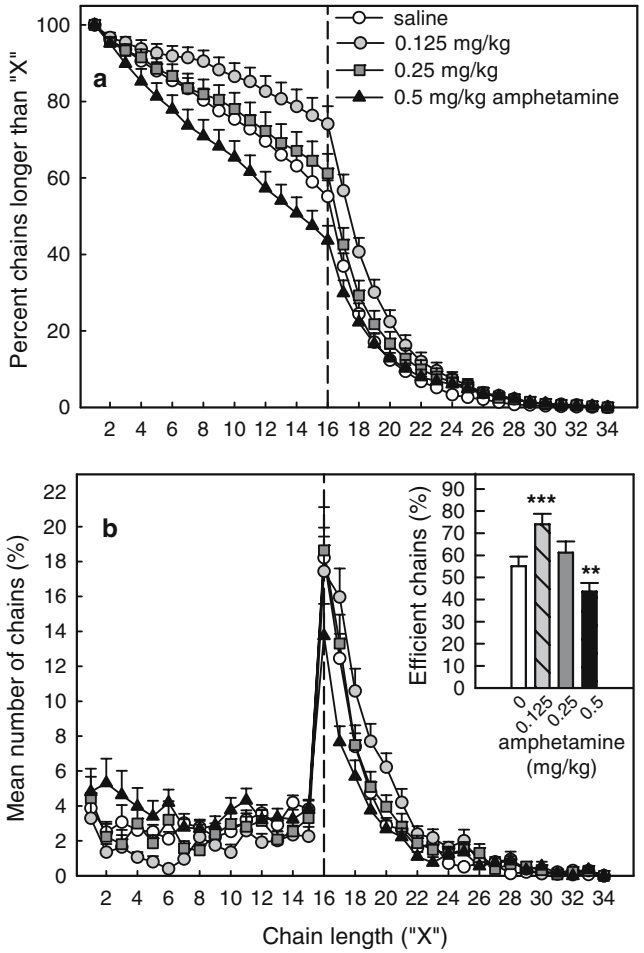

Fig. 4 Dose–effect of amphetamine on the chain length distribution of rats responding under an FCN16$_{cue}$ schedule of reinforcement. The *horizontal axis* shows the chain length, and the *vertical axis* shows **a** the percentage of chains achieving at least that number of responses or **b** the number of chains of each length as a percentage of the total number of chains. At the lower dose, amphetamine decreased the number of short chains and increased the number of long ones as shown by the shift in the peak of the chain length distribution. At the higher dose, opposite effects were observed. *Inset*: dose–effect of amphetamine on percentages of rewarded chains. Statistical comparisons (Dunnett's t test): ***p<0.001, **p<0.01

Correlations between response rate and proportion of rewarded chains were also found in each pharmacological condition (saline injection, r=0.69, df=37; p<0.001; amphetamine challenge, 0.125; 0.25 and 0.5 mg/kg, r= 0.65; 0.69 and 0.72, respectively, df=37; p<0.001).

Similar reduction in unrewarded chains by amphetamine compared to saline injection were observed for IMP, NIMP and INT groups at the lower dose (proportion of unrewarded chains, interaction group x treatment, $F_{2,\ 35}$=0.21, ns; SME, p<0.001 whatever the group).

Discussion

This work was designed to show that non-specific factors could be an important bias in a task aimed at measuring impulsive behaviour, thus evidencing the major role of procedural contingencies on the behavioural performances in impulsivity that these tasks intend to measure. The original version of the FCN schedule of the reinforcement task (Evenden 1998a) requires behavioural inhibition of switching to the other lever before the required number of presses to obtain food has been reached. It probably also requires the control of internally generated behaviours involving timing given that no indication of the number of lever presses made is available. Animals have to evaluate the passage of time, probably associated with the effort made to perform the chain of presses, to avoid premature responding. Deficits in these distinct processes would lead to premature responses.

These experiments enabled us to establish a more specific task for behavioural inhibition that suppresses internally generated behaviours non-specific to impulsivity associated with conditions that permit the expression of inhibitory deficits. This task based on the FCN8 appetitive schedule is particularly suitable because it demands little

attention and thus allowing more rapid measurement of inhibitory capacities compared to other behavioural inhibition tasks (go/no-go DRL, five-choice serial reaction time task). Moreover, it allows us to check the motivational state of the animal given that it freely decides to start a chain of lever presses. Premature responding cannot be related to learning deficits given that scores of inhibition are attributed after stabilisation of the scores and that no relationship between learning of the task and premature responding was observed.

The role of non-specific parameters in the FCN schedule of reinforcement

To examine the role of these non-specific aspects of behavioural control on the measure of impulsivity in the FCN schedule, the performances of rats trained with or without a cue light were compared. The cue light is a signal that clearly avoids time and performance estimation by indicating optimal performance. The light is only switched off when the reward can be obtained and thus constitutes a discriminative stimulus that makes the food availability status unambiguous. A possible stimulus-seeking effect of the cue is avoided by associating the optimal response being reached with switching off the light.

As expected, premature endings of the chain of responses were markedly reduced by the presence of the cue. The drastic change in the curve shapes of chain length distribution during the cued and uncued FCN schedules indicates that the behaviour of the rats was radically modified by the cue and that rats paid attention to it. Moreover, rats drew benefit from the presence of the cue during training, given that proportion of rewarded chains during FCN8 was lower when rats were trained with no cue (42.3±3.1, n=36, data from Dellu-Hagedorn 2006), compared to rats trained with a cue (73.8%). However, despite the easiness of the task, some unrewarded chains (premature responses) were still observed in some individuals. The proportion of these unrewarded chains increased progressively as their length approached the optimal number, reflecting a deficit in inhibitory control rather than random presses on the levers. To amplify the proportion of premature responses and to favour the measurement of behavioural inhibition capacities, the difficulty of the task was enhanced by doubling the number of lever presses required to obtain food. By chance, the enhanced difficulty of the task ($FCN16_{cue}$) yielded similar scores for rewarded chains to that of the unsignalled FCN8 schedule (26%) and thus allowed inter-task comparisons. Significant correlation was obtained between $FCN8_{cue}$ and $FCN16_{cue}$ performance, attesting that similar capacities were measured by these two tasks, differing only in the level of difficulty. However, this was not the case between scores obtained in signalled vs unsignalled conditions. Behavioural performance measured in these two schedules probably involves distinct processes. A more detailed analysis of individual scores was made that consisted in extracting subgroups of individuals according to their proportion of rewarded chains in the $FCN16_{cue}$ schedule: IMP, NIMP and INT groups. This method has already demonstrated its usefulness to describe extreme behaviours in various animal models of psychopathology (Blondeau and Dellu-Hagedorn 2007; Dellu-Hagedorn 2005, 2006; Dellu-Hagedorn et al. 2004; Dellu et al. 1996; Taghzouti et al. 1999). Similar performances in the three groups in FCN8 corroborate the absence of any relationship between the processes measured in these tasks. The $FCN16_{cue}$ task is expected to be less demanding on time estimation and monitoring behaviour but more demanding on inhibitory control compared to the uncued FCN8 task. By examining the gain vs loss of efficient presses between FCN8 and $FCN16_{cue}$ schedules, it was observed that among the individuals presenting impulsive-like behaviour in the uncued FCN8 schedule, (1) some improved their scores in the cued task. Their difficulties in the uncued task were then probably more related to time or performance estimation. (2) Inversely, other poor performers under the uncued FCN8 schedule worsened their scores under the $FCN16_{cue}$ schedule. Because of this task being less demanding on time estimation and monitoring behaviour but more demanding on inhibitory control, these individuals may rather present inhibitory deficits. Rats with no change in rewarded chain scores between the two schedules exhibited no particular deficit in capacities required in both conditions. Thus, different capacities in independent processes, such as time estimation and impulsivity, could explain differences in behavioural performances within individuals according to the FCN schedule used.

Interestingly, six rats performed a large proportion (above 30%) of very long chains of presses, whatever the schedule used, as shown by a similar shape in the curve tails of the two functions, with and without cue, represented on Fig. 1. This perseverative behaviour in the FCN schedule has previously been pointed out under the effect of psychostimulants (Evenden and Ko 2005). It cannot be related to an underestimation of time, given that this behaviour is similarly observed with and without the signal of optimal performance. Excessive length chain execution, not accompanied by any attempt to collect a reward even when a clear signal announces its availability, could be reminiscent of symptoms of compulsive behaviour: It may be related to the excessive and unreasonable behaviour seen in obsessive compulsive disorder (DSM-IV 1994). However, it cannot be excluded that this behaviour reflects a reinforcing and gratifying effect of the task by the effort produced (Clement et al. 2000) or by the control that the animal is able to exert on its environment (Maier 1991).

Effects of *d*-amphetamine

The present study is the first to clearly show a reduction in impulsivity by the use of a low dose of amphetamine (which, unfortunately, Evenden and colleagues never used) in a FCN reinforcement schedule. By introducing a cue light signalling that the optimal number of lever presses has been reached, amphetamine clearly increased the mean chain length and proportion of rewarded chains. Analysis of the chain length distribution revealed that most unrewarded chains were reduced, although among rewarded chains, mainly efficient ones were increased. This effect was consistent across subjects. The possibility of this effect being related to an increase in perseverative responding can be ruled out: the number of efficient rewarded chains being increased more than the inefficient ones, leading to a higher response efficiency. The reduction in impulsivity by *d*-amphetamine could be related to enhanced salience and value of the discriminative stimulus, as it has been hypothesised previously (Laties et al. 1981).

In previous experiments using similar FCN schedules (Evenden and Meyerson 1999; Evenden 1998a,b; Laties 1972; Laties et al. 1981), amphetamine consistently reduced chain length by inducing premature switches to the reinforcement lever. When a low dose such as 0.1 mg/kg of amphetamine was used in the $FCN8_{cue}$ task (Laties 1972; Laties et al. 1981), no increase in rewarded chains was observed probably because of a ceiling effect: About 90% of reinforced chains were obtained under this schedule. It is noteworthy that the same dose of amphetamine had no effect on responding under a FCN8 uncued task, corroborating the hypothesis that the beneficial effect of amphetamine is specifically related to the FCN-cued task in conditions that increase the occurrence of impulsive responses.

Increased mean chain length with amphetamine was also observed in an FCN avoidance schedule in which premature ending led to a punishment (electric shock) associated with food delivery (Evenden and Ko 2005). Rats had reduced impulsive-like responses than they did in an equivalent appetitive procedure: Most of the responses allowed avoidance of the punishment. The stress induced by footshock punishment may have led to enhanced sensitivity to the risk of punishment. Amphetamine did not globally improve performance, given that both response efficiency and proportion of rewarded chains were reduced. Moreover, no shift of the chain length distribution to the right was observed, but the number of both very long and very short chains was increased. These paradoxical effects of amphetamine on chain distribution contrast with the regularity of the beneficial effect of amphetamine on behaviour in the appetitive FCN_{cue} schedule. These differences are certainly related to stress induced by aversive stimuli and a higher sensitivity to their negative effects induced by psychostimulants, effects that are not relevant to the present experiments.

As observed previously (Evenden 1998a), there is a relationship between proportion of rewarded chains and response rate: A decrease in impulsivity brought about by amphetamine (0.125 mg/kg) is accompanied by a higher response rate under FCN16 compared to saline injection. This relationship can be explained because the performance itself in this task requires a high level of activity with long chains of presses. A higher responding rate may reflect higher motivation of rats performing mainly rewarded chains rather than hyperactivity (Dellu-Hagedorn 2006; Dellu-Hagedorn et al. 2004).

A version of the FCN8 task, the paced FCN schedule (Evenden 1998b), allows a better control over the responding rate by retracting the response lever for short periods between presses and may improve timing by reducing response rate variability. This effect could explain why the same dose of amphetamine (0.4 mg/kg) is less efficient in increasing premature responses compared to the FCN8 original version (Evenden 1998a).

It might be hypothesised that the cue may also have played the role of a conditioned reinforcer in the FCN cue schedule. A dose-related increase in responding for a conditioned reinforcer has sometimes been observed after injection of amphetamine (Mazurski and Beninger 1986; Ranaldi and Beninger 1993; Robbins et al. 1983). However, this effect was observed at higher doses (0.5 to 2 mg/kg) for which no improvement in performances in the FCN_{cue} was seen and not at lower doses (0.1–0.25 mg/kg), when amphetamine reduces impulsive responses, thus suggesting that the influence of amphetamine on conditioned response was not responsible for this beneficial effect.

Another effect of signalling time interval in an impulsive choice task, a cognitive process involving the evaluation of delayed vs immediate outcomes (Ainslie 1975), has also been hypothesised. Impulsive choice is measured in animal studies by the choice between a large delayed reward and a small immediate reward. Unlike the FCN experimental paradigm, the delayed reinforcement task does not require an estimation of the delay length but depends on relative comparisons of delay lengths increased within a session. Even if quite different processes are involved, it is noteworthy that the presence of a signal during the delay reduced impulsive responses (Cardinal et al. 2000). As proposed by the authors, the cue could serve as a conditioned reinforcer that facilitates acquisition of delay sensitivity by bridging temporal gaps between the animal's action and the primary reinforcement. This effect could be potentiated by amphetamine and related compounds as they also decreased impulsive responses. The speeding effects of amphetamine on time estimation could also participate in

this effect given that animals should be able to wait longer under the effect of psychostimulants as time passes more quickly.

The dose for which a beneficial effect of amphetamine was observed on impulsivity in signalled conditions was lower than those described by the literature (Cardinal et al. 2000; Feola et al. 2000; Laties et al. 1981; Richards et al. 1999; Wade et al. 2000). The comparison of the dose–effects of amphetamine on behaviour between studies is difficult, given that variations in the protocols may play an important role in the differences observed (strain of the rat, route of administration, time of injection of the drug before the beginning of the test and duration of the test). It is noteworthy that performances within a session in the FCN task improve with time, thus selection of the first half of the experiment to assess drug effects may have been more suitable to show a beneficial effect of the drug. Moreover, testing during a short period of time (15 min) under the peak of behavioural response to amphetamine ensures an optimal effect of the drug, especially at low doses (Gaytan et al. 1998).

Whatever the process by which psychostimulants modulate impulsive responses, this work extends previous findings (Cardinal et al. 2000; Evenden and Ko 2005; Laties et al. 1981) and confirms that signals during impulsive task procedures are of great importance and govern the behavioural effects of psychostimulants (beneficial to detrimental). These data corroborate the hypothesis that a signal may supplement the subject's discrimination of time and performance, capacities involved and required in several impulsivity tasks for laboratory animals and which are unrelated to inhibitory capacities. This work opens new perspectives for a better understanding of the effects of psychostimulants on impulsivity and will help refine the modelling of impulsivity in animal models.

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