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ORIGINAL INVESTIGATION

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The pharmacology of impulsive behaviour in rats III: the effects of amphetamine, haloperidol, imipramine, chlordiazepoxide and ethanol on a paced fixed consecutive number schedule

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Abstract The behavioural trait of impulsivity may be made up of different components, including rapid decision making, intolerance to the delay of reward and a tendency to terminate chains of responses prematurely. It has been proposed to measure the last of these in rats using fixed consecutive number (FCN) schedules. The present study uses a modified version of the FCN procedure in which responding was paced by retracting the response lever for short periods between presses. In this way, the experimenter could control the maximum rate of responding. The procedure was made up of two components based on an FCN 8 schedule of food reinforcement. In the Fast component, lever presses were spaced by a minimum of 2 s and in the Slow component by a minimum of 5 s. The average chain length was significantly shorter, and the rats were less efficient in the Slow component. Five drugs were tested on this baseline, imipramine (1.0–10.0 mg/kg), ethanol (300–3000 mg/kg administered PO), haloperidol (0.01–0.1 mg/kg), chlordiazepoxide (1.0–10.0 mg/kg) and *d*-amphetamine (0.2–0.8 mg/kg). All the drugs reduced responding at the highest dose, but imipramine was different from the others in that it increased the average number of responses in the chain and produced a shift in the chain length distribution to the right, possibly reflecting a reduction in impulsivity. The other four drugs reduced chain length at the highest dose, although in the case of ethanol this effect was very small and, unlike the other three drugs, did not result in a shift in the distribution to the left. The paced FCN procedure can differentiate the effects of different drugs on one aspect of impulsivity, and is likely to be a useful procedure for further study of this aspect of behaviour.

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Introduction

The behavioural trait of impulsivity may be made up of different components, including rapid decision making, intolerance to the delay of reinforcement and a tendency to terminate chains of responses prematurely. Evenden (1998) has proposed that it is possible to measure the last of these using fixed consecutive number (FCN) schedules. Under such a schedule, food-deprived rats are required to press one lever (FCN lever) in a two-lever operant chamber a fixed minimum number of times before pressing the other lever (Reinf. lever) to deliver food. If the rat presses the Reinf. lever before completing the response chain requirement, a brief time-out occurs and it is required to start the chain again. Termination of the chain by premature choice of the Reinf. lever resulting in inefficient performance can be considered as impulsive behaviour. In the present study, a refinement of the FCN schedule was used, exploiting the availability of retractable levers in the apparatus. This has been termed a paced-FCN schedule. The schedule contingencies are the same, but after each response the levers are withdrawn from the box for a short, fixed period, before being re-inserted. The rat can only make one response each time the levers are presented. In this way, the maximum rate at which the rat can respond is determined by the experimenter. In principle, it is possible for the rat to respond more slowly, but in practice, the insertion of the levers has a powerful eliciting effect on responding which maintains good schedule control. As well as limiting the variability in the rate of responding, this refinement also allows the experimenter to control the rate of responding. This was exploited in the present study by dividing the session into two components, using an alternating ABABA design. In the Fast component (A), the minimum time between two lever presses was fixed at 2.5 s, and in the Slow component (B) at 5 s.

The paced-FCN procedure has been used to examine the effects of some of the drugs previously tested by Evenden (1998) using two FCN schedules, FCN 8 and FCN 32, in which the rats were required to make either eight or 32 consecutive responses on FCN lever. Although it proved to be more difficult to maintain stable performance under the FCN 32 schedule, the efficiency of the rats and the distribution of the chain lengths was similar to that under FCN 8, and there was no obvious increase in the sensitivity to the impulsivity-increasing effects of a range of drugs. Thus the FCN 32 schedule did not seem to have advantages over FCN 8 for assessing impulsive behaviour. Although several of the drugs used in that study appeared to increase impulsivity, this was generally accompanied by a reduction in response rate. In this respect, the results obtained by Evenden (1998) resembled those of several other reports in the literature (see Discussion). The broad coincidence of reductions in response rate and increases in impulsivity calls into question the behavioural specificity of the effects. Even if a conventional FCN schedule is based on response choice, which should minimise the effects of general activation or arousal on performance, alterations in response rate will still affect the amount of time taken to complete the schedule requirement, thus not entirely eliminating the potential influence of timing on choice behaviour. This problem is minimised in the paced-FCN, where the rate of responding is primarily under the control of the experimenter, not the experimental subject.

The separation into two components involving different response rates was intended as a control procedure for the comparison between the FCN 8 and the FCN 32 used previously. By forcing the rats to respond slowly but keeping the FCN requirement at 8, it is possible to vary the time taken to complete the chain to some extent independently of the number of responses required. An effect of this manipulation was that the average chain length in the Slow component was consistently shorter than that in the Fast component. Thus, varying the time required to complete the eight-response chain, either 17.5 or 35 s, using the pacing procedure also proved an effective way of making the task more difficult.

Materials and methods

Subjects

The subjects were eight male Sprague-Dawley rats (BK Universal, Sollentuna, Sweden) aged 3 months at the start of the experiment and about 11 months at the end. One rat died during the course of the experiment. The rats were housed in two groups of four, under normal light/dark cycle (12:12 h, lights on 0600 hours), with free access to water. The rats were each fed 15 g laboratory chow every day (i.e. 60 g/cage).

Apparatus

The apparatus consisted of two sets of four operant chambers (Campden Instruments, Model 4102). The chambers were 26.5×22×20 cm, and were fitted with two retractable levers and a food pellet dispenser. Food pellets (45 mg, Campden Instruments) were delivered to a tray placed centrally between the two levers. Access to the tray was by opening a hinged Perspex flap. The food tray could be illuminated by a 24 V, 1 W lamp, while a second 24 V, 2.8 W lamp placed centrally in the roof of the chamber served as a houselight. Each chamber was housed in a separate soundproof box, with a ventilator fan providing low-level background noise. Each set of chambers was controlled by a Paul Fray microcomputer using the Spider programming language. Programs for controlling the apparatus and collecting the data were written by the author.

Procedure

Training

The food was removed from the home cage of the rats on the day before the first test. Thereafter, they were fed in the evening after testing. On day 1, the rats were exposed to the operant chambers with a large number of food pellets placed in the food tray. At this time the levers were retracted. On day 2, they were tested under a fixed time schedule of reinforcement in which 30 food pellets were delivered one at a time every 60 s in a non-contingent manner. On day 3 of training, the left lever was inserted into the chamber, and every press resulted in the delivery of a food pellet. On the following day, the right lever was inserted and the same schedule of reinforcement was employed. This alternation procedure was continued until all the rats had pressed both levers at least 100 times in a 20-min session.

Fixed consecutive number training was then begun. In this procedure, the rats were trained to make a certain minimum number of consecutive responses on the left lever before pressing the right lever to deliver food. Responses on the right lever before completion of the minimum requirement resulted in a short time-out, and the rat was required to restart the sequence of consecutive response. Sequences of responses exceeding the minimum also resulted in food delivery the first time the rat pressed the right lever. On day 1, the rats were required first to press the left lever (FCN lever) only once and then press the right lever (Reinf. lever) to obtain food. (FCN 1). On day 2 the FCN minimum requirement was increased to 3 and, after 4 days further training, it was increased to 8.

Paced-FCN 8

After five sessions of FCN training, the pacing procedure was introduced. After each response the two levers were retracted for a short period so that there was a minimum time between two consecutive responses, but no maximum time. Each time the lever was extended into the chamber a timer was started for this minimum time (e.g. 2.2 s). If the rat responded within this time, as was usual, the lever was retracted until the timer ran out, and after an additional period of 0.3 s it was re-extended into the chamber. If the rat waited for longer than 2.2 s, the lever was retracted and re-extended after 0.3 s. The fixed period of 0.3 s was chosen, as it was slightly longer than the time taken to retract and extend the lever. The training of the rats was adjusted to suit each individual. Initially, the minimum time was set to 2.5 s, and the schedule to FCN 3. After 2 days, the FCN was gradually increased to 4, 5, 6, 7 and eventually 8. Good responding on the paced FCN schedule (2.5 s) was obtained after between 1 and 12 sessions. The rats were then trained for ten consecutive sessions under FCN 8 (2.5 s) before the session was divided into two components with differing minimum times. The unexpected finding that the rats did not perform so well when they were forced to respond more slowly meant that it took some sessions to establish appropriate parameters. Consecutive sessions were run with the following parameters: three sessions, 2.5/10.0 s (Fast/Slow component, respectively), three sessions 2.5/5.0 s, three sessions 2.5/3.5 s, three sessions 2.5/4.0 s, and finally 12 sessions 2.5/5.0 s again. Sessions 10–12 of these have been used to calculate the Baseline data (see Results below).

The final testing procedure consisted of five blocks: an initial 10 min of FCN 8 (2.5 s), followed by a 1-min time-out. Thereafter followed 20 min FCN 8 (5 s), 10 min FCN 8 (2.5 s), 20 min FCN 8 (5 s), completed by a final 10-min FCN 8 (2.5 s). Each block was started by the delivery of a non-contingent food and the illumination of the houselight, and the blocks were separated by a 1-min time-out, during which time the houselight was turned off. Data for the three FCN 8 (2.5 s) blocks were averaged for the purposes of data analysis, as were the data from the two FCN 8 (5 s) blocks.

Measurements and statistics

First, for each measure, vehicle values were obtained from the means of the 3 or 4 vehicle treatment days. These were then combined with the drug treatment data in separate one-way analyses of variances for the Fast and Slow components. The components were separated in this way in all but the analysis of the Baseline data because some rats rarely completed any chains in the Slow component and, coupled with drug-induced reductions in chain length and response rate, sometimes no data were available for the measures of efficiency or chain time. In this case, the empty cell has been treated as a missing value by the statistical analysis program, Sigmastat, and the degrees of freedom adjusted accordingly. Differences between drug treatments and control were tested using Dunnett's *t*-tests. All comparisons were made at the 5% level.

The measures submitted to analysis of variance were defined as follows:

FCN lever responses: the total number of responses made on the FCN lever during each component. Since the length of the components was fixed, this gives a measure of the average rate of responding.

Chain time: the average length of time taken to complete a chain of eight responses on the FCN lever. This provides a measure of the local rate of responding during the time at which the rats were completing response chains.

Chain length: the average length of the chain of responses made on the FCN lever before a response was made on the Reinf. lever. A reduction in this value indicates a shortening of the average chain length and an increase in impulsivity, an increase in this value, an increase in chain length and thus a reduction in impulsivity.

Response efficiency: the number of responses made on the FCN lever divided by the number of pellets obtained. This shows the average number of FCN lever responses required to obtain a food pellet.

Chain efficiency: the number of responses made on the Reinf. lever divided by the number of pellets obtained. This shows which proportion of chains resulted in food delivery.

First response food: the proportion of the total Reinf. lever responses not preceded by a response on the FCN lever.

A selective effect on impulsivity would be expected to produce an effect on the chain length in the absence of changes in the number of responses and chain time. Changes in impulsivity can lead to alterations in response efficiency, particularly if the rats make many short chains, but this is not necessarily the case.

In addition to these measures for statistical analysis, the distribution of chain lengths was also analysed using a "survival" analysis. That is, the proportion of the total number of chains greater than length 1, length 2, length 3 and so on was calculated. Obviously all chains have a length of at least 1, thus the curve always begins at 100%. Statistical analysis of these data was carried out by two-way analysis of variance on cuts through the data at chain lengths ≥6, ≥8, ≥10 and ≥12 with factors of treatment and component. By the nature of the analysis, it is meaningless to carry out a three-way analysis on these data, including a factor "length of chain", since the proportion of chains surviving each length inevitably diminishes. A rat had to have made at least five chains to be included in this analysis.

Drugs

The drugs employed in these experiments were *d*-amphetamine sulphate (Sigma Chemical Company), imipramine hydrochloride (Ciba-Geigy), chlordiazepoxide hydrochloride (Sigma Chemical Company), haloperidol ("Haldol" injection solution, Janssen), and ethanol (Kemetyl). Amphetamine, imipramine and chlordiazepoxide were dissolved in 0.9% saline, whereas the "Haldol" solution and ethanol were diluted with distilled water. The doses, treatment times and routes of injection are given in the Results section. The results are presented in the order in which the experiments were carried out. All doses were given in a Latin-square design at 1-week intervals.

Ethical comment

The experiments described here were approved by Södra Stockholms Djuretisknämnd in accordance with Swedish national law.

Results

Baseline data

There were marked differences in the performance of the rats in the Fast and Slow components, above and beyond the expected difference in the time to complete a chain of eight responses [$F(1,7)=31.1$ $P<0.001$, Table 1]. The number of responses made on the FCN lever did not differ significantly between the two components [$F(1,7)=4,33$, NS], whereas the rats entered the food tray

Table 1 The differences between performance in the Fast (2 s inter-response time) and Slow (5 s inter-response time) components of a paced-FCN procedure. After analysis of variance, significant differences were observed in all measures except response efficiency. Results are means ± SEMs

Component	Total FCN responses	Pellets earned	Panel entries[a]	Chain length	Response efficiency[b]	Chain efficiency	Chain completion time	% First resp. on food
Fast	141.2±15.7	11.2±1.6	8.6±2.8	7.42±0.5	13.4±0.8	1.6±0.2	29.4±3.3	8.1±2.8
Slow	108.8±18.0*****	6.3±1.5*****	17.3±2.6*****	5.01±0.5*****	22.9±5.1	8.2±2.5*****	46.7±1.7*****	35.5±11.4*****

[a] Note, unlike the other measures, the number of panel entries (i..e. the number of times the rats opened the Perspex door giving access to the food tray) is not corrected for the difference in the length of the components (5 min vs 12 min). If this is taken into account there is no difference in the mean number of panel entries (1.7/min vs 1.4/min)

[b] Because of the large variation in the response efficiency in the slow component, the data was also analysed after log transformation, in which case there was a statistically significant difference (1.12 vs 1.31, $P<0.05$)

* $P<0.05$ vs Fast component after analysis of variance

Table 2 Summary of the effects of imipramine, haloperidol and ethanol on a paced FCN 8 schedule of reinforcement. The data shown are the mean ± SEMs. Note that the SEM indicates the amount of variation between subjects, whereas the statistical analysis was carried out on a within-subject design

Drug	Dose mg/kg	Number of FCN responses	Chain time (s)	Chain length	First response food	Response efficiency	Chain efficiency
Imipramine	Veh	147.7±14.2	28.9±3.0	7.6±0.3	4.9±2.6	12.2±0.3	1.49±0.1
IP 60 min	1.0	144.0±16.3	28.7±2.9	8.2±0.3	8.9±4.8	11.5±0.3	1.41±0.2
Fast	3.0	140.6±13.8	29.5±2.7	7.9±0.4	7.0±3.2	11.6±0.3	1.28±0.1
component	10.0	100.2±16.9*	43.9±5.5*	8.8±0.5*	10.1±5.4	12.1±0.6	1.37±0.1
Slow	Veh	110.5±19.8	50.0±4.3	5.4±0.6	24.1±8.5	18.3±1.6	4.11±0.4
component	1.0	88.6±26.7	47.5±5.4	5.4±0.6	30.5±11.5	16.8±3.0	3.08±0.7
	3.0	114.6±25.5	48.7±2.2	5.3±0.8	30.1±10.6	16.7±3.1	3.20±0.9
	10.0	89.1±20.4	62.2±6.7*	6.1±0.4	19.9±7.0	14.2±10.2	2.43±1.0
Haloperidol	Veh	148.1±15.4	29.7±4.1	7.5±0.3	2.1±0.9	11.9±0.6	1.5±0.1
SC 60 min	0.01	143.5±14.4	32.1±4.2	7.6±0.3	1.1±0.8	12.4±0.8	1.6±0.3
Fast	0.03	83.1±16.5*	31.7±4.2	5.8±0.7*	18.3±5.3	15.2±1.6	3.1±0.9
component	0.1	22.1±4.4*	30.6±4.4	3.3±0.4*	30.4±8.5*	27.7±6.3*	6.2±2.0*
Slow	Veh	106.1±25.6	51.4±3.5	5.2±0.7	30.3±10.2	17.1±3.6	2.68±0.5
component	0.01	108.5±29.2	50.1±3.8	4.7±1.2	36.5±14.4	15.7±1.9	2.51±0.3
	0.03	48.6±16.1*	47.9±3.0	3.2±0.5	50.1±11.4	20.2±2.4	5.45±0.5*
	0.1	7.1±3.6*	–	1.2±0.3*	78.1±9.8*	–	–
Ethanol (5 ml/kg)	Veh	135.3±19.3	28.1±3.2	6.9±0.4	7.3±3.8	13.6±1.0	1.76±0.2
PO 15 min	300	133.8±19.4	28.8±2.6	7.9±0.6	2.7±2.7	12.3±0.6	1.49±0.1
Fast	1000	139.1±16.4	29.4±3.5	6.9±0.2	2.5±2.0	12.8±1.0	1.92±0.4
component	3000	63.7±17.9*	33.0±5.4	5.1±0.9*	30.2±9.4*	12.3±0.4	1.54±0.1
Slow	Veh	89.9±27.0	–	4.5±0.8	36.5±11.4	–	–
component	300	85.8±29.7	–	4.5±1.0	36.1±16.2	–	–
	1000	96.4±35.2	–	4.0±1.1	31.0±15.7	–	–
	3000	34.4±17.1*	–	2.3±0.8*	43.0±14.0	–	–

* $P<0.05$ vs vehicle after Dunnett's *t*-test. Vehicle was 0.9% saline for all drugs except ethanol, in which case it was tap water
– Measure could not be calculated due to insufficient data

roughly twice as many times in the Slow component [$F(1,7)=9.73$, $P<0.05$], in proportion to the longer duration of that component. However, the efficiency of the rats was clearly lower in the Slow component. They earned significantly fewer food pellets [$F(1,7)=9.47$, $P<0.05$], and they made significantly shorter chains [$F(1,7)=16.7$, $P<0.01$], with the result that fewer chains resulted in the delivery of food [$F(1,7)=6.54$, $P<0.05$]. However, the number of responses per food pellet did not differ significantly between the two components [$F(1,7)=3,71$, NS]. In addition, the rats pressed the Reinf. lever more frequently without having first pressed the FCN lever [$F(1,7)=6.28$]. Thus, making the rats respond more slowly decreased their efficiency. A calculation based upon the time taken to complete an eight-response chain and the average chain length suggests that the rats pressed the Reinf. lever approximately 27 s after initiating a chain in the Fast component and approximately 29 s after in the Slow component – a strikingly similar interval.

Analysis of the difference in the distribution of the chain lengths is shown in Fig. 1. There were significantly more chains in the Fast component at all of the lengths examined [≥6: $F(1,7)=10.1$, $P<0.05$, ≥8: $F(1,7)=17.8$, $P<0.01$, ≥10: $F(1,7)=15.1$, $P<0.01$ and ≥12: $F(1,7)=7.5$, $P<0.05$].

Imipramine

Imipramine (Table 2 and Fig. 2) was administered IP 60 min before testing at 1.0, 3.0 and 10.0 mg/kg. The average time taken complete a ratio of eight responses was significantly increased by 10.0 mg/kg for both the Fast component [$F(3,21)=20.7$, $P<0.0001$] and the Slow component [$F(3,14)=7.78$, $P<0.01$]. The analysis of chain length revealed a significant effect of the drug in the Fast [$F(3,21)=3.99$, $P<0.05$], but not the Slow component [$F(3,21)=1.0$, NS]. Post-hoc tests on the data for the Fast component revealed a significant difference between saline and a dose of 10.0 mg/kg, indicating that the average chain length at 10.0 mg/kg imipramine was significantly longer than that after saline treatment (see Table 2). The efficiency of the rats was unaffected by the drug, measured either by Response [Fast $F(3,21)=0.91$, NS and slow $F(3,16)=0.35$, NS] or Chain efficiency [Fast, $F(3,21)=0.54$, NS and Slow, $F(3,16)=0.38$, NS]. Imipramine did not significantly increase the proportion of first responses made on the Reinf. lever [Fast: $F(3,21)=0.45$, NS and Slow: $F(3,20=0.95$, NS].

Analysis of the distribution of chain lengths after imipramine treatment (Fig. 2, upper panel) confirmed that the drug shifted the distribution to the right, an increase

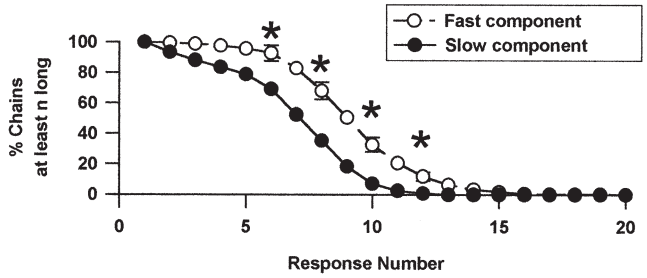

Fig. 1 Comparison of the distribution of chain lengths in the Fast (○) and Slow (●) components in the absence of drug treatment. The *horizontal axis* shows the chain length up to and greater than 20, and the *vertical axis* shows the percentage of the total chains greater or equal to each length, a so-called "survival" plot. Differences between the curves were tested at chain lengths ≥6, ≥8, ≥10, and ≥12. Significance at the $P<0.05$ level after post hoc tests is shown by *asterisks*

in chain length and a reduction in impulsivity. There was no significant effect of the drug at the two shortest lengths examined [Main effect of treatment, ≥6: $F(3,21)=1.3$, NS), and ≥8: $F(3,21)=1.9$, NS]. The percentage chains ≥10 was significantly increased in both Fast and Slow components [Main effect of treatment $F(3,21)=4.8$, $P<0.05$], whereas when the criterion was increased to ≥12, a statistically significant interaction was obtained [$F(3,21)=4.6$, $P<0.05$], post-hoc tests revealing a significant increase in the number of chains only in the Fast component.

Haloperidol

Haloperidol (Table 2 and Fig. 2) was administered SC 60 min before testing. Inspection of the data revealed that, at the two higher doses, the drug reduced the number of responses made in both components [Fast: $F(3,18)=40.1$, $P<0.0001$, Slow: $F(3,18)=12.7$, $P<0.001$], but had no significant effect on the average time taken to complete a chain in the Fast component [$F(3,15)=1.57$, NS] or in the Slow component [$F(2,7)=0.31$, NS). However, there was insufficient data at 0.1 mg/kg in the Slow component to include in the statistical analysis. In contrast, the drug had a marked effect on chain length. Haloperidol, at doses of 0.03 and 0.1 mg/kg, significantly reduced the chain length in the Fast [$F(3,18)=27.1$, $P<0.0001$] and at 0.1 mg/kg in the Slow components [$F(3,17)=10.4$, $P<0.001$, see Table 1]. At the higher of these doses there was also a significant reduction in efficiency measured by both the Response and Chain efficiency in the Fast component [$F(3,16)=8.6$, $P<0.01$, and $F(3,16)=7.0$, $P<0.01$, respectively], whereas in the Slow component, where there was insufficient data at the highest dose to calculate the quotients, there was no significant effect of the drug on the Response efficiency [$F(2,7)=0.88$, NS] but a significant effect of the dose of 0.03 mg/kg on the Chain efficiency [$F(2,7)=10.7$, $P<0.01$]. The rats also made more first responses on the Reinf. lever at a dose of 0.1 mg/kg in both Fast and Slow components [$F(3,18)=8.94$, $P<0.001$, and $F(3,17)=10.57$, $P<0.001$, respectively].

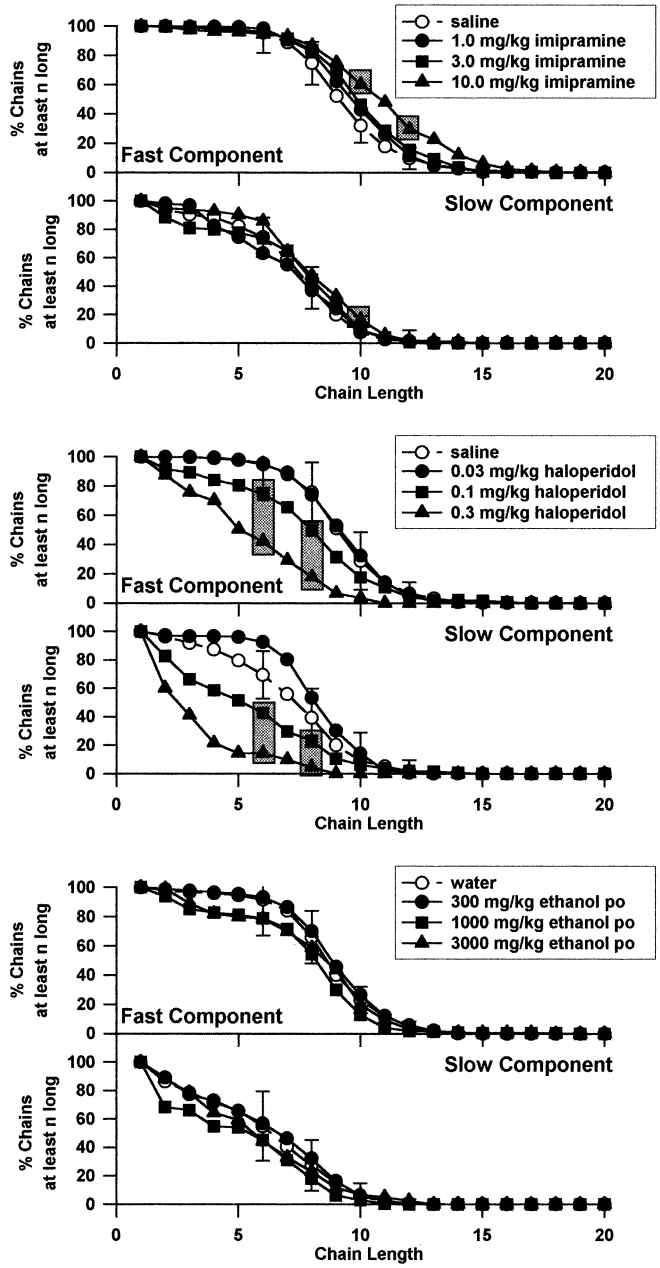

Fig. 2 Distribution of chain lengths after treatment with imipramine (*upper panel*), haloperidol (*centre panel*) and ethanol (*lower panel*). Each panel consists of two graphs showing performance in the Fast and Slow components of the schedule. The *horizontal axis* shows the chain length up to and greater than 20, and the *vertical axis* shows the percentage of the total chains greater or equal to each length, a so-called "survival" plot. Differences between the curves were tested at chain lengths ≥6, ≥8, ≥10, and ≥12. Significance at the $P<0.05$ level after post hoc tests is shown by the *hatched rectangles*

The reduction in chain length produced by haloperidol can be clearly seen in the distribution analysis (Fig. 2, lower panel). A significant reduction in the number of chains of lengths ≥6 and ≥8 was found at 0.1 and

0.3 mg/kg [post hoc tests after significant main effect of treatment ≥6: $F(3,18)=22.4$, $P<0.0001$ and ≥8: $F(3,18)=21.0$, $P<0.0001$]. There was also a significant main effect of treatment at chain lengths ≥10 [$F(3,18)=3.8$, $P<0.05$], but no individual dose differed from vehicle after post-hoc tests. There was no significant effect of the drug at chain lengths ≥12 [$F(3,18)=0.2$, NS].

Ethanol

Ethanol was administered PO 15 min before testing at doses of 300, 1000 and 3000 mg/kg. The number of responses made on the FCN lever was significantly reduced by the highest dose used (3000 mg/kg) in both the Fast [$F(3,15)=10.94$, $P<0.001$] and the Slow [$F(3,15)=4.31$, $P<0.05$] components. Chain time analysis was only possible for the Fast component, and there was no significant effect of ethanol [$F(3,13)=2.67$, NS]. Ethanol reduced the chain length in both components at the highest dose [Fast: $F(3,15)=4.54$, $P<0.05$ and Slow: $F(3,14)=3.88$, $P<0.05$]. However, this chain shortening did not result in a decrease in efficiency in the Fast component measured by either Response efficiency [$F(3,13)=0.84$, NS] or Chain efficiency [$F(3,13)=0.92$, NS]. The proportion of first responses made on the Reinf. lever was significantly increased at 3000 mg/kg ethanol in the Fast component [$F(3,15)=8.69$, $P<0.01$], but the drug had no effect in the Slow component [$F(3,15)=0.17$, NS].

Although ethanol reduced chain lengths at the highest dose in both component, this was not reflected by any significant change in the chain length distribution (Fig. 2, lower panel). There was no significant main effect of treatment at any of chain lengths tested [≥6: $F(3,15)=2.8$, NS, ≥8: $F(3,15)=1.5$, NS, ≥10: $F(3,15)=0.8$, NS and ≥12: $F(3,15)=1.0$, NS]. There were no significant treatment by component interactions, either.

Chlordiazepoxide

Chlordiazepoxide was administered IP at doses of 1.0, 3.0 and 10.0 mg/kg 15 min before testing. The highest dose markedly sedated the rats and affected almost all measures of responding. The total number of FCN lever presses was significantly reduced in both Fast and Slow components [$F(3,15)=33.0$, $P<0.0001$, and $F(3,15)=8.3$, $P<0.01$, respectively]. However, the chain time was not significantly affected in either component [Fast: $F(3,13)=0.94$, NS and Slow: $F(3,8)=3.28$, NS]. The chain length was significantly reduced by the dose of 10.0 mg/kg in the Fast [$F(3,15)=22.3$, $P<0.0001$] but not the Slow [$F(3,12)=2.51$, NS] components. Response efficiency was significantly reduced, particularly in the Fast component [$F(3,13)=35.4$, $P<0.0001$] but also in the Slow component [$F(3,8)=4.40$, $P<0.05$], whereas Chain efficiency was only significantly reduced in the Fast component [$F(3,13)=24.7$, $P<0.0001$]. The proportion of responses made first on the Reinf. lever was significantly increased in the Fast component [$F(3,15)=6.26$, $P<0.01$] but not in the Slow component. In this component the overall analysis of variance was statistically significant, but no dose differed significantly from vehicle. From Table 3, it can be seen that there was a tendency for the proportion of first responses on the Reinf. lever to be reduced at 1.0 and 3.0 mg/kg in this component, but the effect of neither individual dose reached statistical significance.

The reduction in average chain length produced by chlordiazepoxide in the Fast component was mirrored by a significant shift in the distribution to the left (Fig. 3, upper panel). However, unlike in the statistical analysis of average chain length, above, the distribution in the Slow component was shifted to an equal extent. This was supported by a significant main effect of treatment at chain lengths ≥6 [$F(3,12)=53.5$, $P<0.0001$] and ≥8 [$F(3,12)=36.2$, $P<0.0001$]. In both cases, after post-hoc tests, it was the highest dose of 10 mg/kg chlordiazepoxide which differed from vehicle. There was no effect of the drug on chain lengths ≥10 or ≥12 [$F(3,12)=2.7$, NS and $F(3,12)=0.4$, NS, respectively].

Amphetamine

d-Amphetamine was the last of the drugs tested in these rats (Table 3 and Fig. 2). It was administered IP 15 min before testing at doses of 0.2, 0.4 and 0.8 mg/kg ($n=6$). The drug reduced the number of responses made on the FCN lever at the dose of 0.8 mg/kg in both components [Fast: $F(3,15)=12.3$, $P<0.001$, Slow: $F(3,15)=5.3$, $P<0.05$]. Only two rats completed any chains greater than eight responses in the Slow component after the drug treatment, so further analysis of that component is limited. There was no alteration in chain time in the Fast component [$F(3,12)=1.02$, NS]. Amphetamine also reduced the chain length at the dose of 0.8 mg/kg in both components [Fast: $F(3,13)=7.5$, $P<0.01$ and Slow: $F(3,13)=5.4$, $P<0.05$]. Efficiency was not, however affected by the drug where this could be assessed in the Fast component [Response eff.: $F(3,11)=2.79$, NS, Chain eff.: $F(3,12)=1.3$, NS]. Finally, the rats made significantly more first responses on the Reinf. lever, again at 0.8 mg/kg and again in both components [Fast: $F(3,14)=9.05$, $P<0.01$ and Slow: $F(3,14)=4.39$, $P<0.05$].

Analysis of the distribution data for amphetamine was complicated by the fact that at 0.8 mg/kg of the drug, only two rats started five chains or more in the Slow component. A balanced analysis of variance including all doses was thus impossible. Instead, two complementary analyses were carried out – a two-way analysis including the doses of 0.2 and 0.4 mg/kg amphetamine, and a one-way analysis including all three doses for the Fast component alone. The two-way analysis revealed no significant effect of 0.2 or 0.4 mg/kg of the drug at chains ≥6 [main effect $F(2,10)=3,2$. NS], chains

Table 3 Summary of the effects of chlordiazepoxide and amphetamine on a paced FCN 8 schedule of reinforcement. The data shown are the mean ± SEMs. Note that the SEM indicates the amount of variation between subjects whereas the statistical analysis was carried out on a within-subject design

Drug	Dose mg/kg	Number of FCN responses	Chain time (s)	Chain length	First response food	Response efficiency	Chain efficiency
Chlordiazepoxide	Veh	136.5±18.8	29.9±3.5	7.2±0.4	2.4±2.1	12.3±0.8	1.5±0.2
IP 15 min	1.0	143.9±16.2	29.0±3.4	7.2±0.2	1.4±0.9	11.4±0.4	1.4±0.1
Fast	3.0	129.9±18.6	60.8±31.7	7.2±0.5	4.6±3.6	12.3±0.6	2.0±0.5
component	10.0	35.2±14.5*	43.9±5.5	3.9±0.8*	27.7±11.7*	30.1±3.8*	6.0±1.1*
Slow	Veh	87.8±29.8	48.6±3.0	5.2±0.6	41.1±15.8	17.2±2.7	3.4±0.7
component	1.0	100.9±26.0	51.1±3.2	4.6±0.6	31.6±9.3	22.5±3.5	3.6±0.8
	3.0	103.0±31.5	56.4±14.6	4.8±0.6	18.2±8.2	29.2±10.7	5.3±2.4
	10.0	15.4±6.7*	139.6±78.1	3.6±0.8	54.2±14.4	68.0±14.0*	11.0±1.0
Amphetamine	Veh	131.7±17.4	34.6±4.0	7.1±0.3	3.3±1.6	14.7±2.16	1.85±0.3
SC 15 min	0.2	101.8±23.7	29.6±4.0	6.0±0.7	17.3±10.8	13.3±1.64	1.56±0.2
Fast	0.4	109.2±16.1	31.3±5.3	6.8±0.7	22.2±5.8	13.0±1.24	1.85±0.4
component	0.8	29.8±134*	28.6±4.1	2.8±1.1*	60.9±15.3*	21.6±7.1	2.64±1.1
Slow	Veh	91.1±30.3	–	5.1±0.9	41.4±15.5	–	–
component	0.2	85.3±30.2	–	4.4±0.9	39.3±18.9	–	–
	0.4	71.6±29.1	–	3.3±0.9	45.2±18.3	–	–
	0.8	16.3±9.3*	–	1.5±1.0*	78.3±11.9*	–	–

* $P<0.05$ vs vehicle after Dunnett's *t*-test. Vehicle was 0.9% saline for both drugs
– Measure could not be calculated due to insufficient data

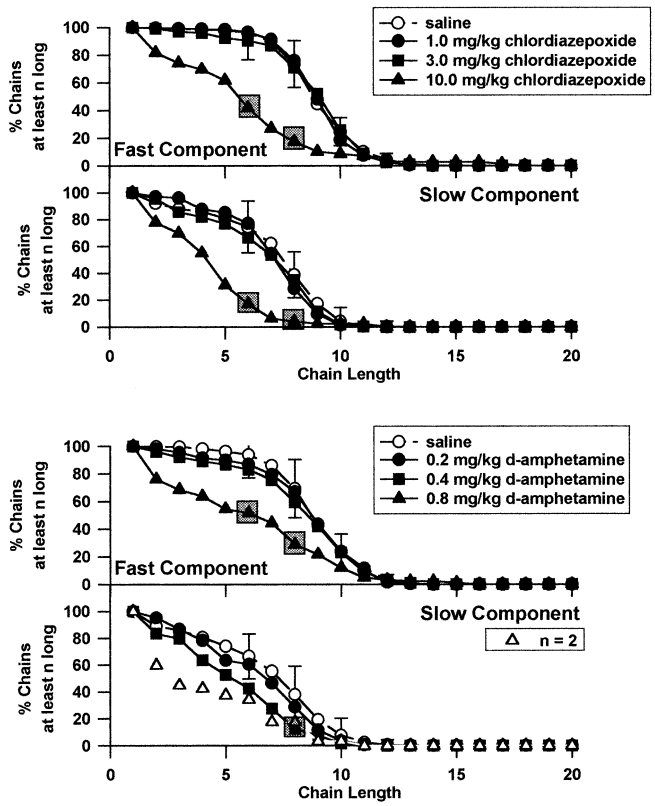

Fig. 3 Distribution of chain lengths after treatment with chlordiazepoxide (*upper panel*) and *d*-amphetamine (*lower panel*). For further description of the layout, see legend to Fig. 2

≥10 [$F(2,10)=0.8$, NS] or chains ≥12 [$F(2.10)=0.4$, NS]. However, there was a treatment by component interaction at chains ≥8 [$F(2,7)=9.3$, $P<0.05$] which revealed a significant effect in the Slow component at 0.4 mg/kg after post hoc tests. The one-way analysis on the Fast component revealed significant effects of 0.8 mg/kg amphetamine at chains ≥6 and chains ≥8 [post hoc tests after $F(3,12)=7.0$, $P<0.01$ and $F(3,12)=6.6$, $P<0.01$, respectively]. There was no significant effect on longer chains [≥10: $F(3,12)=0.5$, NS and ≥12: $F(3,12)=0.1$, NS].

Discussion

The effects of the drugs may be summarised as follows. Haloperidol, amphetamine, imipramine and ethanol reduced the average chain length but only at doses which also produced a reduction in response rate. The effect of ethanol was rather small, since there was no significant change in the chain length distribution. Thus these drugs did not have a selective effect on response choice. Imipramine also reduced the rate of responding, but this reduction was accompanied by an increase in the average chain length and a shift to the right of the chain length distribution. Thus imipramine did not have a selective effect on response choice either. However, there was a dissociation between the effects on response rates, shared by all the drugs, and the effects on chain length, on which the drugs had different effects.

As noted in the Introduction, the paced-FCN procedure employed here was designed to give the experimenter control over the rate of responding, thus reducing the possibility for drug to influence response choice

measures (chain length and efficiency of responding) by affecting response rate. Evidence that this succeeded is provided by the effect of imipramine, which had no effect on chain length under conventional unpaced FCN 8 and FCN 32 schedules of reinforcement (Evenden 1998), but increased chain length in the present study. Splitting the procedure into two components was designed to act as a comparison with this previous study, the Fast component as a parallel to the FCN 8 schedule, and the Slow component to the FCN 32. However, increasing the length of the FCN and forcing the rats to respond slowly had quite different effects on the pattern of performance of the rats. In the former case, the behaviour of the rats was well controlled by the schedule, and clearly resembled the normal, unpaced performance at FCN 8 (Evenden 1998). In contrast, in the present study, analysis of the Baseline data showed that slowing the rats disrupted control by the schedule requirements, so that efficiency was impaired. In the Slow component the rats made, on average, shorter chains than in the Fast component, although the schedule requirement was exactly the same. This suggests that even in this well-trained "ratio-based" schedule, the behaviour of the rats is at least partly controlled by the passage of time. This is supported by the observation that the average time to terminate a chain was roughly similar in the two components. Interestingly, too, the rats did not improve in performance with repeated training, as might reasonably be expected due to their continued success and good performance in the Fast component. Thus it would appear that some fundamental aspect of rats' operant performance underlies the impairment induced by forced slow responding. Loosely, it may be that rats use a timing to regulate their behaviour in this procedure (see discussion in Davis and Pérusse 1988, p. 575) where counting would be a better strategy, and that they have great difficulty in doing otherwise. Mechner and Guevrekian (1962) manipulated the response rate under an FCN schedule by altering the deprivation state of the rats. They found that a faster response rate did not lead to an increase in the modal in the response chain length, which suggests that the rats were not using time-on-lever to estimate the chain length. Possibly a difference between internally and externally generated changes in response rate is responsible for this difference in outcomes. Of course, these results do not rule out the possibility that animals responding under operant procedures can use numerical information under other circumstances, for example, the elegant work of Roberts and Mitchell (1994), carried out in pigeons. One interesting procedural difference between that study and the present is that in Roberts and Mitchell (1994) numerical or timed discriminative stimuli were used to control response choice (a single act), whereas in the present study the information was used to regulate a series of acts making up a chain. As Roberts and Mitchell (1994) point out, the exact influence of either numerical or timing information may depend the subjects previous experience and on the training and testing procedures employed.

The difference between the Fast and Slow components is illuminating in another sense, in that the effects of slowing the rats down resemble the effects of several of the drugs, haloperidol, chlordiazepoxide, ethanol and amphetamine: the chain length was shorter, the efficiency impaired, and the rats made more first responses on the Reinf. lever rather than the FCN lever. Since the effects of the drugs were also accompanied by a reduction in the number of responses made, it could be assumed that their effects were directly due to slowing of responding, i.e. sedation (cf. the discussion of the effects on unpaced FCN responding; Evenden 1996). However, two observations make this hypothesis untenable: first, amphetamine which is not sedative, had the same effects (although it did disrupt responding), and second, imipramine, which also reduced the number of FCN lever responses and increased the time taken to complete a chain of eight responses at the highest dose used here, had the opposite effect.

Of the five drugs tested in this experiment, the most interesting effect was that of imipramine, since the chain length was significantly increased when the rats were treated with this drug, and the distribution of chain lengths was clearly shifted to the right. By the definition provided in the Introduction, this effect is proposed to reflect a reduction in impulsivity. Note, however, that this shift did not result in improved performance measured by a reduction in the number of responses or chains made per pellet delivered. Imipramine (and related drugs) has previously been found to improve performance in the differential reinforcement of low rates of responding procedure (DRL; McGuire and Seiden 1980; O'Donnell and Seiden 1983), in which rats are required to wait for 72 s between consecutive lever presses to maximise the number of pellets obtained. Criticism has been directed against these findings in the DRL-72, since other sedative drugs can sometimes also produce a similar effect (e.g. haloperidol; Pollard and Howard 1986, or diazepam; C.N. Ryan, personal communication). A mild sedation might be expected to improve performance by reducing the rate of responding. However, this is obviously not an adequate explanation for the findings here, since there is no link between low response rate and increased chain length: quite the contrary. In fact, in the conventional, unpaced FCN schedule, where low response rates appear to be associated with reduced chain length, the dose of 10.0 mg/kg imipramine shifted the chain length distribution to the left, the opposite of the effect seen here, although mean chain length was not affected. Furthermore, other drugs which generally reduce activity, including haloperidol and chlordiazepoxide, had the opposite effect to imipramine in the present procedure.

Recently, Ho et al. (1996) have demonstrated that desipramine (a selective inhibitor of noradrenaline reuptake) and fluvoxamine (a selective inhibitor of serotonin reuptake) have no effects in two procedures measuring timing ability: the fixed interval peak procedure and the interval bisection task. Thus neither the effects of imipra-

mine (which inhibits reuptake of both noradrenaline and serotonin) in the DRL-72 nor in the present procedure are likely to be due to effects of this drug on timing performance. These authors suggest that such compounds may affect the animal's ability to inhibit or postpone a reinforced response, and that a task which requires response inhibition but does not entail temporal differentiation of responding might be used to verify this hypothesis. The present procedure was designed to fulfil this role, although it appears that empirically the rats do use temporal pacing of behaviour in this test, even if it is not explicitly required (the same might also be true of other "omission" procedures).

Given that it is possible to rule out mediation of the imipramine effect via changes in response rate and that drugs with related mechanisms of action appear not to affect timing behaviour, it is reasonable to conclude that, at least on acute administration, imipramine reduces impulsivity in rats. As noted in the Introduction, this procedure is designed to assess only one aspect of impulsivity, and Evenden and Ryan (1996) did not find that imipramine increased preference for delayed reinforcers. That test was also based upon lever pressing, reinforced by food, so that the basic motor response and motivation are the same in the two procedures. Instead, it is likely that the difference in effect of the drug in the two procedures depends upon the different aspects of decision making involved: in the present procedure, completing a chain of behaviour made up of several independent responses, and in the delayed reinforcement paradigm, choosing which of two responses to make depending upon the delay associated with their outcome.

The remaining four drugs, ethanol, chlordiazepoxide, haloperidol and amphetamine, shared the effect of reducing the chain length. Chlordiazepoxide, haloperidol and amphetamine produced a shift to the left in the chain length distribution, whereas ethanol had no obvious effect on the distribution. On this basis it can be concluded that the effect of ethanol in this procedure is minimal, and secondary to the general effect of the doses used here on motor performance. Interestingly, Evenden (1996) has recently found that ethanol does reduce preference for a large delayed reinforcer in the task described by Evenden and Ryan (1996), reflecting an increase in impulsivity in that test. Thus it seems that there is also a dissociation of the effects of ethanol between the two procedures. Chlordiazepoxide has not been tested in that delayed reinforcement procedure, although diazepam, perhaps surprisingly, increased preference for the large, delayed reinforcer (Evenden and Ryan 1996). Seen in the context of impulsivity, the shift to the left in the chain length distribution produced by chlordiazepoxide in the present procedure corresponds better to the effect seen in the T-maze delayed reinforcement test used by Thiébot and colleagues (Thiébot et al. 1985) than to the effects of diazepam reported by Evenden and Ryan (1996). The effects of haloperidol reported here resemble those seen in the unpaced FCN 8 procedure (Evenden 1998) and support the suggestion that this class of drugs appears to increase impulsivity in this test at the same time as they reduce response rate rather than increasing average chain length as suggested by Picker (1988).

Finally, the effect of amphetamine also resembled that seen in the unpaced FCN procedures with one interesting and important difference. A close examination of the distribution of responding shows little or no gradual shift in the peak of the distribution as seen under the unpaced FCN schedules. Instead, borrowing from the analysis carried out by Evenden (1998), the rats appear to shift abruptly from "normal" behaviour to behaviour based upon single independent responses, reflecting a preference for the lever most closely associated with food delivery. The most likely explanation of this is that response chain shortening, which is responsible for this shift in the peak, does not occur during paced FCN responding, since successive responses must be separated by the withdrawal and extension of the lever. If this is so, it would support the suggestion that response chain shortening when responding is continuous is largely due to "telescoping" of two or more responses into one unit so that the animal initiates two responses but in effect performs only one. Instead, in the present procedure, the primary effect appears to be a shift in the level of control from "pattern" to "act" (Rachlin 1995), with a resultant predominance of the preference for the lever most closely associated with reinforcement. This shift in the level of control reflects a qualitative alteration in behaviour which may be related to impulse control, but which is not well captured in the definitions given in the Introduction. Such considerations support the suggestion that pharmacological tools can provide valuable insights when investigating behavioural phenomena such as impulsivity.

The study of impulsive behaviour in animals is as yet in its infancy, and, naturally, considerable caution should be taken in extrapolating concepts derived from human studies to other animal species. Nonetheless, animal experiments will most probably be needed to contribute to the study of biological factors influencing impulsivity, in which case it will be desirable to develop and validate appropriate test methods. The paced FCN procedure appears to offer more analytical power than the unpaced version of the test since it was possible to differentiate the effects of drugs which all reduced response rate but either increased or decreased impulsivity. For this reason, the test may be useful for further studies of biological factors influencing impulsive behaviour, including serotonergic drugs (Evenden, manuscript in preparation), and a proposed genetic model of attention deficit/hyperactivity disorder, the spontaneously hypertensive rat (Evenden 1997).

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Appendix 1

Two-way treatment by component analyses were carried out on three of the measures used in the main paper for which results were almost always available for all rats in both components: number of responses on the FCN lever (*NFL*), chain length (*CL*) and percentage first response on Reinf. lever (*1stR*). The results of these analyses (*F* ratio, probability, *df*) are presented below to supplement those given in the main body of the text

	Treatment	Component	Treatment x component
Imipramine			
NFL	4.00 (<0.05) *df* = 3,21	4.48 (NS) *df* = 1,7	4.07 (<0.05) *df* = 3,21
CL	3.31 (<0.05) *df* = 3,21	27.31 (<0.01) *df* = 1,7	0.36 (NS) *df* = 3,21
1stR	0.29 (NS) *df* = 3,21	11.59 (<0.05) *df* = 1,7	1.94 (NS) *df* = 3,20
Ethanol			
NFL	6.35 (<0.01) *df* = 3,15	3.65 (NS) *df* = 1,4	0.46 (=0.058) *df* = 3,12
CL	1.76 (NS) *df* = 3,12	11.9 (<0.05) *df* = 1,4	0.81 (NS) *df* = 3,11
1stR	1.82 (NS) *df* = 3,12	4.12 (NS) *df* = 1,4	3.01 (NS) *df* = 3,12
Haloperidol			
NFL	36.8 (<0.0001) *df*= 3,18	3.18 (NS) *df* = 1,6	0.58 (NS) *df* = 3,18
CL	17.1 (<0.0001) *df*= 3,18	17.9 (<0.01) *df* = 1,6	0.37 (NS) *df* = 3.17
1stR	19.0 (<0.0001) *df*= 3,18	10.4 (<0.05) *df* = 1,6	0.64 (NS) *df* = 3,17
Chlordiazepoxide			
NFL	38.0 (<0.0001) *df*= 3,15	4.40 (NS) *df* = 1,5	0.52 (NS) *df* = 3,15
CL	16.0 (<0.0001) *df*= 3,15	50.8 (<0.001) *df*= 1,5	0.39 (NS) *df* = 3,12
1stR	6.05 (<0.01) *df*= 3,15	8.61 (<0.05) *df*= 1,5	2.23 (NS) *df* = 3,14
Amphetamine			
NFL	9.87 (<0.001) *df* = 3,15	2.64 (NS) *df* = 1,5	1.36 (NS) *df* = 3,15
CL	7.30 (<0.01) *df* = 3,9	7.60 (NS) *df* = 1,3	1.50 (NS) *df* = 3,9
1stR	9.48 (<0.01) *df* = 3,12	6.36 (NS) *df* = 1,4	1.35 (NS) *df* = 3,12

The major conclusion from this analysis is the relative lack of difference in the effects of the drugs in the two components. Only one statistically significant interaction was seen, the effect of imipramine on the total number of FCN lever responses, and this reflects the lack of the effect of this drug in the Slow component (see Results). Note, however, that the degrees of freedom in these analysis are in some cases rather low, and lower than for the corresponding one-way analyses in the Results section due to the exclusion of animals with excessive missing values from analysis by the statistical analysis program, Sigmastat. Thus, in a few cases where the *F* ratio approaches that required for statistical significance, negative results should be interpreted cautiously

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