Reinforcing behavioral variability: An analysis of dopamine-receptor subtypes and intermittent reinforcement

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ABSTRACT

Variability is a functional and modifiable component of behavior that is necessary for learning or behavior change to occur. An appreciation of drug effects on reinforced variability could contribute to a fuller understanding of potential drug effects on behavior change. Dopamine agonists were examined under conditions that produced low and high levels of variability. In Experiment 1, D-amphetamine and dopamine-receptor-specific agonists (quinpirole and SKF 38393) were examined with rats that lever-pressed under a Multiple VARY 8:4 Fixed Ratio 4 (FR 4) schedule. In the VARY 8:4 condition, reinforcement followed every four-response sequence that differed from the previous eight sequences. Any four-response sequence was reinforced in the FR 4 component. Experiment 2 was an attempt to hold overall reinforcement rate constant by reinforcing criterion sequences under a Variable Interval 60″ schedule of reinforcement. In Experiment 1, D-amphetamine and quinpirole (D_2 agonist) increased variability in the FR 4 component while having no effect on variability in the VARY 8:4 component. SKF 38393 (D_1 agonist) had little effect on variability, even at doses that lowered total responding. In Experiment 2, intermittent reinforcement elevated variability in the FR 4 component and attenuated differential effects of D-amphetamine. Thus, D-amphetamine and quinpirole increased variability when it was low under the FR 4 component in Experiment 1. The high variability under the VARY 8:4 schedule was unaffected by D-amphetamine, except for modest effects at high doses. These observations suggest that dopamine and specifically D_2-type receptors are involved in the production of behavioral variability, and that the drug effects depend upon the baseline level of variability.

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The reinforcement of behavior is classically thought to result in repetitive responding that shows little variation since a reinforcer, by definition, strengthens or selects the response that produces it. Reinforcement, however, can also produce variation in behavior when variability is directly targeted, and operant variability is now a well-established response class (Neuringer, 2002). Variability is a functional component of behavior and is necessary for learning or behavior change to occur. An appreciation of drug effects on variability could contribute to a fuller understanding on potential drug effects on behavior change.

Page and Neuringer (1985) produced highly variable responding in pigeons by implementing a "lag procedure" that reinforced only those eight-response sequences that differed from the previous 50 sequences. Reinforced, or operant, variability persists in the face of disruptors such as pre-feeding or non-contingent food (Doughty and Lattal, 2001), reinforcer delay (Odum et al., 2006; Wagner and Neuringer, 2006), extinction (Eckerman and Lanson, 1969; Stokes, 1995; Neuringer et al., 2001), and drugs that act on dopamine (Mook

and Neuringer, 1994) or GABAergic (Cohen et al., 1990; McElroy and Neuringer, 1990; Ward et al., 2006) neurotransmitter systems.

The influence of psychomotor stimulants on reinforced variability may depend on the conditions maintaining behavior. For example, Ward et al. (2006) reported that D-amphetamine increased variability in pigeons when a single, pre-defined response sequence was reinforced. This drug had little effect, however, on the high variability seen when variability was reinforced explicitly. Daily administration of D-amphetamine increased variability in Wystar–Kyoto rats (WKYs) when variability was reinforced using a procedure that compared a sequence with only four of the previously produced sequences (Mook and Neuringer, 1994). When a restricted set of sequences was reinforced, however, D-amphetamine produced a small decrease in variability. This suggests that a drug's effect on a sequence depends on how that sequence is produced, and this is important in designing the control condition. A "nonvariable" component for which variability can increase or decrease could be sensitive to drug effects that would not be detected when the comparison condition is a single, pre-defined sequence and variability can only increase.

Variable responding in the lag procedures entails switching between response devices (Machado, 1997) and D-amphetamine increases switching. For example, this drug was examined using a fixed-consecutive-number-8 (FCN-8) procedure, in which a sequence

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of eight consecutive responses on one lever followed by a switch to a second lever is reinforced (Laties et al., 1981; Rees et al., 1985). D-amphetamine produced premature switching and greater variability in sequence lengths in this procedure. Evenden and Robbins (1983) examined the effects of D-amphetamine on switching and repetition by presenting rats with a choice between two levers. Before a trial began, a reinforcer was randomly assigned to one of the levers. A trial continued until a reinforcer was delivered. Switching was defined as responding on both levers during a trial, whereas perseveration was defined as responding on a single lever despite the absence of the reinforcer. D-amphetamine's effects depended not only on drug dose but also on the baseline probability of switching. Lower doses increased switching and higher doses increased perseveration. The magnitude of the increase in switching was highest when baseline rates of switching were lowest.

The present study was designed to examine the role that baseline levels of variability play in the effects of a non-specific dopamine agonist, D-amphetamine, and then of specific dopamine D_1 and D_2 agonists. High and low levels of variability were established under a multiple schedule, so effects could be examined on a within-subject basis during the same experimental session. In one component, variability was reinforced under what we term a "VARY 8:4" schedule: a four-response sequence was reinforced if it differed from the previous eight sequences. In the other component (termed Fixed Ratio four or "FR 4"), any four-response sequence was reinforced. Thus, the two components differed in whether variability was explicitly reinforced. Drugs administered were D-amphetamine, which increases synaptic dopamine, SKF 38393, a direct D_1-receptor agonist, and quinpirole, a direct D_2-receptor agonist.

1. Experiment 1

1.1. Method

1.1.1. Subjects

The subjects were 19, five-month-old male Long-Evans rats purchased from Harlan. They were housed two per cage, but separated by a plastic divider, in an AAALAC-accredited facility. The colony was on a 12-hr light–dark cycle (lights on at 6:00 am). The rats had free access to water except during the experimental session. The animals were 200 g upon arrival at the colony and their weight was allowed to increase to 300 g. This weight was maintained using caloric restriction with afternoon feedings (at least one-hour after experimental sessions) of a measured allotment of food given to them for an unlimited time. All procedures were approved by the Auburn Institutional Animal Care and Use Committee.

1.1.2. Apparatus

The experiments were conducted in 10 commercial operant chambers (Med-Associates Inc. model #Med ENV 007) containing two levers, each calibrated so that about 0.20 N registered a press. A pellet dispenser was situated midway between the two front levers and the reinforcer was a 45 mg sucrose pellet (Purina Mills, Inc., St. Louis, MO). Sonalert tones™ (2900 and 4500 Hz, nominally) were calibrated to an amplitude of 70 dbC. A house light (28 V 100 ma) was located midway at the top of the back wall, opposite the levers, and a light emitting diode (LED) was above each lever. The standard grid floor was covered with a secured piece of Plexiglas, which covered all but the back inch of the floor. This piece of Plexiglas was required for other experiments conducted in these chambers. Each chamber was surrounded by a sound-attenuating cabinet with built-in ventilating fan that circulates air into the experimental environment and provides masking noise. Programs for experimental procedures and data collection were written using MED-PC IV (Med-Associates, Georgia, VT). Session events are recorded with 0.01-second resolution.

1.1.3. Behavioral Procedure

Lever-pressing was established using autoshaping on the left (L) and right (R) retractable levers separately as described elsewhere (Reed et al., 2006). Then, any four-response sequence was reinforced regardless of how the responses were distributed between the two levers. This is called the FR 4 component because any four-response sequence was reinforced. This differs from the usual implementation of FR 4 schedules but the nomenclature is technically accurate (Lattal, 1991). The reinforcement cycle commenced immediately when the animal met the four-response requirement and began by turning off the lever lights and low tone, sounding a high tone (4500 Hz) for 0.5 s, and delivering a 45-mg sucrose pellet. A 2900 Hz tone (the low tone) sounded during this component.

Next, the VARY contingency, in the absence of the low tone, was introduced. Initially, a four-response sequence had to differ from the previous sequence (VARY 1:4). This is called a "lag 1" criterion because only one previous sequence is considered. The reinforcement cycle commenced immediately when the animal met the lag requirement and began by turning off the lever lights, sounding a high tone (4500 Hz) for 0.5 s, and delivering a 45-mg sucrose pellet. Once an animal received 50 reinforcers during a one-hour session under the VARY 1:4 condition, a lag 2 criterion was imposed, making the schedule a VARY 2:4. Then a VARY 3:4 schedule was imposed, and so on to a VARY 8:4. The criterion for increasing the lag was 50 reinforcers delivered (i.e., 50 criterion sequences) during a single one-hour session. All animals reached the VARY 8:4 schedule in 8–10 sessions. Then the multiple schedule containing a FR 4 and a VARY 8:4 component was initiated.

Under the VARY 8:4 schedule, a four-response sequence was reinforced if it differed from the previous eight sequences. For example, if the current sequence was LLRL then a reinforcer would be delivered if none of the previous eight sequences had been LLRL. Non-criterion trials ended in a 15-second time-out, during which all lights in the chamber darkened and no reinforcer was delivered. No tone sounded during the VARY 8:4 component. The VARY 8:4 and a FR 4 components were presented in strict alternation as a multiple schedule, beginning with the VARY 8:4 component. Components changed after 10 reinforcers. All training and testing sessions began with illumination of both the house and lever lights. The sessions ended after one hour or after 100 reinforcers were presented, whichever occurred first.

1.1.4. Drug administration

D-amphetamine sulfate (0.3–3.0 mg/kg), SKF-38393 HCL (1.0–17.0 mg/kg), and quinpirole HCL (0.01–0.3 mg/kg) were dissolved in 0.9% saline solution (all from Sigma-Aldrich Co., St Louis, Missouri, USA). All doses were measured as the salt. The saline vehicle served as a control for the drug injections. Rats were placed into the experimental chamber immediately after injection and the session began 10 min later to allow time for drug distribution. Drugs were administered intraperitoneally (i.p.) on Tuesday and Fridays. Mondays and Wednesdays served as non-injected control days and Thursdays served as a vehicle control. Drug administrations were separated by two or more days so as to minimize the possibility of sensitization developing.

Rats were divided into two separate groups of nine and ten each. Groups were approximately matched for total responding and variability as denoted by individual U-values (described below). The first group received injections of D-amphetamine (in the order of 0.3, 1.0, 3.0, 1.7 mg/kg) and then the lower range of quinpirole doses of quinpirole (0.01, 0.03, and 0.056 mg/kg) and the second group received injections of SKF 38393 and then the higher range of quinpirole doses (0.1, 0.17, and 0.3 mg/kg). The use of two groups for the quinpirole dosing was done to span a wide range of doses in a reasonable amount of time. The broad range was necessary because low doses of quinpirole stimulate autoreceptors and higher doses

stimulate post-synaptic receptors (Arnsten et al., 1995) and the shape of its dose-effect curve can be multiphasic (Reed and Newland, 2009).

The determination of acute dose-effect curves began once variability and total responding did not show any increasing or decreasing trends. This occurred after approximately six sessions on the terminal *mult* VARY 8:4 FR 4 schedule. Every dose was administered once and there were three to four vehicle administrations for each drug. If a dose decreased total responding to less than 20% of baseline rates, then a higher dose was not given.

1.1.5. Statistical analyses

The dependent measures were: 1) the total number of responses (total number of lever presses) performed in each component, and 2) the U-value, or entropy, an index of variability in the sequences produced. The U-value is an index of overall sequence variability (Page and Neuringer, 1985). The U statistic is denoted by the following equation:

$$U = \frac{-\sum\limits_{i=1}^{16} (p_i \cdot \log_2 p_i)}{\log_2 n}$$

where p is equal to the probability of a given sequence i, and n is the total number of sequences possible, or 2^N. A U value of 1.00 signifies each sequence occurred $1/16^{th}$ of the time and a U value of 0.00 signifies that only one sequence was produced.

A repeated-measures analysis of variance (RMANOVA) was performed for each dependent variable for each drug, with dose and component as the within-subjects factors. When a dose X component interaction was significant, post hoc tests were performed using the Sidak adjustment for multiple comparisons. A p value of 0.05 or less was considered statistically significant. F-values are not reported for non-significant effects ($p > 0.1$). To deal with violations of sphericity, Huynh-Feldt corrections were used if the Huynh-Feldt epsilon was less than 0.7. If fewer than 25% of the animals responded after a specific dose, then this dose was dropped from all entropy analyses but not from the total response analyses since zero responding is measurable. Calculating entropy requires that an animal must respond during a session. All statistical analyses were performed using SYSTAT® 11 (SYSTAT Software Inc. Richmond, CA, USA).

1.2. Results

1.2.1. Baseline

A repeated measures ANOVA showed that baseline values for total responding ($F (1, 18) = 53.16$, $p < 0.001$) and entropy ($F (1, 18) = 70.51$, $p < 0.001$) during the FR 4 were different from that during the VARY 8:4 component. Visual inspection revealed that the differences were seen in every animal (not shown). Total responding was higher in the VARY 8:4 component ($mean = 592$ $SD = 236$) than in the FR 4 component ($mean = 196$ $SD = 31$). Fig. 1 shows histograms from one session to illustrate the response distribution associated with high and low U-values. Overall, in the VARY 8:4 component, U-values ranged from 0.58 to 0.90 across all animals ($mean = 0.83$ $SD = 0.08$), signifying a high degree of variability in the sequences produced. In the FR 4 component ($mean = 0.40$ $SD = 0.25$), U-values were lower and showed more inter-subject variability, ranging from 0.025 to 0.74 across all animals. The most common sequences during the FR 4 component differed across animals but the preferred sequences usually had no (e.g., LLLL) or one (e.g. LLRR) changeover. Table 1 illustrates this by showing sequence preferences for each animal from a single session immediately prior to commencing drug administration in the FR 4 component. These preferred sequences were not stable through the course of the experiment but tended to drift as the experiment progressed (not shown).

1.2.2. D-amphetamine

For total responding (Fig. 2, left), there was a main effect of component ($F (1, 8) = 26.51$, $p < .01$) and dose ($F (4, 32) = 2.99$, $p < 0.1$) but no interaction. Entropy (Fig. 2, right) was influenced by component ($F (1, 6) = 43.29$, $p < .01$) and dose ($F (3, 18) = 10.51$, $p < .01$). D-amphetamine's effect on entropy depended on the component (dose × component interaction, $F (3, 18) = 8.61$, $p < .05$) with an increase in entropy in the FR 4 component only ($p < .05$).

1.2.3. SKF 38393

Total responses decreased in a dose-dependent manner ($F (4, 36) = 17.99$, $p < .001$), and were higher during the VARY 8:4 component ($F (1, 9) = 51.89$, $p < .001$). Responding decreased more in the VARY 8:4 component (dose × component interaction, $F (4, 36) = 7.62$, $p < .01$) (Fig. 3, left). For entropy, there was a main effect of component ($F (1, 8) = 31.43$, $p < .01$) but no significant effect of dose and no significant interaction (Fig. 3, right). The highest dose was not included in the repeated-measures analysis of entropy because some animals did not respond at this dose.

1.2.4. Quinpirole

1.2.4.1. Low dose group (0.01–0.056 mg/kg) (n = 8).
One animal was removed from this group due to illness. Total responding was influenced by dose ($F (3, 21) = 12.56$, $p < .01$), and component ($F (1, 7) = 25.01$, $p < .01$). Responding decreased more in the VARY 8:4 component at higher doses (dose × component interaction, $F (3, 21) = 7.01$, $p < .05$) (Fig. 4, left). Low doses of quinpirole decreased responding in the VARY 8:4 component. For entropy, there was a main effect of dose ($F (3, 18) = 4.09$, $p < .05$) and component ($F (1, 6) = 42.95$, $p < .01$) but no significant interaction (Fig. 4, right).

1.2.4.2. High dose group (0.10–0.30 mg/kg) (n = 10).
Total responding was influenced by both dose ($F (3, 27) = 13.88$, $p < .001$), and component ($F (1, 9) = 45.93$, $p < .001$). Responding decreased more in the VARY 8:4 component at higher doses (dose × component interaction, $F (3, 27) = 3.15$, $p < .1$) (Fig. 4, left). For entropy, there was a main effect of dose ($F (3, 24) = 7.41$, $p < .01$) and component ($F (1, 8) = 92.12$, $p < .001$). Quinpirole's effects on entropy were specific to component, with high doses increasing entropy in the FR 4 component ($p < .05$) but having no significant effect on entropy levels in the VARY 8:4 component (dose × component interaction, $F (3, 24) = 7.85$, $p < .01$) (Fig. 4, right).

1.3. Discussion

Behavioral variability, as indicated by the entropy score, was high when reinforced in the VARY 8:4 component and low when it was merely permitted in the FR 4 component. There were individual differences in the preferred sequences, but most animals showed a preference for sequences that had zero (e.g., LLLL) or one (e.g., LLRR) changeover in the FR 4 component (Table 1). There were more responses during the VARY 8:4 component than during the FR 4 component because components changed after 10 reinforcers and it took only 10 sequences to meet that criterion during the FR 4 component but more than that during the VARY 8:4 component.

Operant variability appeared to be resistant to the effects of D-amphetamine. Under the VARY 8:4 component, variability was high and D-amphetamine exerted no effect. When variability was low and responding was more stereotyped during the FR 4 component, D-amphetamine increased variability. Quinpirole, like D-amphetamine had no effect on variability in the VARY 8:4 component but increased variability in the FR 4 component. This interaction was statistically significant only at the higher doses. SKF 38393 had no effect on variability at doses up to 10.0 mg/kg. SKF 38393 decreased total responding in both the VARY 8:4 and the FR 4 components at 3.0 mg/kg (and
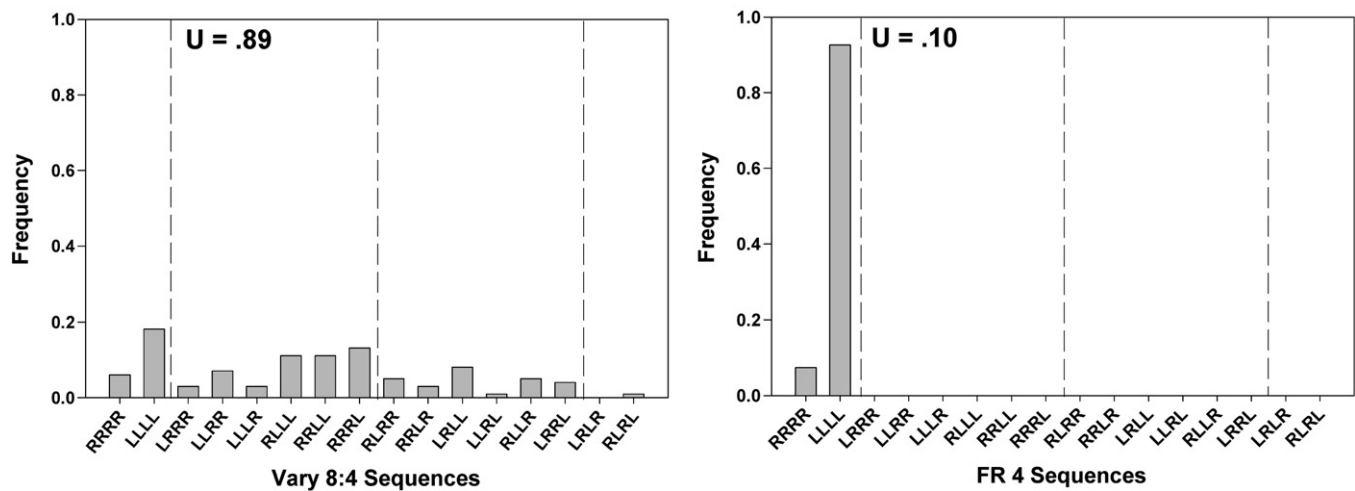
Fig. 1. Histograms for RAT 624 displaying entropy and frequency of responding for each of the 16 possible sequences in the VARY 8:4 (left panel) and the FR 4 (right panel) components on a non-injected control day. The number of changeover responses required for each sequence is represented by the dotted lines (i.e. from zero up to three changeovers required to complete the sequences).

higher) doses. Taken together, the dissociation of drug effects on responding and variability suggests that these are independent properties of behavior and that D_2-type receptors play an important role in response variability.

2. Experiment 2

In Experiment 1, every criterion sequence was reinforced and the number of reinforcers per component and reinforcers per criterion sequence were held constant at ten, but reinforcer delivery per unit of time was free to vary. Inspection of cumulative records and data collected on individual animals indicated that the reinforcer rate (reinforcers per min) was higher during the FR 4 component than during the VAR 8:4 component. This is because animals required more time and produced more sequences to obtain the 10 reinforcers in the VARY 8:4 component since non-criterion sequences could, and did, occur. During the VARY 8:4 component, an average of 2.5 sequences occurred for every correct (and reinforced), sequence (data not

reported). In contrast, *every* four-response sequence was reinforced in the FR 4 component.

Reinforcing every criterion sequence had the intended advantage of producing a large difference in variability between the components and equating the relationship between the criterion response class (four-response sequence vs. variable four-response sequence) and reinforcer delivery. This, however, resulted in different overall rates of reinforcement per unit time and per four-response sequence (including unreinforced ones in the VARY 8:4 component). It is possible that the discrepancies noted in D-amphetamine's effects between the two components may have been due to differences in the reinforcer rate rather than the VARY 8:4 contingency itself. This issue is addressed in Experiment 2 by delivering primary reinforcers for a criterion response sequence (four-response sequence or variable four-response sequence) under a Variable Interval 60 s (VI 60 s) schedule. Technically, the VARY 8:4 and FR 4 schedules could be viewed as unit schedules of a second order VI schedule of reinforcement, with the designation *mult* VI 60-second (Vary 8:4) VI 60-

Table 1

Percentage of sequences emitted and entropy during the FR 4 component prior to drug administration. Sequences that occurred over 50% of the time are shaded.

| Rat | Entropy | 0 Changeovers | | 1 Changeover | | | | | | 2 Changeovers[1] | 3 Changeovers[1] |
		RRRR	LLLL	RRRL	RRLL	RLLL	LLLR	LLRR	LRRR		
R623	0.00	100									
R613	0.08	6	94								
R622	0.10	7	93								
R624	0.10	7	93								
R614	0.15	7	89							4	
R631	0.26	81		4			6	7		2	
R626	0.27	63	34			3					
R621	0.29	7	80		4	4	4			2	
R635	0.43	24	61	2		2	4	2	4	2	
R625	0.44	7	65	2	7	11	6			2	
R615	0.45	7	65		4		13	4		7	
R616	0.47	7	56		2		21	7		7	
R612	0.53	6	53		3	9	16			13	
R632	0.56	7	44			26	12			12	
R633	0.56	7			6	33	6	7		41	
R636	0.73	13		20	13	4		4	20	24	2
R634	0.78	30	11	11	13	7	9	6	2	11	
R640	0.78	9	30		2		15	7	6	30	2
R611	0.80	6	22	6	9	6	13	6		31	

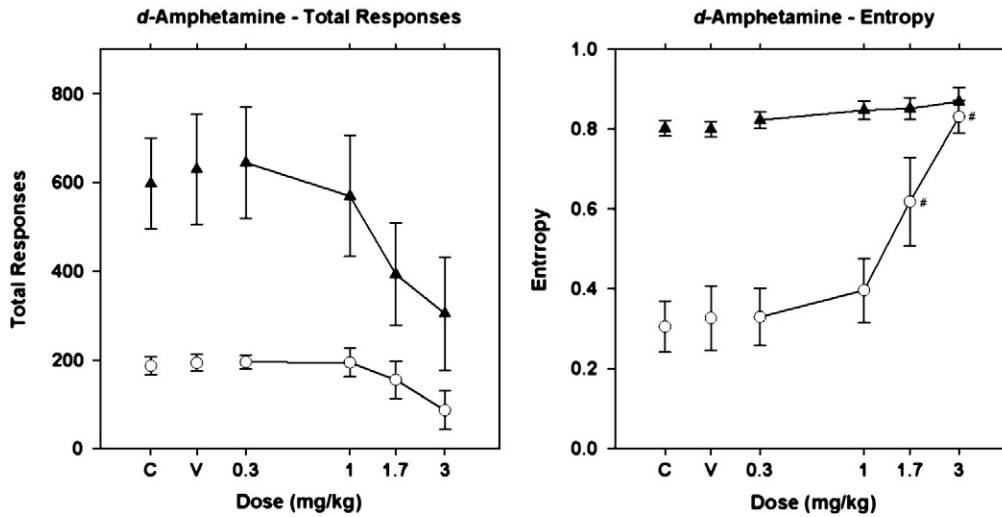[1]Includes all possible 2- and 3-changeover chains.

Fig. 2. Dose–response functions for total responses (left panel) and entropy (right panel) under D-amphetamine for the VARY 8:4 (filled triangles) and the FR 4 (unfilled circles) components. The highest dose includes only the four animals that responded. This dose was not included in statistical analyses. # denotes significance ($p < .05$) from the FR 4 vehicle dose. Error bars = 1 S.E.M.

second (FR 4). This approach held constant the number of reinforcers per unit time (about 1/min) and per criterion response sequence.

2.1. Method

The same eighteen rats and apparatus in Experiment 1 were used for Experiment 2. One rat had to be dropped from Experiment 2 due to apparatus problems that were discovered too late to remedy.

2.1.1. Preliminary training and drug administration

This experiment began approximately six weeks after Experiment 1 ended. A VI 60-second schedule was imposed so that on average, a criterion sequence was reinforced once every 60 s but the actual interreinforcer interval varied unpredictably from 3.1 to 198 s using a Fleshler and Hoffman (1962) sequence. Correct sequences that occurred before the time interval had passed were followed by the same 0.5 s tone that was a component of the reinforcement cycle. In order to equate reinforcer rates between the two components, the VI timer continued to run during the time-out period in the VARY 8:4 component. The components alternated after 10 reinforcers were

delivered. The session lasted one-hour. Other details, including drug administration, are as in Experiment 1.

The determination of acute dose-effect curves began approximately 30 sessions after the last session in Experiment 1. Only D-amphetamine was used; the modest drug effect seen with D-amphetamine and similarity in entropy under the FR 4 and VARY 8:4 baselines suggested that further investigation with the other drugs were unlikely to be informative. As in Experiment 1, if fewer than 25% of the animals responded during a specific dose, then this dose was dropped from all entropy analyses but not from the total response analyses since zero responding is measurable. Calculating entropy requires that an animal respond during a session.

2.2. Results

2.2.1. Baseline

A repeated measures ANOVA was used to compare total responding and entropy levels in the two components during control conditions. Total responding was, again, significantly higher in the VARY 8:4 component ($mean = 940$ $SD = 402$) than in the FR 4 component
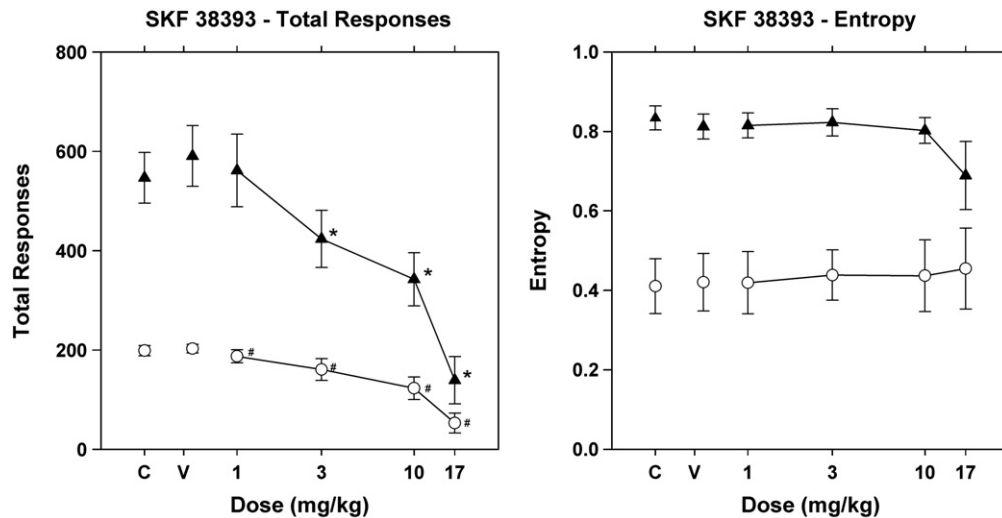

Fig. 3. Dose–response functions for total responses (left panel) and entropy (right panel) under SKF 38393 for the VARY 8:4 (filled triangles) and the FR 4 (unfilled circles) components. The highest dose includes only the six animals that responded. This dose was not included in statistical analyses. # denotes significance from the FR 4 vehicle dose. * denotes significance from the VARY 8:4 vehicle dose. Error bars = 1 S.E.M.
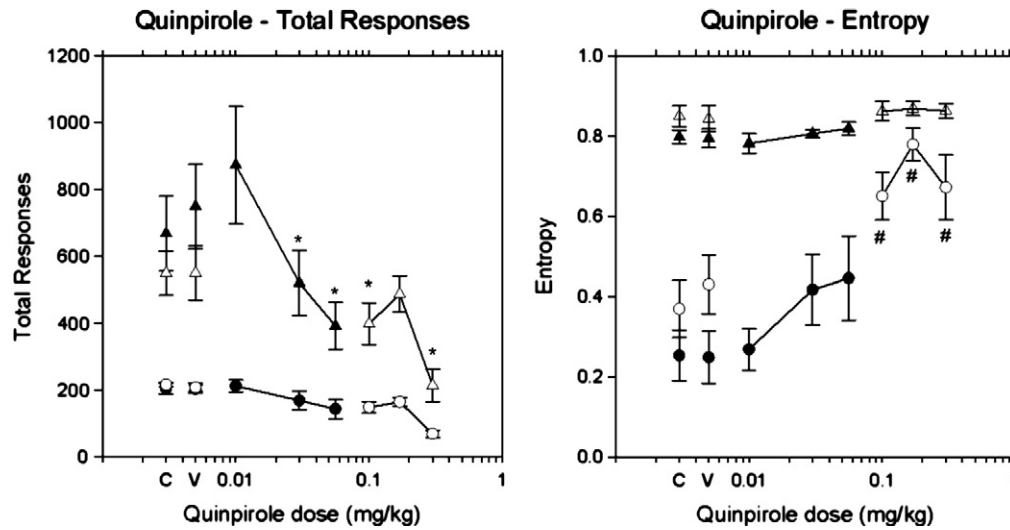
Fig. 4. Dose–response functions for total responses (left panel) and entropy (right panel) under quinpirole for the VARY 8:4 and the FR 4 components. Triangles represent the VARY 8:4 component with filled being the low dose group and unfilled being the high dose group. Circles represent the FR 4 component with filled being the low dose group and unfilled being the high dose group. # denotes significance from the FR 4 vehicle dose. * denotes significance from the VARY 8:4 vehicle dose. Error bars = 1 S.E.M.

($mean = 399\ SD = 136$) ($F(1, 17) = 61.65$, $p < 0.001$). Entropy in the FR 4 component was significantly lower ($mean = 0.63\ SD = 0.18$) than in the VARY 8:4 component ($mean = 0.83\ SD = 0.06$) ($F(1, 17) = 32.12$, $p < 0.001$). The joint role of intermittency (Experiment 1 vs. Experiment 2) and variability requirement (FR vs. VARY) was examined by analyzing the baseline values from the two experiments using a 2 (intermittency) by 2 (variability requirement) ANOVA with repeated measures on each factor. There was a significant interaction ($F(1, 17) = 24.74$, $p < 0.001$), suggesting that the difference between the FR and VARY components was much smaller under intermittent reinforcement than under the FR 1 schedule used in Experiment 1. This can be visualized by comparing (Fig. 5, right panel, values over "C") with the entropy values over C from Experiment 1. The U-values during the FR 4 in Experiment 1 were around 0.3 but they were above 0.6 in Experiment 2, signifying a higher degree of variability in the sequences produced in this component. Overall, there were more responses during the VARY 8:4 component than during the FR 4 component because of the occurrence of non-criterion responses during the VARY 8:4 component (Fig. 5, left panel, over "C"). Total

reinforcers over a session were indistinguishable between the two components (not shown).

2.2.2. D-amphetamine

Total responding was higher in the VARY 8:4 component than in the FR component at all doses ($F(1, 17) = 82.51$, $p < .001$). Total responding decreased significantly at the highest dose for both components ($F(4, 68) = 4.57$, $p < .01$) but there was not a significant interaction with component (Fig. 5, left). For entropy, there was a significant main effect of component ($F(1, 15) = 34.98$, $p < .001$) and of dose ($F(3, 45) = 2.56$, $p < .1$). A dose × component interaction was also found for entropy ($F(3, 45) = 6.43$, $p < .01$). Entropy increased in the FR 4 component at the 1.7 mg/kg dose, whereas it was unaffected in the VARY 8:4 component (Fig. 5, right).

2.2.3. Overall reinforcement rate

Reinforcement rates (reinforcers/minute in a specific component) were calculated for both Experiments 1 and 2. A paired-samples t-test was conducted to compare the reinforcement rates in the VARY 8:4
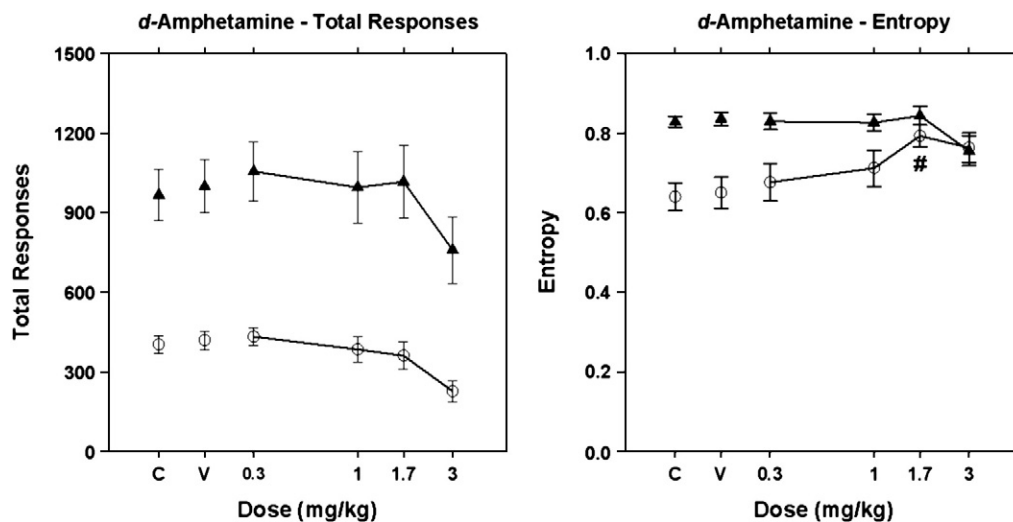

Fig. 5. Dose–response functions for total responses (left panel) and entropy (right panel) under D-amphetamine for the VARY 8:4 (filled triangles) and the FR 4 (unfilled circles) components under a VI 60 s schedule. The highest dose includes only the 13 animals that responded. This dose was not included in statistical analyses. # denotes significance ($p < .05$) from the FR 4 vehicle dose. Error bars = 1 S.E.M.

and FR 4 components. In Experiment 1, there was a significant difference between the rates for the VARY 8:4 component ($mean = 1.3$ $SD = 0.42$) and the FR 4 component ($mean = 7.2$ $SD = 2.1$); $t(18) = -12.89$, $p < .001$. In Experiment 2, the overall reinforcement rate in the VARY 8:4 component ($mean = 0.58$ $SD = 0.08$) was much more similar to that in the FR 4 component ($mean = 0.90$ $SD = 0.11$), but still statistically different $t(17) = -10.32$, $p < .001$.

2.3. Discussion

Experiment 2 was an attempt to hold the reinforcement rates constant between both the VARY 8:4 and FR 4 components by reinforcing criterion sequences under a VI 60″ schedule. The overall reinforcement rate in the VARY 8:4 component was still a little lower than that in the FR 4 component, probably because reinforcers could not be collected during the 15″ timeout periods. The VI 60″ schedule attenuated the difference in variability between the FR 4 and VARY 8:4 components in Experiment 2, as compared with what was seen in the same animals during Experiment 1. It did this by increasing variability in the FR 4 component, while leaving variability during the VARY 8:4 component roughly the same as in Experiment 1. The increased variability during the FR 4 component may have been due to the weakened link between the completion of a response sequence and the delivery of a reinforcer. When every sequence was reinforced during the FR 4 component in Experiment 1, the preferred sequence occurred relatively frequently, and its execution would be strengthened by frequent reinforcer delivery. When sequences were reinforced intermittently, however, there was more opportunity for drift in what sequences were executed and, accordingly, for the reinforcement of a greater variety of sequences during the FR 4 component. A similar outcome has been noted in earlier papers on variability in interresponse time distributions (Blough, 1966; Blough and Blough, 1968). With the imposition of intermittent reinforcement under a time-based schedule, there was greater variability in the production of interresponse times than seen when each criterion sequence was reinforced.

It should be noted that in Experiment 2 the animals had a history of acute drug administration that was not present during Experiment 1. Because of the precautions taken to minimize the likelihood of sensitization or tolerance occurring, we do not feel that this drug history was a significant factor in the key differences between Experiment 1 and Experiment 2. In support of this we first note that the VI 60″ schedule was a powerful intervention that, during baseline sessions, increased entropy in the FR 4 component and total responding during both components in Experiment 1. In contrast, many of D-amphetamine's effects were relatively similar between the two studies. A dose of 3.0 mg/kg of D-amphetamine decreased total responding during the FR 4 component in both experiments. D-amphetamine increased entropy to a value of about 0.7–0.8 at 1.7 mg/kg during the FR 4 component in both experiments, despite the large difference in baseline entropy. Reinforcing every criterion sequence during Experiment 1 resulted in large differences in variability between the FR 4 and VARY 8:4 components and therefore D-amphetamine's effects were more clearly visible when examined against that baseline.

3. General discussion

The effects of dopamine agonists on variability in operant behavior depended upon the conditions under which variability was established and the specific actions of the drugs. In Experiment 1 when variability was low (as in the FR 4 component), it was increased by D-amphetamine and the D_2 agonist, extending observations that behavioral disruptors, such as ethanol and D-amphetamine (Cohen et al., 1990; McElroy and Neuringer, 1990; Ward et al., 2006), prefeeding, and non-contingent food (Doughty and Lattal, 2001),

increase variability under conditions in which variability is low. In those studies, the "low-variability" condition entailed reinforcing a specific and pre-defined reinforcement sequence. The present study extends this to a condition in which any four-response sequence was reinforced, so the effect depends on the presence of low-variability and not on the reinforcement of a specific sequence.

The three drugs exerted little or no effect on entropy when variability was explicitly reinforced in the VARY 8:4 component in Experiment 1. This also extends previous reports that reinforced variability is relatively resistant to disruption. In Experiment 2, in which a time-based intermittent schedule of reinforcement was used, the intermediate to high levels of variability that occurred during the FR 4 component were relatively less influenced by D-amphetamine than were the low levels seen during Experiment 1.

Drug effects on response rate and entropy were separable and independent of each other. Increasing doses of quinpirole decreased total responding, increased entropy in the FR 4 component and had no effect on entropy in the VARY 8:4 component. Increasing doses of SKF 38393 also decreased responding but had little effect on entropy. The effects of D-amphetamine in Experiments 1 and 2 also suggest an independence of these two behavioral measures.

Variability is the inverse of perseverative or highly repetitive activity, so if such effects were to result from drug administration then they would likely appear in the VARY 8:4 component. None of the drugs used affected variability during this component, however, even though some form of perseverative responding has been associated with all of these drugs. Therefore, variability in operant behavior is likely a different response class from motor stereotypies, including perseverative oral behaviors such as licking and sniffing (Horvitz et al., 2001; Salmi et al., 2000), or repetitive motor behaviors such as movements confined to small areas (Ben-Pazi et al., 2001; Szechtman et al., 1994) that are provoked by quinpirole or self-grooming or other repetitive behaviors reportedly provoked by SKF-38393 (Bratcher et al., 2005; Eilam et al., 1992; Wachtel et al., 1992). These behaviors would be inconsistent with lever-pressing so they may have been related to the lowered response rates reported, although this cannot be conclusive since systematic observations were not made of these behaviors.

Quinpirole's selective increase of variability in Experiment 1 mimicked those of D-amphetamine. In contrast, the D_1 agonist SKF 38398 had no effect on variability in either component even as it reduced total responding. These data suggest that promoting activity at the D_2-receptors, but not D_1 receptors, increases variability when it is low and that D-amphetamine's effects on variability was mediated by D_2 receptors.

A mechanistic question that deserves some attention is whether variability is the response class being reinforced or whether it is the outcome of the indirect reinforcement of something else, such as switching levers. One could view the procedure as comprising four operants, responding on each lever and changing from left to right and from right to left levers. Machado (1997) sought to synthesize variable responding by directly reinforcing switching. In that study, the reinforcement of switching increased variability but it did not reach the levels obtained when variability was reinforced directly. It seems plausible that reinforced switching is a component of variable behavior, but not necessarily the only contingency operating.

This proposed behavioral mechanism, the reinforcement of switching, is relevant because D-amphetamine increases switching when it is not explicitly reinforced (Evenden and Robbins, 1983; Laties, 1972). As Machado noted, reinforcing variable responding and switching go hand-in-hand with increased variability. Therefore, it is possible that D-amphetamine produced the effects that it did in the FR 4 component by increasing switching between these levers.

Two other potential drug effects, the possible impairment of memory or of the discrimination between the FR 4 and VARY 8:4 components, may be ruled out. Since behavior in the two components remained distinct across drug doses, it is unlikely that the drugs

impaired discrimination between the FR and VARY components. Working memory was not directly investigated in the present study, but Page and Neuringer (1985) investigated it by increasing the number of previous sequences against which the current sequence is compared (here that value was 8). In their investigation, target performance was an eight-response sequence that had to differ from the previous 50 sequences. They reasoned that if behavior was under the control of which sequences had previously been executed, then increasing the number of sequences that must be remembered should degrade performance. In fact, they found that increasing the look-back window to 50 only increased variability, suggesting that something other than memory is involved in the reinforced variability task used here. If memory were playing a role in the current experiment, we would expect to see a decrease in entropy in the VARY 8:4 component and no change in the FR 4 component. This was not the case, however, entropy remained constant throughout the VARY 8:4 component.

One issue that should be noted is the time-out in the VARY 8:4 component. No time-outs occurred in the FR 4 component because any four-response sequence during this component resulted in a reinforcer. Time-outs did occur in the VARY 8:4 component, however, when there was an incorrect sequence. The specifics of how this may influence the drugs' effects are not clear but the distinction should be noted since time-outs are often used in studies of reinforced variability.

The examination of drug effects using a within-subjects design, as done here, increases power and reduces the number of animals used in a study. Such a design does result in the administration of multiple doses of a drug to the same animal. The present study was designed to minimize the likelihood that sensitization or tolerance from repeated dosing could occur. It has been shown that behavioral sensitization may occur in the form of increased stereotypies with chronic treatment of both D-amphetamine (Yetnikoff and Arvanitogiannis, 2005; Salomon et al., 2006; Anagnostaras and Robinson, 1996) and SKF 38393 (Hu et al., 1992; Mattingly et al., 1993). However, the doses used in those studies were close or equal to our highest doses and they were administered consecutively over as many as 42 sessions. In the present study, at least two days elapsed between drug administrations and each dose was administered only once. The dose of 1.7 mg/kg was administered last and the effects lay between those seen at 1.0 and 3.0 mg/kg, suggesting that sensitization did not occur. Approximately six weeks, and 30 sessions, elapsed between Experiments 1 and 2 and the acute dosing regimen was similar to that in Experiment 1. In Experiment 2, some animals received a second round of D-amphetamine doses, and no evidence of sensitization was seen during that second round of dosing. Thus, sensitization or tolerance to amphetamine is not likely to be a significant confound in the present study.

Identifying the appropriate control for a reinforced variability task is not a straightforward endeavor, and the ultimate approach will depend upon the experimental question. The approach taken in Experiment 1, in which every sequence of four responses was reinforced, was selected in order to meet several goals. First was that the two components differ on as few dimensions as possible. Here, every criterion four-response sequence was reinforced, so the major difference was the requirement of variability in the VARY component and the absence of such a requirement in the control component. A second goal was that the two components require similar training time, a goal that is consistent with the first one of keeping the components similar on important dimensions. The third goal was that there should be a large difference in variability between the VARY component and the control component so that drug effects could be detected with greater power.

A fourth goal is that the two components be similar in the relationship between the operant (here, a criterion four-response sequence) and the consequence. All possible goals cannot be satisfied simultaneously, however. Because of our interest in how a reinforcing consequence influenced the variability of responding, the number of reinforcers per criterion sequence was held constant in Experiment 1. In Experiment 2, the goal was to hold the rate of reinforcement

(reinforcers per min) approximately constant by reinforcing all criterion responses under a VI 60″ schedule. As mentioned earlier, however, the reinforcer rates were not identical because timeouts were counted during the VI schedule. Despite this, the use of an intermittent schedule meant that in both components, only some criterion chains were reinforced. This had the effect of blunting the distinction between the two components, undermining the third goal.

The use of a FR 4 as a procedure bypasses problems that arise when a pre-defined chain is selected, problems that may be especially acute if the control response sequence is relatively un-preferred or difficult to execute. As reported in studies of repeated acquisition, behavior chains can vary in difficulty (Wright and Paule, 2007) and difficult chains are often those that require greater travel or more changeovers among response devices. Moreover, which chains are more difficult can differ across animals. We noted that there were individual differences in which chains were produced during the FR 4 component in Experiment 1 but the preferred chains entailed zero or one changeover(s). In Experiment 2 we also observed a preference for chains with small numbers of changeovers (data not shown). In contrast, the control response sequence used in many studies involved one or more changeovers and these sometimes required extensive training to establish, so they can be viewed as difficult chains. The control chains used were LRRLL in Page and Neuringer (1985), RRLR in Mook and Neuringer (1994), LLRR in Neuringer (1991), McElroy and Neuringer (1990), Cohen et al. (1990), and RRLL in Odum et al. (2006) and Ward, et al. (2006). Even a one changeover chain like RRLL can require much training to produce since RLLL and RRRL, which would likely occur, would be an error.

Procedures that yoke reinforcers in the control component to those received in the VARY component have been developed to equate reinforcer rates in the two components. This has been done when an explicit sequence is required (e.g., Page and Neuringer, 1985) or with a four-response sequence distributed across two levers in which the probability of reinforcement is fixed and is yoked to the prior VARY component (e.g., Ward et al., 2008). The second approach is similar to the FR 4 component in Experiment 2 because both of these approaches equate the number and timing of reinforcers per sequence between the two components. The number of reinforcers per criterion sequence, however, is still different. The approach used in Experiment 1 equated the number of reinforcers and of reinforced criterion sequences in the two components and resulted in a large difference in variability. The approach used in Experiment 2 approximately equated the timing of reinforcement and delivered reinforcement intermittently. The intermittent reinforcement appears to have led to greater similarity in variability in the two components.

In summary, the present study examined behavioral variability under a multiple schedule in which it was either required or allowed. Pharmacologic actions of D-amphetamine and quinpirole on behavioral variability appeared to be baseline dependent. When baseline levels of variability were low, these drugs increased variability suggesting that the D_2 receptor subtype was influenced by baseline levels. When baseline levels of variability were high, these drugs appeared to have no effect on operant variability. SKF 38393, however, showed no such influence on baseline levels as they stayed consistent until the highest doses in which variability decreased in the VARY 8:4 component only.

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