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A stimulus-control account of dysregulated drug intake

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ABSTRACT

Drug self-administration typically occurs in a regular temporal pattern, with a consistent pause following each injection. We have proposed that this patterning results from differential reinforcement of post-injection pausing. In this view, even when every response produces an injection, some injections are not reinforcing because they occur when the level of drug effect is already maximal; consequently, drug reinforcement occurs on an intermittent schedule, and the interoceptive drug effect functions as a cue, indicating when another injection will be reinforcing. Previously, we emulated this situation with rats by using food reinforcement; each response was recorded as delivering a "virtual" injection, and a visual cue tracked the virtual drug level to indicate availability of reinforcement. This emulation schedule produced response patterns strikingly similar to actual drug self-administration. In the present study, the emulation schedule was modified to determine whether reinforcement of pausing is sufficient to produce these patterns, or whether a cue is necessary. Without a cue, response patterns were irregular and virtual drug intake was escalated. These results suggest that a failure of interoceptive cues to control pausing might contribute to the dysregulated drug intake that is associated with addiction.

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In using drug self-administration as an animal model of drug abuse, it is assumed that drugs have reinforcing effects similar to those of other positive reinforcers, but that they also have unique features that are worth understanding. One such feature that has often been noted is that drugs tend to be self-administered in a regular temporal pattern. When each response delivers a fixed dose of a drug, a fairly consistent pause follows each injection. When the dose is manipulated, higher doses produce longer pauses. This feature of drug self-administration is important because understanding why it occurs–and how it might change when drug use escalates–could lead to improved strategies for treating drug abuse.

We have recently proposed that regular patterns of intake result from underlying contingencies of reinforcement that are inherent in drug self-administration (Panlilio et al., 2008). This account is based on the following premises:

(1) *Drug reinforcement is inherently intermittent.* That is, even when every response produces an injection, not every injection is reinforcing. This premise is based on the possibility that high levels of drug may produce a maximal effect that cannot be incremented by injecting more drug (Ranaldi et al., 1999; Zernig et al., 2007). In this view, injections are only reinforcing if they occur when the level of drug in the system is below a certain threshold. Once the threshold is surpassed, a pause must occur before the level drops and another injection can have a reinforcing effect.

(2) *The level of interoceptive drug effect prior to an injection signals whether the injection will be reinforcing.* It is well established that drugs of abuse can have dose-dependent discriminative-stimulus effects. These effects may be reliable predictors of whether the current drug level is above or below threshold. According to this account, the animal learns to cease responding when the interoceptive drug effect signals that another injection will not be reinforcing, then to respond again when the effect has dropped to a sufficiently low level. (Note that this interoceptive signal must be predictive of whether the reinforcing effect is already maximal, but does not necessarily involve direct sensing of the state of the reinforcement substrate.) Although this potential role of stimulus control has not been recognized previously, this hypothesis is consistent with evidence that the self-administration response tends to occur when the level of drug effect drops to a specific point (Ahmed and Koob, 1998, 1999, 2005; Panlilio et al., 2003; Ranaldi et al., 1999; Tsibulsky and Norman, 1999; Yokel and Pickens, 1974).

This stimulus-control account of regulated drug intake proposes that, even though drug reinforcers may be unique in their ability to rapidly and directly saturate the reinforcement substrate, they still affect behavior following the same basic principles as non-drug reinforcers. Therefore, to evaluate the hypothesis that contingencies of reinforcement inherent to drugs are responsible for the distinctive

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patterns of responding that typically occur during self-administration, we emulated these contingencies using a schedule of food reinforcement in rats (Panlilio et al., 2008). A visual stimulus (light) was used to model the interoceptive drug effect. Nose-poke responding produced food pellets only when the light was present. Even though no actual drug was delivered at any time, each response was recorded as producing a "virtual" drug injection. Based on pharmacokinetic principles, the virtual drug level was tracked throughout the session, and the light was presented only when the virtual drug level was below a designated threshold. Specific doses of cocaine and the short-acting opioid, remifentanil, were emulated.

This emulation schedule produced response patterns that were quite similar to patterns of drug self-administration. Pause durations were consistent within sessions, and the average pause duration was a monotonic function of the virtual dose. When response patterns under the emulation schedule were quantified using a variety of measures, the results not only replicated the general profile of results obtained with drug self-administration, but for most measures gave reasonable approximations of the actual values obtained with cocaine and remifentanil by Panlilio et al. (2003).

These findings are consistent with the hypothesis that regular patterns of drug self-administration result from stimulus control. However, an alternative possibility is that the discrete discriminative stimulus (i.e., the interoceptive drug effect) is not a necessary determinant of this behavior. That is, responding might be controlled by the passage of time since the last response (differential reinforcement of inter-response times), or by the development of "mediating" behavior (Laties et al., 1969) that prevents responding between injections. This possibility was evaluated in the present study by modifying the emulation schedule so that there was no explicit discriminative stimulus. Responding only produced food while the virtual drug level was below the threshold, but there was no stimulus change to indicate whether the level was above or below the threshold. A comprehensive battery of tests was used to compare response patterns obtained with this schedule to those obtained earlier with actual drug self-administration (Panlilio et al., 2003) and with the emulation schedule when a visual discriminative stimulus was provided (Panlilio et al., 2008).

1. Method

1.1. Subjects

Twenty male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 380–425 g, were individually housed with free access to water. Food was restricted to approximately 15 g/day to maintain stable body weights. Lights in the cage room were on from 1800–0600 h (reversed light cycle), and experiments were conducted between 0900–1500 h. The facilities were fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care , and all procedures were approved by the Animal Care and Use Committee of the NIDA-IRP and followed the guidelines of the National Research Council (1996). Ten of the rats were experimentally-naive, and the other ten had been trained earlier as described by Panlilio et al. (2008).

1.2. Apparatus

Ten experimental chambers, each with two nose-poke holes and a food trough, were controlled by MED-Associates (St. Albans, VT) software. These were the same chambers used for cocaine and remifentanil self-administration by Panlilio et al. (2003). A green LED served as a houselight, and a shielded light bulb (type 1820, 24V) on the wall above the nose-poke holes served as the light stimulus.

1.3. Procedure

Experimental sessions were conducted 5 days/week for 2.5 h or until 100 pellets were delivered, whichever came first. Responses in the inactive nose-poke hole had no scheduled effect at any time. For the rats that were used only in the present study, all training was the same as in the study by Panlilio et al. (2008), except that a visual discriminative stimulus was used in that study but not in the present study. For these experimentally-naive rats, there were two training sessions prior to training with the emulation schedule. In these two sessions, responses in the active hole immediately produced a 45 mg food pellet (F0021; Bio-Serv, Frenchtown, NJ); the cue light remained on throughout the session, and responses had no scheduled effect if they followed a reinforced response by less than 5 s. Each rat received 100 pellets during both of these sessions.

Under the emulation schedule, no actual drug was given at any time. However, when a response occurred, the computer calculated what the drug level would have been if a drug injection had been delivered. If a response occurred while the virtual drug level was below the designated threshold, the light was extinguished for 5 s, and a food pellet was delivered 2.5 s into this period. This 2.5 s delay was intended to mimic the delivery of a drug injection over several seconds. Responses during the 5 s period while the light was off had no programmed effect and, consistent with the previous food and drug self-administration studies (Panlilio et al., 2003, 2008), were not considered in the response latency measure. If a response occurred while the virtual drug level was above the designated threshold, the light was not extinguished and food was not delivered, but the response did increase the virtual drug level and was followed by an unsignaled 5 s period in which further responses did not increase the virtual drug level. Thus, responding produced food as long as the virtual drug level was below the threshold; once the threshold was surpassed, responding could not be reinforced again until the level dropped below the threshold. These contingencies were the same in the previous emulation study (Panlilio et al., 2008), except that in the previous study the stimulus light was only presented while the virtual drug level was below the threshold, and a response in the presence of the light always produced a pellet.

Virtual drug levels were calculated based on pharmacokinetic principles using the equation, $B_n = (B_{n-1} + D) \cdot e^{-KT}$, where B_n = drug level at the current time, B_{n-1} = drug level at the time of the previous calculation, D = amount of drug delivered since the previous calculation, K = elimination rate constant (halflife/0.693), and T = time since the previous calculation. During each session, there was a fixed virtual dose of cocaine (30, 100, 300, or 1000 μg/kg/injection) or remifentanil (1,4,16, or 32 μg/kg/injection). Each rat was tested at four virtual doses of a drug in counterbalanced order, for four consecutive sessions at each dose. Mean latencies over the last three sessions of each virtual dose were within about 10% of each other for virtual remifentanil and about 15% of each other for virtual cocaine. The stimulus and no-stimulus conditions did not differ with respect to this consistency (p's>.33). All data presented below were taken from the last session under each virtual dose.

The halflife and threshold parameters used for the emulation schedule were obtained through nonlinear regression based on the mean pause duration (latency) at each unit dose in previous drug self-administration studies (for details, see Tsibulsky and Norman, 1999). During initial training with the emulation schedule with no discriminative stimulus, the 10 naive rats of the present study were trained with cocaine parameters (halflife = 492 s; threshold = 1720 μg/kg) reported by Tsibulsky and Norman (1999). Then, these rats were divided into two groups of 5; one group was trained with cocaine parameters (halflife = 762 s; threshold = 1190 μg/kg) and one with remifentanil parameters (halflife = 42 s; threshold = 1.01 μg/kg) reported by Panlilio et al. (2003). The other 10 rats had been divided into two groups of 5 and trained with these cocaine or remifentanil

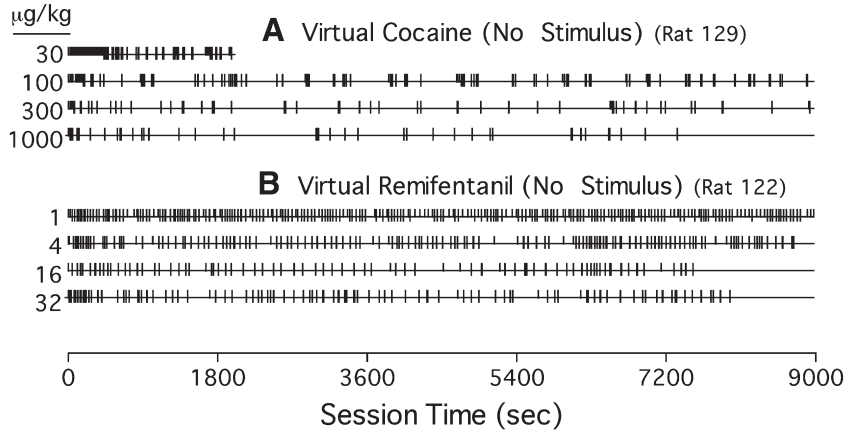

Fig. 1. Representative event records for (A) Rat #129 under virtual cocaine parameters and (B) Rat #122 under virtual remifentanil parameters with the emulation schedule with no added stimulus. Each horizontal timeline represents the record for a complete session. Each vertical mark above the horizontal line represents one response that was counted as a virtual drug injection. Each vertical mark extending both above and below the line indicates that the response was counted as a virtual injection but did not produce a food pellet. At the lowest virtual dose of cocaine, the session was ended when 100 food pellets had been delivered.

parameters in the previous study (Panlilio et al., 2008) using the emulation schedule with a discriminative stimulus; for these rats, the same virtual doses were re-tested in the present study without the discriminative stimulus. Since the major features of behavior under the emulation schedule were similar under the initial Tsibulsky and Norman (1999) training parameters and the Panlilio et al. (2003) cocaine parameters, the initial training data are not presented here. Under these parameters, if a response occurred at the threshold, the minimum number of seconds that had to pass before the next response could be reinforced was 28, 90, 248, or 672 s for virtual cocaine doses of 30, 100, 300, or 1000 μg/kg, respectively, and 43, 99, 173, or 213 s for virtual remifentanil doses of 1, 4, 16, or 32 μg/kg.

Each rat was also tested with a variable-dose version of the emulation schedule. The variable-dose schedules were the same as the fixed-dose schedules described above, except that instead of each virtual injection in the session being the same dose, each of the four virtual doses was made available in pseudo-random order within the session. That is, each time a response occurred, a dose was chosen without replacement from a list in which each dose appeared twice, and this dose was used to calculate the virtual drug level. The list was replenished as many times as necessary during the 2.5 h session. Rats were trained on this schedule for 8 sessions, and data were analyzed for the final session. The ten rats that had originally been trained with the fixed-dose emulation schedule with the visual stimulus in the previous study were tested with the variable-dose emulation schedule with the visual stimulus, prior to training with the no-stimulus, fixed-dose procedure described above. One rat in this group was dropped from the study during this phase due to an equipment malfunction. The other ten rats, which were naive before being trained with the fixed-dose emulation schedule without the stimulus in the present study, were trained with the variable-dose schedule without the stimulus; this was done after the completion of their training with the no-stimulus, fixed-dose procedure described above.

1.4. Data analysis

The presentation and analysis of the data in Figs. 1–6 of the present study parallel those of the same-numbered figures in both the actual

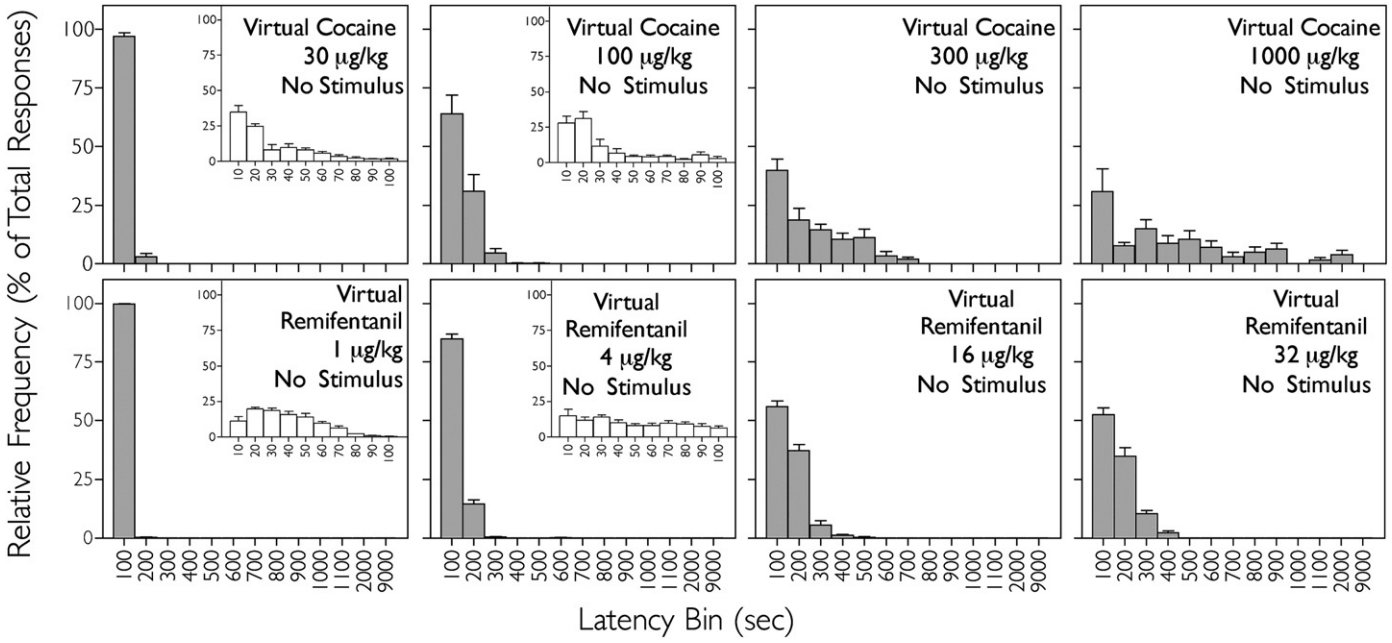

Fig. 2. Frequency distributions of response latencies for virtual cocaine (upper row) and virtual remifentanil (lower row) under the emulation schedule with no added stimulus.

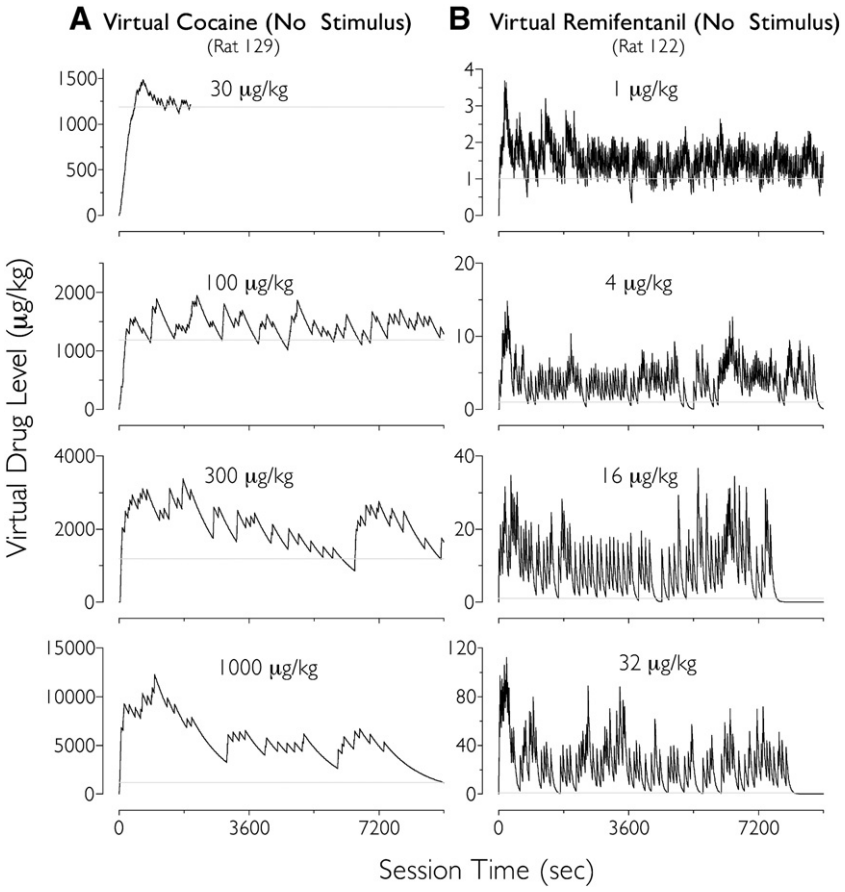

Fig. 3. Virtual drug levels during whole sessions with (A) cocaine and (B) remifentanil parameters under the emulation schedule with no added stimulus. Data are from the same sessions shown in Fig. 1. Horizontal lines represent value of threshold. At the lowest virtual dose of cocaine, the session was ended when 100 food pellets had been delivered.

drug self-administration study by Panlilio et al. (2003) and the study by Panlilio et al. (2008) using the emulation schedule with a discriminative stimulus. Fig. 7 of the present study also parallels Fig. 7 of Panlilio et al. (2008). For comparison between responding under the emulation schedule with versus without the added visual stimulus, some data from the study by Panlilio et al. (2008) are included here in Figs. 4–7. All measures in Figs. 2–7 (except those in Fig. 7b and c) were based on virtual injections without regard to whether a food pellet was delivered. As in both previous studies (Panlilio et al., 2003, 2008), there was a brief "loading" phase during which virtual drug levels steadily increased and virtual injections were spaced more closely in time than in the remainder of the session. Therefore, data from the first 500 s of the session were not included in the analyses or figures, except for Figs. 1 and 3, which represent whole sessions, and Fig. 7B and C, which represent responding at the beginning of the session.

Data in Figs. 4–8 were analyzed using Proc Mixed (SAS Institute, Cary, N.C.), with stimulus condition (added stimulus vs. no added stimulus) and dose as factors. Separate analyses were performed for virtual cocaine and virtual remifentanil. Planned comparisons were performed using the Tukey–Kramer procedure, maintaining a significance level of .05 within each set of tests. These comparisons were (1) within conditions, comparing all pairs of virtual doses within the no-stimulus emulation condition for each virtual drug, to assess dose-dependency of the behavior; and (2) between conditions, comparing the stimulus and no-stimulus conditions for each virtual dose, to assess the effects of the stimulus. Outcomes of these statistical tests are presented in the figure captions for within-condition comparisons and as asterisks in the figures for the between-conditions comparisons. Within-condition statistical comparisons assessing dose-dependence under the emulation schedule with the added stimulus were already presented by Panlilio et al. (2008) and are not repeated here. For all measures, data for the emulation schedule with no added stimulus showed no significant differences between the group trained with this schedule originally versus those trained with the stimulus before being switched to the no-stimulus schedule; therefore, to simplify the presentation, the data for these two no-stimulus groups were combined.

In Table 1, to compare data obtained with the stimulus and no-stimulus versions of the emulation schedule to data obtained with actual drug self-administration, simultaneous confidence intervals were calculated for the difference between each measure under the emulation schedule and the mean value obtained with actual drug self-administration by Panlilio et al. (2003); differences were considered significant if the interval did not include zero. For further discussion of the root mean square of successive deviations (rMSSD, a measure of within-subject variability), autocorrelation (a measure of how each latency is affected by the preceding latency), and whole-body drug level measures, see Panlilio et al. (2003). All figures that show group data depict mean ± s.e.m., but in many cases the error bars are covered by the symbol.

2. Results

Visual inspection of the event records (Fig. 1) of individual rats under the emulation schedule without a discriminative stimulus indicated that the response patterns were not as regular as those seen with actual drug self-administration (Panlilio et al., 2003) or with the emulation schedule with a discriminative stimulus (Panlilio et al., 2008). Although responding was dose-dependent under these conditions, with fewer responses occurring at higher virtual doses, at all virtual doses there were large numbers of "early" responses that

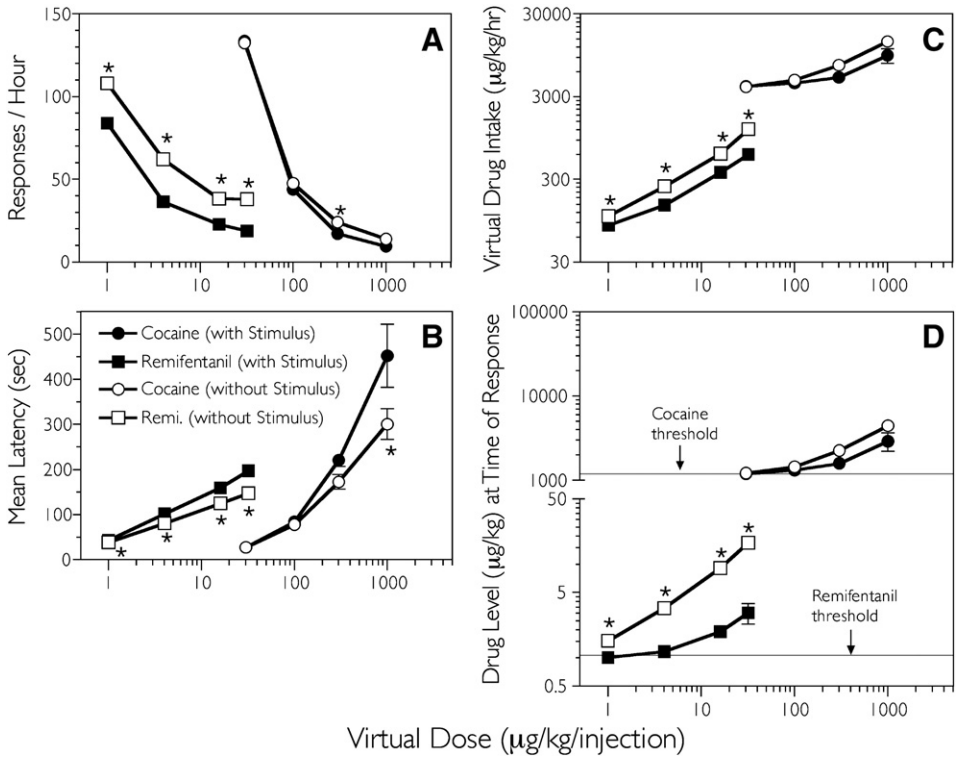

Fig. 4. Dose-effect functions for virtual cocaine and virtual remifentanil without the stimulus (open circles and open squares, respectively) and with the stimulus (filled circles and filled squares, respectively). Asterisks indicate doses where the stimulus and no-stimulus conditions were significantly different ($P<.05$). A: Response rates. Within the no-stimulus condition for each drug, all pairs of doses (μg/kg) differed significantly from each other except 16 vs. 32 for remifentanil. B: Response latencies. Within the no-stimulus condition for each drug, all pairs of doses were significantly different from each other except 30 vs. 100, 100 vs. 300, and 300 vs. 1000 for virtual cocaine and 16 vs. 32 for virtual remifentanil. C: Drug intake. Within the no-stimulus condition for each drug, all pairs of doses were significantly different from each other except 30 vs. 100 and 100 vs. 300 for virtual cocaine. D: Mean drug level at the time of response. Horizontal lines represent thresholds. Within the no-stimulus condition for each drug, all pairs of doses were significantly different from each other except 30 vs. 100, 30 vs. 300, and 100 vs. 300 for virtual cocaine.

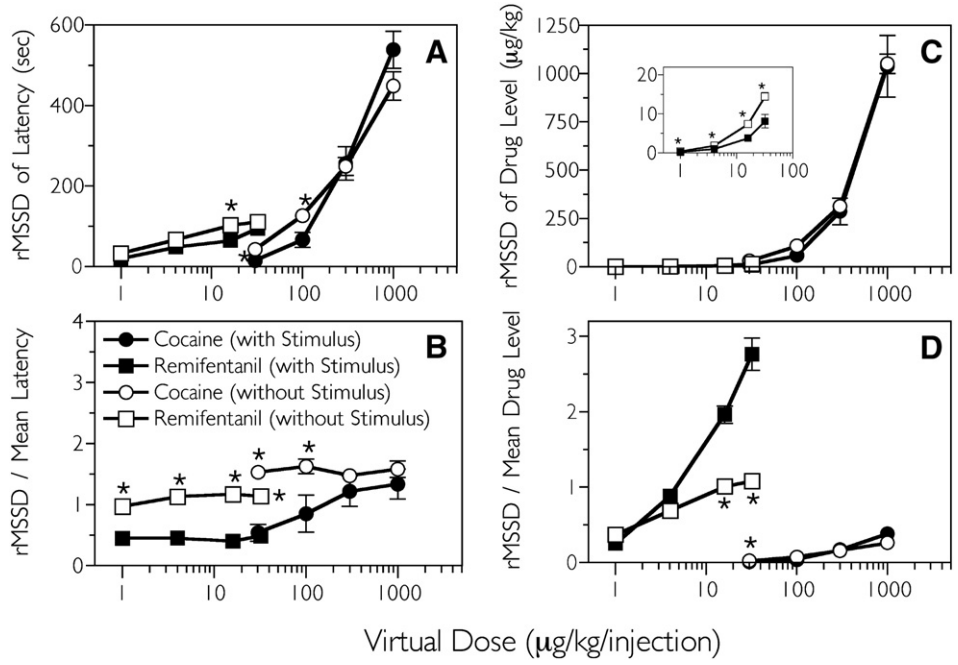

Fig. 5. Within-subject measures of variability as a function of dose of virtual cocaine and virtual remifentanil without the stimulus (open circles and open squares, respectively) and with the stimulus (filled circles and filled squares, respectively). Asterisks indicate doses where the stimulus and no-stimulus conditions were significantly different ($P<.05$). A: Absolute within-subject variability (rMSSD) of latencies. Within the no-stimulus condition for each drug, all pairs of doses (μg/kg) were significantly different from each other except 16 vs. 32 for virtual remifentanil. B: Relative within-subject variability of latencies (rMSSD of latencies divided by mean latency). Within the no-stimulus condition for each drug, none of the pairs were significantly different from each other. C: Absolute within-subject variability (rMSSD) of drug level (μg/kg) at the time of response. Inset shows results for remifentanil with the y-axis expanded. Within the no-stimulus condition for each drug, all pairs of points were significantly different from each other except 30 vs. 100 for virtual cocaine. D: Relative within-subject variability (rMSSD/mean) of drug level at the time of response. Within the no-stimulus condition for each drug, all pairs of points were significantly different from each other except 16 vs. 32 for virtual remifentanil.

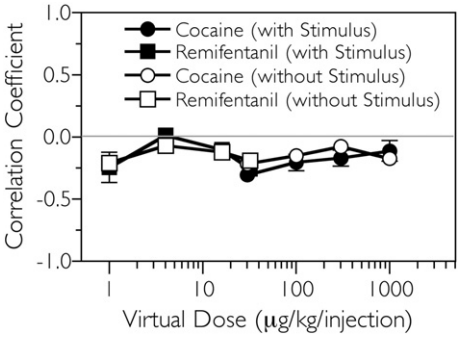

Fig. 6. Autocorrelation of sequential latencies for virtual cocaine and virtual remifentanil without the stimulus (open circles and open squares, respectively) and with the stimulus (filled circles and filled squares, respectively). Within the no-stimulus conditions, these values were significantly less than zero for virtual doses (μg/kg) of 30 and 100 for virtual cocaine, but not significantly different from zero for the other doses of cocaine or any doses for virtual remifentanil.

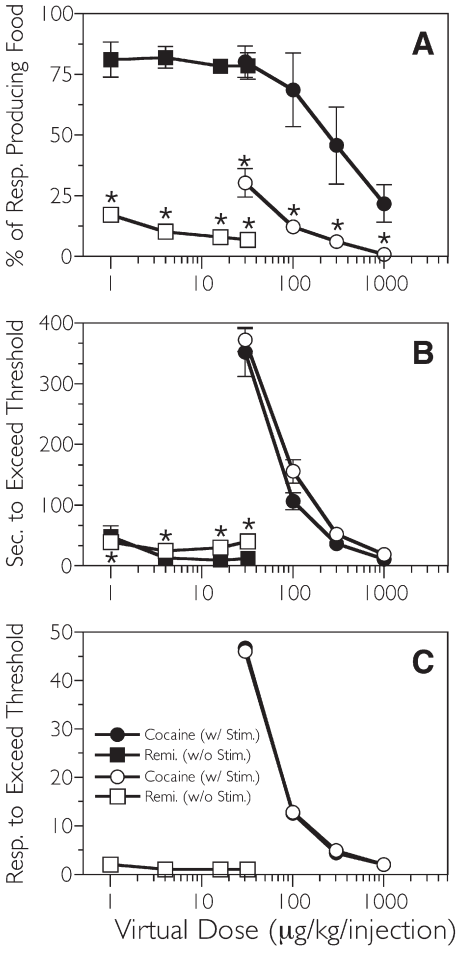

Fig. 7. A. Percentage of responses that produced a food pellet, as a function of virtual dose (μg/kg) of virtual cocaine and virtual remifentanil without the stimulus (open circles and open squares, respectively) and with the stimulus (filled circles and filled squares, respectively). Asterisks indicate doses where the stimulus and no-stimulus conditions were significantly different ($P<.05$). Within the no-stimulus condition for virtual cocaine, all pairs of points were significantly different from each other except 30 vs. 100. This measure did not differ significantly within the no-stimulus condition for virtual remifentanil. B. Number of seconds before the threshold was first exceeded in the session. Within the no-stimulus condition for virtual cocaine, all pairs of points were significantly different from each other except 300 vs. 1000. This measure did not differ significantly within the no-stimulus condition for virtual remifentanil. C. Number of responses before the threshold was first exceeded in the session. Within the virtual cocaine condition, all pairs were significantly different from each other. This measure did not differ significantly within the no-stimulus condition for virtual remifentanil.

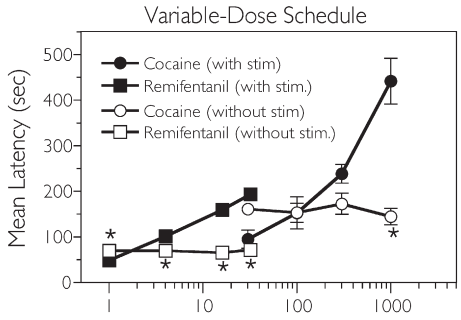

Fig. 8. Response latencies under the variable-dose schedule as a function of dose (μg/kg) of virtual cocaine and virtual remifentanil without the stimulus (open circles and open squares, respectively) and with the stimulus (filled circles and filled squares, respectively). Within the added-stimulus conditions, latencies under all virtual doses were significantly different from each other ($p<.0001$) except for .03 vs .1 and .1 vs .3 within the virtual cocaine schedule. In the no–stimulus conditions, none of the virtual doses produced latencies that differed from each other within the curve for either drug (all P's$<.59$).

did not produce food (indicated by "pip" marks that extend both above and below the horizontal timeline in Fig. 1).

In the previous studies, under both actual drug self-administration (Panlilio et al., 2003) and the emulation schedule with an added stimulus (Panlilio et al., 2008), frequency distributions of the latencies between responses had showed a well-defined peak at the lower virtual doses and a less-defined peak, progressively shifted to the right, at the higher virtual doses. In contrast, under the emulation schedule without a discriminative stimulus (Fig. 2), the range of latencies was greater at higher virtual doses of both cocaine and remifentanil, but the peak did not shift; that is, the modal response latency was shorter than 100 s at all virtual doses without the stimulus.

When the emulation schedule did include a discriminative stimulus (Panlilio et al., 2008), individual records of virtual drug levels throughout the session had showed that once the threshold was surpassed and the cue light was turned back on, most virtual injections were followed by a pause that lasted until the drug level returned to the threshold. Thus, those records looked similar to the records obtained with actual drug self-administration (Panlilio et al., 2003), in which drug levels had repeatedly dropped to about the same level over the course of the session. In contrast, individual records of virtual drug levels in the present study (Fig. 3) did not show a consistent level at which responding occurred. Instead, there were often series of responses that occurred while the level was well above the threshold, and at other times there were extended pauses that caused the level to fall below the threshold.

For the various measures of responding and virtual drug levels depicted in Fig. 4, we found earlier (Panlilio et al., 2008) that the emulation schedule with an added visual stimulus produced results that were quite similar to those obtained with actual drug self-administration. Under the no-stimulus emulation schedule of the present study, the measures in Fig. 4 were still dose dependent, and the general profiles still resembled the basic shape of actual drug curves. However, it is clear that the stimulus had profound effects on the response patterns. As described in detail below, all of the values obtained under the no-stimulus condition in Fig. 4 were significantly different from the added-stimulus condition for virtual remifentanil. The stimulus had qualitatively similar effects on responding under the virtual cocaine schedule, although fewer of these changes were significant.

When the visual discriminative stimulus was provided, dose-response functions (Fig. 4A) for virtual cocaine and remifentanil under the emulation schedule resembled the descending limb of the inverted U-shaped dose-response functions typically obtained with actual drug self-administration. When the stimulus was not provided,

Table 1
Comparison of data obtained with the emulation schedule vs. with actual drug self-administration.

	Cocaine								Remifentanil							
	With stimulus				Without stimulus				With stimulus				Without stimulus			
	30	100	300	1000	30	100	300	1000	1	4	16	32	1	4	16	32
Response rate	↑			↑	↑	↑		↑	↑		↑		↑	↑	↑	↑
Latency	↓	↓			↓	↓	↓	↓			↑		↓	↓	↓	↓
Intake	↑				↑	↑		↑			↑	↑	↑	↑	↑	↑
Drug level					↑	↓		↑					↑	↑	↑	↑
Resp. variability	↓	↓	↓	↓	↓		↑	↑	↓	↓	↓	↓	↓	↑	↑	↑
Drug level var.					↓	↓		↑		↓			↓	↓	↓	↓
Autocorrelation								↓				↓			↑	

Arrows indicate measures where the difference between the values obtained with the emulation schedule were significantly ($p<.05$) greater (↑) or less (↓) than the mean obtained by Panlilio et al. (2003) with actual drug self-administration under the same nominal dose. Numerals indicate dose (μg/kg). "Drug level" refers to the drug level at the time of response. Variability ("Var.") of response latencies ("Resp.") and drug level at the time of response were measured as root mean square of successive deviations (rMSSD) divided by the mean latency or drug level, respectively.

the virtual remifentanil curve was shifted upward, with significantly higher rates of responding at all virtual doses, but the shape of the curve was maintained. The virtual cocaine dose-response curve was also shifted upward when no stimulus was provided, but only slightly. Consistent with the changes in response rate, which are inverse to latencies, the dose-latency curves (Fig. 4B) for virtual remifentanil showed significantly shorter latencies at all virtual doses when the stimulus was not provided. Latencies for virtual cocaine were shorter at the two highest virtual doses when no stimulus was provided, and this was significant at the highest virtual dose.

The rate of virtual drug intake (Fig. 4C) and the mean virtual drug level at the time of response ("observed threshold"; Fig. 4D) also showed differences between the stimulus and no-stimulus conditions. Values for both of these measures were higher when no stimulus was provided. These changes were significant at every dose for virtual remifentanil but did not reach significance for virtual cocaine. We reported earlier that, under the emulation schedule with an added stimulus, the observed threshold was greater than the actual threshold due to "early" responding (Panlilio et al., 2008). In the present study without the stimulus, this discrepancy between the actual and observed threshold was even greater.

Within-subject variability of response latencies was used as a formal measure of the regularity of response patterns. For absolute levels of variability in latencies (Fig. 5A), the general shape of the curve was essentially unaffected by the stimulus. But, more importantly, when this variability measure was scaled by the mean latency to allow direct comparisons between conditions (Fig. 5B), the relative variability of latencies was much higher without the stimulus for virtual remifentanil and the two lowest doses of virtual cocaine.

Within-subject variability of virtual drug levels at the time of response was analyzed to assess the consistency with which responding was controlled by the virtual drug level. Absolute variability of this measure (Fig. 5C) increased monotonically as a function of dose for virtual cocaine and virtual remifentanil both with and without the added stimulus. However, when scaled by the mean to allow comparisons between conditions with remifentanil (Fig. 5D), this variability was less dose dependent when no stimulus was provided. This reduced sensitivity to the virtual drug level when no stimulus was provided is consistent with the failure to effectively regulate virtual remifentanil levels, as seen in Fig. 4D, where the mean virtual drug level at the time of response was 9 and 17 times greater than the threshold for the two highest virtual doses, respectively.

Autocorrelations (Fig. 6) were performed on sequential latencies to determine whether the latency of a given response was affected by the latency of the previous response. This measure was used to determine whether sequential latencies were adjusted in a way that compensated for deviation of previous latencies from the average; this would be indicated by a negative correlation. As with actual drug self-administration and the emulation schedule with an added stimulus, the correlations for cocaine and remifentanil were near zero or slightly negative. Unlike food-reinforced responding under a continuous-reinforcement, non-emulation schedule (Panlilio et al., 2003), none of the correlations in Fig. 6 were significantly positive.

Under the no-stimulus condition, at all virtual doses for both drugs, the majority of responses occurred above the threshold and therefore failed to produce a food pellet (Fig. 7A). Above-threshold responding was much less prevalent when the stimulus was provided, although the percentage of above-threshold responses did increase at the higher doses of virtual cocaine (when longer pauses were required between reinforced responses). The amount of time to first exceed the threshold within the session (Fig. 7B) was slightly increased for the three highest virtual doses of remifentanil when no stimulus was provided. For these virtual remifentanil doses, which were all higher than the threshold, this measure equates to the amount of time before the first response. For virtual cocaine, where all virtual doses were lower than the threshold, the amount of time and responses required to first exceed the threshold increased as a function of virtual dose and did not differ between the stimulus and no-stimulus conditions. The number of responses to first exceed the threshold (Fig. 7C) did not differ between the stimulus and no-stimulus conditions for either drug.

Table 1 provides a summary and assessment of how closely the data in Figs. 4–6 coincide with those obtained with actual drug self-administration. For virtual cocaine with the added stimulus, 67.9% of the dose-measure combinations in Table 1 successfully approximated the value obtained with actual cocaine; without the stimulus, this success rate dropped to 28.6%. For virtual remifentanil, these values were 60.7% and 10.7% for the stimulus and no-stimulus conditions, respectively. These differences between the stimulus and no-stimulus conditions reflect the facts that, when no stimulus was provided: (1) response rates were higher, and therefore latencies were shorter, than with actual drugs; (2) many responses occurred when the virtual drug level was not near the threshold; and (3) response patterns were more variable. Although the variability of responding differed from actual drug self-administration for both the stimulus and no-stimulus conditions of the emulation schedule, it is important to note that the added-stimulus conditions tended to produce response patterns that were less variable than actual drug self-administration, while the no-stimulus conditions tended to produce response patterns that were more variable than actual drug self-administration.

Under the variable-dose versions of the emulation schedule for both virtual cocaine and virtual remifentanil (Fig. 8), response latencies in the added-stimulus condition increased monotonically as a function of virtual dose and were comparable to those obtained with the fixed-dose added-stimulus schedule (Fig. 4B). In contrast, when the variable-dose schedule was implemented without the visual stimulus, the latency functions were flat (i.e., not dose-dependent) for both virtual cocaine and virtual remifentanil. Under the variable-dose

schedule with a discriminative stimulus provided, the mean (±s.e.m.) percentages of responses that produced a food pellet were 67.2±6.2 for cocaine and 87.1±1.5 for remifentanil; with no added stimulus, these percentages were much lower, at 8.4±2.3 and 7.0±1.2 respectively.

3. Discussion

The schedule of food reinforcement studied here was designed to emulate the underlying contingencies that might be inherent in drug self-administration. The goal of this approach was to test the hypothesis that such contingencies are capable of producing the patterns that have so often been observed with actual drugs. Although these experiments do not provide direct proof for this mechanism, they provide "proof of concept." In our previous study with this emulation schedule (Panlilio et al., 2008), reinforcement was differentially associated with a visual stimulus that was intended to model the interoceptive effects of a drug that signal when the drug level is already so high that additional drug would not have a reinforcing effect. That schedule produced patterns of operant responding that were similar to those typically observed with drug self-administration. In the present study, the same basic schedule was implemented without the visual stimulus, to assess the possibility that the schedule of intermittent reinforcement might be sufficient to produce these response patterns.

With or without the stimulus, the emulation schedule produced monotonically descending dose-response curves, with the same general shape as the descending limb of the inverted-U shaped curves typically obtained with drug self-administration. However, when the stimulus was provided, these curves more closely approximated the actual values of the curves obtained with drug self-administration. Furthermore, the modal latency was only dose-dependent when the stimulus was provided. When no stimulus was provided, the response patterns were more variable than actual drug self-administration, rates of virtual drug intake were higher, and most responses occurred while the virtual drug level was already above the threshold. Although the increase in virtual cocaine intake did not reach statistical significance, the general upward shift at the higher virtual doses was similar to that seen with virtual remifentanil. Thus, without the added stimulus, the emulation schedule failed to produce the highly-regular, dose-dependent response patterns and precise titration of drug levels that are seen with actual drug self-administration. These findings suggest that stimulus control might contribute to the development of this behavior in both the emulation schedule and actual drug self-administration.

The added-stimulus version of the emulation schedule appears to provide a reasonable model of the contingencies of reinforcement inherent in drug self-administration. Although the response patterns were even more consistent under this schedule than under actual drug self-administration, this discrepancy is quantitative rather than qualitative. There are several potential reasons for this difference, and they suggest ways that the parameters of the schedule might be refined. One possibility is that fluctuations in the effect level of actual drugs are less regular than described by the idealized formula used to calculate virtual drug levels. Another possibility is that the on vs. off state of the visual stimulus may be more discriminable than the above-threshold vs. below-threshold state of interoceptive drug effects. As we discussed in our earlier paper (Panlilio et al., 2008), using a binary visual stimulus to emulate interoceptive drug effects is justifiable because, even though drug levels vary continuously, there is evidence that different drug levels can produce qualitatively different interoceptive effects (Colpaert, 1991, 1999). Nonetheless, modifying the discriminability of the light might make it a more accurate analog of the interoceptive drug effect. It should also be mentioned that there are known aspects of drug self-administration that are not incorporated into the emulation schedule (see Panlilio et al., 2008), such as: (1) the reduced reinforcing efficacy of low doses, which may be responsible for the ascending limb of inverted U-shaped dose-response curves; and (2) the unconditioned effects of intravenous delivery of high drug doses, which may disrupt ongoing operant behavior immediately after the injection.

Variable-dose schedules of drug self-administration (Gerber and Wise, 1989; Panlilio et al., 2006, 2007; Panlilio and Schindler, 2000) provide some of the most convincing evidence that post-injection pausing is highly sensitive to the amount of drug currently in the system. Under the variable-dose version of the emulation schedule with a stimulus provided, response latencies were dose-dependent and about the same as the latencies produced by the same subjects under fixed-dose conditions. However, responding was not dose-dependent under the variable-dose emulation schedule when no stimulus was provided. In a previous study of variable-dose cocaine self-administration in squirrel monkeys, we found that, after extensive exposure to the cocaine schedule (hundreds of sessions), response latencies became insensitive to dose (Panlilio et al., 2006). In fact, these "dysregulated" dose-latency curves in monkeys were quite similar to the flat curves obtained in the present study under the no-stimulus variable-dose schedule, where latencies were short regardless of the dose.

The phenomenon of highly-regular drug self-administration is important in large part because addiction may represent a breakdown of the regulation process. It is well-established that extensive access to drugs can lead to dysregulation and escalation of drug intake (e.g., see Ahmed and Koob, 1998, 1999, 2005; Bozarth and Wise, 1985; Fitch and Roberts, 1993; Tornatzky and Miczek, 2000), and various accounts of this phenomenon have been proposed (see Zernig et al., 2007, for a review). The stimulus-control hypothesis proposed here suggests an additional account, that escalation may represent a failure of the interoceptive drug stimulus to control a cessation of responding when the threshold has been surpassed. In essence, self-administration in an addicted individual may be analogous to the behavior exhibited under the emulation schedule when no stimulus is provided. Intake is increased, but the functional rate of reinforcement is actually reduced. According to this analysis, escalated drug intake could be due to changes such as: (1) reduced intensity of or sensitivity to the interoceptive discriminative stimulus; (2) increased control by exteroceptive discriminative stimuli associated with drug availability; (3) increased control by response-contingent, exteroceptive, drug-paired conditioned reinforcers; (4) disruption of inhibitory control; (5) decreased sensitivity to nonreinforcement; or (6) a general decrease in sensitivity to the antecedents and/or consequences of the response as the response becomes more "habitual" or "compulsive."

4. Conclusion

In our earlier study of the variability of drug self-administration (Panlilio et al., 2003), we concluded that regular patterns of drug self-administration were not due to precise titration of drug levels in an automatic (i.e., unlearned) manner, but seemed to involve a less stringent process. Our subsequent work with the emulation schedule supports the hypothesis that this process is discrimination learning that is based on interoceptive effects of the drug. Highly-regular response patterns only developed under the emulation schedule when the availability of reinforcement was signaled. When no signal was provided, the pauses between responses were irregular, virtual drug intake was increased, and there was a dramatic increase in the incidence of responses that occurred when the virtual drug level was already above threshold. This profile of behavioral changes resembles the escalation in drug use that is considered a hallmark of addiction. These findings suggest that dysregulation of drug intake may be due to a failure of the interoceptive drug stimulus to control a cessation of responding when the level of drug effect is already maximal.

Drug reinforcers may be unique in their ability to rapidly saturate the substrates involved in reinforcement. Nonetheless, it appears that once this is taken into account, drug self-administration behavior can still be

described in terms of the same general principles that apply to non-drug reinforcers. Although further research with actual drug self-administration will be required to confirm that contingencies of reinforcement are responsible for highly-regular patterns of drug intake, the results obtained with the emulation schedule demonstrate that the stimulus-control account of regulated and dysregulated drug intake provides a plausible explanation for this ubiquitous phenomenon.

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