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Differential Effects of Ketamine On Schedule-Controlled Responding and Motility[1,2]

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MELISKA, C. J. AND A. J. TREVOR. *Differential effects of ketamine on schedule-controlled responding and motility.* PHARMAC. BIOCHEM. BEHAV. 8(6) 679–683, 1978. – Male Sprague-Dawley rats were trained to bar press for food reinforcement on an FI–300 sec schedule. Ketamine (7.5 mg/kg, IP) significantly increased response rates of both drug-naive and drug-experienced rats for the first 10 min after injection. With a 15.0 mg/kg dose of ketamine, response rates decreased significantly during the first 10 min after injection, irrespective of prior drug experience, but increased significantly above control thereafter in drug-experienced animals. Both doses of ketamine enhanced spontaneous locomotor activity significantly, irrespective of prior drug experience. Differences in the time course and dose dependency of these effects suggest that ketamine stimulates schedule-controlled responding and spontaneous locomotor activity via different neuropharmacologic mechanisms.

Ketamine Schedule-controlled responding Locomotor activity Prior drug experiencce

KETAMINE 2-(0-chlorophenyl)-2-(methylamino) cyclohexanone HCl is a potent anesthetic and analgetic agent which is similar to phencyclidine in its chemical and pharmacological properties [9]. Clinically, recovery from ketamine anesthesia is often associated with post-hypnotic sequalae, including hallucinations, restlessness, and psychomotor agitation [4,7]. In laboratory animals, these stimulant actions of ketamine are manifested by increased spontaneous locomotor activity [3, 11, 14].

Ketamine can also stimulate schedule-controlled behaviors. For example, the responding of pigeons during the FI–300 sec component of a multiple FI–FR schedule was increased by ketamine [19]. Similar effects were also observed in the mouse [20]. Because lower rates of responding were stimulated proportionally more than higher rates, it was concluded that ketamine had "amphetamine-like" actions. And although mice were tested repeatedly with doses of ketamine ranging from 1.0 to 180 mg/kg, IP, no evidence of response depression with the higher doses, nor of tolerance development as a consequence of repeated drugging was reported. By contrast, others [5,8] have observed that in rats, as little as 30 mg/kg, IP induced sleep, with tolerance to the hypnotic actions of the drug occurring after repeated administration of 60 mg/kg.

The present studies were designed to further explore the behavioral effects of ketamine in the laboratory rat. The time-course of the behavioral actions of the drug was followed in order to compare its effects on conditioned, schedule-controlled responding with its effects on spontaneous locomotor activity (motility). Also, in order to control for and statistically evaluate the effects of repeated exposure to the drug, a balanced, Latin Square order of administration was employed.

METHOD

Tests on Operant Responding

Animals. Nine male Sprague-Dawley rats, 80–90 days of age, were acquired from Simonsen Laboratories (Gilroy, California). They were partially fasted and gradually reduced to 80% of their ad lib body weights, and maintained on Purina Lab Chow under standard laboratory conditions with a 12 hr light/dark cycle, at a temperature of approximately 24°C. Body weights ranged from 180 to 205 g during testing.

Apparatus. A standard Skinner box (inside dimensions: 18 cm × 18 cm × 37 cm long) was enclosed in a ventilated, sound-attenuating chamber. Programmed apparatus dispensed 37 mg Noyes food pellets on an FI–300 sec schedule. Automatic counters provided for the accumulation of bar press frequencies, which were printed out, automatically, 300 sec after each reinforced bar press. Additionally, during the first 300 sec of testing, cumulative

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bar presses/60 sec were printed each minute, providing for close analysis of the initial effects of the drug. Reinforcement was given for the first press of each session, which lasted for 10 additional reinforcements (approximately 50 min). Rats were tested Mondays through Saturdays, between 0800 and 1700 hr, each rat being tested at about the same time each day.

Procedure. Bar pressing was shaped manually on a CRF (FR1) schedule, which was gradually shifted to the FI–300 sec schedule. Shaping of stable performance was carried out over a two month period.

Drug tests were carried out after response rates had become relatively stable. In preliminary experiments done with a group of similarly food-deprived rats, spontaneous locomotor activity was significantly increased at ketamine doses in the range of 8–20 mg/kg, IP. At the lower end of this dose range, activity was initially increased with a relatively rapid return to control levels in 10–15 min; by contrast, the higher doses produced intermediate levels of stimulation initially, with greatly increased stimulation beyond 20 min post-injection. For this reason, doses characteristic of these two behavioral patterns – 7.5 mg/kg and 15.0 mg/kg – were chosen for further investigation.

Ketamine hydrochloride (KETASET) was obtained from Bristol Laboratories and diluted with saline for injection. Drugs were administered, IP, in volumes of 1.0 ml/kg, immediately before placing rats into the Skinner box. All drug tests were separated by four days. As a control for the injection procedure, saline was injected on non-drug test days.

Design. In order to control for and evaluate effects of order of administration, drugs were given according to a Latin Square design, so that one third of the rats (N = 3) received each dose on each of the three drug test days. The particular Latin Square employed generated three orders of administration: (1) Saline, 7.5, 15.0; (2) 7.5, 15.0, Saline; and (3) 15.0, Saline, 7.5.

Tests on Spontaneous Locomotor Activity (Motility)

Animals. Fifteen male, Simonsen rats, 80–90 days of age at the start, were partially fasted to reduce body weights to 80% of ad lib, and were maintained for two weeks under the same conditions described above. Body weights ranged from 190 to 215 g during testing.

Apparatus. Locomotor activity was measured with a motility meter (Motron Produkter, Model Fc40, Stockholm, Sweden). The device contains a series of photocells, arranged beneath a translucent Plexiglas platform. Interruption of the light from a 100 W lamp, overhead, falling on any cell produced a digital output, which drove an electromechanical counter located in an adjacent room. Programmed circuitry provided for the automatic printing of these motility units (photocell interruptions) at 10 min intervals.

Procedure. Rats, fasted for 20 hr prior to testing, were transferred from their individual, wire cages to plastic containers, transported to the test room, and allowed approximately 30 min to acclimatize to their new surroundings. Animals were randomly assigned to one of three groups, based upon their first drug dose: Saline, 7.5 mg/kg, or 15.0 mg/kg ketamine.

An injection of drug or saline was administered, IP, exactly as in the operant responding test, and the rat was immediately placed into a 29 cm x 18 cm x 13 cm high Plexiglas container, which was positioned on the motility meter. After 10 min of testing, the first container was removed, a second rat was injected, and his container positioned for motility recording. The procedure was repeated for a third animal. After 10 min of testing, the third animal was set aside, and the first rat's container was returned to the motility meter. This procedure was repeated with the other two rats, so that each rat was tested three times, for 10 min each, at 0–10, 30–40, and 60–70 min post-injection – times which corresponded approximately to the intervals where the greatest changes in FI responding had occurred. Testing took place between 0800 and 1700 hr on three separate occasions, with drug tests separated by four days as in the operant responding test.

RESULTS

Ketamine and Conditioned, Schedule-Controlled Responding

Bar pressing rates during the first five consecutive minutes of testing were analyzed separately from subsequent responding, using a Latin Square Analysis of Variance (ANOVA). During this time period, the effect of Order of Administration approached, but did not reach statistical significance, $F(2,12) = 3.12$, $p<0.10$. And since all interactions with Order of Administration were small and also non-significant, scores were collapsed across the three administrations for further analyses. As Fig. 1 shows, the two different doses produced opposite effects during the first 5 min of testing, with differences between the doses and saline being highly significant, $F(2,12) = 32.40$, $p<0.001$. With a dose of 7.5 mg/kg, bar pressing increased to double the saline control value from minutes 3–5, while at 15.0 mg/kg, bar pressing was depressed significantly below control levels, being reduced to almost zero from minutes 3–5.

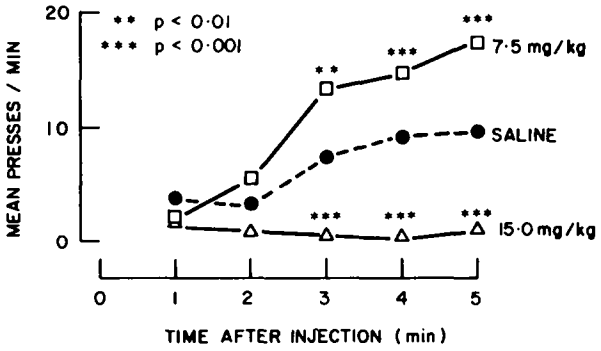

FIG. 1. Effects of two doses of ketamine on bar pressing rates during the first five consecutive minutes after drugging. Data points are based on means for N = 9 rats. The *p*-values refer to significance of differences from saline control.

To evaluate this drug/dosage effect across the entire 50 min session, a similar Latin Square ANOVA was performed. Raw response rates were transformed to difference scores, based on the difference between response rates made on the drug test and those of the previous (saline) control day, and analysed at five consecutive, 10-min intervals. This analysis revealed a significant inter-

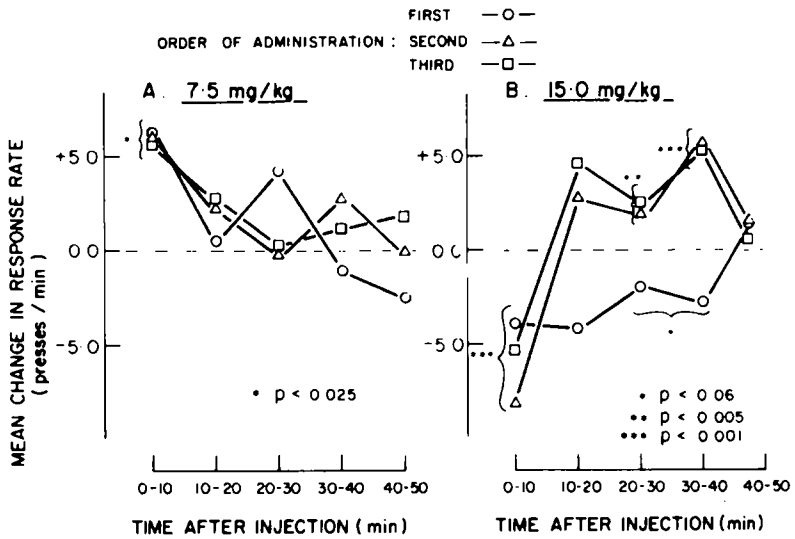

FIG. 2. Changes in bar pressing rates after (A) 7.5 mg/kg and (B) 15.0 mg/kg ketamine, at three different orders of drug administration: "FIRST" indicates no previous druggings; "SECOND" indicates that the 7.5 mg/kg dose was preceded by Saline, while the 15.0 mg/kg dose was preceded by 7.5 mg/kg; "THIRD" means that the 7.5 mg/kg dose was preceded by 15.0 mg/kg, then Saline, while the 15.0 mg/kg dose was preceded by Saline, then 7.5 mg/kg. Scores represent differences between drug day and previous day's Saline control. Data points are based on means for N = 3 rats. The p-values indicate significance of differences from a separate Saline control (not indicated in the figure). Brackets enclose statistically equivalent means.

action of Drug Dose, Test Interval, and Order of Administration, $F(8,48) = 2.27$, $p<0.05$. Further analyses suggested that rats which received 15.0 mg/kg ketamine as their first injection reacted differently from those which received 15.0 mg/kg on second or third injections. Reanalysis of the data using an ANOVA which tested the influence of previous drug "experience," rather than order of drug administration, confirmed a highly significant interaction of Drug Dose, Time Interval, and Prior Drug Experience, $F(4,28) = 5.56$, $p<0.001$ for the 15.0 mg/kg dose. This interaction is illustrated in Figs. 2A and 2B. With 7.5 mg/kg ketamine (Fig. 2A), response rates were significantly higher, $F(1,35) = 6.71$, $p<0.025$ during the first 10 min after injection, than with saline, irrespective of previous drug administration. Pressing rates returned to saline control levels between minutes 10 and 20, $F(1,35) = 0.03$, $p>0.05$.

Figure 2B shows that prior drug exposure had a significant effect on animals' response to 15.0 mg/kg of ketamine. At this dose, responding was reduced, $F(1,56) = 19.03$, $p<0.001$, during the first 10 min after injection, irrespective of previous drug administration. However, rats which had previously received 7.5 mg/kg ketamine resumed control rates of responding, $F(1,35) = 0.28$, $p>0.05$, during minutes 10–20, pressing at significantly higher rates, $F(1,35) = 16.05$, $p<0.001$, than drug-inexperienced animals. Thereafter, responding increased significantly above control in drug-experienced rats during minutes 20–30, $F(1,35) = 10.29$, $p<0.005$, and 30–40, $F(1,35) = 231.7$, $p<0.001$. During this time period, rats which had not received prior injections of ketamine continued to press at somewhat lower rates, the difference from saline being of borderline significance, $t(35) = 2.01$, $p<0.06$. Groups did not differ significantly, $F(1,35) = 0.13$, $p>0.05$, during minutes 40–50 post-injection.

Ketamine and Spontaneous Locomotor Activity

To evaluate the effects of prior drug experience on motility, scores per 10 min interval were analyzed using the same ANOVAs as employed above. As Fig. 3A shows, rats were significantly more active with 7.5 mg/kg ketamine than with saline during the first 10 min after injection, $F(1,39) = 120.46$, $p<0.001$, but returned to control levels by minutes 30–40, $F(1,39) = 0.46$, $p>0.05$. Prior drugging with 15.0 mg/kg of ketamine four days before the test with the 7.5 mg/kg dose did not affect motility significantly, $F(1,13) = 0.11$, $p>0.05$. A separate Latin Square ANOVA and subsequent analyses indicated that 7.5 mg/kg ketamine increased motility significantly more than the 15.0 mg/kg dose, $t(72) = 2.74$, $p<0.01$, during the first 10 min after administration.

As Fig. 3B illustrates, the pattern of locomotor response to 15.0 mg/kg ketamine appears to be partly dependent upon earlier drug experience. Because the expected interaction of Drug, Time Interval, and Previous Drug Experience approached significance, $F(2,26) = 1.99$, $p<0.16$, it was deemed appropriate to perform post hoc analyses of simple effects on this interaction. These showed that drug-naive and drug-experienced groups did not differ significantly during the first 10 min of testing, $F(1,78) = 2.46$, $p>0.05$. However, while motility at 30–40 min after drug injection was elevated in both drug-naive, $F(1,39) = 14.97$, $p<0.001$ and drug-experienced groups, $F(1,39) = 5.05$, $p<0.05$, those which had previously received the 7.5 mg/kg dose of ketamine were significantly less motile, $F(1,78) = 4.83$, $p<0.05$, than those which had not been previously drugged. Apparently, prior exposure to ketamine reduced the "late" stimulation component of ketamine's effect on motility, without affecting the "early" component.

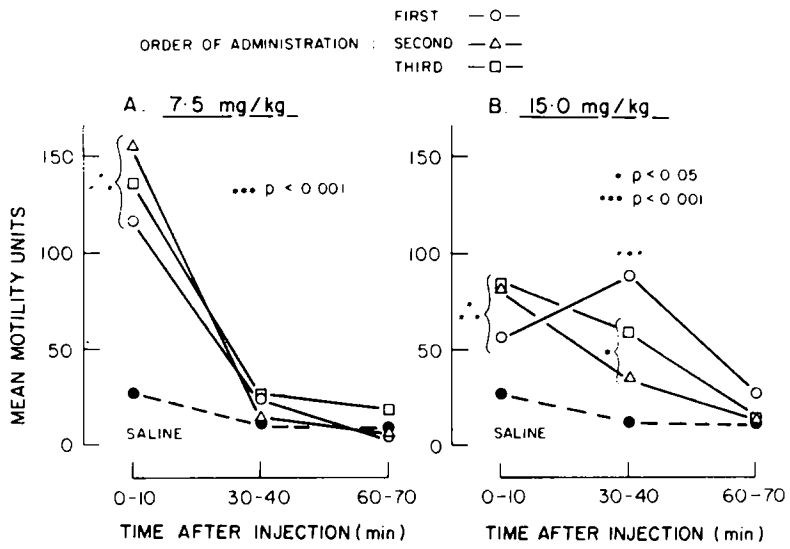

FIG. 3. Effects of (A) 7.5 mg/kg and (B) 15.0 mg/kg ketamine and three different orders of drug administration on spontaneous locomotor activity (motility). Orders of administration are the same as in Fig. 2. Data points are based on means for N = 5 rats. The *p*-values indicate significance of differences from appropriate Saline controls. The Saline mean for all N = 15 rats is indicated. Brackets enclose statistically equivalent means.

DISCUSSION

Stimulation of FI–300 sec response rates with ketamine has been reported previously in experiments with pigeons [19] and with mice [20]. The present study demonstrates a similar effect in Sprague-Dawley rats which is both time and dose dependent, and also influenced by prior drug exposure. A dose of 7.5 mg/kg ketamine increased FI response rate (Fig. 2A) for a period roughly coincident with increased spontaneous locomotor activity (Fig. 3A). However, with the 15 mg/kg dose FI responding was depressed (Fig. 2B) during the time when locomotor activity was increased significantly over control. These results suggest that there is no direct relationship between the effects of ketamine on motility and its effects on FI responding, and that any drug-induced stimulation of the latter response is not merely a sequalae of increased random locomotor activity. While it may be anticipated that neural mechanisms involved in the performance of coordinated behaviors required in bar pressing are more complex than mechanisms underlying spontaneous locomotor activity, the extent of the dissociation of the effects of ketamine on these two behaviors suggest that these mechanisms are quite dissimilar.

However, an alternative interpretation, similar to one offered to account for the dose dependency of some of amphetamine's actions [10], is also tenable. Specifically, with the lower ketamine dose, increased general activity – including random exploratory and manipulatory behaviors – could also be reflected in enhanced FI responding. But with higher doses, increased irrelevant responding would tend to intrude upon and interfere with the performance of the operant response, thereby reducing the FI rate. Furthermore, with time, recovery to a lower but still-somewhat-elevated level of activity could produce a period of enhanced FI responding, comparable to that produced initially by the lower ketamine dose. Such an interpretation could explain the initial depression, followed by enhancement of FI responding at 30–40 min, after drugging with 15.0 mg/kg ketamine, in drug-experienced rats. Nevertheless, this interpretation is flawed by its failure to adequately account for the fact that with 7.5 mg/kg, the period of maximal motility enhancement coincided with elevated, rather than depressed FI responding. Thus, changes in FI rate are not consistently related to changes in motility.

The effect of prior drug exposure on FI responding was also dose dependent. At the 7.5 mg/kg dose, FI response rate was not altered significantly by prior administration of the drug. When the dose was increased to 15.0 mg/kg, FI response rate was depressed initially (0–10 min) in both naive and drug-experienced animals, but the latter groups exhibited enhanced response rates at subsequent time intervals. Dose-dependent stimulation and depression of schedule-controlled behavior has been described widely in neuropsychopharmacologic research [15], and is quite common with anesthetic and central depressant drugs [1, 6, 17, 20]. The present study provides an example of depression of schedule-controlled behavior, followed by response rate enhancement, only in animals that have received prior exposure to the drug. The dependence of this effect on prior drug administration implies that adaptation or tolerance to this behavioral depressant action of ketamine occurred following only a single exposure to the drug, and with a dose (7.5 mg/kg) that in itself produced only behavioral stimulation. Such adaptation to the effect of the drug on FI response rate occurred only in the case of its rate-suppressing actions, since the rate-enhancing actions of a 7.5 mg/kg dose were not diminished by prior drug experience.

Other cases of changed responsiveness to ketamine following repeated administration have been reported. Among these are instances of reduced sleeping times in rats [5,8] and reductions in analgesia and in duration of ataxia [12] with hypnotic doses of ketamine. Changes in drug

metabolism, mediated by self-induction of hepatic microsomal enzyme systems [12,13] may account for some of these adaptation effects. However, the behavioral adaptations observed in the present study – which occurred following only a single exposure to 7.5 mg/kg of the drug – seem unlikely to involve enhancement of the rate of ketamine metabolism by self-induction of hepatic microsomal enzyme systems, since a single dose of 50 mg/kg has been shown to have no effect on hepatic metabolism of ketamine in the rat [2].

A plausible interpretation for the type of behavioral adaptation seen in the present study has been suggested [16,18]. Whenever an animal learns to perform a particular response, the presence or absence of some drug during training becomes one of the stimulus conditions for making that response. When the response is learned in the absence of some drug, that drug may later alter response vigor or frequency simply because its presence changes the stimulus characteristics of the test situation, rather than because the drug acts specifically on the motivation and/or learning of the animal. Therefore, when a drug depresses responding when first given, but not on subsequent administrations, the apparent increase in performance is not necessarily attributable to physiological tolerance development. Instead, the increment may actually derive from a reduction of the disruptive novelty of the drug resulting from prior exposure to it. Thus, the failure of 15.0 mg/kg ketamine to stimulate FI responding in drug-naive rats may simply reflect a non-specific disruption of performance due to the novelty associated with the first administration of the drug.

The experiments involving prior drug treatments also reveal a further apparent dissociation of the influence of ketamine on FI response rate compared with locomotor activity. At the 30–40 min time period, the increase in locomotor activity caused by a ketamine dose of 15.0 mg/kg was smaller in rats which had prior exposure to the drug than in naive animals. In this case, the reduction in motility can be interpreted as an adaptation (or tolerance) to a stimulatory action of ketamine, following a single exposure to the drug. However, this adaptation occurred only in conjunction with, or subsequent to, depression of other behavioral responses. It did not occur during the initial period (0–10 min) of locomotor stimulation caused by the drug (Fig. 3A); nor does this adaptation occur in the absence of a depression in FI response rate.

These data suggest the possibility of heterogeneity in the neuropharmacologic bases of ketamine's stimulatory effects. For example, the initial increase in locomotor activity after administration of 7.5 mg/kg (which is not subject to adaptation/tolerance) may represent direct actions of the parent drug. On the other hand, the behavioral stimulation following administration of a ketamine dose that depresses FI response rate (which is subject to adaptation/tolerance) may occur through different neuronal mechanisms. In view of the fact that the latter stimulation is delayed, occurring some 30–40 min after injection of the 15.0 mg/kg dose, this behavioral enhancement could reflect the actions of some late-appearing ketamine metabolite(s). Among these, the N-demethylated derivative ("Metabolite I") and/or its cyclohexenone oxidation product ("Metabolite II") are logical candidates [14].

This hypothesis would appear to be subject to a variety of empirical tests. For example, one could determine whether the onset of the late behavioral stimulation produced by ketamine was correlated with the appearance of particular metabolites in brain and/or plasma. A more direct test could be accomplished by assessing the effects of direct injection of metabolite(s) upon various behaviors. According to the hypothesis presented above, such treatment should produce behavioral stimulation without producing prior depression.

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