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BRIEF COMMUNICATION

A Silicone Pellet for Continuous Cocaine: Comparison With Continuous Amphetamine

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LIPTON, J., S. ZEIGLER, J. WILKINS AND G. ELLISON. *A silicone pellet for continuous cocaine: Comparison with continuous amphetamine*. PHARMACOL BIOCHEM BEHAV **38**(4) 927–930, 1991.—An inexpensive silicone pellet is described for the continuous administration of cocaine for up to 5 days. Rats implanted with this pellet show minimal skin irritation and go through distinct behavioral stages, with an initial period of hyperactivity followed by motor stereotypies. Then, at 3–4 days after implantation, a variety of hallucinogen-like ("late-stage") behaviors appear, including limb flicks, sudden startle responses, and repetitive mid-air grasping movements. Compared to continuous d-amphetamine, continuous cocaine induces decreased motor stereotypies but heightened "late-stage" behaviors.

Cocaine Amphetamine Drug regimen Psychosis Rats

AN established analog of paranoid schizophrenia is the psychosis associated with the chronic administration of d-amphetamine in humans (1, 2, 9, 21). The development of a slow-release silicone pellet for chronic amphetamine administration (11) enabled the study of the stages of continuous amphetamine administration in rats, for it allowed modeling of the 'speed runs' of amphetamine addicts by continuously releasing low levels of amphetamine for many days. A number of experiments (4, 5, 7, 8) showed how continuous exposure to low concentrations of d-amphetamine initially produced a greater behavioral response than a correspondingly low dose administered acutely to drug naive animals, and eventually led to a distinctive "late-stage" of hallucinogen-like behaviors accompanied by biochemical alterations in brain distinctly different from those following intermittent amphetamine (4, 6, 10).

Cocaine abuse has also been reported to lead to a paranoid psychosis, with psychotropic effects ranging from tactile sensations to intricate visual events (17, 22, 23). According to Manschreck et al. (13) the freebase form of cocaine can produce paranoid psychosis in humans. The strong reinforcing effect of freebase is a factor in its binge-like abuse pattern, which induces elevated levels of cocaine for as long as the addict continues to indulge (17,23).

Most studies concerned with the effects of chronic cocaine administration do not truly mimic the drug schedule of the freebase cocaine abuser. Injection regimens that are used to model chronic abuse of cocaine show wide fluctuations of serum cocaine levels over a 24-hour period (14), whereas freebase smokers continue to frequently self-administer drugs in order sustain euphoric effects (as well as to prevent dysphoric effects), and tend to show smaller plasma fluctuations than those seen in experimental paradigms (23). Therefore, an uninterrupted (i.e., continuous) administration may more closely characterize the intake patterns of an addict than an intermittent administration.

Attempts to administer continuous cocaine using subcutaneous osmotic mini-pumps have had mixed results due to the vasoconstrictive properties of cocaine at the release site, which can progressively alter absorption rates into the bloodstream and often induces a marked local tissue necrosis which further decreases serum levels of the drug. We have developed a silicone pellet for use in the chronic administration of cocaine freebase which shows none of the necrotic properties associated with the osmotic minipumps and thereby provides an easy, inexpensive method to study the effects of chronic cocaine administration.

METHOD

Subjects

Thirty male Sprague-Dawley CDF rats (250–300 g) were placed

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into individual transparent-front observation cages and allowed to habituate to the new environment for two days. The 12-h light/dark cycled room was equipped with black and red fluorescent fixtures which illuminated the cages during the dark cycle so observers could record behavioral data. Observers sat directly in front of the cages, but were not easily seen by the animals because of the low level illumination facing the cage fronts.

Pellet Design

The cocaine pellet was based on a modification of the previous amphetamine pellet design (11) and consisted of a 4 cm section of Silastic Medical Grade tubing (Dow Corning 601-405) filled with 80 mg of cocaine freebase (Sigma Pharmaceuticals) in a suspension of PEG capped on each end with medical grade silicone elastic polymer. Control pellets were identical to cocaine pellets but were filled exclusively with PEG. Pellet release was determined by high pressure liquid chromatography (HPLC) assay of 80 mg pellets implanted for various time periods and then removed ($n=4$ for days 0, 1, 3, and $n=8$ for 5 days). The amphetamine pellets used in the behavioral experiments release 20 mg of amphetamine base over the first five days [details in (11)].

Pellet Implantation and Removal

A general anesthetic (Halothane) was used to anesthetize the animal and a 15 mm incision was made on the back of the animal. The connective tissue under the skin was separated, forming a 5×1 cm cavity, and a pellet was inserted into the space, the wound was sutured and a topical antibiotic was applied. For the behavioral experiments, a second pellet was implanted by repeating this procedure on the contralateral flank. On day 6 pellets were removed from all groups under Halothane anaesthesia. A cut was made just adjacent to the old wound, topical anesthetic applied, the connective tissue was cut, pellet(s) removed, and the wound was then closed as described previously.

Behavioral Scoring

Each animal was observed twice daily for 5 min at 1 and 5 h after the onset of the dark cycle. During these periods behavioral data was recorded with the aid of a 10-s interval timer and frequency/duration sampling observation sheets. A frequency score was recorded every time an animal was observed making a behavioral response; duration scores were recorded (at 5- or 10-s intervals) for as long as the response occurred. Any behavior which lasted more than 10 seconds, or was bisected by a 10-second time interval was given another tally (i.e., if animal continually sniffs without stopping for 1 minute, it would receive 6 tallies: one under each 10-second interval of that minute). Observation sheets listed the eight behaviors which were reliable indicators (based on pretest observations) of the progression of symptoms seen in animals exposed to cocaine, amphetamine and LSD (limb-flicks and wet-dog shakes). The categories were: *Lying Motionless* (rat immobile and not supported by any limbs for at least 5 s); maximum number of tallies possible in a 5-min session was 60; *Stand and Rear* (animal standing on hind legs and rearing); scored every 5 s if continuous, or for each rear if it lasted for less than 5 s; *Cage Crossing* (animal's head and shoulders cross the midline of the cage); *Body Bite* (using teeth on body to groom or scratch); *Stereotyped Cage Biting* (chewing on metal floor incessantly for 10 s); *Sudden Startle* (a sudden jump, usually accompanied by vocalization, with no apparent external cue); *Limb Flick* (animal flicks fore-paws), and *Wet-Dog Shakes*. Other competing behaviors were noted on the observation sheets but were either too low in frequency to be quantified or were similar to those listed above and offered redundant information.

These behaviors have been shown previously to occur during the "late stage" of continuous amphetamines and in similar work involving cocaine in both monkeys (8,18) and rats (14). Two trained observers who were not informed of treatment conditions each watched the animals for one of the two observation periods during the day. Totals were averaged across observers each day, and a total of seven observation days were recorded, including one day of both predrug and postdrug.

In order to assess the degree to which declining pellet output vs. tolerance to the drug accounted for the appearance of "late-stage" behaviors in the cocaine animals, a second set of animals was habituated to the automated activity cages previously described (3) and then implanted with pellets. After 5 days the pellets were removed and, 12 h later, fresh pellets reimplanted. Activity measurements continued throughout the 7 days of this experiment.

TABLE 1

MEAN COCAINE SERUM LEVELS ($\pm$SEM, N=6) AND MEAN RELEASE RATE ($\pm$SEM, N=6) OF COCAINE PELLETS (TWO PER RAT) FOR DAYS 1, 3 AND 5 AFTER IMPLANTATION

	Day 1	Day 3	Day 5	Day 7
Serum levels ($\pm$SEM; ng/ml)	270 $\pm$ 33	369 $\pm$ 58	187 $\pm$ 18	0 $\pm$ 0
Amt released by pellets ($\pm$SEM; mg)	17 $\pm$ 2.2	56 $\pm$ 3.7	30 $\pm$ 3.2	N/A

The levels indicate that the pellets gradually increase their release through day 3 and slowly decline through day 5. Serum levels roughly correspond to the frequency of stereotypic behaviors (see Fig. 1).

RESULTS

Cocaine pellet assays indicated the mean release rate from two pellets of cocaine freebase $\pm$ SEM was 17 ± 2.2 mg in the first 24 h, 56 ± 3.7 mg over the next 48 h and 30 ± 3.2 mg in the final 48 h (see Table 1). Thus each rat received 103 mg (51.5 mg per pellet) over the five days, or about 65% of the total amount implanted. HPLC analysis of serum collected from animals 1, 3 and 5 days after implantation of cocaine pellets and 2 days after removal (i.e., day 7) indicated persisting cocaine levels over the 5- day administration (see Table 1).

As reported previously, the amphetamine group initially showed marked increases in stereotypy [stereotyped biting, $F(3)=3.246,128$, $p<0.05$ and cage-biting, $F(3,128)=5.786$, $p<0.005$, compared to controls; see Fig. 1]. This activity then declined and was not significantly different from controls during days 3 and 4, when the amphetamine animals exhibited the characteristics of the amphetamine "crash" stage. During this period virtually all behaviors were attenuated [except lying motionless, $F(3,128)=12.981$, $p<0.0001$, cf. Fig. 1]. Day 5 witnessed a recovery of some stereotypic behaviors (sniffing, cage biting) as well as a peak in frequency for standing and rearing. Because of the brevity of the observation periods, no significant "late-stage" behaviors were evident in the amphetamine group when compared with controls.

Increased cage crossing [$F(3,128)=28.643$, $p<0.0001$; two-tailed t-test vs. controls, $p<0.0001$, Fig. 1] was the first noticeable change in the behavior of the cocaine group on day 1, these peaked on day 3. Rearing also peaked on day 3 [$F(3,128)=$

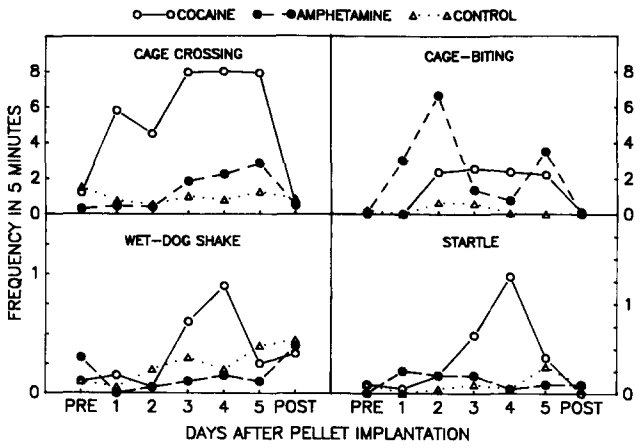

FIG. 1. Mean frequencies of two 5-minute trials per day over seven days (one pre- and one postimplantation). Increased locomotion is evident for cocaine animals (see cage crossing), whereas stereotypic behavior is more characteristic of amphetamine animals (see cage-biting). Late-stage behaviors (wet-dog shake, startle) are only evident in cocaine animals.

16.408, $p<0.0001$, t-test vs. controls $p<0.0001$]. Figure 1 shows that the cocaine group showed significant increases in spontaneous "startle" responses [$F(3,128)=4.511$, $p<0.01$; t-test, $p<0.006$] and wet-dog shakes [$F(3,128)=4.383$, $p<0.02$; t-test $p<0.03$] by day 4. On the final day of drug administration, the cocaine group showed a diminution in the frequency of "late-stage" responses with a return to stereotypic behaviors (sniffing, biting, rearing). Removal of cocaine pellets led to a rapid decline in all "late-stage" and stereotypic behaviors.

Controls showed very little fluctuation in the frequencies of their behaviors. The lying motionless category showed marked fluctuations because animals were found to be either sleeping or active on different days, with no discernable pattern. Controls also showed a systematic increase in body biting behaviors, most likely due to pellet wound irritation. Upon removal of the drug pellets, all behavior frequencies fell to control levels, or below, with the exception of lying motionless, which was much higher on day 6 for both drug groups when compared to controls.

The measurements in the activity cages indicated that in drug-naive animals, implantation of the cocaine pellets resulted in a gradual increase in activity levels. The cocaine pellet animals were significantly more active than the controls at 24 hr after pellet implantation [mean activity $\pm$ sem in arbitrary units described previously (3) for 2 preimplantation days was 1805 ± 463 as compared to 2908 ± 744 for the 1st day after implantation; Dunnett, $p<0.01$]. Activity levels peaked on Day 2 (4215 ± 726) and began to decline on subsequent days. However, upon reimplantation of fresh pellets on day 5, the cocaine animals showed no change in the gradual decline of their activity levels (day 4: 3378 ± 635; day 6: 3052 ± 734), and by day 9 (2477 ± 500) the activity of the animals was not significantly different from preadministration levels. Thus reimplantation with a second pellet produced much smaller increases in activity, indicating that some tolerance to the drug had developed.

DISCUSSION

The development of this inexpensive pellet for the chronic administration of cocaine offers a reliable mode of delivery while avoiding the local tissue necrosis, with its attendant alteration in release rates, often encountered with osmotic mini-pumps. Unlike daily injections, which typically induce a progressive sensitization of motor stereotypies, the cocaine pellet leads to gradual tolerance to the drug. Thus, with cocaine, as with amphetamine (15, 19, 20), the mode of delivery is a key factor in the production of hyperactivity, stereotypies and "late-stage" behaviors. The uninterrupted flow of cocaine from the pellets may mimic some critical aspects of the schedule of drug intake of chronic "binge" abusers better than do either repeated injections or minipumps. According to the HPLC analysis of the pellets it appears that during the first 3 days there is a gradual increase in cocaine release; this slowly tapers off during the remaining 2 days. It is possible that this initial gradual increase in release models an addict's progressively increasing usage at the beginning of a binge, while the gradual decrease during days 4 and 5 may mimic an addict's gradual weaning from the drug as dysphoric effects increase.

While the progression of behaviors associated with chronic cocaine exposure differs somewhat from that of amphetamine, both produce a similar "late-stage" (16). Cocaine-induced hyperactivity and motor stereotypies were increased over controls in all categories, as was seen by the heightened cage crossing in the cocaine animals. This was not true in the amphetamine animals because their intense and focussed motor stereotypies dominated their activity, limiting the expression of other behaviors tremendously, including those typically seen in the "late-stage."

The fact that the frequency of "late-stage" behaviors produced by the cocaine pellet were significantly greater than those produced by the amphetamine pellet might be taken to indicate that the "late-stage" behaviors induced by amphetamine were lessened due to its selective damage to caudate dopamine terminals (6). Since cocaine does not appear to cause comparable caudate DA terminal damage as does amphetamine animals (12,25), the present findings indicate that caudate DA neurotoxicity is not integral to the onset of "late-stage" behaviors. However, we have recently (25) reported that while the amphetamine pellet induces alterations in dopamine D1 and D2 receptors in caudate and the cocaine pellet does not, with continuous cocaine there is a sizable and persisting alteration in benzodiazepine and muscarine receptors in several brain regions. The fact that these changes in benzodiazepine and muscarine binding are primarily in dopamine-rich areas suggests that dopamine may act as a modulator for the induction of these unique neurotoxic effects.

In order to discern the mechanisms which precipitate "late-stage" behaviors, the attributes shared by the amphetamine and cocaine pellets must be identified. Both pellets cause incessant dopaminergic hyperstimulation, progressive sleep loss over time, and persisting receptor changes in dopamine-rich brain regions. Which of these effects is essential for the appearance of "late-stage" behaviors remains unknown, but the answer to this question has clear implications for the biochemistry underlying stimulant-induced psychoses.

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