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Intravenous self-administration of heroin, cocaine, and the combination in Balb/c mice

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Abstract

Polydrug abuse, including the abuse of cocaine + heroin combinations (or 'speedballs') is an increasingly significant problem. The use of genetically defined populations of mice has the potential to add considerably to the study of polydrug abuse. Balb/cByJ (Balb/c) mice have been shown to self-administer opiates, but not cocaine, therefore these mice were chosen for the initial characterization of intravenous self-administration of cocaine + heroin combinations. Mice were implanted with chronic indwelling jugular catheters and given the opportunity to self-administer heroin, cocaine or heroin + cocaine combinations. Heroin was self-administered, while, under the same conditions, none of the mice tested acquired cocaine self-administration. However, heroin + cocaine combinations were self-administered in naive mice as well as in mice that had failed to self-administer cocaine alone. The heroin + cocaine combination dose–effect curve resembled the heroin dose–effect curve. It is hypothesized that heroin may interact with effects of cocaine that function to limit self-administration in Balb/c mice, facilitating the acquisition and maintenance of self-administration of cocaine + heroin combinations.

Keywords: Balb/c mouse; Cocaine; Heroin; Self-administration, intravenous; (Mouse); Speedball

1. Introduction

The use of genetically defined populations of mice such as inbred, recombinant inbred, transgenic and knockout strains along with behavioral genetic analytical techniques has added considerably to the study of drug abuse (Crabbe et al., 1994). The Balb/cByJ (Balb/c) inbred mouse strain has been highly utilized in the study of drugs of abuse such as cocaine, heroin and ethanol. These mice are unique in that they do not appear to acquire intravenous cocaine self-administration (Deroche et al., 1997) at doses equal to and lower than those that support acquisition in other mouse strains (Carney et al., 1991; Grahame et al., 1995; Grahame and Cunningham, 1995). In addition, a blunted locomotor response to cocaine has been observed in Balb/c mice as compared to other inbred strains (Shuster et al., 1977; Ruth et al., 1988; Weiner and Reith, 1990; Reith and Selmeci, 1991; Seale and Carney, 1991; Elmer et al., 1996; Deroche et al., 1997). It has been suggested that this may be due to lower brain concentrations of cocaine in Balb/c mice relative to other strains (Weiner and Reith, 1990), but this has not been reliably observed (Ruth et al., 1988). In fact, it appears that cocaine is quite potent in these mice as low doses are associated with significant increases in locomotor activity. However, its efficacy is low, as activity is only increased 100–150% over baseline (Elmer et al., 1996; Deroche et al., 1997). It does not appear that Balb/c mice are hyporesponsive to all behavioral effects of cocaine and other dopaminergic manipulations as they show pronounced cocaine-induced conditioned place preference (Seale and Carney, 1991) and haloperidol-induced catalepsy (Fink et al., 1982; Kanes et al., 1993). Thus, the phenomenon of low efficacy despite moderate potency of cocaine's effects in Balb/c mice may be related to an increased influence of cocaine-induced competing behaviors which disrupt measures of locomotion and self-administration.

In contrast to the effects of cocaine, opioids are self-administered and produce profound behavioral effects in Balb/c mice. For example, these mice have been shown to self-administer the opioid, etonitazene, orally in an operant paradigm (Elmer et al., 1995). They also self-administer morphine directly into several discrete brain regions in a site- and dose-specific manner (David and Cazala, 1994a,b, 1996; Cazala and David, 1995). One report noted a failure

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of Balb/c mice to intravenously self-administer morphine in a single 30 min trial; however, conditioned place preference to this drug was exhibited (Semenova et al., 1995). Conditioned place preference has also been observed to the opioid etonitazene (Seale and Carney, 1991). Balb/c mice appear to be quite sensitive to several other behavioral effects of opiates including analgesia, respiratory depression, and enhanced locomotion (Castellano and Oliverio, 1975; Pick et al., 1991; Semenova et al., 1995; Elmer et al., 1995).

A difference with regard to behavioral sensitivity to cocaine versus opioids is evident from previous studies with Balb/c mice. Therefore this strain offers a unique model for examining the self-administration of combinations of cocaine and the opioid, heroin. Co-injection of cocaine and heroin, referred to as a 'speedball', is increasing in popularity (Pollack et al., 1989; Schütz et al., 1994). The precise nature of the simultaneous use of two pharmacologically distinct substances remains unclear. However, discussions with speedball users suggest that speedballs enhance the positive effects of opiates and/or decrease the aversive properties of cocaine (Rosen and Kosten, 1991). These observations are supported by empirical evidence from a human behavioral pharmacology study indicating that speedballs have subjective properties which are unique to either cocaine or heroin alone (Foltin and Fischman, 1992).

The goal of the present experiments was to characterize intravenous heroin self-administration in Balb/c mice and to examine self-administration of combinations of heroin + cocaine. Based on the literature, it was predicted that a model of intravenous heroin self-administration could be developed in this mouse strain. Furthermore it was assumed that these mice would not acquire cocaine self-administration using criteria similar to those used previously in our laboratory (Deroche et al., 1997). Thus the Balb/c mouse strain offers a unique model for investigation of the nature of intravenous self-administration of combinations of heroin and cocaine. Furthermore, this study forms the basis for further development of mouse models of polydrug self-administration, allowing for a detailed investigation of the contribution of genetic and neuropharmacological influences to this type of drug abuse.

2. Materials and methods

2.1. Animals

Adult male Balb/cByJ mice weighing 24.5 ± 0.4 g at the start of the experiments and 25.4 ± 0.4 g at their completion were obtained from The Scripps Research Institute breeding colony. Following surgery mice were housed 4–6 per cage ($44 \times 23 \times 19.5$ cm). Food and water were available ad libitum and lights were on a 12 h light/dark cycle with lights off at 10:00 a.m. Testing occurred between 7:30 and 9:30 a.m. daily. All procedures were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.2. Drugs

Cocaine hydrochloride and 3,6-diacetylmorphine HCl (cocaine and heroin: NIDA, Rockville, MD, USA) and their combination were dissolved in sterile 0.9% saline solution. The concentrations of cocaine used were 0.05 and 0.1 mg/ml and concentrations of heroin varied from 0.0019 to 0.03 mg/ml depending on the desired unit dose. Doses were calculated as μg/kg per injection based on an average body weight of 25 g and were 100 and 200 μg/kg for cocaine and 1.875–60 μg/kg for heroin per 50 μl injection.

Brevital Sodium (Ely Lilly, Indianapolis, IN, USA) was administered intravenously every 5–7 days to evaluate catheter patency. This barbiturate was dissolved in sterile 0.9% saline to obtain a concentration of 5 mg/ml. Approximately 0.015 ml was administered resulting in a Brevital dose of 3 mg/kg.

2.3. Catheterization surgery

The chronic intravenous catheter used presently was similar to that described previously for use in the rat (Caine et al., 1993) except with minor modifications in sizing and materials. Briefly, Silastic tubing (0.3 mm i.d./0.64 mm o.d.) was dilated with Hemo-De solvent (Fisher Scientific, Pittsburgh, PA, USA) and fit onto the curved end of a 22 gauge cannula complete with a plastic screw-threaded pedestal. A small piece of Silastic tubing (0.64 mm i.d./1.175 mm o.d.) was slipped over the connection for protection. Dental cement was used to encase the cannula and a round piece of fine mesh was secured to the bottom for skin attachment. Catheters were soaked in 70% ethanol and then washed in sterile saline prior to surgical implantation.

The mice were anesthetized with a halothane/oxygen vapor mixture and kept at all times on a heating pad in order to prevent hypothermia. Following shaving and cleansing, incisions were made in the mid-scapular region of the back and on the ventral surface, anteromedial to the forearm above the right jugular vein. A catheter was passed subcutaneously from the dorsal incision to the ventral incision and the catheter mount was positioned on the mouse's back. The thin Silastic was cut at a bevel to the appropriate length and was inserted into the jugular vein. Suture was used to gently tie the vein around the tubing in two places. Appropriate catheter placement was verified at this time by gently aspirating blood into the tubing using a syringe connected to the exit point of the catheter. Approximately 0.01 ml sterile saline was flushed

through the catheter to displace the blood and minimize clotting. The incisions were then sutured closed and antibacterial ointment was applied externally. The exit point of the catheter was capped using a small segment of Tygon tubing (0.5 mm i.d./1.5 mm o.d.) encased in slightly larger copper tubing. Mice were allowed 48 h recovery time prior to the first verification of catheter patency with Brevital and another 24 h before the initiation of self-administration testing. When approximately 3 mg/kg of Brevital is flushed through the catheter it produces overt signs of sedation within seconds with complete recovery within 5 min. Brevital tests occurred every 5–7 days throughout the experiments. Animals showing no immediate signs of sedation were removed from the experiments.

2.4. Intravenous self-administration

Twelve Plexiglas chambers ($14.9 \times 15.2 \times 18.3$ cm) were each contained within larger exterior boxes equipped with exhaust fans to ventilate the chambers as well as to mask background noise. Each chamber was equipped with two small holes (0.9 cm diameter), equipped with photocell emitters and detectors, located 4 cm apart and 1 cm above the grid floor. A small Plexiglas divider ($5.4 \times 6.4 \times 0.3$ cm) separated the two holes, thus requiring the mouse to walk around or climb over the divider to go from one hole to the other. On the outside of the exterior box a Razel syringe pump (Razel Scientific Instruments, Stamford, CT, USA) equipped with a 5 rpm motor was set to deliver 50 μl solution per 2 s from a 10 ml syringe. The syringe was connected via Tygon tubing (0.5 mm i.d./1.5 mm o.d.) to a swivel (Brown et al., 1976) mounted on a balance arm above the Plexiglas chamber. Another piece of tubing attached to the swivel and entered the operant chamber for connection to the exit site of the mouse's catheter.

One hole was assigned to be 'active' and the other 'inactive' for each mouse. The assignment was counterbalanced between mice in each experiment and then remained constant throughout the study. A single nose poke in the active hole resulted in the disruption of a photocell beam which activated the pump to deliver the drug solution. Once the pump was triggered, nose pokes in the active hole were recorded, but no further drug was administered for 20 s. Nose pokes in the inactive hole were recorded, but were without scheduled consequences at any time. The numbers of responses in each hole and the numbers of reinforcers earned were recorded over the entire 2 h session. Mice were tested 5–7 times per week with at least 1 day off every 10 days.

2.5. Specific experimental designs

2.5.1. Heroin self-administration

The purpose of this experiment was to characterize intravenous self-administration of heroin in Balb/c mice. Acquisition was tested at 15 and 60 μg/kg per injection doses. For each of these doses, 8 mice were tested for up to 8 days. The three criteria for acquisition were: (1) at least 6 injections over the 2 h access period, (2) discrimination index ≥ 0.7 ($\geq 70\%$ of total nose pokes in active hole) and (3) number of injections within 20% of each other over two consecutive sessions. These criteria as well as the number of days employed in the acquisition phase of this and the subsequent experiments were similar to those previously used in our laboratory (Deroche et al., 1997). Mice that reached the criteria were entered into the dose–effect portion of the experiment.

Doses of heroin were varied such that a new dose was introduced and presented for two consecutive days before changing the dose again. Drug substitutions, including the training dose, were randomized according to a Latin square design to permit an evaluation of potential order effects. Doses of heroin used were 1.875, 3.75, 7.5, 15 and 30 μg/kg per injection. Saline was substituted for drug at the completion of the dose–effect portion of the experiment. Total number of injections taken on the second day at each dose, including saline, was used in the dose–effect analyses.

2.5.2. Cocaine versus heroin + cocaine cross-over

The goals of this experiment were to examine further the acquisition of cocaine self-administration in Balb/c mice and to investigate potential differential responding for cocaine and cocaine + heroin in the same mice. Six experimentally naive mice were trained on cocaine (100 or 200 μg/kg per injection) for 5 days in order to examine acquisition of cocaine self-administration at doses lower than those used previously (250 and 1000 μg/kg per injection: Deroche et al., 1997). On day 6, the mice were switched to heroin + cocaine (15/100 μg heroin/cocaine/kg per injection) for another 5 days. The four mice which were started with 100 μg cocaine/kg per injection were then returned to this dose of cocaine for an additional 4 days.

2.5.3. Heroin + cocaine self-administration

The purpose of this experiment was to further characterize the self-administration of heroin + cocaine in experimentally naive Balb/c mice. Acquisition was tested at 15/100 μg heroin/cocaine/kg per injection in 8 naive mice. The same acquisition criteria were used as for heroin self-administration. For dose–effect analysis, the dose of cocaine was kept constant and the dose of heroin was varied. Thus doses of 0/100, 3.75/100, 7.5/100, 15/100, 30/100 and 60/100 μg/kg heroin/cocaine per injection were made available, randomized according to a Latin square design, with saline being substituted for drug as the final step. Again, responding on the second day at each dose, including saline, was used in the dose–effect analysis.

2.6. Statistics

Mice which did not successfully complete the initial acquisition phases of the experiments because of ill health, death, or a negative Brevital test were excluded from the experiments. Mice which successfully acquired self-administration of heroin or heroin + cocaine were examined in the dose–effect portion of each experiment. In the heroin + cocaine cross-over experiment, a one-way analysis of variance (ANOVA) was used with the within-subjects factor day to analyze the numbers of injections taken by the 6 mice across the cocaine and heroin + cocaine acquisition phases (days 1–10) of the experiment. The discrimination indexes defined as the number of active pokes/total number of pokes were analyzed across the 10 day period in all six mice as well as in the subset of four mice over days 11–14 using one-sample *t*-tests in which the mean from each day was compared with chance responding (discrimination index of 0.5).

Dose–effect data (numbers of injections taken on the second day of presentation of each dose) were analyzed by repeated measures ANOVA using all of the drug doses except saline. Post-hoc Tukey(a) tests were used when appropriate. The discrimination indexes (No. active pokes/total No. of pokes) were analyzed using one-sample *t*-tests in which responding for each drug dose was compared with chance responding (discrimination index of 0.5). Dose-dependent responding for heroin and heroin + cocaine was compared by separate ANOVA at the overlapping heroin doses (i.e., 3.75 μg/kg per injection heroin vs. 3.75/100 μg heroin/cocaine/kg per injection, etc.).

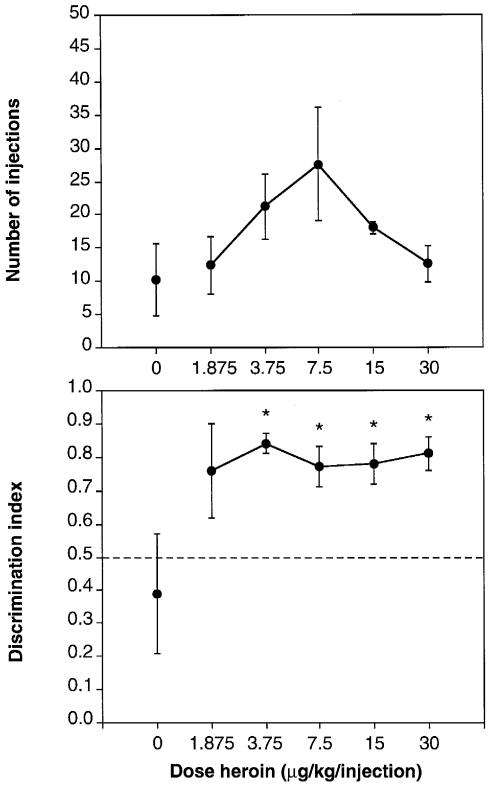

Fig. 1. Number of injections taken (2 h means ± S.E.M.) of various doses of heroin (top) and corresponding discrimination indexes (bottom) in all mice that met the acquisition criteria ($n = 5$). Discrimination indexes are defined as the number of nose pokes in the active hole/total number of nose pokes. The symbol * denotes statistical difference from chance responding (0.5, $P < 0.05$).

3. Results

Overall, 39 mice were catheterized to obtain the 30 employed for the entire study. Six mice were excluded from the heroin groups, 0 from the cocaine to heroin + cocaine cross-over group, and 3 from the heroin + cocaine group.

3.1. Heroin self-administration

Eight mice were initiated on 15 and 8 on 60 μg heroin/kg per injection. None of the mice initiated at the 60 μg/kg dose met the criteria for acquisition despite having been tested for at least 8 days. These mice generally displayed very low rates of responding for this dose suggesting that it was too high. In contrast, 5 of the 8 mice initiated on 15 μg/kg heroin (62.5%) acquired self-administration based on our three criteria. As a group, these mice took 20.2 ± 5.7 injections and displayed discrimination indexes of 0.95 ± 0.02 on the final day of the acquisition phase.

The dose–effect curve for heroin-maintained responding and discrimination indexes of the 5 mice that successfully acquired self-administration are shown in Fig. 1. There was no significant effect of dose on the numbers of injections of heroin taken by these mice ($F(4,16) = 1.5$, $P = 0.2$). However, the dose–effect curve has a shallow inverted U-shape which is characteristic of self-administration studies (Meisch, 1987). Discrimination indexes for responding maintained by 3.75, 7.5, 15 and 30 μg/kg per injection heroin were significantly above chance levels ($P < 0.05$), whereas those obtained for saline and the lowest dose of heroin were not. Taken together, these results suggest that although this drug did not support high rates of responding, Balb/c mice self-administered heroin according to the presently employed criteria.

3.2. Cocaine to heroin + cocaine cross-over

None of the mice trained to self-administer 100 μg/kg per injection cocaine ($n = 4$) or 200 μg/kg per injection cocaine ($n = 2$) met any one of the criteria for acquisition of cocaine self-administration after 5 days of testing. However, by the second day of heroin + cocaine exposure all mice met all three acquisition criteria. The results of this experiment are shown in Fig. 2. There was a significant main effect of day on the number of injections taken

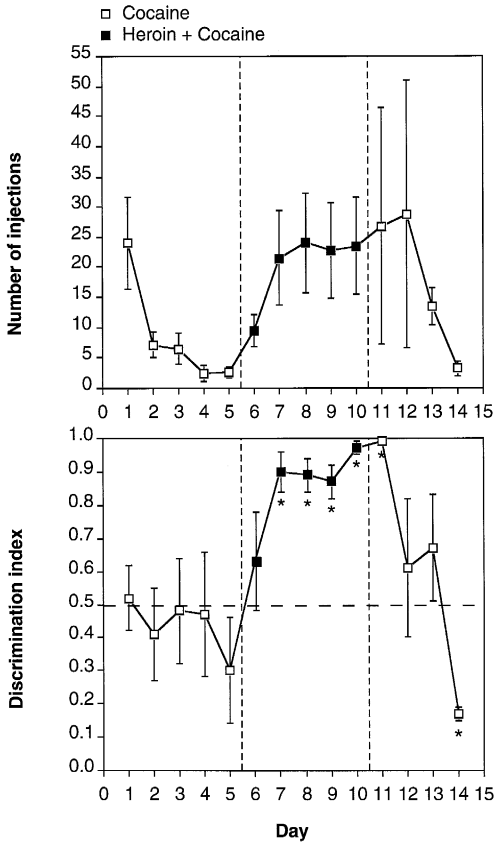

Fig. 2. Number of injections taken (2 h means ± S.E.M.) of cocaine (days 1–5 and 11–14) and cocaine + heroin (days 6–10; top) and corresponding discrimination indexes (bottom). Data for all mice are shown for days 1–10 ($n = 6$), while a subset of these mice were re-tested with cocaine on days 11–14 ($n = 4$). The symbol * denotes statistical difference from chance responding (0.5, $P < 0.05$).

across the first 10 days in all 6 mice ($F(9,59) = 3.7$, $P < 0.01$). Responding for cocaine was qualitatively different from that when cocaine and heroin were combined. Discrimination indexes for responding on days 7, 8, 9 and 10 were significantly greater than chance (t values > 7.03, P values < 0.001). These data indicate that mice that failed to meet the criteria for acquisition of cocaine self-administration were capable subsequently of acquiring self-administration of the combination of heroin + cocaine.

Four of the 6 mice were then returned to conditions under which cocaine was available (100 μg/kg per injection) for an additional 4 days. One of these mice responded for over 80 injections on the first two days back on cocaine, contributing to the large variability on days 11 and 12. In fact, although the discrimination indexes for cocaine self-administration on day 11 were significantly greater than the chance level, by day 14 these were actually significantly lower than chance. This suggests that even following acquisition of heroin + cocaine self-administration, cocaine did not maintain self-administration when presented alone.

3.3. Heroin + cocaine combinations

Six of the 8 mice trained on the 15/100 μg/kg dose (75%) met the criteria for acquisition. The dose–effect curve and discrimination indexes for responding maintained by heroin + cocaine are shown in Fig. 3. There was a significant overall effect of dose on the number of injections taken by these mice ($F(5,25) = 10.7$, $P < 0.001$). The mice took significantly more injections of 7.5/100 and 15/100 μg/kg heroin/cocaine than the two doses on either side. The discrimination indexes for all of the dose combinations except 60/100 μg/kg per injection and saline were significantly greater than chance at the $P < 0.05$ level.

There was no significant difference in responding for saline between the heroin experiment (Fig. 1) and the heroin + cocaine combination experiment (Fig. 3). Numbers of injections of 3.75, 7.5, 15, and 30 μg/kg heroin were compared to the same doses given in combination with 100 μg/kg cocaine. The only significant difference was observed at the 15 μg/kg doses where the number of

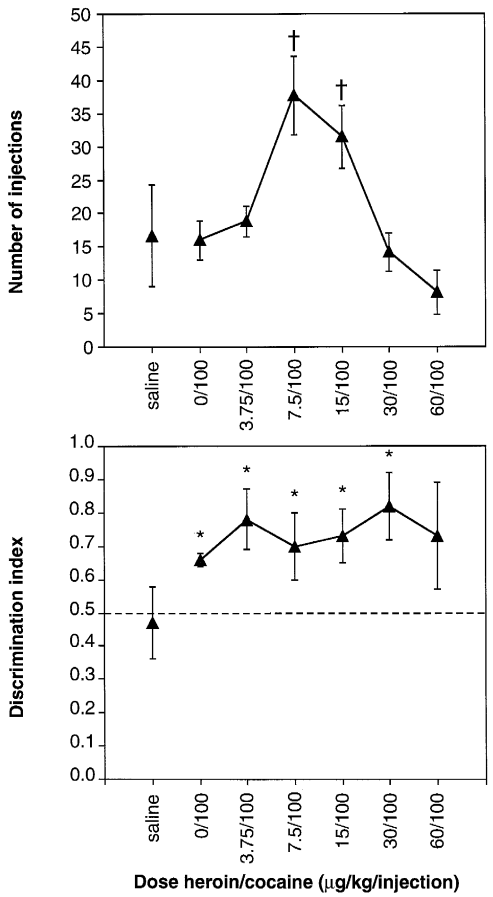

Fig. 3. Number of injections taken (2 h means ± S.E.M.) of various doses of cocaine + heroin (top) and corresponding discrimination indexes (bottom) in all mice that met the acquisition criteria ($n = 6$). The symbol † denotes statistical difference from the number of saline injections taken ($P < 0.05$) and * denotes statistical difference from chance (discrimination index of 0.5) responding ($P < 0.05$).

heroin + cocaine injections was greater than heroin alone. The results of this experiment suggest that heroin + cocaine combinations are self-administered by Balb/c mice using the criteria defined herein, and that under particular conditions the combination of heroin + cocaine may support higher levels of responding than heroin alone.

4. Discussion

Heroin at a dose of 15 mg/kg per injection supported self-administration in 62.5% of the Balb/c mice tested as determined by the criteria established by this study. This is consistent with previous reports of opiate self-administration (oral or intracranial) in Balb/c mice (Elmer et al., 1995; David and Cazala, 1994a,b, 1996; Cazala and David, 1995). In the present study, the mice responded preferentially in the active hole for all but the lowest dose (and saline), suggesting that heroin 'guides' responding to the active hole and therefore is acting as a reinforcer in Balb/c mice. The absence of a significant overall effect of dose on responding maintained by heroin may be due to the greater variability on the ascending limb of the dose–effect curve. However, the somewhat flat dose–effect function suggests that heroin maintains stable, but not high, rates of responding, much like those observed in rats (Ettenberg et al., 1982).

The lack of cocaine self-administration in Balb/c mice is consistent with a previous report from our laboratory (Deroche et al., 1997) in which these mice did not acquire self-administration at either 250 or 1000 μg/kg cocaine/injection under similar test conditions. Interestingly, in the present study even mice which had acquired heroin + cocaine self-administration did not subsequently self-administer cocaine alone. In fact, by the final day of the experiment the mice actually displayed a preference for the inactive hole (i.e., discrimination indexes < 0.5), suggesting an avoidance of the hole associated with cocaine delivery had developed. Although it could be argued that these mice would acquire cocaine self-administration given longer than 5 days, the fact that responding decreased when cocaine alone was re-introduced suggests that a qualitative difference exists in the ability of cocaine versus heroin + cocaine to maintain responding. Other strains have been found to acquire self-administration of cocaine using 100–2000 μg/kg per injection (Carney et al., 1991; Grahame et al., 1995; Grahame and Cunningham, 1995; Deroche et al., 1997), indicating that cocaine can support self-administration in mice, as has been shown for other strains of laboratory animals. Thus it appears that Balb/c mice are less responsive than other mouse strains to cocaine in self-administration paradigms perhaps due to effects of cocaine which compete with this behavior.

Heroin + cocaine combinations were self-administered in almost all of the mice tested as well as mice that failed to self-administer cocaine under the presently employed conditions. These results are consistent with previous reports of reliable self-administration of heroin + cocaine combinations in monkeys (Mello et al., 1995) and rats (Hemby et al., 1996), and represent, to the best of our knowledge, the first demonstration of heroin + cocaine self-administration in mice.

In the present study, heroin + cocaine supported self-administration while cocaine alone did not. This suggests that the drug combination may possess properties different from those of cocaine alone. The likelihood that a particular drug solution will be self-administered is determined by the ratio of positively reinforcing versus punishing properties as well as direct effects on rates of responding. For example, the addition of heroin may attenuate competing behaviors produced by cocaine and thereby result in the self-administration of the combination. There was also evidence that cocaine may have increased the number of injections taken of 15 μg/kg per injection heroin. However, because only one dose was significantly affected by the addition of cocaine, it cannot be argued that cocaine shifted the dose–effect curve for heroin either upwards or laterally.

In other preclinical studies, Mello et al. (1995) showed that heroin + cocaine combination self-administration was similar to that of either cocaine or heroin alone. This led the authors to suggest that, in rhesus monkeys, heroin and cocaine did not potentiate each other's reinforcing effects. Hemby et al. (1996) found that, in rats, the addition of heroin resulted in a downward shift in the cocaine self-administration dose–effect curve. A potentiation of dopamine neurotransmission with combined cocaine and heroin self-administration (Hemby et al., 1995) led these authors to suggest that the rate-altering effects of cocaine were influenced by heroin. While cocaine may be reinforcing to Balb/c mice as suggested by conditioned place preference (Seale and Carney, 1991), cocaine's response-altering effects may interfere with its ability to be self-administered in an operant paradigm. In the present studies, the addition of heroin, rather than altering cocaine's reinforcement potential, may have interacted with its rate-altering effects, resulting in self-administration of the combination.

There appears to be a subset of human speedball abusers who only take cocaine in the presence of heroin. For example, the opiate buprenorphine used in the treatment of heroin abuse has been reported by speedball users to decrease cocaine use (Rosen and Kosten, 1991). One of the reasons for this effect reported by the patients is that they 'never liked' to take cocaine alone and now that they were not taking heroin they were no longer interested in cocaine. This implies that at least a subset of speedball users do not self-administer cocaine alone, but enjoy the combination of cocaine + heroin. The Balb/c mouse strain appears to model this type of polydrug abuse quite well.

In the present experiments heroin + cocaine combination self-administration was acquired and maintained in Balb/c mice. Further studies will be required to examine

the neuropharmacological mechanisms mediating this drug combination self-administration in these mice. It is becoming clear from the human and animal studies that there is likely more than a single factor underlying speedball intake. Therefore, the examination of heroin + cocaine combination self-administration in other mouse strains will extend our knowledge regarding the relative contributions of cocaine and heroin to speedball self-administration and allow further investigation of the unique properties of heroin + cocaine combinations. In addition, the wide variety of strains and other genetic tools such as knockout and transgenic mice allow for a detailed investigation of the contribution of genetic and neuropharmacological influences on polydrug abuse.

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References

Brown, Z.W., Amit, Z., Weeks, J.R., 1976. Simple flow-thru swivel for infusions into unrestrained animals. Pharmacol. Biochem. Behav. 5, 363–365.

Caine, S.B., Lintz, R., Koob, G.F., 1993. Intravenous drug self-administration techniques in animals. In: Sahgal, A. (Ed.), Behavioural Neuroscience: A Practical Approach. Oxford University Press, Oxford, pp. 117–143.

Carney, J.M., Landrum, R.W., Cheng, M.S., Seale, T.W., 1991. Establishment of chronic intravenous drug self-administration in the C57BL/6J mouse. NeuroReport 2, 477–480.

Castellano, C., Oliverio, A., 1975. A genetic analysis of morphine-induced running and analgesia in the mouse. Psychopharmacology 41, 197–200.

Cazala, P., David, V., 1995. Dose-discrimination performance of mice for self-administration of morphine into the lateral hypothalamus. Pharmacol. Biochem. Behav. 51, 49–55.

Crabbe, J.C., Belknap, J.K., Buck, K.J., 1994. Genetic animal models of alcohol and drug abuse. Science 264, 1715–1723.

David, V., Cazala, P., 1994a. Differentiation of intracranial morphine self-administration behavior among five brain regions in mice. Pharmacol. Biochem. Behav. 48, 625–633.

David, V., Cazala, P., 1994b. A comparative study of self-administration of morphine into the amygdala and the ventral tegmental area in mice. Behav. Brain Res. 65, 205–211.

David, V., Cazala, P., 1996. Preference for self-administration of a low dose of morphine into the ventral tegmental area rather than into the amygdala in mice. Psychobiology 24, 211–218.

Deroche, V., Caine, S.B., Heyser, C.J., Polis, I., Koob, G.F., Gold, L.H., 1997. Differences in the liability to self-administer intravenous cocaine between C57BL6×SJL and BALB/cByJ mice. Pharmacol. Biochem. Behav. (in press).

Elmer, G.I., Pieper, J.O., Goldberg, S.R., George, F.R., 1995. Opioid operant self-administration, analgesia, stimulation and respiratory depression in μ-deficient mice. Psychopharmacology 117, 23–31.

Elmer, G.I., Gorelick, D.A., Goldberg, S.R., Rothman, R.B., 1996. Acute sensitivity vs. context-specific sensitization to cocaine as a function of genotype. Pharmacol. Biochem. Behav. 53, 623–628.

Ettenberg, A., Pettit, H.O., Bloom, F.E., Koob, G.F., 1982. Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. Psychopharmacology 78, 204–209.

Fink, J.S., Swerdloff, A., Reis, D.J., 1982. Genetic control of dopamine receptors in mouse caudate nucleus, relationship of cataleptic response to neuroleptic drugs. Neurosci. Lett. 32, 301–306.

Foltin, R.W., Fischman, M.W., 1992. The cardiovascular and subjective effects of intravenous cocaine and morphine combinations in humans. J. Pharmacol. Exp. Ther. 261, 623–632.

Grahame, N.J., Cunningham, C.L., 1995. Genetic differences in intravenous cocaine self-administration between C57BL/6J and DBA/2J mice. Psychopharmacology 122, 281–291.

Grahame, N.J., Phillips, T.J., Burkhart-Kasch, S., Cunningham, C.L., 1995. Intravenous cocaine self-administration in the C57BL/6J mouse. Pharmacol. Biochem. Behav. 51, 827–834.

Hemby, S.E., Dworkin, S.I., Smith, J.E., 1995. Extracellular dopamine concentrations in the nucleus accumbens during cocaine, heroin and speedball self-administration. College on Problems of Drug Dependence.

Hemby, S.E., Smith, J.E., Dworkin, S.I., 1996. The effects of eticlopride and naltrexone on responding maintained by food, cocaine, heroin and cocaine/heroin combinations in rats. J. Pharmacol. Exp. Ther. 277, 1247–1258.

Kanes, S.J., Hitzemann, B.A., Hitzemann, R.J., 1993. On the relationship between D_2 receptor density and neuroleptic-induced catalepsy among eight inbred strains of mice. J. Pharmacol. Exp. Ther. 267, 538–547.

Meisch, R.A., 1987. Factors controlling drug reinforced behavior. Pharmacol. Biochem. Behav. 27, 367–371.

Mello, N.K., Negus, S.S., Lukas, S.E., Mendelson, J.H., Sholar, J.W., Drieze, J., 1995. A primate model of polydrug abuse: cocaine and heroin combinations. J. Pharmacol. Exp. Ther. 274, 1325–1337.

Pick, C.G., Cheng, J., Paul, D., Pasternak, G.W., 1991. Genetic influences in opioid analgesia sensitivity in mice. Brain Res. 566, 295–298.

Pollack, M.H., Brotman, A.W., Rosenbaum, J.F., 1989. Cocaine abuse and treatment. Compr. Psychiatry 30, 31–44.

Reith, M.E.A., Selmeci, G., 1991. Cocaine binding sites in mouse striatum, dopamine autoreceptors, and cocaine-induced locomotion. Pharmacol. Biochem. Behav. 41, 227–230.

Rosen, M.I., Kosten, T.R., 1991. Buprenorphine: Beyond methadone?. Hosp. Community Psychiatry 42, 347–349.

Ruth, J.A., Ullman, E.A., Collins, A.C., 1988. An analysis of cocaine effects on locomotor activities and heart rate in four inbred mouse strains. Pharmacol. Biochem. Behav. 29, 157–162.

Schütz, C.G., Vlahov, D., Anthony, J.C., Graham, N.M.H., 1994. Comparison of self-reported injection frequencies for past 30 days and 6 months among intravenous drug users. J. Clin. Epidemiol. 47, 191–195.

Seale, T.W., Carney, J.M., 1991. Genetic determinants of susceptibility to the rewarding and other behavioral actions of cocaine. J. Addict. Dis. 10, 141–162.

Semenova, S., Kuzmin, A., Zvartau, E., 1995. Strain differences in the analgesic and reinforcing action of morphine in mice. Pharmacol. Biochem. Behav. 50, 17–21.

Shuster, L., Yu, G., Bates, A., 1977. Sensitization to cocaine stimulation in mice. Psychopharmacology 52, 185–190.

Weiner, H.L., Reith, M.E.A., 1990. Correlation between cocaine-induced locomotion and cocaine disposition in the brain among four inbred strains of mice. Pharmacol. Biochem. Behav. 36, 699–701.