SYNERGISTIC EFFECTS OF ETHANOL AND COCAINE ON BRAIN STIMULATION REWARD

MICHAEL J. LEWIS AND HARRY L. JUNE

HOWARD UNIVERSITY

The effects of two widely abused drugs, ethanol and cocaine, were examined alone and in combination on intracranial reward processes. In agreement with previous research, higher doses of both cocaine and ethanol alone produced facilitation of behavior maintained by brain stimulation reward. Low intraperitoneal doses of ethanol and cocaine, which alone did not affect performance, were found to reduce stimulation reward threshold and modestly increase response rate. The enhancement of brain stimulation reward by the combination of ethanol and cocaine suggests that both drugs may produce their rewarding effects through common neuronal substrates and that they may potentiate the abuse of each other.

Key words: ethanol, cocaine, brain stimulation reward, threshold, lever press, rats

Both ethanol (EtOH) and cocaine produce pleasant subjective effects that undoubtedly play a major role in the abuse of both substances. Although the former is a well-known depressant and the latter a psychomotor stimulant, EtOH (Carlson & Lydic, 1976; Lewis & June, 1990; Lewis & Phelps, 1987; Vrtunski, Murray, & Wolin, 1973) and cocaine (Crow, 1970; Esposito, Motola, & Kornetsky, 1978) have been found to enhance electrical brain stimulation reward (BSR). Moreover, increases in locomotor activity have been found with injections of EtOH (Carson, Engel, & Svensson, 1972; Lewis & June, 1990; Pohorecky, 1977) as well as cocaine (Yeh & Haertzen, 1991). These increases in locomotor activity usually occur soon after administration of EtOH and during the period when blood alcohol concentrations (BAC) are increasing (Lewis & June, 1990).

Increasingly, both drugs have been abused in combination with each other (Grant & Harford, 1990). The reasons behind the motivation

for this abuse pattern remain at the level of conjecture. The purpose of the present research was to examine the possibility that EtOH and cocaine may have some common reward effects. We, therefore, examined the effects of both of these drugs alone and in combination on brain reward mechanisms.

BSR has been used to examine the rewarding properties of many drugs of abuse, including EtOH (Lewis & June, 1990; Lewis & Phelps, 1987) and cocaine (Crow, 1970; Esposito et al., 1978). The brain site most frequently investigated in this research has been the lateral hypothalamus (LH), a site that supports high response rates. The brain systems that project to and from the LH are believed to be most important in mediating BSR and also to play an important role in the reinforcing functions of drugs of abuse (Lewis, 1990). This region contains a heterogeneous group of neurotransmitter systems including dopamine (DA), which has been shown to play a significant role in the reinforcing effects of both cocaine (Fischman, 1987) and EtOH (Di Chiara, Acquas, & Carboni, 1992). Given this research literature, we thought it appropriate to examine the effects of the combination of EtOH and cocaine on BSR at lateral hypothalamic sites.

METHOD

Subjects

Twenty-one adult male Charles River albino rats, weighing 250 to 300 g at surgery, were individually housed under controlled

The research reported here is presented in honor of Joseph V. Brady for his many contributions to behavioral research and his long and sustained nurturance of our field. We wish to acknowledge his encouragement of the research reported here. Support for the research was provided by grants from the National Institute of Alcohol Abuse and Alcoholism (AA06263 and RR08016) and RIII8505430 from the National Science Foundation (M.J.L.). Preliminary data were presented at the annual meeting of the Society for Neuroscience (Lewis, 1985).

Address correspondence and reprint requests to Michael J. Lewis, Neurobehavioral Laboratory, Department of Psychology, Temple University, Philadelphia, Pennsylvania 19119.

temperature, humidity, and noise conditions. Each was individually housed and remained under a reverse light/dark condition throughout the experiment. Lights were off from 8:00 a.m. to 8:00 p.m.

Surgery and Histology

All animals were anesthetized with pentobarbital sodium (50 mg/kg, intraperitoneal [ip] injection) and ketamine (20 mg/kg, ip) and then surgically implanted with chronic indwelling bipolar platinum electrodes (0.3 mm diameter) aimed at the lateral hypothalamus-medial forebrain bundle area (LH-MFB). Stereotaxic coordinates were -3.5 mm anterior-posterior, 1.5 mm medial-lateral, and -8.1 to -8.3 mm dorsal-ventral from the top of the skull; bregma and lambda were level. The electrodes were insulated with Teflon®, except for the cross section of the tips.

Upon completion of behavioral testing, the rats were sacrificed with an overdose of anesthesia (pentobarbital sodium) and perfused with normal saline and buffered formalin. The brains were removed from the skull, frozen, and $20\text{-}\mu\text{m}$ sections taken. The sections were mounted and examined microscopically to determine electrode placement.

Stimulation and Apparatus

Subjects were tested in an operant conditioning chamber with a single lever at one end. Above the cage, a mercury swivel commutator allowed the animal free movement while connected to the stimulation apparatus. The stimulation equipment, software, and hardware are described in greater detail in Lewis and Phelps (1987).

A 0.2-s train of 100-Hz biphasic rectangular pulses (1.0 ms duration) was controlled by a microcomputer that was interfaced with a constant-current stimulator. The stimulator functioned as a voltage follower-regulator with continuous feedback; instantaneous samples of the resistance (impedance) to the electrical current were made and resulted in voltage adjustments to maintain constant current intensity. The current intensity was controlled by the computer and varied according to the following threshold technique, which is a modification of that of Huston and Mills (1971).

Data on total time to complete the session, BSR threshold, and average impedance values were collected by the computer and were an-

alyzed by a statistical program that summarizes, calculates means and standard errors of the mean, and correlates each measure of an animal's performance over the current range tested. Overall rate of response and rate during the first current intensity were also calculated.

BSR Training

The lever-press response was initially shaped under a schedule of continuous BSR reinforcement. Responding then was maintained under a fixed-ratio (FR) 10 schedule of reinforcement using the method of Huston (1968). This added training requires a longer period of time to train each animal, but provides more information about rewarded behavior and changes produced by aging and drug effects. Of the 21 rats initially implanted with electrodes, lever pressing was shaped in 12, and response rates of 50 responses per minute or higher were maintained. Nine of these 12 performed reliably under FR 10 during the 3-week computer-controlled BSR testing schedule. One rat lost its implant during experimentation and was eliminated from the study. Reliable threshold and response rates were recorded for all experimental conditions for 6 and 8 rats, respectively.

Threshold Determination

Two concurrent schedules of reinforcement were used to determine BSR threshold. The first was the FR 10 schedule, which was used principally to maintain the animal's behavior throughout the period of testing so that responding did not extinguish or falter. A continuous reinforcement (CRF) schedule was superimposed upon the FR 10 schedule (i.e., every response resulted in stimulation). The FR current intensity was maintained at a level (60 to 70 μA) well above the threshold that maintains responding. The CRF intensity varied over a range established during training to be symmetrical about the threshold of each animal. It was systematically decreased in discrete and equal steps over the range.

The criterion for determining threshold was based upon the proportion of postreinforcement pauses (PRPs) that appeared at each of the CRF intensities tested. The PRPs, brief respites in responding that are characteristic of FR schedule performance, are defined as interresponse intervals of two standard deviations above the mean for each current inten-

sity. This measure is independent of rate, in that the determination of PRPs was modified at each CRF intensity to reflect the rate of response at the CRF intensity. As the CRF intensity is reduced, the presence of these pauses begins to appear more frequently. This indicates a change in the response pattern: It is no longer characteristic of a CRF schedule of reinforcement and takes on the characteristics of an FR schedule of reinforcement. The number of PRPs per FR was recorded at each CRF current level by the computer. These proportions were analyzed as a function of current intensity using a least squares regression analysis that applies the best fitting line to the proportions arrayed across the CRF intensities. Proportions of 0 and 1.0 were omitted from the analysis. Threshold of BSR is the current level at which the ratio of PRPs to the number of FRs at each current level is .5.

FR stimulation current intensities were 75 μ A, and CRF stimulation intensities ranged from 20 to 55 μ A. Completion of 10 FR 10 was required at each CRF stimulation intensity level. There were four equally spaced CRF stimulation intensities chosen to be symmetrical around each rat's reward threshold based upon the animal's performance during initial training. These values varied with each animal.

Response rate was determined during BSR sessions by measuring the elapsed time to complete the programmed number of responses for the first two CRF current intensities and dividing this value into the total number of responses produced. Because CRF current intensities descended in intensity, this provides a measure of response rate to suprathreshold stimulation that is comparable to other studies using response rate to measure reward changes.

Drugs and Injections

EtOH was administered as a 20% solution (v/v) prepared from 95% pure EtOH in saline. Cocaine HCl was also prepared in saline. Animals were initially given intraperitoneal injections of saline vehicle. They were then given low doses of EtOH (0.1, 0.25, and 0.50 g/kg) 5 min and cocaine (0.10, 0.25, and 0.50 mg/kg) 10 min before BSR testing. With concurrent administration of EtOH and cocaine, cocaine was injected first, followed approximately 5 min later by injection of EtOH. BSR testing occurred 5 min after EtOH injection.

Data Analysis

Reward threshold (μ A) ($n = 6$) and response rate (responses per minute) ($n = 8$) were each analyzed using a one-way repeated measures analysis of variance. Design conditions for each measure were two control levels (no injection and saline) and six drug treatment levels (0.10 g/kg EtOH, 0.25 g/kg EtOH, 0.10 mg/kg cocaine, 0.25 mg/kg cocaine, 0.50 mg/kg cocaine, and 0.10 g/kg EtOH plus 0.25 mg/kg cocaine). Reward threshold and response rates of drug treatments were compared with performance during the two control conditions. The Newman-Keuls test was used on all post-hoc comparisons of group means.

RESULTS

Microscopic examination of the electrode placements revealed that all electrode tips were found within the LH-MFB region of the diencephalon. Most were found immediately lateral to the descending portion of the fornix. Analysis of the rate of response and threshold data with electrode placements did not yield any systematic performance differences attributable to the site of stimulation.

Figure 1 shows the effects of the eight treatment conditions on BSR threshold. The 0.25-g/kg dose of EtOH reduced BSR threshold in comparison to the control conditions ($p < .01$); however, the 0.10-g/kg dose of EtOH did not affect this parameter of BSR. The 0.50-mg/kg doses of cocaine also reduced BSR threshold ($p < .01$) below control, as did 0.25 g/kg of EtOH ($p < .05$). The effects of 0.10- and 0.25-mg/kg doses of cocaine did not differ from the control conditions.

Concurrent measurement of response rate in these animals shows (Figure 2) that the 0.25-g/kg dose of EtOH produced a slight increase in performance when compared to control conditions that approached statistical significance ($p < .10$). There was no statistically significant change in response rate with 0.10 g/kg of EtOH. Cocaine's effects on response rate appeared to be dose dependent. The 0.50-mg/kg dose of cocaine produced a statistically significant (though small) increase in rate that was greater than that following 0.25 g/kg of EtOH ($p < .01$). Inspection of individual response rates with the 0.25-mg dose of cocaine showed that the failure to reach

Fig. 1. Group mean effects of ethanol, cocaine, and ethanol plus cocaine on BSR threshold. Values are expressed as mean microamperes $\pm$ SEM of $n = 6$ animals per group. * indicates statistically significant differences in comparison to the two control conditions. + indicates statistically significant differences in comparison to the 0.25-g/kg EtOH condition.

statistical significance with the post-hoc test with this dose was due primarily to the high lever-press variability of 2 animals. The 0.10-mg/kg dose of cocaine yielded response rates that remained at the level seen with control injections ($p > .10$).

After the completion of testing of EtOH and cocaine alone, the injections of both given in combination produced enhancement of both BSR measures. The 0.10-g/kg dose of EtOH combined with the 0.25-mg/kg dose of cocaine reduced BSR threshold (Figure 1) ($p < .01$) and increased response rate (Figure 2) ($p < .05$) in comparison to control levels of each parameter. The reduction in threshold was of the same magnitude as that of the 0.50-mg/kg dose of cocaine alone and significantly lower ($p < .05$) than that of the 0.25-g/kg dose of EtOH alone. The increase in response rate was not as dramatic; however, it was of the same magnitude as that of 0.50 mg/kg of cocaine.

DISCUSSION

The major findings of the present research are that low doses of EtOH and cocaine, which did not alter BSR threshold and response rate when administered alone, did reduce BSR threshold and increase response rate when given concurrently. Previous preliminary research (Lewis, 1985) from our laboratory showed similar reduction of BSR threshold, but not increases in response rate, with concurrent administration of both drugs. More recently, Moolten and Kornetsky (1990) found that EtOH and cocaine enhanced BSR when animals self-administered EtOH. The dose of EtOH varied in this research because of differences in voluntary consumption by the rats. In the present study, the doses of both cocaine and alcohol were carefully selected in an attempt to evaluate possible synergistic effects of both substances of abuse. The 0.1-g/kg dose of EtOH was found to produce no effects on

Fig. 2. Group mean effects of ethanol, cocaine, and ethanol plus cocaine on BSR response rate. Values are expressed as mean lever presses per minute of $n = 8$ animals per group. * indicates statistically significant differences in comparison to the two control conditions.

BSR threshold or response rate when given alone. These effects are in agreement with our previous research (Lewis & June, 1990; Lewis & Phelps, 1987) showing that this dose produced no effects; however, a 0.25-g/kg dose of EtOH did lower threshold. The 0.25-g/kg dose did not affect response rate in the present experiment. These data indicate that the threshold for effects of EtOH on BSR using our system appears to be at approximately 0.25 g/kg with Charles River male rats. Preliminary research in our laboratory with these animals showed that the dose of cocaine that reliably lowers threshold is in the range of 0.5 mg/kg. The present data show, in agreement with these preliminary studies, that a 0.25-mg/kg dose of cocaine alone does not lower threshold. The 0.1-g/kg dose of EtOH and the 0.25-mg/kg dose of cocaine were selected to be just below the threshold for effects on BSR. We reasoned that this strategy would permit the determination of any synergistic effects of the two substances.

The effects of concurrent EtOH and cocaine administration on passive avoidance performance have been reported to be antagonistic (Aston-Jones, Aston-Jones, & Koob, 1984). These investigators showed that EtOH (1.0 g/kg) alone produced anticonflict action and ataxia, as measured in the Geller-Seifter paradigm. The concurrent administration of cocaine (10 mg/kg) with EtOH antagonized the anticonflict effect while enhancing the ataxia. The doses of both EtOH and cocaine were higher, and the time of injections prior to the task of this study was different from that used in the present research. The differences between BSR (generally regarded as a positive reinforcement paradigm) and passive avoidance (a negative reinforcement or punishment paradigm) as well as the differences in dose and time of administration make comparison of these studies difficult.

An enhancement of BSR-maintained performance by the combination of intraperitoneal cocaine and an orally self-administered

EtOH-sucrose solution has been reported (Moolten & Kornetsky, 1990a). These investigators, however, did not find any effects with the combination of intraperitoneal injections of both cocaine and EtOH. Kornetsky and his collaborators (Bain & Kornetsky, 1989; Moolten & Kornetsky, 1990b) have also failed to find enhancement of BSR when EtOH alone was intraperitoneally injected. The differences in the effects of EtOH on BSR between the work of Kornetsky and his collaborators and that in this laboratory may be due to several possible differences in BSR methodology. The differences may be due to the differences in our procedures for determining BSR threshold and the temporal parameters of testing ethanol's effects on BSR (Lewis, 1990; Lewis & June, 1990). In previous studies in this laboratory (Lewis & June, 1990; Lewis & Phelps, 1987), BSR threshold was determined in less than 20 min and was measured during the ascending BAC. In addition to time of testing and different BSR procedures, the stock of rat differed: Charles River (Sprague-Dawley) rats were used in our research, and F-344 rats were used by Kornetsky and his collaborators.

Our results suggest that both EtOH and cocaine may share a common neural substrate, related to reward, that is activated by each compound at low doses. This common mechanism may involve the ventral striatal pathways in the brain. Lewis, Perry, June, Garnet, and Porrino (1990) found that low doses of EtOH increase functional brain activity, as measured by 2-deoxyglucose methods in the olfactory tubercle during the time when BAC is ascending and enhancement of BSR occurs. Cocaine, at doses comparable to those of the present study, and BSR have also been found to increase functional brain activity in this brain region (Kornetsky, Huston-Lyons, & Porrino, 1991). It is tempting to speculate that this common action on the ventral striatum and perhaps interconnected brain areas may mediate the enhancement of reward by EtOH in combination with cocaine.

The discovery (Raffa & Epstein, 1979) of the formation of cocaethylene, an active metabolite of the combination of EtOH and cocaine, suggests another reason that the combination of EtOH and cocaine acts directly on the same neurochemical mechanism. It is conceivable that the formation of this compound may act on neurochemical systems mediating

reward. If this is correct, one would expect that cocaethylene would be highly rewarding itself and would be self-administered. A recent report (Jatlow et al., 1991) has shown maintenance of intravenous cocaethylene self-administration in primates and enhanced locomotor activity in rats. Moreover, these investigators report that cocaethylene was equipotent with cocaine in blocking the reuptake of dopamine into synaptosomes and increasing extracellular dopamine in the nucleus accumbens. It is also conceivable that the production of cocaethylene may be an additional mechanism of action to the direct effects of EtOH and cocaine on brain reward systems.

In conclusion, our data show that EtOH and cocaine can have synergistic effects on BSR. Further investigation of the neuropharmacological basis of these actions may increase our understanding of the concurrent abuse of EtOH and cocaine as well as the abuse of each alone.

REFERENCES

- Aston-Jones, S., Aston-Jones, G., & Koob, G. F. (1984). Cocaine antagonizes anxiolytic effects of ethanol. *Psychopharmacology*, 84, 28-31.
- Bain, G. T., & Kornetsky, C. (1989). Ethanol oral self-administration and rewarding brain stimulation. *Alcohol*, 6, 499-503.
- Carlson, R. H., & Lydic, R. (1976). The effects of ethanol upon threshold and response rate for self-stimulation. *Psychopharmacology*, 50, 61-64.
- Carson, A., Engel, J., & Svensson, T. H. (1972). Inhibition of ethanol-induced excitation in mice and rats by alpha-p-tyrosine. *Psychopharmacologia*, 26, 307-312.
- Crow, T. J. (1970). Enhancement by cocaine of intracranial self-stimulation in the rat. *Life Sciences*, 9, 375-381.
- Di Chiara, G., Acquas, E., & Carboni, E. (1992). Drug motivation and abuse: A neurobiological perspective. In P. Kalivas & H. H. Samson (Eds.), *The neurobiology of drug and alcohol addiction* (pp. 207-219). New York: New York Academy of Sciences.
- Esposito, R. U., Motola, A. H. D., & Kornetsky, C. (1978). Cocaine: Acute effects on reinforcement thresholds for self-stimulation behavior in the medial forebrain bundle. *Pharmacology Biochemistry and Behavior*, 8, 437-439.
- Fischman, M. W. (1987). Cocaine and the amphetamines. In H. Y. Meltzer (Ed.), *Psychopharmacology: The third generation of progress* (pp. 1543-1553). New York: Raven.
- Grant, B. F., & Harford, T. C. (1990). Concurrent and simultaneous use of alcohol and cocaine: Results of national survey. *Drug & Alcohol Dependence*, 25, 97-104.
- Huston, J. (1968). Reinforcement reduction: A method for training ratio behavior. *Science*, 159, 444.
- Huston, J., & Mills, A. W. (1971). Threshold of re-

- inforcing brain stimulation. *Communications in Behavioral Biology*, 5, 331-340.
- Jatlow, P., Elsworth, J. D., Bradberry, C. W., Winger, G., Taylor, J. R., Russell, R., & Both, R. H. (1991). Cocaethylene: A neuropharmacologically active metabolite associated with concurrent cocaine ethanol ingestion. *Life Sciences*, 48, 1787-1794.
- Kornetsky, C., Huston-Lyons, D., & Porrino, L. J. (1991). The role of olfactory tubercle in the effects of cocaine, morphine and brain stimulation reward. *Brain Research*, 541, 75-81.
- Lewis, M. J. (1985). Ethanol and cocaine show additive effects on BSR. *Alcoholism: Clinical and Experimental Research*, 9(2), 204.
- Lewis, M. J. (1990). Alcohol effects on brain-stimulation reward: Blood alcohol concentration and site specificity. In R. E. Meyers, G. F. Koob, M. J. Lewis, & S. Paul (Eds.), *Neuropharmacology of ethanol: New approaches* (pp. 163-178). Boston: Birkhauser.
- Lewis, M. J., & June, H. L. (1990). Neurobehavioral studies of ethanol reward and activation. *Alcohol*, 7(3), 213-219.
- Lewis, M. J., Perry, L. B., June, H. L., Garnet, M. L., & Porrino, L. J. (1990). Regional changes in functional brain activity with ethanol stimulant and depressant effects. *Society for Neuroscience Abstracts*, 16, 459.
- Lewis, M. J., & Phelps, R. W. (1987). A multifunctional on-line brain stimulation system: Investigation of alcohol and aging effects. In M. A. Bozarth (Ed.), *Reinforcing properties of abused drugs* (pp. 463-478). New York: Springer-Verlag.
- Moolten, N. M., & Kornetsky, C. (1990a). Cocaine potentiates ethanol threshold lowering effects on brain-stimulation reward. *Society of Neuroscience Abstracts*, 16, 765.
- Moolten, M., & Kornetsky, C. (1990b). Oral self-administration of ethanol and not experimenter-administered ethanol facilitates rewarding brain stimulation. *Alcohol*, 7, 221-225.
- Pohorecky, L. A. (1977). Biphasic action of ethanol. *Biobehavioral Reviews*, 1(4), 231-240.
- Rafla, F. K., & Epstein, R. L. (1979). Identification of cocaine and its metabolites in human urine in the presence of alcohol. *Journal of Analytical Toxicology*, 3(2), 59-63.
- Vrtunski, P., Murray, R., & Wolin, L. R. (1973). The effect of alcohol on intracranially reinforced response. *Quarterly Journal of the Studies of Alcohol*, 34, 718-725.
- Yeh, S. Y., & Haertzen, C. A. (1991). Cocaine-induced locomotor activity in rats. *Pharmacology Biochemistry and Behavior*, 39, 723-727.