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Microinjections of Dopamine Agonists in the Nucleus Accumbens Increase Ethanol-Reinforced Responding

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HODGE, C. W., H. H. SAMSON AND M. HARAGUCHI. *Microinjections of dopamine agonists in the nucleus accumbens increase ethanol-reinforced responding.* PHARMACOL BIOCHEM BEHAV **43**(1) 249–254, 1992.—Long-Evans rats (N = 3) were trained to lever press on a fixed-ratio 4 (FR 4) schedule with ethanol (10% v/v) presented as the reinforcer. Each rat received a total of six bilateral nucleus accumbens microinjections, one per week. They were tested with one physiological saline control, three 20.0-μg/brain *d*-amphetamine, and two 6.0-μg/brain quinpirole injections given 10 min prior to operant sessions. Ethanol-reinforced responding terminated after approximately 10 min during control sessions. Microinjections of the D_2 agonist quinpirole and the nonspecific dopamine (DA) agonist *d*-amphetamine increased total responding but produced slowed response rates that continued for 45–60 min. The slowed response rate produced by *d*-amphetamine resulted in a peak increase in interresponse times (IRTs) between 8–10 s, whereas quinpirole increased IRTs in the 14- to 16-s range, indicating that nonspecific DA activation resulted in higher rates of ethanol-reinforced responding than specific D_2 activation although both drugs decreased local response rates. These data indicate that the amount and temporal extent of ethanol-reinforced responding are increased by microinjections of DA agonists in the nucleus accumbens and support the hypothesis that DA activity in this region is involved in the regulation of ethanol-reinforced responding.

Ethanol reinforcement Dopamine Nucleus accumbens Oral ethanol self-administration
d-Amphetamine Quinpirole Rats

UNDERSTANDING the CNS mechanisms that control the onset and offset of excessive intake of ethyl alcohol (ethanol) are key to the development of both preventative and therapeutic interventions in alcoholism. The mesolimbic-mesocortical dopamine (DA) system has been implicated in reinforcement by many drugs of abuse including *d*-amphetamine (12,24,26), cocaine (3,17,18), and opiates (11,23), as well as the integration of behavioral activity in general (10). However, the degree to which this system is involved in the regulation of ethanol self-administration remains to be elucidated (9).

Recently, we have shown that bilateral microinjections of the nonspecific DA agonist *d*-amphetamine (20.0 μg/brain) and the D_2 agonist quinpirole (4.0 μg/brain) in the ventral striatum (nucleus accumbens) increase ethanol-reinforced lever pressing, whereas the D_2 antagonist raclopride (0.5 and 1.0 μg/brain) significantly decreases responding (21). Increased number of responses was due to a protracted pattern of responding that continued for the duration of 30-min operant sessions. The results suggested that the mesolimbic DA system is involved in regulation of ethanol-reinforced behavior and that increased DA activity produced a disruption of the mechanisms responsible for the offset of drinking.

It could be hypothesized that responding might continue for as long as the agonist-induced DA activation was in effect. However, procedural constraints imposed an upper limit of 30 min on responding and the temporal extent of this effect was not explored. Thus, this role of the mesolimbic DA system in the regulation of ethanol-reinforced behavior remains to be clarified.

The present experiment was designed to a) replicate the initial finding of prolonged ethanol-reinforced responding produced by DA agonists and b) determine if the increased responding would continue for longer periods.

METHOD

Animals

Three male Long-Evans rats weighing 300–350 g were obtained from the Psychology Department's breeding facility at the University of Washington. Rats were housed individually

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in standard stainless steel hanging cages with food (Wayne Rodent Blox 8604, Wayne Laboratories, Bartonville, IL) always available. Water access was restricted during the initial 2 days of lever press shaping but was otherwise available continuously. The colony room was maintained on a 12 L:12 D cycle with lights on at 0700 h. Temperature and humidity were maintained within NIH guidelines. All experimental sessions were run during the light portion of the cycle.

Apparatus

The apparatus used in this study has been previously described (21,22). Briefly, operant sessions wee conducted in Plexiglas chambers (27 × 37 × 37 × 21 cm) located in sound-attenuating cubicles. The chambers were equipped with two liquid dispensers (Ralph Gerbrands Corp., Model B-LH, Arlington, MA) that presented fluid in a 0.1-ml dipper for 3 s during each operation. Responses on a lever located on the left wall resulted in activation of the left dipper. The right dipper was inactive. Apple IIe microcomputers, interfaced with the chambers, were programmed to record lever-press responses and initiate dipper presentations. Microinjector cannulae were connected with PE-20 tubing to 1-μl syringes (Hamilton, Reno, NV) mounted on a single microdrive pump (Harvard Apparatus, Model 22).

Procedure

Rats were given 1 week to adapt to individual housing conditions, during which time they were handled and weighed daily. They were then trained to orally self-administer 10% ethanol (v/v) on a fixed-ratio 4 (FR 4) schedule of reinforcement using a sucrose-substitution procedure as previously described (19). When ethanol-reinforced responding stabilized, cannula guides were surgically implanted. Daily 30-min operant sessions were resumed immediately after recovery from surgery.

Microinjections began when presurgery response rates and patterns were reestablished. Experimental sessions were run Monday–Friday. Microinjections were conducted once per week on Thursdays, with sham injections occurring each Wednesday. The data from Tuesdays were used as noninjection controls. Operant sessions following noninjection and sham controls were 30 min in duration during the first 3 weeks and 1 h in duration during the final 3 weeks of the experiment. Operant sessions following all injections of *d*-amphetamine and quinpirole were 1 h in duration. The duration of saline sessions was 30 min. Operant sessions began 10 min after microinjections and sham injections.

Surgery

Rats were anesthetized with equithesin (3.0 ml/kg, IP) and placed in a stereotaxic device (David Kopf Instruments, Tujunga, CA, Model 1204 with rodent adaptor) with the incisor bar 5 mm above the interaural line. Stainless steel cannula guides (26 ga) were implanted bilaterally to terminate 1 mm dorsal to the injection site. The guide cannulae were secured to the skull with dental cement and stainless steel cranial screws. Removable wire obturators (33 ga) were inserted in the full length of the cannulae to prevent obstruction by foreign substances and limit infection. Plastic caps were affixed around the cannula area to prevent animals from disrupting the obturators. The stereotaxic coordinates used for nucleus accumbens placements were 3.7 mm anterior to the bregma, 1.8 mm lateral to the midline, and 5.0 mm ventral to the cortical surface (14).

Microinjection Procedure

Prior to injections, unanesthetized animals were placed in a plastic tub (30 cm in diameter by 14 cm deep) to minimize movement. Obturators were removed and the cannula area was swabbed with sterile physiological saline. Bilateral saline and drug injections were performed through 33-ga stainless steel hypodermic tubing lowered to 1 mm below the end of guide cannulae. The pump delivered 0.5 μl over 60 s. Injectors were left in place for 30 additional s to allow drug diffusion. New sterile obturators were inserted after removal of the injectors.

Sham injections were conducted similarly with two exceptions. First, the injectors were the same length as the guide cannulae to prevent brain penetration. Second, although the pump was operated the syringes were not driven.

Drugs and Dosing

All rats received one bilateral microinjection of physiological saline, three microinjections of the DA agonist *d*-amphetamine (20 μg/brain), and two injections of the D_2 agonist quinpirole (LY171555, 6.0 μg/brain). Injections were in a total volume of 1.0 μl/brain (0.5 μl/side). Drugs were dissolved in physiological saline and shaken on a mechanical shaker. New drug solutions were prepared immediately prior to each injection session.

Histology

Rats were deeply anesthetized with pentobarbital sodium and perfused transcardially with a sodium phosphate buffer solution (pH 7.5) followed by 10% formaldehyde. Brains were removed immediately and stored in 10% formaldehyde for at least 5 days, and were then cut into 90-μm coronal sections and stained with cresyl violet.

Data Analysis

Total responses. Total number of ethanol-reinforced lever-press responses were computer recorded during each session. Average number of responses and ethanol intake (g/kg) for *d*-amphetamine and quinpirole injection sessions were statistically compared to corresponding sham conditions by repeated-measures analysis of variance (ANOVA) using a commercially available package (SYSTAT, Evanston, IL).

Response pattern. Computer-generated cumulative response records displayed the temporal distribution of responses. Drug-induced changes in response patterns were analyzed by comparing drug vs. sham interresponse time (IRT) distributions as previously described (20). Briefly, IRTs up to 30 s in duration were counted in 15 2-s bins. IRTs greater than 30 s were counted in a 16th bin. Relative frequencies were then derived by dividing the number of IRTs in each bin by the total. Changes in IRT distributions were quantified by calculating relative differences between each bin in the average drug and sham distributions.

RESULTS

Histological examination found that all injections were bilateral in the nucleus accumbens (Fig. 1). Although presurgery response patterns recovered prior to the beginning of microinjections, total responding decreased following surgery. Therefore, average ethanol intake (g/kg) on the 5 days prior to surgery was compared to average intake on the 5 days immediately preceding microinjection sessions. Postsurgery intake

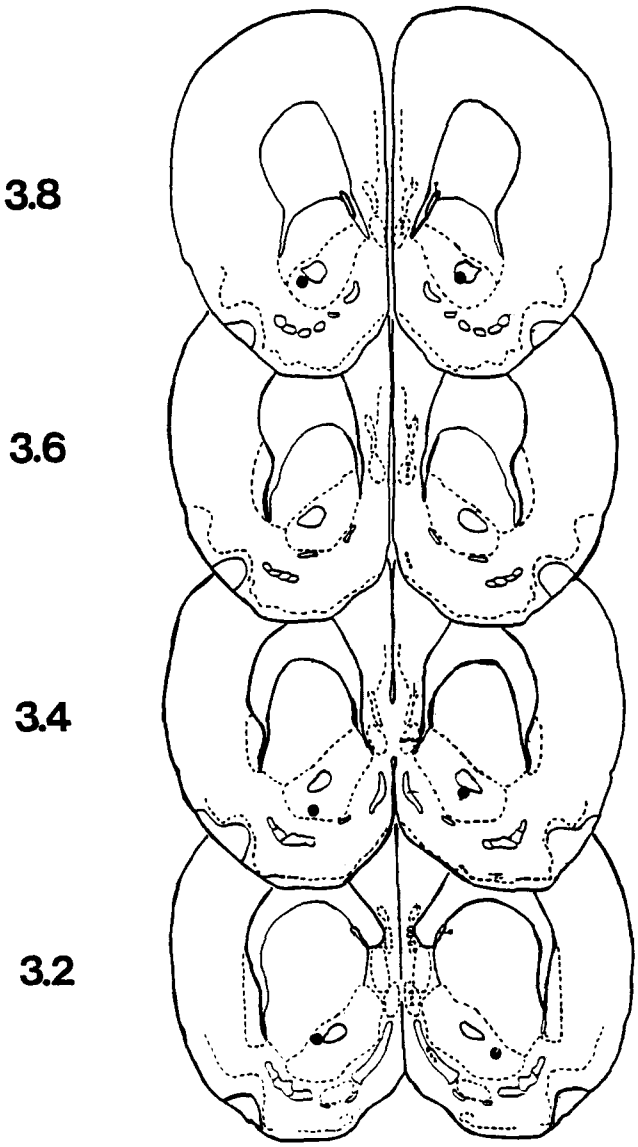

FIG. 1. Histological representations of microinjection sites within the ventral striatum (nucleus accumbens). Numbers on the left represent distance from bregma in mm (14).

levels (mean g/kg = 0.52) were significantly decreased from presurgery levels (mean g/kg = 0.67), $t(14) = 2.48$, $p = 0.026$.

Total Responding

Figure 2 shows that total responses increased slightly when noninjection and sham injection sessions were increased in duration from 30 to 60 min but differences were not statistically significant. Microinjections of *d*-amphetamine (20.0 μg/brain) increased total number of responses over 1-h sham injection controls although the amount of increase was less with each subsequent injection, $F(1, 8) = 13.30$, $p = 0.007$. Quinpirole (6.0 μg/brain) produced a similar increase in total number of responses that was also less marked on the second injection. Although the increases in total responding produced by quinpirole were in the same direction as those produced by *d*-amphetamine, they were not statistically significant due to intersubject variability.

Response Pattern

Figure 3 shows representative cumulative response records from one animal for saline, *d*-amphetamine (20.0 μg/brain), and quinpirole (6.0 μg/brain). The top two graphs show response patterns during 30-min sessions and the bottom four graphs show response patterns during 1-h sessions. Noninjection (data not shown) and sham injection response patterns were characterized by an initial high response rate followed by a period of little or no responding for the remainder of the session. This response pattern occurred regardless of session length. Saline injections produced no significant changes in response pattern. Microinjections of *d*-amphetamine and quinpirole, however, decreased the initial high response rate at the beginning of the session and also maintained this lower rate for much longer. This continuous pattern of responding that lasted for the duration of the 1-h sessions was similar to the effect previously reported following nucleus accumbens injections of *d*-amphetamine (21,22) and quinpirole in 30-min sessions (21). However, response rates occasionally slowed at approximately 45–50 min into the session. This occurred across animals during four of the nine *d*-amphetamine sessions and two of the six quinpirole sessions.

Changes in response pattern were analyzed by comparing sham and drug IRT distributions. The left side of Fig. 4 shows representative IRT distributions following microinjections of saline, *d*-amphetamine (20.0 μg/brain), and quinpirole (6.0 μg/brain) in the nucleus accumbens. The sham distributions associated with each drug injection were characterized by the majority of IRTs falling between 2–6 s (bins 1–3) and in the bin containing IRTs greater than 30 s. This indicates a biphasic response pattern (as shown in Fig. 3). Microinjections of *d*-amphetamine and quinpirole resulted in a decrease in the number of IRTs in the first bin (0–2 s) with increases distributed over the longer time bins, indicative of the slowed but continuous response rate.

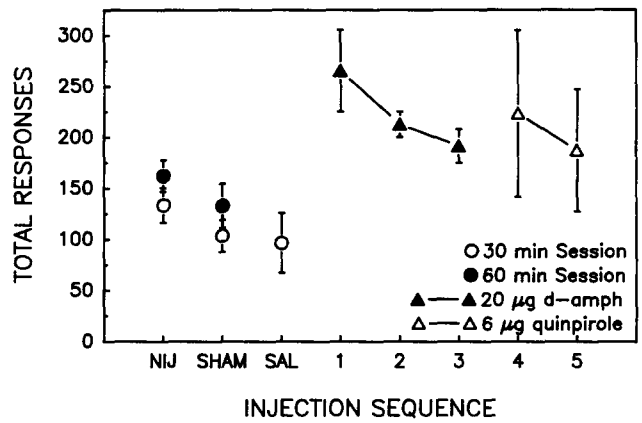

FIG. 2. Total number of ethanol-reinforced responses plotted as a function of injection sequence. Data points for no injection (NIJ) and sham injection (SHAM) represent the means from 6 days for each rat ($n = 18$). Data points for saline injections (SAL) and each drug injection are means from one injection for each rat ($n = 3$). Saline and drug injections were conduced in the order shown on the *x*-axis. Error bars are ± SEM.

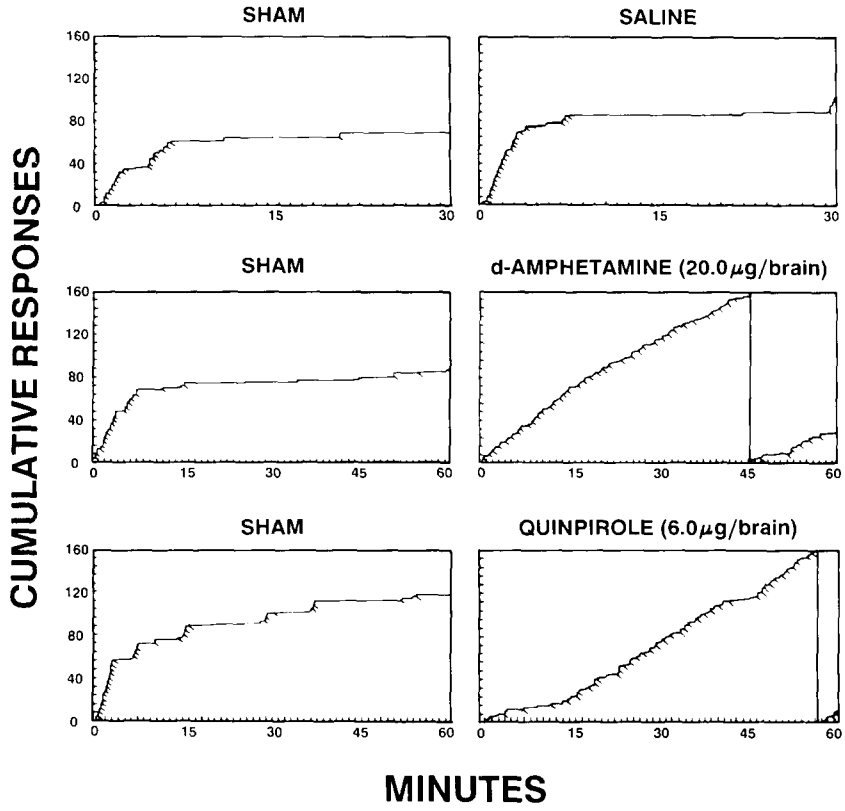

FIG. 3. Representative computer-generated cumulative response records for sham injections (left) and corresponding vehicle or drug injections (right) showing the pattern of ethanol-reinforced responding. Hatch marks on the graphs indicate delivery of 0.1 ml ethanol (10% v/v).

The right side of Fig. 4 shows relative change (drug/sham) in each IRT bin for saline, *d*-amphetamine, and quinpirole. Saline injections produced no relative change in the shape of the IRT distributions generated under sham conditions. Microinjections of *d*-amphetamine resulted in a peak relative increase in bin 5 (8–10 s) with additional spread occurring through bin 10. Quinpirole resulted in a similar effect with the peak increase occurring in bin 8 (14–16 s), which corresponds with the slightly slower response rate than that produced by *d*-amphetamine (Fig. 3). In addition, quinpirole shifted more IRTs to longer bins (bins 12–16) than *d*-amphetamine, producing a significantly different peak-shift location, $t(5) = 7.0, p = 0.001$.

Ethanol Intake

Increased total responding produced by microinjections of 20.0 μg/brain *d*-amphetamine increased possible ethanol intake (g/kg) over 1-h sham, $F(1, 8) = 14.58, p = 0.005$, but 6.0 μg/brain quinpirole failed to statistically increase intake. However, as previously reported in 30-min sessions (21), informal observation of rats indicated that not all of the presented ethanol was consumed following drug injections.

DISCUSSION

The purpose of this experiment was to test the effects of microinjections of DA agonists in the nucleus accumbens on ethanol-reinforced responding when operant sessions were increased from 30 min to 1 h. Both the nonspecific DA agonist *d*-amphetamine and the D_2 agonist quinpirole increased total ethanol-reinforced responding by producing a slow continuous response pattern that lasted for most of the 1-h sessions. This finding corresponds with previous observations of DA agonist-induced continuous responding for the duration of 30-min sessions (21,22).

It has been hypothesized that DA activity regulates behavior controlled by primary reinforcers or stimuli paired with primary reinforcers (2) and that increases in nucleus accumbens DA activity enhances control by these stimuli (7). In the operant behavioral situation, environmental stimuli occasion response sequences that produce the reinforcing stimulus and related events (e.g., blood ethanol levels, ataxia, satiety, etc.). It is assumed that these associated events most likely control the offset of responding. The increased responding produced in the present experiment suggests that agonist-induced increases in nucleus accumbens DA activity disrupts these behavioral sequences.

The occasional failure of animals to drink presented ethanol indicates that the behavior of approaching the dipper and drinking was disrupted but not the response that produced the reinforcer (i.e., the lever press). Thus, total session lever pressing increased but did not appear to always be controlled by the primary reinforcer. This suggests that the changes in DA activity resultant from agonist injection may have inter-

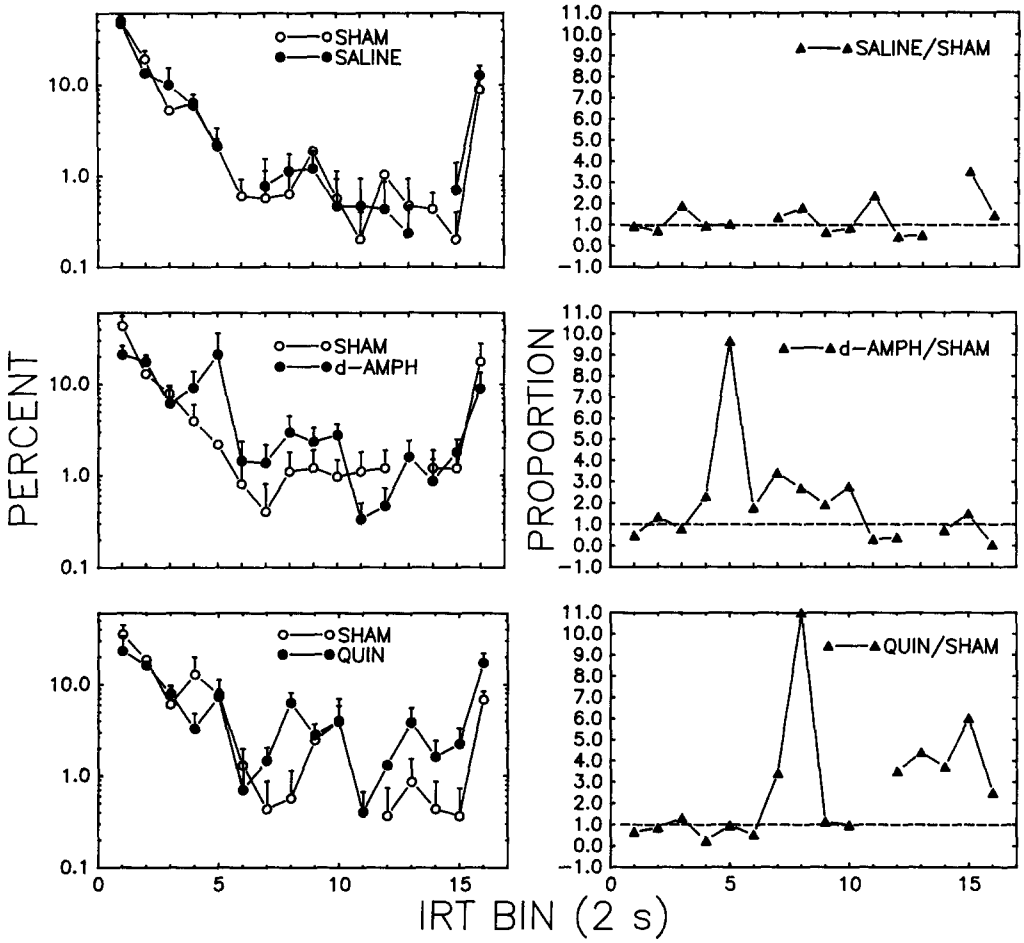

FIG. 4. IRT distributions for sham and drug sessions (left panel) and relative change in distributions (right panel) plotted as a function of 2-s bins. IRT distributions are plotted as the percent of total IRTs in each bin averaged over all subjects and injections. Error bars represent SEM. Relative change graphs were plotted by dividing each drug point by each corresponding sham point in the average IRT distributions. Horizontal lines indicate no change from sham. Missing data points indicate that no IRTs occurred in that bin.

fered with the normal regulatory role of the nucleus accumbens in initiating or terminating appropriate moment-to-moment response sequences (13) rather than altering the reinforcing impact of the ethanol stimulus or stimuli paired with the ethanol that may have acquired motivational significance (7). However, the present data support the hypothesis that changed nucleus accumbens DA activity interferes with the mechanisms that normally control ethanol drinking bouts (21).

Microinjections of quinpirole (0.3–3.0 μg/side) have been shown to potentiate locomotor activity, but increases are not as great as those produced by the D_1 receptor agonist SKF 38393 (5.0 μg/side) or the additive effect produced by the two in combination (4). However, nucleus accumbens injections of higher doses of quinpirole (4.0 μg) and SKF 38393 (10.0 μg) when coadministered result in locomotor stimulation similar to that produced by *d*-amphetamine (10.0 μg) but not when administered separately (16). The present finding that *d*-amphetamine produced higher response rates and totals than quinpirole suggests that locomotor activation produced by combined D_1 and D_2 activation may play a role in the *d*-amphetamine effect (1). Because quinpirole produced similar response patterns but lower response rates (i.e., less locomotor activation), the present data may indicate that the termination of ethanol-reinforced responding is at least partially mediated through a D_2 mechanism.

Further support for this conclusion comes from recent evidence indicating that nucleus accumbens injections of SK&F 38393 (2.0 μg) followed by quinpirole (2.0 μg) result in activation of ventral pallidum neurons, with no changes observed when the two drugs were administered in reverse order or in isolation (25). This suggests that both D_1 and D_2 activation in the nucleus accumbens may be necessary for postsynaptic locomotor effects (16) controlled by the striatal-globus pallidum-thalamus-motor cortex system (8). Thus, the present observation of continued responding for ethanol reinforcement following both nonspecific DA and specific D_2 activation in the nucleus accumbens suggests that the mechanisms regulating the termination of ethanol-reinforced responding may be similar to those that control locomotor activity.

Based upon microdialysis data indicating that systemically administered ethanol increases extracellular concentrations of DA in the nucleus accumbens (5), it may be hypothesized that orally consumed ethanol could have a similar effect on nucleus accumbens DA. Thus, under control conditions, the termination of ethanol-reinforced responding may be correlated with an increase in extracellular DA in the nucleus accumbens. However, because locally applied quinpirole inhibits DA release in the nucleus accumbens (6), the continued response pattern observed in the present experiment may have been due to the inability of ethanol to produce DA release in the presence of the DA agonists. It should be noted, however, that opposite conclusions relating increases in DA with seeking behavior and decreases in DA activity with satiety have been drawn from neurochemical analyses of extracellular DA activity during feeding and sexual behavior in rats (15). Therefore, the issue of whether increases or decreases in nucleus accumbens DA activity are correlated with the termination of ethanol intake cannot be satisfactorily addressed until DA activity is measured during oral self-administration.

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REFERENCES

1. Annett, L. E.; Ridley, R. M.; Gamble, S. J.; Baker, H. F. Behavioural effects of intracerebral amphetamine in the marmoset. Psychopharmacology (Berl.) 81: 18–23; 1983.
2. Beninger, R. J. The role of dopamine in locomotor activity and learning. Brain Res. Rev. 6:173–196; 1983.
3. de Wit, H.; Wise, R. A. Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide; but not with the noradrenergic blockers phentolamine or phenoxybenzamine. Can. J. Psychol. 31:195–203; 1977.
4. Dreher, J. K.; Jackson, D. M. Role of D1 and D2 dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats. Brain Res. 487:267–277; 1989.
5. Imperato, A.; DiChiara, G. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J. Pharmacol. Exp. Ther. 238:219–228; 1986.
6. Kalivas, P. W.; Duffy, P. A comparison of axonal and somatodendritic dopamine release using in vivo dialysis. J. Neurochem. 56:961–967; 1991.
7. Kelley, A. E.; Delfs, J. M. Dopamine and conditioned reinforcement I. Differential effects of amphetamine microinjections into striatal subregions. Psychopharmacology (Berl.) 103:187–196; 1991.
8. Kelly, J. P. Anatomical basis of sensory perception and motor coordination. In: Kandel, E. R.; Schwartz, J. H., eds. Principles of neural science. New York: Elsevier Science; 1985:223–243.
9. Koob, G. F.; Goeders, N. E. Neuroanatomical substrates of drug self-administration. In: Liebman, J. M.; Coopers, S. J., eds. Topics in experimental psychopharmacology: The neuropharmacological basis of reward. vol. 1. Oxford, UK: Oxford University Press; 1989:214–263.
10. Le Moal, M.; Simon, H. Mesocorticolimbic dopamine network: Functional and regulatory roles. Physiol. Rev. 71:155–324; 1991.
11. Leone, P.; Pocock, D.; Wise, R. A. Morphine-dopamine interaction: Ventral tegmental morphine increases nucleus accumbens dopamine release. Pharmacol. Biochem. Behav. 39:469–472; 1991.
12. Lyness, W. H.; Friedle, N. M.; Moore, K. E. Destruction of dopaminergic nerve terminals in nucleus accumbens: Effects of d-amphetamine self-administration. Pharmacol. Biochem. Behav. 11:553–556; 1979.
13. Mogenson, G. J.; Jones, D. L.; Yim, C. Y. From motivation to action: Functional interface between the limbic system and the motor system. Prog. Neurobiol. 14:69–97; 1980.
14. Pellegrino, L. J.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Century Crofts; 1967.
15. Phillips, A. G.; Pfaus, J. G.; Blaha, C. D. Dopamine and motivated behavior: Insights provided by in vivo analyses. In: Willner, P.; Scheel-Kruger, J., eds. The mesolimbic dopamine system: From motivation to action. New York: John Wiley & Sons; 1991: 199–224.
16. Plaznik, A.; Stefanski, R.; Kostowski, W. Interaction between accumbens D1 and D2 receptors regulating rat locomotor activity. Psychopharmacology (Berl.) 99:558–562; 1989.
17. Roberts, D. C. S.; Koob, G. F. Disruption of cocaine self-administration following 6-hydroxydopamine lesions of ventral tegmental area in rats. Pharmacol. Biochem. Behav. 17:901–904; 1982.
18. Roberts, D. C. S.; Koob, G. F.; Klonoff, P.; Fibiger, H. C. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacol. Biochem. Behav. 127:781–787; 1980.
19. Samson, H. H. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol. Clin. Exp. Res. 10:436–442; 1986.
20. Samson, H. H.; Hodge, C. W.; Tolliver, G. T.; Haraguchi, M. Effects of dopamine agonists and antagonists on ethanol reinforced behavior: The involvement of the nucleus accumbens. Brain Res. Bull. (submitted).
21. Samson, H. H.; Toliver, G. T.; Haraguchi, M.; Hodge, C. W. Alcohol self-administration: Role of mesolimbic dopamine. In: Samson, H. H.; Kalivas, P. W., eds. The neurobiology of drug and alcohol addiction. Ann. NY Acad. Sci.; 654:242–251; 1992.
22. Samson, H. H.; Tolliver, T. T.; Haraguchi, M.; Kalivas, P. W. Effects of d-amphetamine injected into the n. accumbens on ethanol reinforced behavior. Brain Res. Bull. 27:267–271; 1991.
23. Smith, S. G.; Davis, W. M. Haloperidol effects morphine self-administration: Testing for pharmacological modification of the primary reinforcing mechanisms. Psychol. Rec. 23:215–221; 1973.
24. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Attenuation of heroin reward in rats by disruption of the mesolimbic dopamine system. Psychopharmacology (Berl.) 79:278–283; 1989.
25. Yang, C. R.; Mogenson, G. J. Ventral pallidal neuronal responses to dopamine receptor stimulation in the nucleus accumbens. Brain Res. 489:237–246; 1989.
26. Yokel, R. A.; Wise, R. A. Increased lever pressing for amphetamine after pimozide in rats: Implication for a dopamine theory of reward. Science 187:547–549; 1975.