NUCLEUS ACCUMBENS DOPAMINE DEPLETIONS MAKE ANIMALS HIGHLY SENSITIVE TO HIGH FIXED RATIO REQUIREMENTS BUT DO NOT IMPAIR PRIMARY FOOD REINFORCEMENT

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Abstract—It has been suggested that dopamine in nucleus accumbens is involved in the process of enabling organisms to overcome work-related response costs. One way of controlling work costs with operant schedules is to use fixed ratio schedules with different ratio requirements. In the present study, the effects of nucleus accumbens dopamine depletions were investigated using six schedules: fixed ratio 5, 20, 50, 100, 200, and 300. In the first three schedules the food reinforcement consisted of one 45 mg food pellet per ratio completed. In the remaining schedules the food reinforcement per ratio completed was increased to two pellets for fixed ratio 100, four pellets for fixed ratio 200, and six pellets for fixed ratio 300. All rats were trained extensively prior to surgery, and rats were able to maintain high levels of responding on all schedules up to the fixed ratio 300. After training, rats were injected with either ascorbate vehicle or 6-hydroxydopamine into the nucleus accumbens. Rats were tested post-surgically on each of the schedules, with 3 days of testing per schedule.

Rats with nucleus accumbens dopamine depletions exhibited behavioral deficits that were highly dependent upon the ratio value. There were small and transient effects of dopamine depletion on fixed ratio 5 lever pressing, but as the ratio value got larger the impairment became greater. On the fixed ratio 20 and 50 schedules, response rates were partially reduced in dopamine-depleted rats. Responding on the fixed ratio 200 and 300 schedules was severely impaired, and on the last day of fixed ratio 300 testing no dopamine-depleted rats obtained a single reinforcer. These data are consistent with previous reports that accumbens dopamine depletions enhance ‘ratio strain’, making rats more sensitive to high ratio values. The induction of ratio strain by dopamine depletions does not appear to be related to a loss of appetite, and seems to be relatively independent of the baseline rate of responding and the overall density of food reinforcement across the session.

We conclude that dopamine in nucleus accumbens may be important for enabling rats to overcome behavioral constraints such as work-related response costs, and may be critical for the behavioral organization and conditioning processes that enable animals to emit large numbers of responses in the absence of primary reinforcement. © 2001 IBRO. Published by Elsevier Science Ltd. All rights reserved.

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The behavioral functions of nucleus accumbens dopamine (DA) continue to be the subject of great interest in neuroscience. It was suggested for many years that accumbens DA mediates the reinforcing effects of drugs of abuse (e.g. Wise, 1982; Caine and Koob, 1994), as well as the primary reinforcing characteristics of natural reinforcers such as food (Wise et al., 1978; Wise, 1982; Hernandez and Hoebel, 1988; Smith, 1995). Yet despite the longevity and popularity of this view, there is now a considerable body of evidence demonstrating that accumbens DA does not mediate primary food reinforcement or motivation (Caine and Koob, 1994; Roberts et al., 1977; for reviews see Salamone, 1987, 1991, 1992; Salamone et al., 1997, 1999). In fact, there is little evidence to indicate that performance on some operant tasks can be affected by accumbens DA depletions because of impairment in primary food reinforcement or motivation. For example, although performance on the continuous reinforcement schedule is highly dependent upon primary reinforcement and food motivation (Salamone et al., 1991; Aberman and Salamone, 1999), overall response output on this schedule was relatively unaffected by accumbens DA depletions (McCullough et al., 1993; Salamone et al., 1995; Aberman and Salamone, 1999). The effects of accumbens DA depletions that have been seen with some operant schedules were shown not to resemble the effects of extinction (McCullough et al., 1993; Salamone et al., 1995; for review see Salamone et al., 1997), and also did not resemble the effects of prefeeding to reduce food motivation (Salamone et al., 1991; Aberman and Salamone, 1999). Depletions of DA in nucleus accumbens did not suppress food intake (Koob et al., 1978; Salamone et al., 1993a), and failed to affect several parameters of feeding behavior, including total food intake, time spent feeding, feeding rate, or forepaw usage during food handling.

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Abbreviations: ANOVA, analysis of variance; DA, dopamine; EDTA, ethylenediaminetetra-acetate; FR, fixed ratio; n.s., not significant; 6-OHDA, 6-hydroxydopamine.
(Salamone et al., 1993a). Intra-accumbens injections of a high dose of the DA antagonist haloperidol did not suppress food intake, although the same dose injected into ventrolateral neostriatum did impair feeding (Bakshi and Kelley, 1991). Several studies involving choice procedures have demonstrated that accumbens DA depletions can affect performance on some instrumental tasks, yet the animals remain directed towards the acquisition and consumption of food (Salamone et al., 1991; Cousins et al., 1993, 1994, 1996; Salamone et al., 1997, 1999; Sokolowski and Salamone, 1998). Accumbens DA depletions did not alter the discrimination of reinforcement magnitude or the allocation of response choice based upon reinforcement magnitude (Salamone et al., 1994; Cousins et al., 1996). Thus, because primary food motivation is seen by many researchers and theorists as a fundamental aspect of food reinforcement (Thordike, 1911; Timberlake and Allison, 1974; Salamone, 1992; Salamone et al., 1997, 1999; Timberlake, 1993; Berridge and Robinson, 1998), considerable evidence now indicates that critical aspects of food reinforcement are left intact after interference with accumbens DA transmission. With the demise of the DA hypothesis of primary food reward, a number of alternative theoretical approaches have been offered in this area (Salamone et al., 1997, 1999; Berridge and Robinson, 1998).

One of the interesting features of this literature is that the effects of accumbens DA depletions on operant responding vary greatly depending upon the schedule being tested. As noted above, accumbens DA depletions have little overall effect on responding supported by continuous reinforcement (McCullough et al., 1993; Salamone et al., 1995; Aberman and Salamone, 1999). Accumbens DA depletions had little or no effect on the performance of interval schedules that generate relatively low baseline rates, such as variable- or fixed interval 30-s schedules (Sokolowski and Salamone, 1998; Cousins et al., 1999; Correa et al., in press). Although performance on a variable-interval 30-s schedule was shown not to be impaired by accumbens DA depletions (Sokolowski and Salamone, 1998; Correa et al., in press), the addition of a ratio requirement (i.e. fixed ratio (FR) 5) to the interval schedule did result in greater sensitivity to the effects of accumbens DA depletions (Correa et al., in press). This latter finding is consistent with recent evidence indicating that some ratio schedules are sensitive to the effects of accumbens DA depletions. Responding on progressive ratio schedules was suppressed by accumbens DA depletions (Aberman et al., 1998; Hamill et al., 1999). With FR schedules, the effect of accumbens DA depletions varies greatly depending upon the ratio value (Aberman and Salamone, 1999). Although FR1 performance is relatively unaffected by accumbens DA depletions, and FR4 or FR5 responding is transiently affected (Salamone et al., 1993b; Sokolowski et al., 1998; Aberman and Salamone, 1999), lever pressing on FR16 and FR64 schedules is much more severely impaired (Aberman and Salamone, 1999). Several different factors, such as dependence upon conditioned stimuli, the baseline rate of responding, the overall density of reinforcement or the work requirement of the schedule, may contribute to make higher value ratio schedules more sensitive to the effects of accumbens DA depletions (Aberman and Salamone, 1999; Salamone et al., 1999; Correa et al., in press). Clearly, additional research is needed to determine the factors that influence the sensitivity of some ratio schedules to the effects of accumbens DA depletions.

The present study was designed to investigate the effects of accumbens DA depletions on the performance of FR schedules across a broad range of ratio values (i.e. FR5, 20, 50, 100, 200, 300). FR64 performance has been studied previously in our laboratory, and in pilot studies we have employed FR80 or 100 schedules. One difficulty with increasing ratio size beyond this point is the phenomenon of ‘ratio strain’: untreated animals are resistant to lever pressing if the ratio value becomes too large (Staddon, 1979; Aberman and Salamone, 1999). The present report details the development of behavioral procedures that were capable of generating reliable performance up to FR300 in untreated rats. This performance was maintained by using an FR50 density of reinforcement for the higher ratio values. Thus, animals on the FR100 schedule received two pellets of reinforcement per ratio, while the FR200 was reinforced by four pellets and the FR300 by six pellets. This schedule enabled us to sustain very high levels of ratio performance, and to maintain the same level of overall reinforcement density (i.e. one pellet per 50 responses) across several different ratio values. In addition, extensive pilot studies showed that the baseline rate of responding was somewhat similar for the FR50, FR100, FR200 and FR300 schedules (i.e. less than 20% variability in mean responding across schedules). These features enabled us to study the effects of accumbens DA depletions on the performance of ratio schedules across a very broad range, while maintaining some similarity in overall reinforcement density and control response rate across the higher value ratio schedules (i.e. FR50–300).

**EXPERIMENTAL PROCEDURES**

**Subjects**

A total of 10 adult male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) were used in this experiment. Rats were housed in a colony maintained at a constant temperature (23°C) with a 12-h light/dark cycle (lights on at 07:00 h). All rats weighed between 290 and 330 g at the beginning of the study. Animals were food-deprived to 85% of their free feeding body weight, but were allowed a modest growth (up to 95% of original body weight) over the course of the study. Water was available *ad libitum* in the home cages at all times. Rats were group-housed until the beginning of the training period. All procedures were approved by the institutional animal care and use committee. All efforts were made to minimize the number of animals used and their suffering.

**Pre-surgical behavioral procedures**

Lever pressing sessions were conducted in operant chambers (28×23×23 cm; Med Associates, Georgia, VT, USA), and were conducted in the light part of the light/dark period (2–7 h after light onset). Animals were trained to lever press after 1 day of magazine training, and were maintained on a continuous rein-
force schedule for an additional 4 days (30-min sessions; reinforcement pellets were 45 mg). Rats were randomly assigned to the groups received bilateral injections of either 6-OHDA (n=5) or ascorbate vehicle (0.1%; n=5). The dose of 6-OHDA was 12.5 μg in 0.5 μl per side, which is identical to several previous studies (Aberman et al., 1998; Hamill et al., 1999; Aberman and Salamone, 1999; Correa et al., in press). Surgically were performed via 30-gauge stainless steel injectors directly into the nucleus accumbens (antero-posterior +2.8 mm, medio-lateral ±1.4 mm, dorso-ventral −7.8 mm; incisor bar 5.0 mm above the interaural line). The injection was driven at a flow rate (0.3 ml/min, for 5 min) by a Harvard Apparatus syringe pump. After post-surgical training, intra-accumbens injections of 6-OHDA were performed with the rats under pentobarbital anesthesia (50 mg/kg). All rats received i.p. injections of 10.0 mg/kg pargyline 30 min prior to surgery. Pargyline is an inhibitor of monoamine oxidase, which is a standard treatment to enhance the effect of 6-OHDA. Rats were randomly assigned to groups that received bilateral injections of either 6-OHDA (n=5) or ascorbate vehicle (0.1%; n=5). The dose of 6-OHDA was 12.5 μg in 0.5 μl per side, which is identical to several previous studies (Aberman et al., 1998; Hamill et al., 1999; Aberman and Salamone, 1999; Correa et al., in press). Post-surgical testing After a 2-day post-surgical recovery period, rats were tested for 3 days on each of the FR schedules using the following order (number of pellets per ratio in parentheses): FR5 (1), FR20 (1), FR50 (1), FR100 (2), FR200 (4), FR300 (6). After completion of the FR300 component, all rats were killed and dissected for tissue assays. Neurochemical analyses for tissue DA After completion of the experiment, rats were exposed to a carbon dioxide chamber for 30 s, decapitated, and then their brains were quickly removed and frozen. A 16-gauge stainless steel tube (inner diameter = 1.19 mm; outer diameter = 1.65 mm) was used to dissect bilateral tissue punch samples from a 0.75-mm thick coronal section through the nucleus accumbens. Tissue samples from each region were placed in 200 μl of chilled 0.1 N perchloric acid, homogenized, and centrifuged. The sample tubes were frozen, and the DA content of the supernatant was later determined using a high-performance liquid chromatography system that has been previously described (Aberman and Salamone, 1999; Cousins et al., 1993). The mobile phase consisted of a sodium phosphate buffer, with 7.0% methanol, EDTA, and 1.4 ml of a 0.4 mM sodium octyl sulfate solution added as an ion pairing agent to 11 of mobile phase. Standards of DA (Sigma Chemical, St. Louis, MO, USA) were assayed before, during, and after the tissue samples. Statistical analyses For the initial analyses, performance on each FR schedule was analyzed separately by a 2 (group) x 3 (day) factorial analysis of variance (ANOVA; Systat version 7.0, Chicago, IL, USA), with repeated measures on the day factor. Additional analyses were performed on the four schedules that had the same overall density of reinforcement (i.e. FR5/0, FR100/2, FR200/4, and FR300/6). For these analyses, the 3-day averages for each animal were used, and the data were analyzed with a 2 (group) x 4 (schedule) ANOVA, with repeated measures on the schedule factor. The significant interactions (P<0.05) were analyzed by analysis of simple effects (Keppel, 1982). RESULTS Figure 1 displays the total number of lever presses per day for 6-OHDA and vehicle-treated rats across all six lever pressing schedules. For the FR5 schedule, there was not a significant overall effect of 6-OHDA (F(1,8)=3.45, P<0.1), but there was a significant effect of days (F(2,16)=62.7, P<0.01) and a significant treatment group x day interaction (F(2,16)=18.43, P<0.01). Analysis of simple effects to determine the source of the interaction demonstrated that 6-OHDA-treated rats differed from the vehicle control group only on the first day of testing (F(1,8)=17.0, P<0.01), and the two groups did not differ on the second or third day of FR5 testing. On the FR20 schedule, there was a significant overall effect of 6-OHDA (F(1,8)=6.4, P<0.05), and there was a significant effect of days (F(2,16)=4.14, P<0.05), but no significant treatment group x day interaction (F(2,16)=2.3, not significant, n.s.). Analysis of the 3 days of FR50 testing showed that there was a significant overall effect of 6-OHDA (F(1,8)=10.5, P<0.01), but there was no significant effect of days (F(2,16)=0.3, n.s.), and no significant treatment group x day interaction (F(2,16)=1.2, n.s.). Over the 3 days of FR100 testing, there was a significant overall effect of 6-OHDA (F(1,8)=33.0, P<0.01), a significant effect of days (F(2,16)=6.14, P<0.05), and a significant treatment group x day interaction (F(2,16)=12.7, P<0.01). With the FR200 schedule, there was a significant overall effect of 6-OHDA (F(1,8)=73.6, P<0.01), and there was a significant effect of days (F(2,16)=3.6, P<0.05), but no significant treatment group x day interaction (F(2,16)=1.2, n.s.). Similarly, with performing on the FR300 schedule, there was a significant overall effect of 6-OHDA (F(1,8)=88.3, P<0.01), and there was a significant effect of days (F(2,16)=4.4, P<0.05), but no significant treatment group x day interaction (F(2,16)=1.4, n.s.). Additional analyses were performed on the data obtained during testing on the FR50/1, FR100/2, FR200/4, and FR300/6 schedules (Fig. 2). These analyses were performed on this group of schedules because they...
all had the same overall programmed density of reinforcement for large units of time (i.e. all schedules had an overall ratio of one pellet per 50 responses). Factorial ANOVA demonstrated that, within this group of schedules, there was a significant effect of 6-OHDA treatment ($F(1,8) = 47.2, P < 0.01$), and a significant effect of schedule ($F(3,24) = 48.3, P < 0.01$), as well as a significant interaction ($F(3,24) = 24.5, P < 0.01$). Analysis of simple effects showed that there was no effect of schedule within the vehicle control group ($F(3,12) = 2.25$, n.s.), which demonstrates that the control rates of responding did not differ across these schedules. Apparently, the predominant source of the interaction effect was the very robust effect of schedule in the 6-OHDA-treated group ($F(3,12) = 137.0, P < 0.001$). This significant effect of schedule reflects the significantly lower level of responding at higher ratio levels in DA-depleted rats.

Day-by-day analyses of the number of food pellets obtained within each schedule yielded the same ANOVA results as those obtained by analyzing the number of responses (data not shown). All reinforcement pellets were consumed by the rats prior to being removed from the test chamber. Table 1 shows the 3-day averages for the number of food pellets obtained on each schedule by DA-depleted and vehicle-treated rats. It can be seen that as ratio value was raised from FR5 to FR20 to FR50, control levels of the number of pellets obtained were highest for the FR5 schedule, and decreased as ratio value increased. As with the data on response number, additional analyses were performed on the data obtained with the FR50, 100, 200 and 300 schedules. Factorial ANOVA demonstrated that, within this group of schedules, there was a significant effect of 6-OHDA treatment ($F(1,8) = 48.0, P < 0.01$), and a significant effect of schedule ($F(3,24) = 53.8, P < 0.01$), as well as a significant interaction ($F(3,24) = 21.54, P < 0.01$). Analysis of simple effects showed that, despite a tendency for pellet number to decrease slightly as ratio value increased, there was not a significant effect of schedule within the vehicle control group ($F(3,12) = 3.3, P < 0.1$). As with numbers of responses, the predominant source of the interaction effect was the very robust effect of schedule in the 6-OHDA-treated group ($F(3,12) = 113.3, P < 0.001$).

Neurochemical analyses of the tissue samples from nucleus accumbens of vehicle- and 6-OHDA-treated rats demonstrated that there was a robust depletion of DA after 6-OHDA injection. The DA levels in the tissue (mean $\pm$ S.E.M.) pg DA/mg tissue) were as follows: vehicle $= 1.58 (\pm 0.22)$, 6-OHDA $= 0.18 (\pm 0.08)$. This difference between groups was statistically significant ($t = 6.0, df = 8$, $P < 0.001$), and these values represent a depletion of approximately 89% in 6-OHDA-treated rats.

**DISCUSSION**

The present results clearly demonstrate that accumbens DA depletions produce effects on lever pressing that are highly dependent upon the ratio requirement of the schedule. Performance on the FR5 schedule was only slightly suppressed by accumbens DA depletions, but the DA-depleted rats recovered rapidly. This is consistent with previous reports of recovery of response rate on FR4 or FR5 schedules after accumbens DA depletions (Salamone et al., 1993b; Aberman and Salamone, 1999). Responding on the FR20 and FR50 schedules also was suppressed in DA-depleted rats, although these animals still maintained a moderate rate of responding. As ratio value increased to FR100, FR200, and ultimately to FR300, DA-depleted rats showed greater impairments as a function of ratio size. Rats with accumbens DA depletions were severely impaired on the FR300 schedule, and by the last day of FR300 testing none of the 6-OHDA-treated rats received a single reinforcement. Of all the operant schedules investigated in previous studies from our laboratory, it is clear that the FR200 and FR300 are the schedules that are
most sensitive to the disruption produced by accumbens DA depletions.

It is extremely unlikely that the present effects are due to an action of the DA depletions on appetite, or a general interference with food motivation or primary food reward. Previous work has shown that depletions of DA in nucleus accumbens did not suppress food intake (Koob et al., 1978; Salamone et al., 1993a), and did not affect several parameters of feeding behavior (Salamone et al., 1993a). In the present study, the schedule with the highest food density (i.e. the FR5; see Table 1) was the schedule that was least affected by accumbens DA depletions. Previous work has shown that low to moderate value FR schedules that have high densities of food presentation, such as FR1 and FR4, are very sensitive to the effects of prefeeding to reduce food motivation (Aberman and Salamone, 1999). In addition, recent evidence from our laboratory has demonstrated that the appetit suppressant fenfluramine substantially reduces FR5 performance using the same type of reinforcement pellet used in the present study (Ariazi et al., 2000). Thus, if the effect of accumbens DA depletions was to suppress appetite or generally reduce food motivation, this effect should have yielded a much larger deficit on the FR5 schedule, which generated by far the largest amount of food reinforcement of any schedule in the present study. Yet despite the relatively large amount of food being presented, the FR5 schedule was only affected by accumbens DA depletions on the first day of testing, with numbers of responses emitted and food pellets obtained by DA-depleted rats showing complete recovery by the second and third day of testing. Recovery of function is a normal feature of the behavioral effects of DA depletions (Zigmond et al., 1984; Wolterink et al., 1990), including accumbens DA depletions (Salamone et al., 1993b; Aberman and Salamone, 1999; Correa et al., in press). The very rapid recovery seen in the present work is somewhat faster than the 1-week recovery period after DA depletions noted in previous papers for performance of similar schedules (Salamone et al., 1993b; Aberman and Salamone, 1999). It is possible that the very rapid recovery seen in the present paper is due to the fact that rats in the present study had experience with much higher ratios (i.e. up to FR300). Thus, experience with very high ratios could reduce the sensitivity to accumbens DA depletions if the animals are tested post-surgically on the low ratio schedule. Future research should investigate directly if prior experience with very large ratios alters the effects of accumbens DA depletions. Yet regardless of the mechanism responsible for recovery of FR5 responding in the present experiment, the mild deficit and rapid recovery on this schedule indicate that reductions in food intake per se do not account for the overall pattern of data seen across all schedules tested.

In a previous study of the effects of accumbens DA depletions upon ratio performance (FR1, 4, 16, 64), it was observed that 6-OHDA had effects that were related to ratio size (Aberman and Salamone, 1999). Thus, the FR1 schedule was unaffected, and the FR4 schedule was transiently effected, while the FR16 and 64 schedules were more substantially affected (Aberman and Salamone, 1999) in DA-depleted rats. In that study, it was recognized that the FR1 and FR64 schedules differ in many important ways. For example, the two schedules generate extremely different baseline rates of responding, with response rates on the FR64 schedule being 8–10 times higher than on the FR1 (Aberman and Salamone, 1999). In a recent review from our laboratory, we suggested that baseline response rate was an important factor in determining the sensitivity of an operant schedule to accumbens DA depletions (Salamone et al., 1999). According to this hypothesis, schedules that generate low control rates would be less sensitive to the effects of accumbens DA depletions than schedules that generate higher control rates. This relation may be valid over the low to moderate range of response rates (i.e. 0–2000 responses per 30 min; see Salamone et al., 1999, for review), or across low ratio values. For example, in the present study the FR20 generated higher control response rates after surgery than the FR5, and the effects of DA depletions were generally greater with the FR20 than with the FR5 schedule (see Fig. 1, days 2–6). Nevertheless, the present data demonstrate that differences in baseline response rate do not explain the sensitivity of the very high value ratio schedules to 6-OHDA, and indicate that other factors must also be important. In the present study, control levels of responding did not differ significantly between the FR50, 100, 200 or 300 schedules (Fig. 2). Yet, DA-depleted rats showed deficits in responding on the FR200 and FR300 schedules that were much larger than the deficits shown on the FR50 schedule. Thus, the extreme sensitivity of the FR200 and FR300 schedules to the effects of accumbens DA depletions cannot be explained simply because of differences in baseline or control rates of responding.

Another difference between FR64 and FR1 responding in the previous study (Aberman and Salamone, 1999) was the density of food reinforcement. The FR64 reinforced by one pellet, which is very sensitive to the effects of accumbens DA depletions (Aberman and Salamone, 1999), has a much lower density of reinforcement than the FR1 reinforced by one pellet. Even though rats on the FR64 adjust to this schedule by pressing faster, they still receive about 1/8 the food that rats on the FR1 receive (Aberman and Salamone, 1999). In the present study, the FR50/1, 100/2, 200/4 and 300/6 schedules were set to have roughly the same molar density of reinforcement (i.e. overall density across the schedule) by increas-

Table 1. Mean (±S.E.M.) number of 45-mg pellets received by vehicle- and 6-OHDA-treated rats on all schedules during the post-surgical test period

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Vehicle</th>
<th>6-OHDA</th>
</tr>
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<tbody>
<tr>
<td>FR5</td>
<td>119.6 (9.9)</td>
<td>86.8 (14.6)</td>
</tr>
<tr>
<td>FR20</td>
<td>58.6 (6.7)</td>
<td>39.3 (3.9)</td>
</tr>
<tr>
<td>FR50</td>
<td>24.1 (2.6)</td>
<td>15.3 (0.9)</td>
</tr>
<tr>
<td>FR100</td>
<td>23.2 (2.2)</td>
<td>8.1 (1.5)</td>
</tr>
<tr>
<td>FR200</td>
<td>20.5 (2.0)</td>
<td>2.4 (1.0)</td>
</tr>
<tr>
<td>FR300</td>
<td>22.0 (1.8)</td>
<td>2.4 (0.7)</td>
</tr>
</tbody>
</table>

Three-day averages are shown for each group and schedule.
ing the number of pellets delivered in proportion to the increase in ratio size. With this procedure, there was not a significant difference in total food obtained by control animals across these four schedules. There was a tendency for rats on the higher ratios to get slightly fewer food pellets because the session would sometimes end while the animals had not yet completed the end of the very large ratios. Nevertheless, the molar densities of reinforcement were programmed to be similar over the course of the session, and Table 1 shows that the actual amounts of food obtained by control animals were indeed quite similar across the FR50, 100, 200 and 300 schedules. Thus, the observations that DA-depleted rats on the FR100 showed progressive decrements in responding over days, and that DA-depleted rats on the FR200 and FR300 were so severely impaired compared to the FR50 schedule, do not seem to be attributable to differences in the molar reinforcement density that is programmed for each schedule. This conclusion is similar to the results of a recent study that compared a variable-interval 30-s schedule with a variable-interval 30-s schedule that had a FR5 ratio requirement attached (Correa et al., in press). Taken together, these studies suggest that the molar density of reinforcement that is programmed for the schedule is not the predominant factor in determining sensitivity to the effects of accumbens DA depletions. Moreover, research with maze tasks has demonstrated that haloperidol and accumbens DA depletions did not alter discrimination or response choice based upon the number of food pellets presented (i.e. choice between four and zero pellets, or between four and two pellets; see Salamone et al., 1994; Cousins et al., 1996). Rats with excitotoxic lesions of nucleus accumbens were shown to be sensitive to changes in reinforcement magnitude (Balleine and Killcross, 1994), and a previous report demonstrated that systemic DA antagonism did not alter the perception of reinforcement magnitude as determined in an operant psychophysical procedure (Martin-Iverson et al., 1987). Thus, it seems unlikely that accumbens DA depletions impaired the perception of reinforcement magnitude.

CONCLUSIONS

In summary, it does not appear as though accumbens DA depletions have substantial effects on operant lever pressing on some schedules because of reduced appetite, or because of sensitivity to differences in molar reinforcement density between different schedules, or impairments in the perception of reinforcement magnitude. Nevertheless, across a broad range of experiments that include the present data, three generalizations can be made. First, on some schedules, such as FR1 or variable-interval 30 s, there is little or no overall effect of accumbens DA depletions on total response output (McCullough et al., 1993; Salamone et al., 1993b; Sokolowski and Salamone, 1998; Aberman and Salamone, 1999; Correa et al., in press). In general, the schedules that are insensitive to accumbens DA depletions are characterized by low response rates, as reflected either by total number of responses or by the interresponse time distribution. Second, responding on some schedules, such as FR5, FR16, or tandem variable-interval/FR5 (Salamone et al., 1993b; Sokolowski and Salamone, 1998; Aberman et al., 1998; Correa et al., in press), is affected by DA depletions in a manner that is best described as response slowing. In studies involving these schedules, which typically generate moderate to high rates of responding, animals with accumbens DA depletions continue to respond, but do so at a relatively low rate compared to controls. Considerable evidence indicates that accumbens DA depletions make animals less active (Koob et al., 1978; Cousins et al., 1993; Correa et al., in press), and several studies have shown that accumbens DA depletions produce a slowing of the interresponse time distribution (Salamone et al., 1993b; Sokolowski and Salamone, 1998). In the present study, a slowing of overall response rate was shown with the FR20 and FR50 schedules. Finally, with large ratio schedules such as FR64 (Aberman and Salamone, 1999), or FR200 and FR300 in the present work, responding is affected in a catastrophic manner by accumbens DA depletions. This effect could be described as ‘breaking’ or ‘ratio strain’ (Aberman and Salamone, 1999), because animals essentially stop responding, or show very long periods without responding. Based upon the present data, the development of ratio strain by DA-depleted rats appears to be independent of the programmed molar reinforcement density and the control rate of responding generated by the schedule. Moreover, it does not appear as though responding on very large ratios is reduced simply because of an absolute ceiling on the number of responses that can be emitted (see also Cousins and Salamone, 1994; Aberman and Salamone, 1999). In the present paper, DA-depleted rats that were capable of pressing 700–900 times in 15 min on the FR20 or FR50 schedules were reduced to much lower levels of responding on the FR200 and FR300 schedules (i.e. 0–300 response). In other words, DA-depleted rats were still capable of responding at a slow, steady rate provided that primary reinforcement is given every 20 or 50 responses. Yet, these DA-depleted rats showed dramatically reduced responding if they were required to press 200 or 300 times in the absence of primary reinforcement. Therefore, accumbens DA depletions do not simply produce a problem with emitting large numbers of responses. Rather, DA-depleted rats appear to be extremely sensitive to how the response requirement is organized. The specific behavioral processes that underlie the enhanced ratio strain shown by rats with accumbens DA depletions still are somewhat uncertain. Depletions of DA in accumbens increase the tendency to take long pauses in responding, which may interact with large ratio requirements to lead to enhanced ratio strain (Salamone et al., 1993b; Sokolowski and Salamone, 1998; Aberman and Salamone, 1999). There is no evidence that rats with accumbens DA depletions are particularly sensitive to the time requirement in intermittent schedules (Correa et al., in press), but they may be very sensitive to the work requirements of each ratio that must be completed in order to receive each unit of reinforcement. Consid-
erable evidence in the behavioral literature indicates that animals are sensitive to work-related response costs in operant procedures (Collier and Jennings, 1969; Staddon, 1979, 1983; Kaufman, 1980; Hursh et al., 1988; Salamone, 1992) and it is possible that accumbens DA depletions enhance this sensitivity (Salamone, 1987, 1991, 1992; Salamone et al., 1997, 1992). Accumbens DA depletions appear to render the animals much more dependent upon the direct feedback or behavioral activation provided by primary reinforcement, perhaps by affecting the higher order sensorimotor and conditioning processes that must sustain responding in the absence of primary reinforcement. For example, it is possible that accumbens DA depletions affect response organization and planning processes, or that they make rats less sensitive to the response-eliciting or response-sustaining effects of contextual cues in the environment (see also Cador et al., 1991; Salamone, 1991, 1992; Salamone et al., 1997; Berridge and Robinson, 1998; Parkison et al., 1999a,b). Considerable evidence indicates that accumbens DA depletions reduce behavioral activation and impair various schedule-induced activities (McCullough and Salamone, 1992; Cousins et al., 1993; Correa et al., in press), which may reflect a lack of activation in response to the temporal cues associated with the schedule. It is possible that several factors related to schedule requirements, including dependence upon conditioned stimuli, temporal factors, and kinetic or energetic requirements, combine to make some schedules particularly sensitive to the effects of accumbens DA depletions. Additional research will be necessary to identify more specifically the functions impaired by interference with accumbens DA transmission. Research on the functions of accumbens DA may yield insights into the neural bases of psychomotor slowing in psychiatric disorders (Salamone et al., 1997, 1999). Moreover, it is possible that the ability of frequent primary reinforcement to elicit FR1 or FR5 lever pressing in DA-depleted rats that do not respond to higher ratios is related to the 'paradoxical kinesias' elicited by sensory stimulation in patients with Parkinson's disease (Schwab and Zieper, 1965).

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