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Dopamine Applied into the Nucleus Accumbens and Discriminative Avoidance in Rats

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BRACS, P. U., D. M. JACKSON AND P. GREGORY. *Dopamine applied into the nucleus accumbens and discriminative avoidance in rats.* PHARMACOL BIOCHEM BEHAV **20**(1) 49–54, 1984.—The actions of dopamine (DA) administered into the nucleus accumbens on motor function and discrimination were examined in rats trained to perform a discriminative conditioned avoidance response (DCAR). α-Methyl-p-tyrosine was found to suppress performance of the CAR although it did not impair discrimination. The administration of DA reinstated CAR performance but it also increased discriminative errors. Multivariate comparisons suggested that both of these effects were closely related to the stimulation of intertrial crossings by DA.

α-Methyl-p-tyrosine Conditioned avoidance response Discrimination Dopamine Multivariate Nucleus accumbens

WE have previously demonstrated that dopamine (DA) applied bilaterally into the nucleus accumbens and caudate nucleus of rats trained to perform a simple conditioned avoidance response (CAR) is able to reverse the suppression of this task by α-methyl-p-tyrosine (α-MT), a tyrosine hydroxylase inhibitor which produces a transient depletion of catecholamines [11,15]. This reversal of the suppressed task lasted for about 8 hours, and a "pseudo" CAR occurred even in rats conditioned to the environment but naive to the unconditioned stimulus [11]. Separate studies indicated that reversal of the α-MT effect was accompanied by significant locomotor activity [11]. These findings confirm the association between DA and the performance of a variety of avoidance paradigms in rodents [1,24]. In studies of this sort, however, it is difficult to distinguish between the effects of DA on "higher" functions such as cognitive, associative, discriminative or memory processes and the effects of DA on performance-related factors such as motor function, sensory perception thresholds or reactivity to footshock. With regard to performance related factors, it is well documented that the nucleus accumbens plays a major role in the control of co-ordinated locomotor activity. Thus, the local application of DA receptor agonists such as DA, apomorphine, *d*-amphetamine and ergometrine produce marked locomotor excitation, which is readily antagonised by DA receptor antagonists [5, 16, 19].

The present study is a further investigation into the role of DA and the nucleus accumbens in learned behaviours. We have utilised a discriminative (D) CAR paradigm since there are a number of conflicting reports of the action of dopaminergic drugs on discrimination [3, 6, 9, 22]. We report here data showing that the local application of DA into the nucleus accumbens of rats reinstates a previously learned DCAR suppressed by α-MT, albeit with a minor impairment of discrimination, and that the effects of DA on the DCAR are highly associated with the locomotor stimulatory effects of DA.

METHOD

Animals

Male Sprague-Dawley rats (220–280 g, from the University of Sydney Animal Farms) were used in all experiments. Approximately 2 weeks before experimentation, the rats were transferred to a holding area, housed at 20–23°C under a 12 hr light–12 hr dark cycle beginning at 07.00 hr, and "gentled" by repeated handling. Rats were housed in groups of 5, and allowed food and water ad lib except during experimentation.

Apparatus

DCAR training was performed in a semi-automated two-way perspex® shuttle-box of length 630 mm, width 250 mm and height 210 mm, divided into two compartments by a central partition into which two openings were cut (80 mm wide, 100 mm high, 70 mm apart). The left side of each surface of the partition was painted black and the right side white as a discriminative cue for the rat. The floor consisted of a grid of stainless steel bars 6.3 mm in diameter connected to a 100 V current-limited supply shock generator-scrambler, and a 24 V house buzzer was mounted at each end as a conditioned stimulus (CS). The apparatus was enclosed within a dimly lit, sound-attenuated, ventilated chamber.

Surgery

The surgery has been described in detail previously

[11,16]. Briefly, guide cannulae were implanted in pentobarbitone-anesthetised rats and aimed bilaterally at the nucleus accumbens. After at least 48 hr recovery, DA (dissolved in 1 μl normal saline) or saline (1 μl) was injected into the nucleus accumbens, using co-ordinates of A 9.2, L 1.1, DV −0.8 [17]. The stated DA dose refers to the amount of DA base (administered as the hydrochloride) applied to each side of the brain. After experimentation was complete, the animals were sacrificed and the placement of the injections assessed histologically.

Behavioural Experiments

Prior to the commencement of training, each rat was randomly assigned the left or right door as the correct door. The onset of the CS marked the beginning of a trial. After 5 sec, the grid floor passed a 1.0 mA footshock to the rat for a maximum of 25 sec. The rat could terminate a trial at any time by traversing the chambers via the correct door. An avoidance response through the incorrect door (CAR−) resulted in the immediate initiation of footshock and an escape through the incorrect door (E−) failed to terminate the trial. A 30 sec intertrial interval was allowed as a rest period. The following responses were recorded: correctly discriminated CAR (CAR+), a passage through the correct door within 5 sec of the presentation of the CS; incorrectly discriminated CAR (CAR−), initial passage through the incorrect door within 5 sec of the presentation of the CS; total CAR, the sum of CAR+ and CAR−; correctly discriminated escape response (E+), initial passage through the correct door more than 5 sec after the presentation of the CS; incorrectly discriminated escape response (E−), initial passage through the incorrect door more than 5 sec after presentation of the CS; response failure (RF), failure to pass through either door within 30 sec of the presentation of the CS; intertrial crossing (ITC), passage through either door during the intertrial interval.

Animals were trained in daily sessions consisting of 40 trials until they reached a criterion of at least 80% CAR+ for 3 consecutive sessions. Following recovery from surgery, trained rats were administered α-MT (150 mg/kg IP) 12 hr before the intracerebral injection. A second dose of α-MT (50 mg/kg IP) plus nialamide (80 mg/kg IP) was administered 11 hr later. One hour after the nialamide, saline or DA (5 or 10 μg) was administered bilaterally into the nucleus accumbens. The animals' performance was examined in 10-trial test sessions at −0.5, 0.5, 1, 2, 3, 4, 8, 12, 24 and 48 hr, relative to the intracerebral injection.

Drugs

Nialamide, DA hydrochloride, α-MT methyl ester hydrochloride (Sigma Chemical Company, St. Louis, MO).

All drugs were dissolved in saline except nialamide which was dissolved in 0.1 M HCl and buffered with Tris to yield a final pH of about 6.5.

Statistical Methods

Avoidance scores were expressed as a percentage of each test session of 10 trials. To avoid the potentially serious limitations inherent in a univariate analysis of variance (ANOVAR) with repeated measures [14], response trends were analysed by multivariate techniques in this study [10]. Firstly, response curves for the time intervals from 0.5 hr to 8 hr were characterised in terms of stochastically independent, orthogonal, polynomial components of trend by multiple regression analysis. Differences between the orthogonal trend components of the treatment groups were investigated by univariate ANOVAR and multivariate ANOVAR using Rao's F-approximation [20] of Wilks' Λ test criterion [26]. Roy-Bargmann step-down analysis [21] was employed to investigate conditional effects in one trend component, adjusting for effects on lower trend components. The DA effect was partitioned into two orthogonal, additive components by means of Helmert contrasts. The first contrast examined the overall DA effect by comparing the saline goup with the mean of the DA 5 and 10 μg groups. The second Helmert contrast examined the dose-dependence of DA by comparing the DA 5 with the DA 10 μg group.

Doubly multivariate analysis [10] was used to relate the effects of DA on the trend components of one response (for example, avoidance) to its effects on another response (ITC).

RESULTS

General Observations

The results are summarized in Figs. 1 to 3. The −24 hr session shows that the rats had achieved a high level of total CAR (Fig. 1), while exhibiting low levels of E, RF (Fig. 2), incorrectly discriminated responses (Fig. 1) and ITC (Fig. 3). The α-MT treatments suppressed avoidance responding, as was evident at the −0.5 and 0.5 hr sessions. This was accompanied by an increase in E and RF, but α-MT did not seem to impair discrimination. The effects of DA administration became apparent within 1 hr when the rats appeared more alert, assumed a more normal posture, and started to engage in locomotion and grooming. The total CAR rate increased dose-dependently to a maximum between 2 and 4 hr and there was a minor increase in discriminative errors. There was also a marked increase in intertrial crossings. As the effects of DA wore off, the influence of α-MT re-emerged and total CAR decreased to a minimum around 12 hr after DA challenge. After 12 hr the action of α-MT waned, and most animals recovered to the control level by 48 hr.

Effect of α-MT

The effects of α-MT were examined by comparing the pre-α-MT response level (−24 hr session) and the mean post-α-MT response level (−0.5 hr and 0.5 hr sessions). The total CAR and CAR− data of the three treatment groups (i.e., saline and the two DA groups) were not significantly heterogeneous at these time periods, multivariate $F(8,42)=1.328$, $p>0.05$.

In the absence of heterogeneity between groups, the actions of α-MT were ascertained from the constant effect for the pooled groups. The multivariate test indicated the presence of highly significant effects, $F(4,21)=927.396$, $p<0.001$. The univariate tests indicated that α-MT produced a highly significant change in total CAR, $F(1,24)=458.834$, $p<0.001$, but no significant change in CAR−, $F(1,24)=0.205$, $p>0.05$. Roy-Bargmann step-down analysis of changes in CAR− demonstrated that α-MT did not impair discrimination, even after the masking effect of the α-MT-induced suppression of CAR in general was taken into account, $F(1,21)=0.510$, $p>0.05$.

Effect of Dopamine

Polynominal regression. The time course of changes in the variables between 0.5 and 8 hr were analysed into ortho-

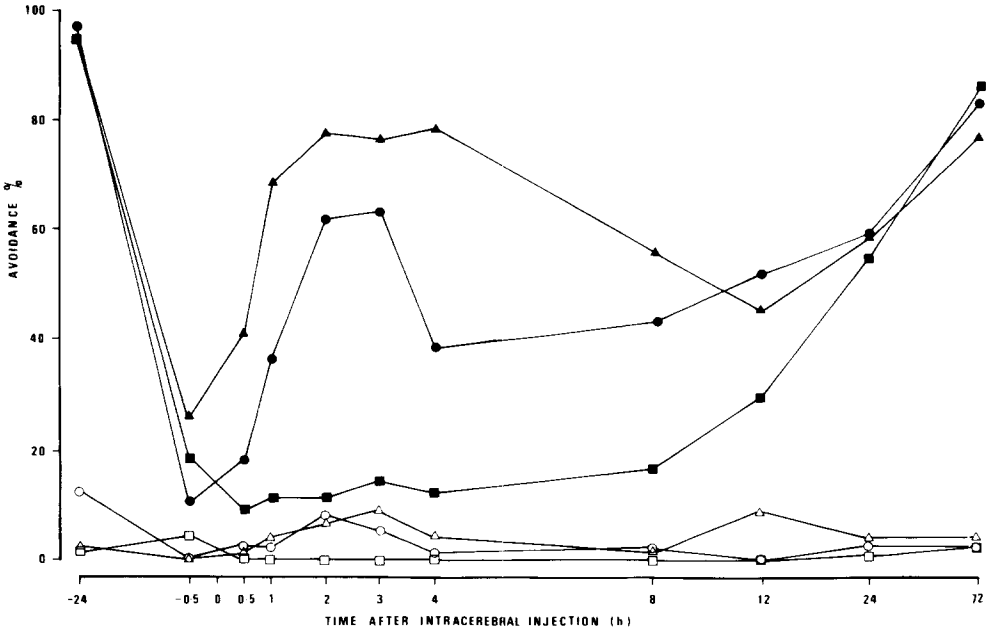

FIG. 1. Effects of the intracerebral administration of saline (■, □), 5 μg DA (●, ○) or 10 μg DA (▲, △) at Oh on total CAR (closed symbols) and CAR− (open symbols) of rats pretreated with α-MT (at −12 hr and −1 hr) and nialamide (at −1 hr).

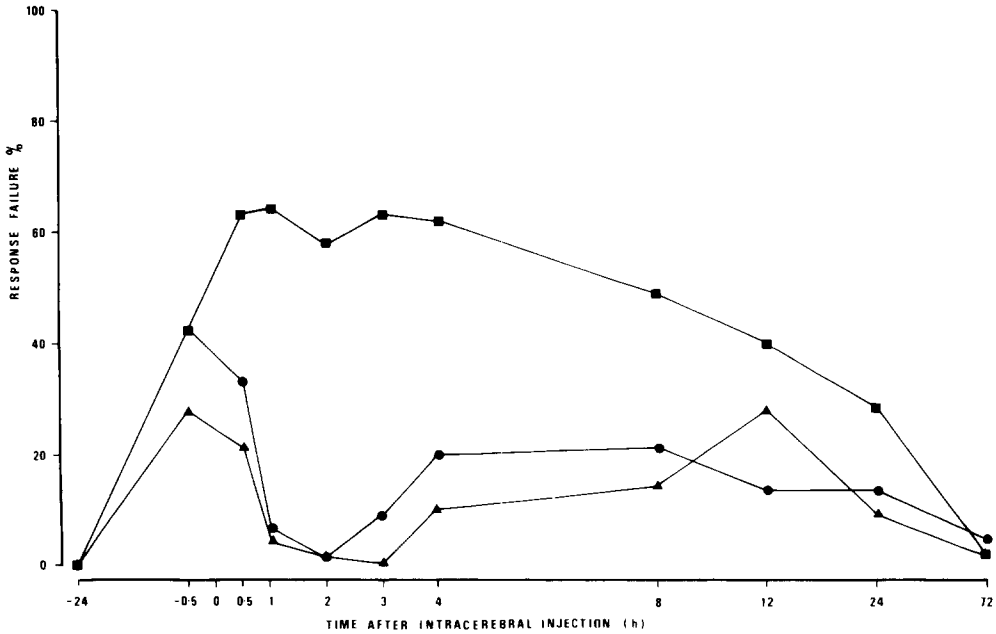

FIG. 2. Effects of the intracerebral administration of saline (■), 5 μg DA (●) or 10 μg DA (▲) at Oh on the response failure of rats pretreated with α-MT (at −12 hr and −1 hr) and nialamide (at −1 hr).

gonal polynomial trend components by multiple regression. One notable feature of the effects of DA on avoidance was the close correlation between CAR+ and total CAR (Fig. 1) over all test sessions from 0.5 to 8 hr, in all groups ($r=0.98$). This suggests that total CAR and CAR+ can be considered identical for all practical purposes and the discussion is therefore limited to total CAR. The flat horizontal time-curves of the saline-challenged groups did not contain any significant trends (see Figs. 1–3). In contrast to the effect of saline, both doses of DA significantly increased total CAR,

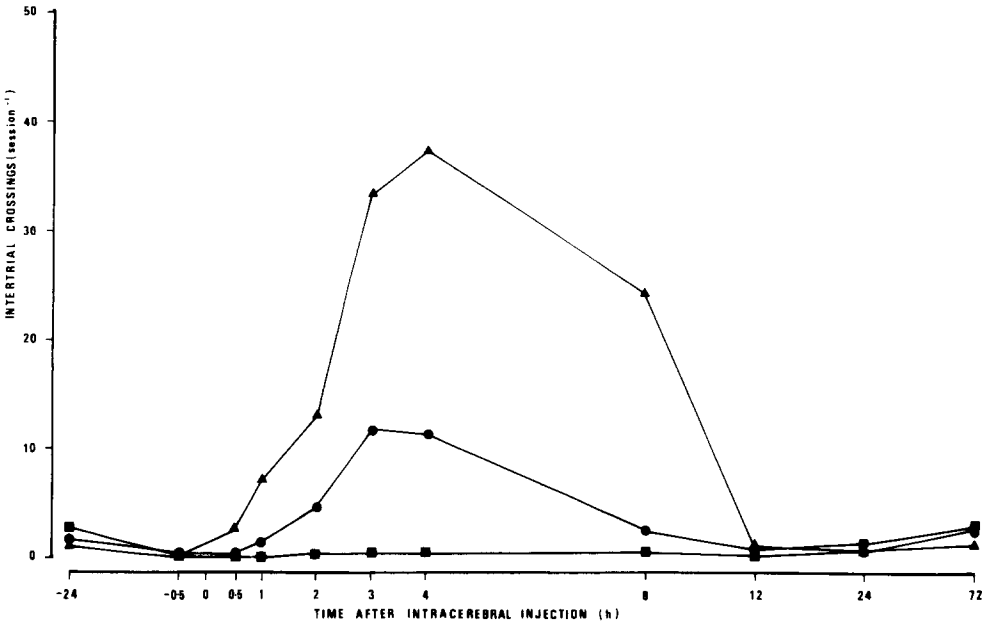

FIG. 3. Effects of the intracerebral administration of saline (■), 5 μg DA (●) or 10 μg DA (▲) at Oh on the intertrial crossings of rats pretreated with α-MT (at −12 hr and −1 hr) and nialamide (at −1 hr).

with significant cubic, $F(1,48)=6.776$, $p=0.012$, and negative quadratic, $F(1,48)=4.771$, $p=0.034$, components characterizing the transient stimulation seen after 5 μg DA, while a significant negative quadratic component, $F(1,48)=7.141$, $p=0.010$, was seen with 10 μg DA. The difference between the two doses of DA was due to the longer duration of the 10 μg dose (see Fig. 1).

E+, E− and total E scores exhibited no significant change in trend after DA treatment and all trend components (other than the constant term) were non-significant in all groups.

RF was decreased by 5 μg DA, cubic component, $F(1,48)=5.732$, $p=0.021$. Ten μg DA also produced a decrease in RF (Fig. 2), but because of a "floor" effect, this change was relatively indistinct, and thus the quadratic, $F(1,48)=2.443$, $p=0.125$, and cubic, $F(1,48)=2.879$, $p=0.096$, trends did not reach significance.

ITC were temporarily increased by both doses of DA and the effect was manifested by parabolic curves with significant quadratic components, 5 μg DA, $F(1,48)=9.288$, $p=0.004$; 10 μg DA, $F(1,48)=5.026$, $p=0.030$.

The curves for CAR− (Fig. 1) showed a similar time course to that for total avoidance, albeit on a lesser scale, with a peak occurring 2–3 hr after DA administration (both DA groups were significantly different from the control at 3 hr, $t(16)=2.289$, $p=0.036$). The transient DA-induced rise in CAR− was manifested as a cubic trend component, $F(1,48)=5.333$, $p=0.025$, in the 5 μg DA group, and as a marginal quadratic trend, $F(1,48)=3.983$, $p=0.052$, in the 10 μg DA group. Since no significant information was contributed by the E− variable, the CAR− variable is used here as indicative of overall discrimination effects in this experiment.

Multivariate ANOVAR. Differences in total CAR scores between the treatment groups were analysed by multivariate and univariate ANOVAR. The multivariate test of linear, quadratic and cubic component differences for the first partition of the DA effect showed that DA significantly affected the avoidance trend, compared to controls administered saline, $F(3,22)=3.132$, $p=0.046$. This was primarily reflected in the cubic, univariate $F(1,24)=8.254$, $p=0.008$, and quadratic, univariate $F(1,24)=4.194$, $p=0.052$, trend components. In addition, DA exerted a highly significant action on the general level of avoidance, univariate $F(1,24)=24.983$, $p<0.001$. The multivariate test of the second contrast of the DA effect indicated that there was only a minor difference between the shapes of the two DA curves, $F(3,22)=2.348$, $p=0.100$, attributable largely to their skewness, cubic component, univariate $F(1,24)=4.500$, $p=0.044$. Note also that the general level of the two DA curves differed significantly, univariate $F(1,24)=5.336$, $p=0.029$, indicating a dose-dependent effect.

DA had no significant effect on RF trends, multivariate $F(3,21)=2.272$, $p=0.108$, first contrast. However, the difference in mean RF scores between DA-treated and control rats was significant, univariate $F(1,24)=27.692$, $p<0.001$. Analysis of the second partition of the DA effect indicated non-significant effects, multivariate $F(3,21)=0.393$, $p>0.05$, due to the near maximal suppression of response failure by both doses of DA.

Because of relatively wide error variation, DA was not found to affect the shape of the ITC curves, multivariate $F(3,21)=1.821$, $p>0.05$, although the general level of crossings by DA-treated rats was greater than that of controls, $F(1,24)=4.511$, $p=0.044$.

The occurrence of CAR− was affected by DA, albeit to a relatively minor degree. The multivariate test of the first contrast which tests the existence of a general DA effect on curve shape was non-significant, $F(3,21)=1.824$, $p>0.05$, which is somewhat surprising in view of the significant cur-

TABLE 1

DOUBLY MULTIVARIATE STEP DOWN ANALYSES OF THE DA EFFECT ON TOTAL AVOIDANCE, ADJUSTED FOR OTHER VARIABLES

Avoidance Trend Component	Unadjusted		Variable used in adjusting avoidance: Total Escapes		Response Failure		Intertrial Crossings	
	F	p	F	p	F	p	F	p
Constant	12.159	< 0.001	17.802	< 0.001	4.887	0.019	2.357	0.120
Linear	2.594	0.096	2.535	0.106	2.228	0.135	2.819	0.085
Quadratic	0.713	0.501	2.042	0.159	0.082	0.921	0.283	0.757
Cubic	5.355	0.013	4.965	0.020	3.348	0.059	1.788	0.197

vilinear trends observed for both DA groups (*vide supra*). This lack of effect is due perhaps to imprecise measurement of the variable at its threshold level of occurrence and the relatively small sample size. Nevertheless, an increase in CAR− was indicated by the significant DA effect on mean response levels, $F(1,24)=6.376$, $p=0.019$, contrast 1, although this effect was not dose dependent, $F(1,24)=0.259$, $p=0.616$, contrast 2.

Doubly multivariate analyses. The results for the step-down analyses of the DA effect on CAR, adjusted for several other variables, are shown in Table 1. The first pair of columns represents total CAR, with each trend component adjusted for lower trend components, but not adjusted for any other response variable. The main action of DA on total CAR was represented by the significant constant and cubic effects.

The second pair of columns presents CAR adjusted for E and shows that the effects of DA on CAR were unrelated to its effects on escape.

The third pair of columns shows that some of the significant variation in CAR was removed by adusting for response failure, suggesting that changes in these two variables were partially interrelated. When avoidance was adjusted for the effect of intertrial crossings (last two columns), no significant effect of DA remained, signifying that the effect of DA on intertrial crossings was sufficient to explain the observed changes in CAR. In this respect it is interesting that there was a high positive correlation between intertrial crossings and total CAR ($r=0.498$, $p<0.001$) reflecting the ability of animals with a high level of locomotor activity to perform the CAR efficiently.

A similar series of step-down analyses examining the effect of DA on CAR− found that changes in CAR− were only slightly related to E, more so with total CAR and RF trends, and most closely related to intertrial crossings changes. An unusual feature of the last analysis was the emergence of a spurious cubic effect arising from the comparison of CAR− curves (which were positively skewed within the time period analysed) and the intertrial crossings curves (negatively skewed).

DISCUSSION

In the present study, α-MT specifically suppressed the performance of CAR, without significantly impairing the ability of the rats to perform a simple spatial discrimination task, suggesting that the suppression was due to a depression of locomotor function. This finding is in agreement with those of other workers [2, 3, 22], although some reports have noted that α-MT can exert disruptive effects on operant responding tasks which require temporal discrimination [12].

DA temporarily reinstated CAR, in agreement with our earlier studies [11,15], without significantly affecting E. The observed increase in ITC reflected a stimulation of locomotor activity produced by the locally applied DA [16,19]. The time course of the latter effect bore a general resemblance to the effect of DA on CAR, although the maximum increase in ITC occurred later. Doubly multivariate analysis disclosed the existence of a close association between ITC and CAR by showing that all the significant effects of DA on avoidance trends could be explained by DA-induced changes in ITC. This was the only variable that could fully explain the DA induced reversal of the α-MT effect. Other reports have also noted an association between stimulation of motor activity by DA agonists and the facilitation of learned responses [2, 6, 7, 13, 15, 23, 24].

The data pertaining to discrimination were affected by several limiting factors, including the relative transience and the low frequency of incorrectly discriminated responses as well as a relatively small sample size, resulting in a proportionally larger error variance and reduced power. Nevertheless, several features indicated a transient disruption of discrimination. Thus, polynomial regression analysis of CAR− trends revealed significant, or marginally significant, curvilinear trend components for the DA groups. Moreover, Student's *t*-tests, indicated a significant increase in CAR− at the 3 hr session. The ANOVAR, however, did not reveal any significant trend effects but we believe this represents a failure to find significant trend changes rather than proof of the absence of changes. Apart from this negative finding, ANOVAR confirmed a significant DA effect on the overall level of CAR−. Taken together, these data are indicative of an impairment of discrimination resulting from DA administration into the nucleus accumbens. Such a conclusion is in agreement with other findings that various DA agonists impair discrimination [2, 3, 4, 25].

The relationship of CAR− trend changes to changes in other variables indicated that the DA-induced increase in CAR− was related to the effect of DA on total CAR, supporting the hypothesis that the increase in avoidance is the result of a general non-specific activation. CAR− exhibited a particularly close association with ITC, a finding consistent with several other studies reporting a correlation between drug-induced hyperkinesia and discrimination errors [7, 8,

25]. However, the comparison of CAR− and ITC disclosed an anomalous cubic term which reflected the different rates of decline of the two variables after their maxima had been reached, with CAR− diminishing more rapidly than ITC. The divergence of these two trends may be due to the fact that CAR−, unlike ITC, was subject to continuing negative reinforcement. In an analogous finding, Barrett *et al.* [7] found that amphetamine initially increased both CAR− and locomotor activity, but that CAR− subsequently decreased while locomotion remained elevated.

The observation that DA produced a large increase in CAR+ but only a minor increase in CAR− may be interpreted in several ways. If the application of DA to the nucleus accumbens produces locomotor activation without the intervention of cognitive functioning, one would expect an equivalent increase in CAR+ and CAR− responses. Hence, the observed discrepancy between changes in CAR+ and CAR− may imply that the nucleus accumbens is not merely responsible for general motor activation, but is also involved in the coordination of motor responses at a higher level of integration. Although some evidence suggests such a role for the nucleus accumbens [18] the present results should not be considered as providing a confirmation of this hypothesis. It is more parsimonious to regard the relatively minor increase in CAR− as a consequence of the fact that α-MT did not impair discrimination in the first instance.

Overall, these data provide evidence suggesting that the ability of DA to reverse the α-MT-induced suppression of a DCAR task is due to locomotor activation. The receptor population being stimulated in the nucleus accumbens would appear to be dopaminergic in nature [16] and studies are underway to substantiate this hypothesis.

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