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A Failure to Support Cross-Sensitization Between Effects of Apomorphine and Lesions of the Habenula Nucleus

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THORNTON, E. W., G. E. BRADBURY, J. A. C. EVANS AND A. WICKENS. *A failure to support cross-sensitization between effects of apomorphine and lesions of the habenula nucleus.* PHARMACOL BIOCHEM BEHAV 32(1) 77–81, 1989.—An interchangeability between the effects of stress and psychostimulants has been reported. The possible common physiological effects of lesions of the habenula, stress and psychostimulant administration of activation of ascending dopamine systems suggested examination of a cross-sensitization between lesions of the habenula and psychostimulant administration. Lesions of the habenula were found to increase baseline activity but there were no significant changes in response to apomorphine in either various categories of stereotypy or locomotor response. Lesioned rats and controls both demonstrated similar dose and time-related effects in various response measures. Although not significant, certain results suggested that changes tended to be in the direction opposite to that of the suggested sensitization. The results are contrasted with previous supporting data and discussed in terms of the potentially diverse manner in which habenular manipulation and psychostimulants may influence dopaminergic activity and subsequent behaviour.

Apomorphine Habenula nucleus Sensitization Activity Stereotypy

SINCE research first suggested a similarity between the psychosis induced by repeated administration of large doses of amphetamine and the symptoms of acute paranoid schizophrenia (3), a considerable amount of research has been directed towards examination of the factors affecting these two syndromes. Among the many discoveries that have been made about the nature of amphetamine-induced psychosis, is the finding that repeated administration of amphetamine to animals produces a sensitization, characterized by a progressive enhancement of drug-induced behaviours (15). Several studies have shown an exacerbation of symptoms in certain schizophrenics by amphetamine, suggesting a sensitization to the drug (6) and hence further linking amphetamine-related syndromes with schizophrenia. An additional link has been suggested by the finding that both amphetamine addicts and schizophrenics show a sensitization to the effects of stress (7,20). These results led to the suggestion of a similarity in the effects of sensitization to stress and amphetamine. In a direct investigation of this hypothesis Antelman *et al.* (2) and MacLennan *et al.* (11) showed that, with regard to amphetamine-induced stereotypy and stress-induced eating or gnawing in rats, stress and amphetamine could cause cross-sensitization. Antelman (1) further reported a cross-sensitization between brief stressors and dopamine agonists and antagonists.

Evidence suggests a selective activation of the mesocorticofrontal dopamine neurones following exposure to stress, given in the form of electric footshock, an effect occurring within minutes of exposure to stress (17). Since research into the neurochemical pathways mediating the effects of psychostimulants has revealed an activation of the ascending dopaminergic systems (5), the possibility exists that activation of ascending dopaminergic pathways originating in the ventral tegmental area may underlie the interchangeability of sensitization between stress and psychostimulants.

Lisoprawski *et al.* (10) have shown that lesioning the habenula nucleus caused a selective activation of mesocorticofrontal dopamine neurons, shown by an enhanced DOPAC/DA ratio in the frontal cortex, six days after lesioning. If activation of the mesocorticofrontal pathway forms the basis for the cross-sensitization between stress and psychostimulants, it may be hypothesized that lesions of the habenula nucleus should also increase the responsiveness to psychostimulants. That lesioning the habenula does produce an altered sensitivity to stress has already been suggested by data which demonstrate altered behavioural responses in such lesioned animals only at higher levels of environmental stimulation or stress (12,19). Some more specific but preliminary data in support of increased sensitivity in response to dopamine agonists in habenular-lesioned animals has recently been reported by Carvey *et al.* (4).

This study was designed to further examine the possibility that lesions of the habenula nucleus could cause an increased sensitization to dopamine agonists.

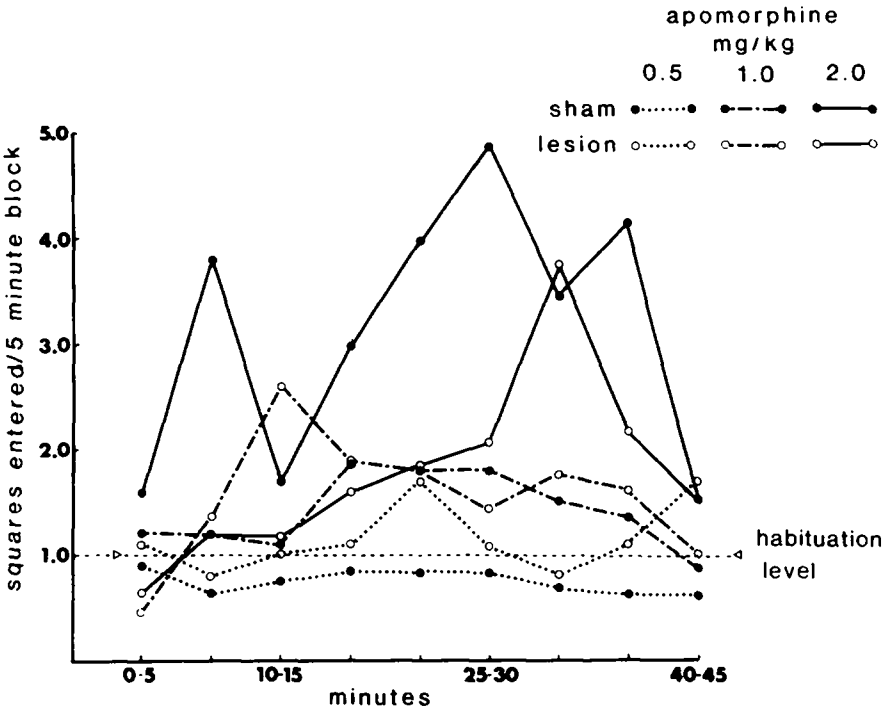

FIG. 1. Group mean (N=7) locomotor activity measured by rectangles entered, in lesion and sham groups, after injection of apomorphine hydrochloride (0.5, 1.0, 2.0 mg/kg IP) over successive 5-minute periods, expressed as a percentage of activity levels under saline on the preceding day.

METHOD

Forty-two male rats, weighing 319±26 g, housed singly in a room maintained on a 12-hour light/dark cycle, at a temperature of 70±2°F, were used as subjects. Animals were matched into pairs by weight, one animal of each pair being allocated to the experimental group, the other to the control group. Animals in the experimental group received bilateral lesions of the habenula. Operations were carried out under pentobarbitone anaesthesia (50 mg/kg IP) with a supplementary injection of 0.2 ml atropine sulphate to reduce respiratory distress. Animals were positioned in a Kopf stereotaxic frame and radio frequency lesions made using a Grass Lesion Maker LM4, at coordinates specified according to the atlas of Pellegrino *et al.* (13) of A-P=3.8, lateral=0.7±0.7, DV=0.7 above stereotaxic zero. Lesions were produced by passing a current of 12 mA for 12 sec through an insulated stainless steel electrode, exposed for 0.4 mm at the tip. Sham-operated control animals were treated in the same way as experimental animals except that no current was passed through the electrode.

Animals were allowed 10 days recovery before beginning behavioural testing.

Behavioural Testing

Testing took place in two stereotypy cages, each consisting of a wooden base 30×45 cm, with 45-cm high wire mesh walls, and a similar wire mesh lid. The wooden base was marked into 6 equal rectangles to enable quantification of locomotion.

The stereotypy boxes were illuminated with 60-W lamps, and flooded with white noise at 70 dB. Tests were forty-five minutes duration and were started with the animal being placed in one corner square of the boxes. All behaviours were recorded on videotape and later scored by one of the experimenters without knowledge of treatment condition.

All animals were given 2 habituation trials of 45 minutes duration on consecutive days, in which the behavioural test procedure followed injections of physiological saline (2 ml/kg IP). The purpose of this was to familiarize the animals with the testing procedure and environment so that on drug test days the drug effect would be less confounded by the animals' reactions to a novel environment. On the third day, lesion and sham groups were divided randomly into 3 equal groups, each receiving one of the three doses of apomorphine hydrochloride (0.5, 1.0 or 2.0 mg/kg IP) injected at a volume of 2 ml/kg, with behaviour recorded immediately after the injection.

Behaviours documented during the 45-minute test sessions were general activity (scored as the number of rectangles marked on the floor entered every minute) and four categories of stereotypy: sniffing, locomotor counts, grooming and rearing. For these stereotyped responses, the procedure proposed by Fray *et al.* (8) was used to score the presence or absence of different response categories without regard for the degree or intensity of response.

Histology

Following completion of all behavioural observations, animals were sacrificed with pentabarbitone and, following perfusion, their brains removed and stored in sucro-

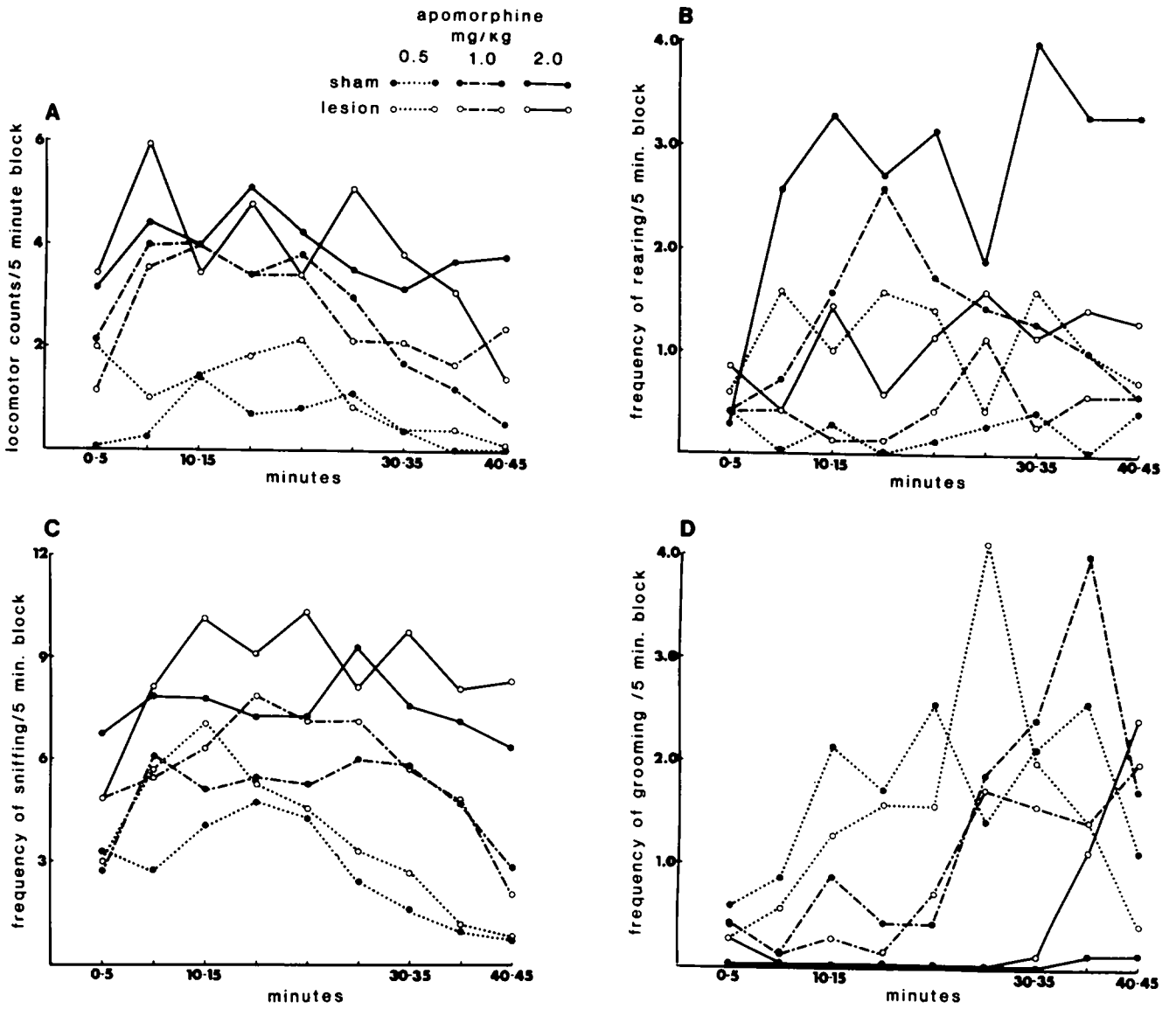

FIG. 2. (A–D) Mean stereotypy measures in lesion and sham groups after injections of apomorphine (0.5, 1.0, 2.0 mg/kg IP) expressed for each 5-minute block. Predominant activity each 20 seconds was given a score of 1, and scores for each measure were summed over 5 minutes. A: Locomotor counts, B: Rearing, C: Sniffing, D: Grooming.

formalin. Frozen sections of 50 μm were taken from each brain and examined for the site and size of lesion.

RESULTS

Considerable variability was seen in baseline locomotor activity during habituation trials and analysis of changes during drug treatment was made by expressing scores as a percentage of each animal's response on the final habituation trial. This derived score is more meaningful since analysis of locomotor activity on the second habituation trial showed a significant effect of lesion [ANOVA, $F(1,40)=12.6$, $p<0.001$]. It is emphasised that such baseline data, whilst derived from activity scores under saline treatment on Day 2 (second habituation trial), were used only for the purpose of minimising the influence of individual response variance and could not be considered as 'control' data for use in comparison with the response to drug treatment. Locomotor activity expressed as this percentage measure for each five-minute period throughout testing is presented in Fig. 1.

Stereotypy was, as described previously, measured by the frequency of occurrence of four behaviours: rearing, sniffing, grooming and locomotor counts. The predominant behaviour category occurring at each 20-second interval was given a score of 1 and scores of each category accumulated over 5 minutes, giving a maximum score of 15 for any one category during each 5-minute period. Scores in habituation were infrequent and changes in the four measures are presented simply as the response to drug over successive five-minute periods in Fig. 2.

As a summary of the results, the mean scores for each

TABLE 1

A SUMMARY OF THE BEHAVIOURAL RESPONSE TO APOMORPHINE AT DOSES OF 0.5–2.0 mg/kg IN HABENULAR-LESIONED AND CONTROL RATS

		A			B			
		Dose Level mg/kg	Sham Control	Lesion	Effect	F	*df*	Significance
Locomotor Activity	Squares Entered	0.5	6.84	16.29	Dose	6.8	2,24	$p<0.01$
		1.0	15.68	17.46	Lesion × Dose	1.8	2,24	NS
		2.0	26.78	32.35	Time	4.7	8,96	$p<0.001$
					Lesion × Time	1.2	8,96	NS
					Dose × Time	2.7	16,192	$p<0.01$
	Rearing	0.5	0.22	1.10	Lesion	2.8	1,12	NS
		1.0	1.25	0.46	Time	1.8	8,96	NS
		2.0	2.71	1.10	Lesion × Time	1.7	8,96	NS
	Grooming	0.5	1.68	1.48	Dose	16.0	2,24	$p<0.001$
		1.0	1.36	0.94	Time	6.0	8,96	$p<0.001$
		2.0	0.14	0.44	Dose × Time	3.5	16,192	$p<0.01$
Stereotypy	Sniffing	0.5	2.75	3.75	Dose	16.4	2,24	$p<0.001$
		1.0	4.91	5.71	Time	7.6	8,96	$p<0.001$
		2.0	7.49	8.54	Dose × Time	2.3	16,192	$p<0.01$
	Locomotor Counts	0.5	0.54	1.14	Lesion	2.0	1,12	NS
		1.0	2.67	2.67	Dose	9.7	2,24	$p<0.01$
		2.0	3.92	3.86	Time	3.0	8,96	$p<0.001$

A. Mean scores for 5-minute time blocks over the entire 45-minute behavioural test session. For stereotypy measures the maximum score is 15.

B. Statistical tests on behavioural scores using ANOVA following appropriate transformations to account for deviance from normality. Effects are shown only when F values <1. Scores for locomotor activity were determined on the basis of percentage measure of baseline activity on the preceding day.

behaviour over the entire 45-minute test are listed for each treatment group in Table 1A.

Analysis of the results was undertaken by ANOVA using percentage scores for locomotor activity and raw scores for stereotypy measures transformed as necessary according to the criteria by Kirk (9) for deviations from normality and the frequency of extreme scores. No measure displayed a significant effect of lesion. More importantly, no measure revealed a significant or near significant lesion by dose interaction that was specifically predicted on the basis of a lesioned-induced sensitisation to apomorphine. Although no significant effects were seen for rearing, there were significant dose, time and dose by time interactions for all other categories of stereotypy (see Table 1B; all $p<0.01$). These dose and time effects were a consequence of increased levels of sniffing and locomotor counts with increases in dose, with a tendency for a decline of both these measures over the test period. Conversely, grooming declined as dose increased and there was a tendency to increase grooming as the test period progressed.

Locomotor activity also showed significant changes of response with drug dose over time with increased activity at the highest dose, and a tendency to increase activity over time but especially during the middle of the test period.

Examination of brain sections revealed lesions in all animals to be confined to the habenula complex with complete destruction of both the medial and lateral nuclei. Minimal intrusion was found in a few animals into the overlying dorsal hippocampus and other adjacent areas. The extent of the lesions was similar to those reported in more detail in a previous study (18). The size of the lesion was not found to correlate with the levels of behavioural activity.

DISCUSSION

The data provide no evidence to support significant differences between habenular-lesioned animals and controls in terms of an increased sensitization of response to apomorphine reflected in changes of either locomotor activity or stereotyped responses. The data demonstrate increased levels of sniffing and grooming, decreased locomotor counts but no change in rearing with increasing dose of apomorphine. There was also a significant tendency to increase locomotor activity especially at the highest dose of 2.0 mg/kg. Although effects were not significant, an examination of the data for locomotor activity (Fig. 1) and rearing (Fig. 2B) indicates the direction of any lesion effect is opposite to that of the experimental prediction with a somewhat increased response in sham-operated controls relative to the habenular-lesioned animals at the highest drug dose. This tendency however was not consistently apparent for the other measures of stereotyped behaviours.

The absence of a differential effect of apomorphine in lesioned and control rats contrasts with the brief report of Carvey *et al.* (4) where doses of 0.35 and 0.75 mg/kg apomorphine significantly increased stereotypic responses (chewing, gnawing and biting) whilst decreasing locomotor activity (neither data nor statistical significance are given). Higher doses of 1.25 and 2.0 mg/kg, however, produced no significant differences although, as for the present findings, there

was a tendency towards hyporesponsiveness in lesioned animals. Specific comparisons between dose effects in the Carvey *et al.* study would be contentious since different categories of stereotypy were scored and lesions in that study were produced by the neurotoxic action of kainic acid. Whilst histological details were not described, the dose of the neurotoxin used together with the author's own comments, suggests incomplete destruction of even their target of the lateral habenula. Moreover, the present data found an increased activity following the destruction of the entire habenula complex (data from the final habituation session) when, in contrast, those from Carvey *et al.* report less locomotor activity following their partial lesion. In previous data we have reported no effects of similar lesions for locomotor activity in the open-field, although at higher levels of ambient environmental stimulation there was a nonsignificant tendency to increased levels of activity (19).

Much of the evidence for cross-sensitization between psychostimulants and stress has been derived from studies of amphetamine and cocaine. The cross-sensitization between psychostimulants, including amphetamine and apomorphine, suggested a common basis for cross-sensitization to stress for apomorphine in terms of activation of dopamine systems. The choice of apomorphine in the present study was made on the basis of its greater selectivity relative to amphetamine for mesolimbic and mesocorticofrontal, as opposed to striatal, dopamine system; and it is in these specific pathways that an habenular influence on the stress response has been documented (10). It remains to be shown, however, that cross-sensitization specifically occurs between apomorphine and stress. Certainly, the differential mode of action of apomorphine and amphetamine in potentiation of dopaminergic effects may be of relevance for the failure to support the predicted sensitization to apomorphine in lesioned animals. There is evidence for a direct feedback dopaminergic pathway from the ventral tegmental areas to the lateral habenula (14,16) and the potentiation of the frontocortical and to a lesser extent the mesolimbic dopamine systems following lesions of the habenula suggests this VTA to habenula pathway provides feedback inhibition. Within the present context, therefore, apomorphine's effect as a postsynaptic receptor agonist may result from a balance between direct effects at terminal sites for ascending dopamine pathways and effects at possible supersensitive sites which remain following habenular destruction. Consequently, whilst the present data do not support the hypothesis of an altered response to the dopamine agonist apomorphine and a previously reported supportive finding, the inference that the habenula plays no role in affecting the response to stimulants in stressed animals is not justified on the basis of the limited data presented. Rather the data suggest that any behavioural interaction between habenular lesions and dopaminergic agents may be complex and relate to the specific psychostimulant, test doses and the primary site of action.

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