Pharmacology Biochemistry & Behavior, Vol. 4, pp. 575–582.

Catecholamines and Self-Stimulation: Evidence Suggesting a Reinforcing Role for Noradrenaline and a Motivating Role for Dopamine

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(Received 30 June 1975)

HERBERG, L. J., D. N. STEPHENS AND K. B. J. FRANKLIN. *Catecholamines and self-stimulation: evidence suggesting a reinforcing role for noradrenaline and a motivating role for dopamine.* PHARMAC. BIOCHEM. BEHAV. 4(5) 575–582, 1976. – Investigation of the role of noradrenaline (NA) and dopamine (DA) in self-stimulation showed that d-amphetamine (which releases more DA than does l-amphetamine, but not more NA) was much more effective than l-amphetamine in enhancing self-stimulation of NA sites in the locus coeruleus and near-lateral hypothalamus. In DA sites in the substantia nigra and far-lateral hypothalamus the effects of the 2 isomers were confirmed to be more nearly equal. Thymoxamine HCl (10 mg/kg IP), a specific α-adrenergic receptor blocker, depressed self-stimulation at all sites, but significantly more severely at DA sites. Thus the drugs most effective in influencing self-stimulation at a particular site were those acting predominantly on the unstimulated system. These findings were interpreted in terms of a hypothesis that DA and NA play complementary roles in self-stimulation and that both are essential; or, more specifically, that DA pathways, implicated in other motivational activities, contribute to a state of drive or arousal necessary for self-stimulation; while response-contingent noradrenergic activity (elicited by the electrodes directly, or indirectly via a transsynaptic route) mediates reinforcement. Further predictions from this hypothesis were tested as follows: (1) Direct pharmacological stimulants of adrenergic α-receptors should disrupt self-stimulation by acting randomly on the reinforcement system and disrupting response-reward contingencies; this was confirmed by the finding that the α-receptor stimulant clonidine HCl (0.05 mg/kg) depressed self-stimulation at all sites tested. (2) Direct stimulants of DA receptors should enhance self-stimulation of NA sites by augmenting dopaminergic motivational activity; but in rats with DA electrodes, noncontingent stimulation of DA receptors would also impose similar noncontingent activity on the transsynaptic noradrenergic reinforcement pathways and thus depress self-stimulation; this was confirmed by the finding that apomorphine (0.3–1.0 mg/kg) was strongly stimulant for NA electrodes but strongly depressant for DA electrodes, and that the degree and direction of these effects was highly correlated with the differential effects of d- and l-amphetamine (rho = .65, $p<0.01$). Neither effect of apomorphine depended on the occurrence of motor stereotypy. These results can be interpreted in terms of 2-component models for self-stimulation, with the predominant transmitter of the drive component being identified as DA and that g the reinforcing component as NA.

Catecholamines Self-stimulation Amphetamine Apomorphine Substantia nigra Noradrenaline
Reinforcement Clonidine Locus coeruleus Dopamine Drive Thymoxamine Hypothalamus

EXPLANATIONS of the self-stimulation phenomenon generally invoke a reinforcement process and a drive or arousal process, the latter being elicited either by the electrodes directly [15], or indirectly as a secondary effect of reinforcing stimulation [53] (for review see Gallistel [20]). The present study sought to relate these views to recent anatomical findings which have revealed a correspondence between sites where self-stimulation may be obtained and the ascending catecholamine (CA) pathways of the brain [8,10].

The anatomical findings have been supported by biochemical [4,47], pharmacological [9, 31, 39, 49] and electrophysiological [34] evidence but the relative roles of noradrenaline (NA) [40,57] and dopamine (DA) [11,31] are disputed and some studies have yielded discordant results. The DA-receptor stimulant, apomorphine, for example, has been reported to suppress responding [28,45] and to enhance it [29,55] seemingly regardless of electrode site [6], leading at least one group of investigators to conclude that the direction of its action in different rats was determined by "accident" [5,6]. Other controversies concern the effects on self-stimulation of NA-synthesis blockers [43,57], and the relative roles of NA and DA in the stimulant action of the amphetamines [7,39]. The present study sought to reconcile these findings in terms of an hypothesis that self-stimulation was equally dependent on both NA and DA, acting in concert and respectively mediating reinforcement and drive. Thus the drugs most

effective in influencing self-stimulation at a particular site would be those acting on the less strongly stimulated system of the two. In other words, self-stimulation of noradrenergic sites should be particularly sensitive to drugs acting on DA receptors, and vice versa.

To test this hypothesis and identify the role of each transmitter we have reinvestigated the effects on self-stimulation at different sites of each of 3 categories of drug: direct CA stimulants, indirect (i.e. presynaptic) CA stimulants, and an adrenergic α-receptor blocking agent. We have reasoned that indirect stimulants, administered in moderate doses, might be expected to enhance information transfer in reinforcing pathways by selectively facilitating ongoing neural activity, including neural activity generated by the reinforcing stimulus. Direct stimulants, on the other hand, would excite postsynaptic receptors in a quasi random pattern, poorly correlated with presynaptic activity and largely independent of the animal's ongoing activity. Although noncontingent stimulation might intensify tonic neural processes and raise the level of activities such as feeding behaviour [3,23] or general activity [2], it would tend to disrupt phasic processes involved in the reinforcement of discrete operants such as lever-pressing for food [1] or for brain stimulation [22]. We therefore compared the effects on self-stimulation of direct and indirect stimulants with the purpose of distinguishing the possible reinforcing and drive inducing functions of different transmitter systems.

METHOD

Animals

Twisted bipolar stainless steel electrodes were implanted in 1 of 3 brain areas in adult male Wistar rats: (a) the locus coeruleus (LC) (De Groot [14] coordinates: A- 1.8, 0.8–1.2, 7.5), (b) the substantia nigra (SN) pars compacta (A–2.2, 2.0, 8.5) and (c) the near- and far-lateral lateral hypothalamus (LH) (A–5.2, 1.4, 8.8 and A–5.2, 2.0, 8.8). The LC and SN have been shown by histochemical techniques to be largely constituted by noradrenergic and dopaminergic neurones respectively [13,35].

The rats were trained to operate a pedal for 0.5 sec sine wave reinforcing pulses, available at randomly varied intervals of 10 sec mean duration (VI 10 sec). Use of this reinforcement schedule ensures a steady, seizure-free rate of responding on which clear stimulant or depressant effects can be imposed without any appreciable change in the rate at which reinforcing shocks are received [18]. The stimulating current for each rat was fixed at the lowest intensity that elicited sustained responding at a rate between 10 and 20 responses per min, and regular training continued until response rates had become stable over a 2 hr session. Rats with LC electrodes were generally given at least 3 shaping sessions before being rejected as nonresponders. At the end of the experiment the anatomical location of the electrode tips was determined from 10 X enlarged photographic projections of unstained frozen sections.

Drugs

d- and l-Amphetamine suplphate (S.K.F.) were dissolved in physiological saline; apomorphine HCl (Evans Medical), clonidine HCl (Boehringer) and thymoxamine HCl (Warner) were dispensed from pharmaceutical ampoules and diluted with physiological saline as required. Solutions for injection were adjusted to a volume of approximately 0.5 ml and administered intraperitoneally at not less than 72 hr intervals. Table 1 shows the dosages, and the number of rats in different groups receiving each of the drugs administered.

Procedure

Injections took place after self-stimulation had been in progress for approximately 45 min, and the rate of responding during the last 30 min before injection was taken as a pre-injection baseline. Self-stimulation continued for another 60 min after injection, and mean response rates were recorded automatically at 10 min intervals. Except where indicated, drug effects were determined from response rates in the third 10 min period after injection, and were expressed as a percentage of the pre-injection baseline. The relative effects of d- and l-amphetamine on response rates were compared as in a previous investigation [48] by calculating the peak percentage increase in any 10 min period in the hour after l-amphetamine and subtracting it from the corresponding figure for d-amphetamine. The dose level used was that found to yield maximal discrimination between different implantation sites [39], and all rats were scored and ranked in terms of the d–l differential obtained in this way. Rats which stopped responding after injections were encouraged to restart by taps on the lever; if this failed, by the administration of priming shocks, and then by the experimenter placing the rat bodily on the

TABLE 1

NUMBER OF RATS IN EACH GROUP RECEIVING DIFFERENT DOSES OF EACH DRUG

Drug	Dose (mg/kg)	LH-NA n = 6	LH-DA n = 5	SN n = 5	LC n = 3	PVG n = 3	RF n = 1	Total n = 23
d- and l-Amphetamine	0.5	6	5	5	3	3	1	23
Thymoxamine	10.0	6	3	5	2	0	1	17
Clonidine	0.015	5	3	0	0	0	0	8
	0.05	5	3	5	2	0	1	16
	0.15	5	3	0	0	0	0	8
Apomorphine	0.1	6	5	5	3	2	1	22
	0.3	6	5	5	3	3	1	23
	1.0	4	3	5	3	3	1	19

lever. This sequence was repeated at intervals until responding returned.

For analysis of the results, all self-stimulators were assigned to 1 of 4 groups according to their histology or, in the case of LH electrodes, on the basis of the relative effects of d- and l-amphetamine: (1) Group LC comprised rats with electrodes shown histologically to be within the LC as defined by the distribution of the CA-containing cells in the atlas of Palkovits and Jacobowitz [35]. (2) Group SN had electrodes in or on the margin of the SN; (3) Group LH-NA has LH electrodes which yielded d−l differentials equal to or greater than the median value of all LH electrodes (i.e. ⩾ 33); (4) Group LH-DA had LH electrodes yielding d−l differentials less than the median. Rats with electrodes elsewhere in the brain were not assigned to the above groups, and their results were considered separately.

RESULTS

Histology

Locus coeruleus. Of 6 reinforcing electrodes aimed at the LC, 3 terminated in the anterior LC between the dorsal tegmental nucleus and the mesencephalic nucleus of the trigeminal nerve and were assigned to Group LC, and 3 terminated in the adjacent periventricular grey matter (PVG), Fig. 1C. Eighteen electrodes terminating in or above the dorsal tegmental nucleus, and 2 electrodes in the LC failed to support self-stimulation.

Substantia nigra. Of 6 reinforcing electrodes aimed at the SN, 5 terminated within it or on its dorsal margin and were included in Group SN. The other electrode stopped short, in the reticular formation (RF), Fig. 1B.

Lateral hypothalamus. Three electrodes terminated in the far-lateral LH just medial to the internal capsule, 4 were in the perifornical near-lateral LH, and 3 were in an intermediate position, Fig. 1A. One brain was not available for sectioning.

d- and l-Amphetamine

The mean d−l differential for LC electrodes (106 ± 26 SE) was much higher than for SN electrodes (19 ± 12 SE) with overlap (a tie) occurring once. Inspection of individual placements in the SN (Fig. 1B) shows that the lowest differentials were obtained from sites slightly below and lateral to the main concentration of cell bodies constituting Area A9 [13]; more superficial electrodes (and the short electrode in the reticular formation) gave somewhat higher differentials, perhaps because they were in a position to recruit fibres of the noradrenergic tegmental radiation now known to penetrate this area [30].

Differentials recorded from the 3 PVG electrodes (respectively 12, 14 and 43) (Fig. 1B) resembled those generally recorded in the SN rather than in the LC, consistent with other recent reports of functional and pharmacological differences between this area and the LC [29,46].

Differentials in the LH (Fig. 1A) ranged from −6 to 118, and, as elsewhere reported [48] were negatively correlated with the distance of the electrode site from the midline (Spearman rho = −0.7 n = 10, $p<0.05$). Preinjection rates of responding in Groups LC, LH-NA, LH-DA and SN were respectively 18.4, 20.0, 21.7 and 15.2 responses per min for tests with d-amphetamine, and 21.6, 21.8, 21.8 and 15.8 responses per min for l-amphetamine. The differences between groups were not significant (Kruskal-Wallis H = 2.8, $df = 3$, $p>0.3$), and there was no correlation between the mean pre-amphetamine rate and the magnitude of the d-l amphetamine differential (Spearman rho = 0.10, n = 19, n.s.). Mean preinjection rates in each group remained stable near these levels throughout the investigation.

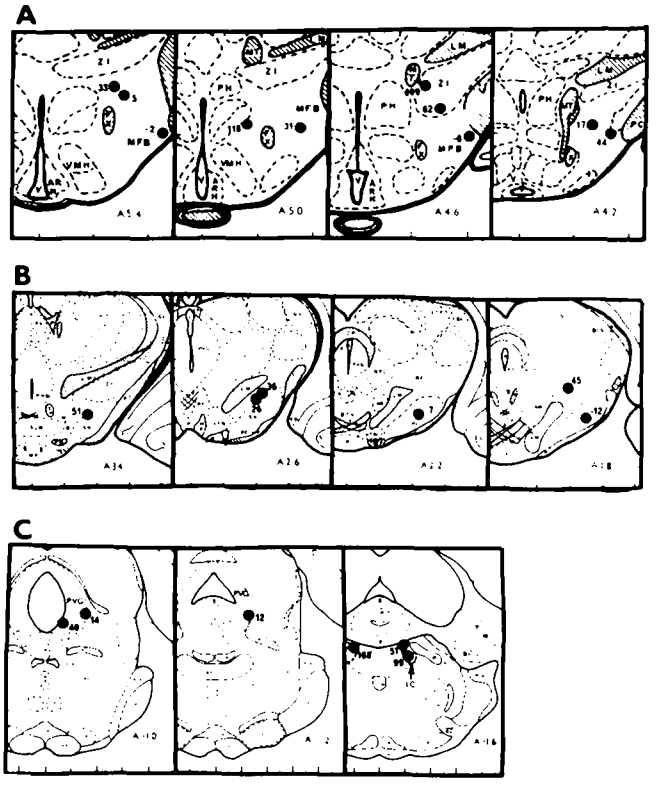

FIG. 1. Coronal sections of rat brain after de Groot [14] (A and B) or Pellegrino and Cushman [37] (C) showing the location of electrodes supporting self-stimulation. Numerals indicate the d−l amphetamine differentials for each rat ranging from extreme dopaminergic values (−12) to extreme noradrenergic values (+168). A: Lateral hypothalamus (wanting one rat with d−l differential = 76). B: Substantia nigra (n = 5) and reticular formation (n = 1). C: Locus coeruleus (n = 3) and periventricular central grey (n = 3).

Thymoxamine

Thymoxamine (10 mg/kg) caused transient flaccidity and a depression of self-stimulation in every rat, followed by a rapid recovery which was usually complete within 20 min. To eliminate zero scores response rates were calculated over 20 min periods, and the average maximal fall in the 1 hr after thymoxamine (70 per cent) proved significantly greater than the average maximal fall after saline (13 per cent) (Wilcoxon T = 4, n = 17, $p<0.01$). The DA groups (Groups SN and LH-DA; mean d−l amphetamine differential = 13.9) were more strongly affected than the NA groups (Groups LC and LH-NA; mean d l differential = 87.9), the respective falls being 79 per cent and 61 per cent (Mann-Whitney U = 16, $n_1 = n_2 = 8$, $p<0.05$), and there was a significant negative correlation between the d−l differentials of all rats tested in all groups and the degree of slowing caused by thymoxamine (Spearman rho = -.52, n =

17, $p = 0.05$). When scores from the DA and NA groups were treated separately, a negative correlation was still apparent within the DA groups (rho = −.80, n = 8, $p<0.05$) but not within the NA groups (rho = −.04, n = 8, n.s.).

Clonidine

Clonidine (0.05 mg/kg) depressed responding in virtually all (14/15) rats regardless of implantation site, the mean response rate in the third 10 min period after injection being reduced to 59.5 per cent of the preinjection rate. Rats which stopped responding were inactive but their eyes stayed open and they retained the ability to make coordinated movements. In the series of LH rats treated with 3 doses of clonidine (0.015, 0.05 and 0.15 mg/kg) the reduction in response rate was significantly dose-dependent (respectively 14.7, 40.9 and 71.9 per cent; Friedman χ_r^2 = 19.5, n = 8, $p<0.01$). There was no significant relationship between the degree of slowing and the d-l differential (Spearman rho = - .29, n = 15, n.s.).

Apomorphine

The initial effect of apomorphine regardless of dose was a brief depression of responding in virtually all (22/23) rats tested, and at the lowset dose (0.1 mg/kg) this was usually the only effect apparent (see Fig. 2B). With doses of 0.3 and 1.0 mg/kg, however, initial depression was followed either by a further fall, or by a sharp increase in responding to well above baseline levels, usually reaching a maximum by the third 10 min period after injection (Figs. 2C and 2D).

A highly significant correlation (Spearman rho = .70, n = 23, $p<0.01$) was present between the changes after apomorphine (especially the 0.3 mg/kg dose) and the values obtained for the d−l differential; thus Fig. 3 shows that the changes in response rate were much greater in Groups LC and LH-NA than in Groups SN and LH-DA, and in opposite directions. All but 2 of the LC and LH-NA rats showed substantial increases after 1 or both higher doses of apomorphine, and all but 2 of the SN and LH-DA rats were depressed by both doses. Two of the PVG rats were depressed by both doses; the third (with a d−l differential = 45), and the RF rat, showed increases. For all rats the changes in response rates after apomorphine were not significantly related to pre-injection levels of responding (Spearman rho = −.047, n = 23, n.s.) or to the effects of either d- or l-amphetamine considered separately (Spearman 0>rho<.34, n = 23, n.s.).

The 1.0 mg/kg dose of apomorphine almost always elicited persistent sniffing and other forms of stereotyped behaviour. Stereotypy sometimes coexisted with lever-pressing activity, but 2 rats, which had been strongly facilitated by 0.3 mg/kg, were possessed by continuous stereotyped movements after the 1.0 mg/kg dose, and would not self-stimulate. Extinction of responding in apomorphine-treated rats was sometimes considerably delayed when the stimulating current was switched off, in one case for as long as 30 min, but similar unreinforced lever-pressing for zero current could not be elicited in rats in which apomorphine depressed self-stimulation, or if the rats were pre-extinguished before being treated with apomorphine. The lower doses or apomorphine did not usually elicit stereotypy, even in rats in which self-stimulation was suppressed.

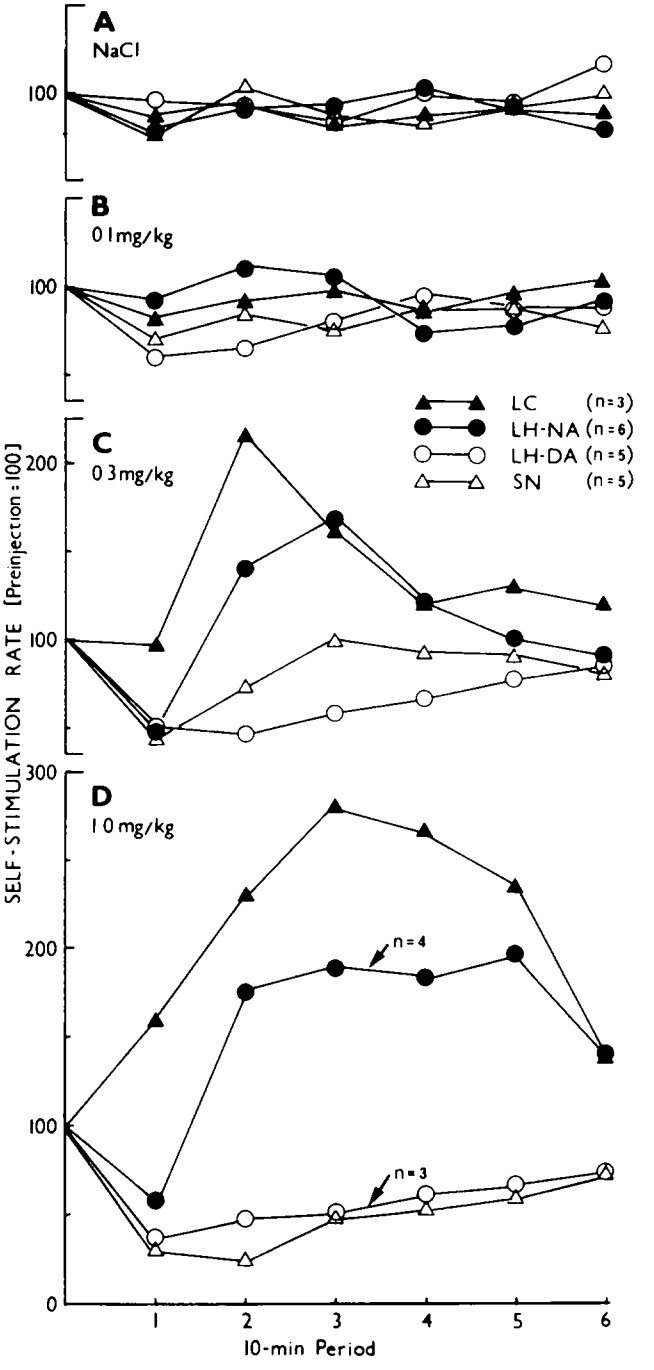

FIG. 2. Self-stimulation rates in 4 groups of rats recorded at 10 min intervals for 1 hr after injection of saline (A), or apomorphine in doses of 0.1 mg/kg (B), 0.3 mg/kg (C), or 1.0 mg/kg (D), and expressed as a percentage of the preinjection rate. Figures in parenthesis indicate the number of rats in each group receiving each treatment.

DISCUSSION

d- and l-Amphetamine

The unambiguous difference between the d−l differentials of Groups LC and SN confirms earlier reports that the 2 amphetamines differentiate reliably between

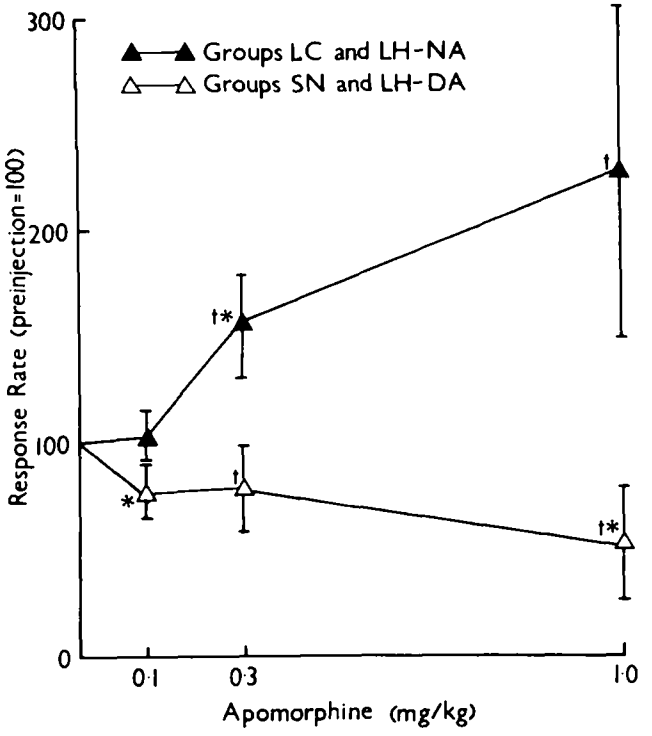

FIG. 3. Changes in self-stimulation rates at noradrenergic (high differential) implantation sites (Groups LC and LH-NA, n = 9) and at dopaminergic (low-differential) implantation sites (Groups SN and LH-DA, n = 10) in the third 10 min period after various doses of apomorphine. For the 1.0 mg/kg dose, sample sizes were n = 7 and n = 8 respectively. *different from preinjection rate ($p<0.05$), +noradrenergic sites different from dopaminergic sites ($p<0.05$).

self-stimulation of dopaminergic and noradrenergic areas of the brain [39]. The use of d- and l-amphetamine for this purpose was originally based on observation that d-amphetamine was 10 times more active than the l-isomer in inhibiting neuronal uptake of NA, but equally active on DA uptake [52]; the same relationships were expected to govern their stimulant effects on self-stimulation. More recent uptake studies, however, have revealed precisely the opposite order of potencies [17, 21, 25], and studies not of CA uptake but of its release (which contributes more than does reuptake inhibition to the stimulant action of amphetamine [44]) have shown the d-isomer to be 10 times more potent in releasing DA [7] and equipotent in releasing NA [51].

It is thus necessary to consider whether the special effectiveness of d-amphetamine at noradrenergic self-stimulation sites may be due to the facilitated release of DA rather than NA. Noradrenergic electrodes would normally release ample NA but would not release DA except by an indirect and relatively less efficient transsynaptic route. Thus, on the present hypothesis, the rate-limiting factor in noradrenergic sites would be the availability of DA, not NA, and this would explain why such sites are particularly sensitive to dopaminergic drugs such as d-amphetamine. The converse picture would apply to dopaminergic sites; they would be particularly sensitive to noradrenergic drugs and thus equally sensitive to d- and l-amphetamine. Further findings discussed in the following sections provide additional support for this interpretation and throw light on the nature of the complementary roles of the 2 transmitters.

Thymoxamine

Thymoxamine reputedly exerts a selective blocking action on the adrenergic α-receptor without incurring the side effects of other α-blocking agents [36], and its depressant action on self-stimulation is thus further evidence that NA may have a specific role in self-stimulation. Furthermore, the finding that thymoxamine acted more strongly in the DA than the NA groups confirms a prediction from the present hypothesis, and would be difficult to explain entirely in terms of the sedative effects apparent in both groups. Nonspecific effects of thymoxamine may have been largely responsible, however, for the relatively milder slowing which occurred in the NA groups, since in the NA groups slowing was unrelated to the d−l differentials.

Clonidine

Stimulating the adrenergic α-receptors with clonidine had the same effect as blocking them with thymoxamine. This finding not only again underlines the importance of NA in self-stimulation but suggests that its specific role may be the transmission of discrete packets of information correlated with the occurrence of reinforcing events; as we have pointed out, this would mean that direct α-stimulants such as clonidine would tend to disrupt performance, regardless of electrode site, by breaking down this correlation and by reinforcing inactivity or irrelevant activity.

One cannot exclude the possibility that sedative [24], hypotensive [24] or other [19] effects of clonidine contributed to its action on self-stimulation but these seem unlikely to have played a major role since the dose of clonidine effective against self-stimulation (0.05 mg/kg) has proved not incompatible with "persistent feeding activity" when administered intraventricularly [24], while the ED_{50} for suppression of a relatively difficult conditioned avoidance response (pole-climbing) was over 40 times greater [27].

Apomorphine

We cannot account for the brief depression that followed most injections of apomorphine or for the depressant effects of the lowest dose (0.1 mg). Similar findings have however been reported for mouse locomotor activity after low doses (0.05 mg/kg) that were subthreshold for central thermogenic effects, and after low doses of DA (which does not cross into the brain), and it has therefore been suggested that nonspecific peripheral effects may contribute to the depressant action of apomorphine in low concentrations [32].

At higher doses, however, apomorphine sometimes caused a very marked increase in self-stimulation, and in this respect differed from clonidine which was everywhere depressant. This finding argues against suggestions [11,49] that DA and NA might play similar roles in similar but independent self-stimulation mechanisms. Moreover, virtually all rats showing enhanced performance with apomorphine belonged to groups classified as noradrenergic (Groups LC and LH-NA), a finding significant in 3 respects: first, it constitutes further evidence that DA is a rate-

limiting factor in self-stimulation of noradrenergic brain sites; second, it shows that enhanced performance after apomorphine was unlikely to have been a simple manifestation of stereotyped motor activity since stereotypy affected all rats, not just rats with noradrenergic implants; and finally, the fact that enhanced performance could be brought about by dopaminergic stimulation of an essentially random nature indicates that in these groups DA was not significantly involved in the transmission of response-correlated reinforcement signals. The alternative is that DA is involved in a tonic motivating process, and this possibility is considered in the next section.

NA-DA Interaction

The interaction of reinforcement and drive or arousal in self-stimulation has been considered in detail in models of self-stimulation proposed by J.A. Deutsch and others, and the logical status of each term has been rigorously defined [15,20]. The present findings can be integrated with these models by an hypothesis that the reinforcing component in such models depends in part on NA, and the motivating component on DA. This interpretation may be illustrated by considering two extreme cases. Fig. 4A indicates schematically how an electrode in a pure noradrenergic structure could maintain self-stimulation. Such an electrode would be in a position to excite optimal levels of adrenergic activity but would not elicit dopaminergic activity except via indirect transsynaptic pathways. Thus activity levels in the motivational component would tend to be suboptimal unless supplemented by possible endogenous motivational activity [16] or, as in the present case, by the effects of apomorphine. This interpretation readily accounts for the characteristically unenthusiastic quality of LC self-stimulation [12,42], as well as for the absence of extinction (or of "drive decay" [26]), after treatment with apomorphine [6] or d-amphetamine [33]. The extraordinary resistance to extinction conferred by these drugs also illustrates that in self-stimulation as in conventional learning tasks, large changes in reward may have relatively little effect on response strength as long as drive is continuously maintained at high intensity [20,41]. The dopaminergic drive component of self-stimulation does not, however, seem the complete equivalent of specific drives such as hunger or thirst since feeding and drinking are not usually evoked by apomorphine alone, or by LH stimulation unless the electrodes involve, in addition, parafornical pathways now known to be predominantly noradrenergic [48]. This is consistent with recent suggestions that the ascending DA pathways mediate a non-specific motivational state [54] which is manifested as specific drive activity only in the presence of a corresponding reinforcer [56].

Electrodes in purely dopaminergic structures are considered in Fig. 4B; with such placements maximal activation of the dopaminergic component would be readily achieved, and self-stimulation of these sites is characterized by obviously excited behaviour with continual sniffing, jumping, and biting of the lever [10]. On the other hand, noradrenergic reinforcement activity would be elicited exclusively via synaptic connexions with the dopaminergic implantation sites and not by the electrodes directly. Randomisation of dopaminergic neural activity by administration of apomorphine would thus have similarly randomizing effects on reinforcing noradrenergic activity, with consequently disruptive effects on self-stimulation.

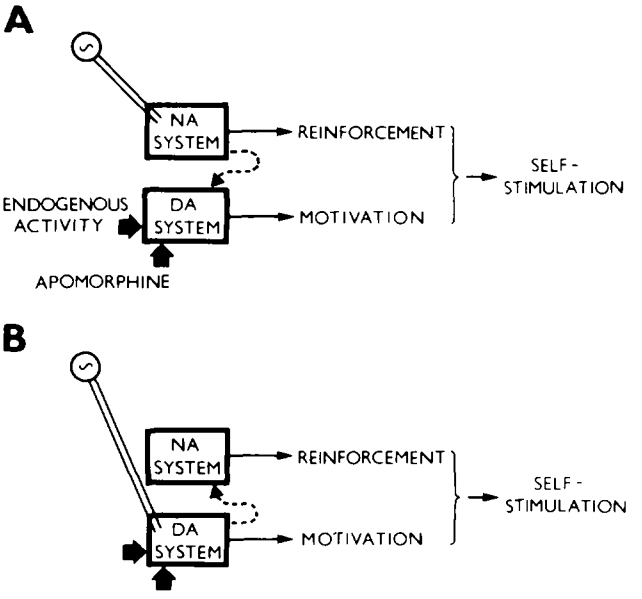

FIG. 4. A: Self-stimulation of a pure noradrenergic structure. Adequate response-contingent reinforcement is generated by the electrodes, and the rate-limiting factor is thus level of dopaminergic activity derived either from the stimulating electrodes via a transsynaptic NA-to-DA route (broken line), or from endogenous motivational processes, and augmented in the present instance by apomorphine. The conjectural NA-to-DA connexion indicated by the broken line could mediate the 'incentive motivation' envisaged in some accounts of self-stimulation [53], but it would not be essential to Deutsch's account [15] in which the motivational component of self-stimulation need not derive exclusively from the stimulating current [16]. B: Self-stimulation of a purely dopaminergic structure. Adequate dopaminergic activity is elicited by the stimulating current, and the rate-limiting factor is thus the level of noradrenergic response-contingent reinforcement, derived transsynaptically from dopaminergic pathways. Randomisation of dopaminergic activity by apomorphine would disrupt the response contingency of the reinforcing signals and suppress self-stimulation. The broken line indicates the DA-to-NA transsynaptic connexion required by the present account. Its existence is supported by recent biochemical findings [38,50].

This prediction was confirmed in virtually all members of the dopaminergic groups; in these rats apomorphine acted like clonidine and depressed responding at all doses, including doses subthreshold for stereotypy. Hence, with these electrodes, response-contingent dopaminergic activity could be regarded as essential for reinforcement even if not constituting part of the reinforcement process proper. The absence of similar findings in previous studies [6,28] may have been due to the inclusion of implantation sites superficial to the SN. A reanalysis of one such set of conflicting data [6] carried out in the light of the present histological findings has uncovered significant site-related apomorphine effects ($n = 10$, $p<0.05$ 2-tailed Mann-Whitney) similar to those reported here (C. L. E. Broekkamp, personal communication, 1975).

Electrodes involving mixed populations of NA- and DA-containing neurones might be expected to give results intermediate between the extreme cases considered above, but the very few electrodes outside the target areas in the present study do not permit generalization to other self-stimulation sites or to areas traversed by other transmitter systems.

ACKNOWLEDGEMENTS

Some of this work was communicated to the First International Conference on Brain Stimulation Reward, Beerse, Belgium, in April 1975. We thank Dr. C. L. E. Broekkamp for generously informing us of the reanalysis of his earlier data and for permitting us to cite his findings. We are grateful to the Medical Research Council, the Elizabeth Hermitage Fund and the Brain Research Trust for support.

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