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Drug-potentiated differential rearing effects on brain stimulation reward

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Threshold and response rate for brain stimulation reward (BSR) were determined in rats reared for 60 days in an enriched or an impoverished environment. No differences in threshold or rate were found. Subsequent injections of amphetamine or scopolamine at 1-week intervals slightly decreased threshold and significantly increased response rate in enriched relative to impoverished rats. Results suggest that subtle manipulation of brain catecholamines and morphology by rearing can have a strong influence on BSR at its site of action in forebrain.

A major research effort in intracranial self-stimulation (ICS) has been directed toward neurophysiological processes underlying reward. The observation that neuronal pathways releasing catecholamines at their terminals are identical to those yielding reward by ICS (German & Bowden, 1974) gave additional impetus to research into these mechanisms. A number of studies employing massive pharmacological manipulation of the catecholamines (CA) conclude that the dopamine (DA) (Phillips & Fibiger, 1973) and/or the noradrenaline (NE) (Crow, 1972; Stein, 1964) systems mediate the rewarding effects, although the precise nature and extent of their mediation are still being debated (German & Bowden, 1974; Mogenson & Phillips, 1978; Wise, 1978).

The present study examines whether procedures that induce more natural manipulation of brain CA levels alter the characteristics of ICS. Earlier work by Rosenzweig, Bennett, and Diamond (1972) demonstrated that differential rearing produces changes in the level of another neurotransmitter, acetylcholine (ACh), as measured by changes in the enzyme cholinesterase. It is of some interest that the course of the CA systems implicated in brain stimulation reward (BSR) (German & Bowden, 1974) are paralleled by ACh fibers (Jacobowitz & Palkovits, 1974; Palkovits & Jacobowitz, 1974). Differential rearing also produces some interesting morphological changes. Basically, rats exposed to enriched (EC) rearing regimens develop heavier cortices and larger somata and nuclei of cortical neurons and develop more and larger synapses than do impoverished (IC) rats (Walsh & Cummins, 1975). The most important differences produced by differential rearing in terms of the present study are neurochemical. The EC rats show higher cortical and lower hypothalamic CA levels (both DA and NE) than do IC rats (Reige & Morimoto, 1970).

The present study examines whether the very subtle but reliable neurochemical and morphological changes produced by differential rearing are sufficient to alter rewarding properties of brain stimulation, specifically self-stimulation threshold and rate. A secondary purpose is to use the reported relative cortical and hypothalamic CA levels (Reige & Morimoto, 1970) to determine the possible locus of reward effects. If the pertinent sites for effects of rewarding medial forebrain bundle (MFB) stimulation are "hypothalamic," then EC rats with lower hypothalamic CA levels than IC rats should show higher brain stimulation threshold and reduced response rates for ICS and should be less responsive to the rate-increasing effects of amphetamine. If the pertinent reward sites are "cortical," then IC rats should show lower thresholds and increased response rates for ICS and should be more responsive to amphetamine.

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METHOD

Subjects

Fourteen experimentally naive male hooded rats (Canadian Breeding Laboratories) were divided into two equal groups for differential rearing at 25 days of age.

Procedure

The animals were placed in one of two rooms of identical size and wall coloring, depending on the group. In one room, IC rats were raised individually in white translucent polypropylene cages (28.5 x 17 x 13 cm) for 60 days. Food and water were available continuously through the removable stainless steel grids covering the cages. A 10-W bulb supplied constant low-level room illumination. Low-level white noise was used to mask extraneous sounds. With the exception of weekly cage cleaning and daily checks for food and water, these animals were not handled or otherwise disturbed.

In the other room, EC rats were housed in a plywood cage (70 x 75 x 45 cm) for 60 days. Food and water were available continuously. Normal fluorescent room lighting was programmed on a 12-h-light/12-h-dark cycle. White masking noise was not used because EC animals were raised intentionally in a non-monotonous, variable environment. These animals were handled daily and exposed in their cages to a randomly selected set of 5 toys from a larger pool of 25. These included, for example, wooden tunnels, blocks, plastic animals, and small children's toys purchased from a variety store. These conditions have been shown to produce cortical morphological and biochemical differences between EC and IC rats (Reige & Morimoto, 1970;

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Rosenzweig & Bennett, 1969; Rosenzweig, Bennett, & Diamond, 1971, 1972).

Surgery

Following differential rearing, all rats were implanted with a single twisted bipolar stimulating electrode (Plastic Products, Roanoke, Virginia), insulated except at the tip. Rats were anesthetized with 65 mg/kg body weight of sodium pentobarbital (Nembutal), so equal body weights received equal volumes of anesthetic. The rat was then fixed in a stereotaxic instrument, the skull was exposed and cleared of connective tissue, and four pilot holes were drilled to receive 0-80 x 3.2 mm self-tapping stainless steel screws. A further hole was drilled over the electrode site. The electrode was lowered to coordinates in the anterior or posterior MFB, either AP .0, L 2.0, DV 8.0 (anterior site) or AP 3.5, L 1.8, DV 8.5 (posterior site) from the top of the skull with the head level (lambda equal to bregma). The electrode was secured to the screws and skull with cold-cure dental cement. The wound was dusted with P.E.P. veterinary antibiotic powder and sutured.

Training

Following 14 days of recovery from surgery, rats were trained to leverpress in a two-lever operant chamber. Each leverpress was reinforced with a 200-msec 60-Hz sinusoidal train of current delivered through a mercury swivel and spring-protected wires attached to the electrode. Training to leverpress proceeded by successive approximations, with reinforcing current set at the minimum that would produce activation, sniffing, and exploration of the chamber (usually 50-150 microA). Rats acquiring the response were allowed to respond freely for 30 min to stabilize rates for subsequent testing. Rats failing to acquire the response in two training sessions were discarded and not replaced.

After this initial training, EC and IC groups were subjected to three successive drug conditions separated by 7 days: a vehicle control condition, an amphetamine condition, and a scopolamine condition. Drugs were mixed so rats would receive 1 ml/kg body weight.

In the vehicle control condition, each rat received a 1-ml/kg body weight injection of .9% NaCl vehicle. Immediately following injection, rats were retested for ICS in the operant chamber. Each leverpress in a 5-min period produced a constant-current 200-msec train of stimulation beginning at 125 microA. This was reduced by 5 microA every 5 min until three successive reductions produced no further response decrement. In several rats, the current had to be increased to get any responding (see Histology). The ICS threshold was defined as the current below which no further reduction in response was observed. Response rate was the number of responses per 5-min period at the current 10 microA above threshold. The rats were then returned to their respective environments for a further 7 days' maintenance.

On the 8th day, rats were returned to the operant chamber, with ICS intensity set at the previously determined threshold plus 10 microA. Following a 5-min warm-up period, response rate was determined in a 5-min predrug baseline condition. Rats were then injected intraperitoneally with 1.0 mg/kg d-amphetamine sulfate. After a 10-min period of free responding, threshold and rate were determined in a manner identical to that used for the vehicle control. The same procedure was followed 1 week later, except the rats were subcutaneously injected with .5 mg/kg scopolamine hydrobromide.

Histology

Histologies were performed on all rats completing surgery. Each rat was injected with a lethal dose of sodium pentobarbital and perfused intracardially with normal saline followed by 10% formol-saline. Brains were extracted and placed in formol-saline. The tissue was then frozen, and a number of 40-micron slices were taken from the area of the electrode tract. These slices were stained with formol thionin (Donovic, 1974) and mounted on slides. The slides were projected on a screen through a projecting microscope and compared with the Konig and Klippel (1963) atlas to determine electrode tip location.

RESULTS

During the surgical recovery period, one of the original 14 rats lost the electrode. Two more rats lost the electrode prior to completion of the drug testing, and one (IC8) had an unusually high threshold. Two of the remaining rats did not self-stimulate. Placements and corresponding ICS thresholds for rats that leverpressed for stimulation are plotted in Figure 1.

With the exception of Rat IC8, all of the placements were within or on the immediate border of the MFB. Because of the abnormally high threshold, this rat was not included in the statistical analysis. Statistical analyses were performed on the remaining four animals in each rearing condition.

ICS rates in the vehicle control condition were not significantly different from the predrug baseline rates for the 2 drug injection days taken at 1-week intervals, indicating that the control rates were highly reliable over the course of the experiment, in spite of intervening treatments [$F(2,12) = .96$, $p = .59$]. Neither the main effect of environment [$F(1,6) = .026$, $p = .63$] nor the Environment by Control Condition interaction was significant. This indicates that prior to any drug treat-

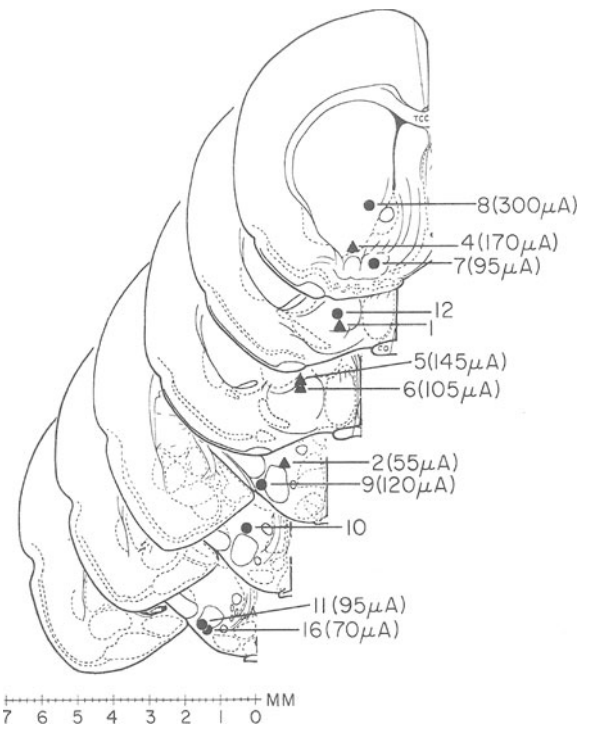

Figure 1. Electrode tip location and self-stimulation threshold (+10 microA) for enriched (▲) and impoverished (●) rats. Numbers on left are animal identification. Rats 1, 10, and 12 did not self-stimulate.

ment, differential rearing failed to alter self-stimulation rate.

As in previous studies employing these drugs (Stein & Ray, 1959), both amphetamine and scopolamine significantly [$F(2,12) = 5.80$, $p = .02$] lowered ICS thresholds relative to the vehicle control from 125 to 72 microA averaged over the two environmental conditions. Orthogonal comparisons showed that the average thresholds for both drug conditions were significantly [$F(1,12) = 9.96$, $p = .01$] lower than vehicle control thresholds, but the amphetamine and scopolamine thresholds were not different from each other [$F(1,12) = 1.64$, $p = .22$]. The lower threshold for EC relative to IC rats seen in Figure 2 was not significant for any of the drug conditions [for the Drug by Environment interaction, $F(2,12) = .45$, $p = .65$].

Response rates for EC and IC rats, illustrated in Figure 3, suggest that both amphetamine and scopolamine increased responding in EC rats and decreased responding in IC rats. Preplanned orthogonal comparisons confirmed that amphetamine and scopolamine produced greater response rates in EC than in IC rats [$F(1,12) = 5.28$, $p = .04$], but the amphetamine was not different from the scopolamine in this respect [$F(1,12) = .37$, $p = .56$].

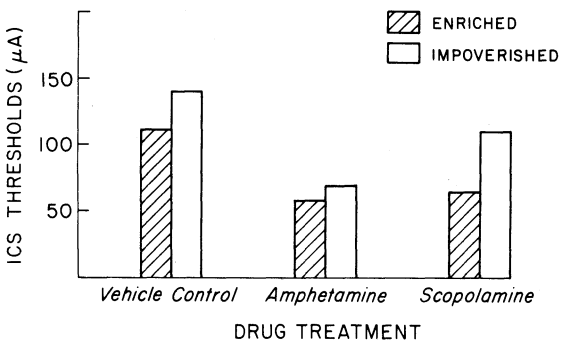

Figure 2. Mean self-stimulation threshold following injections of Ringer's solution (vehicle control), d-amphetamine sulfate, or scopolamine hydrobromide in rats reared in enriched or impoverished environments.

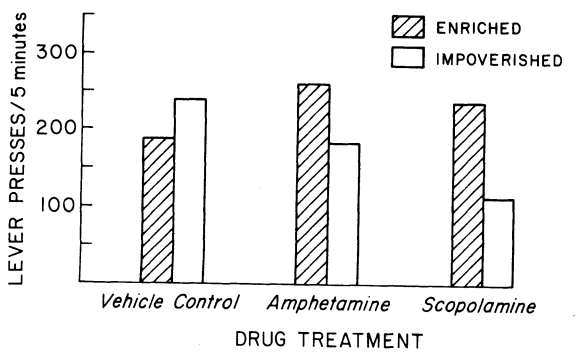

Figure 3. Mean self-stimulation rates following injections of Ringer's solution (vehicle control), d-amphetamine sulfate, or scopolamine hydrobromide in rats reared in enriched or impoverished environments.

DISCUSSION

Modern theory of BSR emphasizes the mediating role of cerebral CA (German & Bowden, 1974; Mogenson & Phillips, 1978; Wise, 1978). Such theories are based on effects of massive pharmacological manipulation of CA metabolism or turnover on ICS parameters such as threshold and rate of responding. The present experiment examined whether more natural and more subtle manipulation of CA levels by differential rearing also affected BSR. Instead of directly observing CA levels, we duplicated the environmental rearing conditions that have been demonstrated to alter cerebral morphological and biochemical (Globus, Rosenzweig, Bennett, & Diamond, 1973; Reige & Morimoto, 1970) substrates of behavior.

Surprisingly, differential rearing in infancy did not alter later threshold or rate of responding for BSR prior to the drug conditions. Such a result might suggest that morphological and biochemical changes produced by differential rearing are too subtle to manifest themselves in more complex behaviors, such as learning and reward. Such a suggestion is premature in light of the preliminary nature of these data, but it is consistent with the difficulty in demonstrating any learning-related, as opposed to experientially induced, biochemical changes (Brown & King, 1971; Rosenzweig, 1966; Walsh & Cummins, 1975). Conceivably, subsequent rearing investigations that circumvent the problems of demonstrating learning differences during growth and maturation may well be able to show direct differential rearing effects on neural substrates of reward.

Alternately, CA or ACh function in the present experiment may not have been affected by rearing. Without direct observation of CA and ACh levels through biochemical assay, this becomes a possibility. However, our drug manipulation results on both threshold and response rate suggest that CA and ACh function was, in fact, altered. Both the CA agonist, amphetamine, and the ACh antagonist, scopolamine, lowered threshold for ICS. Moreover, amphetamine and scopolamine differentially decreased threshold slightly and increased response rates significantly in enriched relative to impoverished rats. Thus, CA and ACh function, normally modified by amphetamine and scopolamine, must have been previously altered by differential rearing from infancy.

The precise nature of CA and ACh function altered by differential rearing resulting in changes in BSR needs elaboration. Increased dendritic arborization (Greenough & Volkmar, 1973), increased number of dendritic spines (Globus et al., 1973), increased nuclear and perikaryonal size (Diamond, 1974), or increased CA or ACh available for release by stimulation in enriched rats (Rosenzweig et al., 1972; Reige & Morimoto, 1970) may have contributed to the observed drug results. However, these morphological and biochemical differences between EC and IC rats were not sufficient by themselves to alter characteristics of BSR, although they may have provided a neuronal substrate upon which drug-enhanced CA and ACh could act.

The possibility remains that effects of differential rearing on brain biochemical function may be helpful in isolating the site of action of BSR. Since CA and ACh synapses occur throughout the brain (German & Bowden, 1974), systemic injection of drugs resulting in widely distributed pharmacological action at many brain and peripheral sites is not particularly useful in determining the ultimate site of action of rewarding stimulation. Such stimulation within the anterior-posterior limits of the MFB might be acting directly through immediate effects on local neurons underlying the electrode site or polysynaptically at remote terminals. However, Reige and Morimoto (1970) demonstrated that hypothalamic and cortical CA levels were differentially altered by rearing. Hypothalamic DA and NE

were increased in enriched relative to impoverished rats. German and Bowden's (1974) review of the literature clearly indicates that increased CA levels are associated with increased reward and decreased CA levels, with decreased reward. Thus, EC rats whose hypothalamic CA levels are lower than IC rats would respond less for BSR than would IC rats if the local diencephalic stimulation were mediating reward. On the other hand, the increased cortical CA content in EC rats would produce greater responding to BSR if telencephalic terminals were involved. This preliminary experiment finds a higher rate of self-stimulation responding in EC rats and supports the conclusion that remote telencephalic terminals are responsible for BSR in intact rats.

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