SEPTAL STIMULATION AS A REINFORCER OF AND AN ALTERNATIVE TO CONSUMATORY BEHAVIOR¹

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Although considerable work has been done recently elucidating the control of behavior by intracranial reinforcement, and exploring the anatomical and stimulus parameters of the reinforcing effect, only a few experiments have attempted to relate ICS-motivational phenomena to deprivation-induced states such as hunger and thirst. With electrodes in the septal region of the rat, Brady *et al.* (1957) found that food and water deprivation increased the response rate for ICS on both CRF and VI schedules. Olds (1958) cites an experiment by Wendt which showed increases in food intake during sessions where unavoidable trains of rewarding ICS were administered.

In the present experiment, I was not primarily concerned with motivational interaction but with the possibility of using septal-area stimulation as a reinforcer of consumatory behavior. A second objective was to explore parameters controlling the choice between septal self-stimulation and rehydration.

METHOD

Subjects

Subjects were two female, albino rats (CD) obtained from the Charles River Breeding Laboratories. They were designated CD-3 and CD-4, and weighed 255 and 250 grams, respectively, at the beginning of the experiment.

Apparatus

The experimental space was a varnished, wooden box 6.5 inches wide, 11.25 inches long, and 14.75 inches high, with a grid floor. A drinkometer whose aperture was 1.25 inches from the floor was attached to the front wall. The physical arrangement of the drinkometer spout and front-wall aperture was as follows: A 100-milliliter-capacity eudiometer tube, fitted with a glass drinking spout, was clipped to the outside of the apparatus. The opening of the spout from which the animal drank was 3 millimeters in diameter and situated about 3 millimeters behind a black, 0.125-inch micarta panel which formed part of the front wall of the apparatus. The front-wall aperture was cut out of the micarta and measured 6 millimeters wide by 11 millimeters high. A Gerbrands rat lever was mounted on one side. Both the drinkometer panel and the lever could be replaced by plain micarta panels. The box was open at the top to permit the animal free movement while the leads

to the implanted electrode were attached. Depression of the lever produced a 0.5-second, 60-cycle electrical stimulation at the uninsulated electrode tip. A response during stimulation prolonged it by 0.5 second from the time of the response. A step-down transformer and variable potentiometer allowed the voltage to be set between 0 and 10 v.r.m.s. An alternate arrangement provided ICS for various fixed ratios of licking on the electronic drinkometer when the lever was removed.

The design of the bipolar stimulating electrode and its carrier is described in a separate note (Falk & Emma, 1961). The implantation operation was similar to the procedure described by Olds and Milner (1954).

PROCEDURE AND RESULTS

Histology² (See Fig. 1.)

Brains were fixed in Bouin's fixative made with 10% formalin, embedded in paraffin; and the anterior two-thirds of the forebrain was serially sectioned at 8 μ frontally and stained mostly with hematoxylin and eosin, occasionally with iron hematoxylin for myelin.

CD-3. The electrode track passed vertically and slightly caudad from the medial-most part of the vertex of the left cerebral hemisphere through the genu of the corpus callosum into the left septum. The track in the fixed sections was about 600 μ wide because the electrode pulled out just before the animal was killed for study. In terms of precisely what structures were destroyed, its width was 200 to 300 μ . Its path coursed through the deepest two-thirds of the cingular cortex, through the genu, then just inside the upper inner angle of the lateral ventricle and on into the center and medial part of the septum. Fresh blood filled most of the track, the freshness was evidenced by no reaction by phagocytes and leukocytes, and no deposition of fibrin. There was older focal coagulation necrosis of brain tissue in the track wall in the cingular region and genu of the callosum, for a distance of about 20 to 50 μ outward. Just above and in the genu of the corpus callosum, in the track, were collections of leukocyte remnants and some lymphocytes. No gliosis was present. Some proliferation of endothelial cells in minute vessels in the cingular cortex medial to the track was seen, and the corresponding neurons in laminas II and III showed acute recent degeneration. A single collection of polymorphonuclear leukocytes, well-preserved (less than 18 hours old), was present in the inferior angle of the ventricle at the track level. Some perivascular lymphocytes were also present in the genu and in the meninges

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²The histological analysis was performed by Dr. Samuel P. Hicks, Dept. of Pathology, Harvard Medical School.

Fig. 1. Frontal sections of the brains of Rats CD-3 and CD-4 are shown at levels almost at the rostral-most extent of the electrode tracks. The fresh hemorrhage (see text) in the track in CD-3 resulted from the pulling loose of the electrode at the end of the experiment (hematoxylin and phloxin, $\times 22$).

closest to the entrance of the track and adjacent to the anterior part of the left lateral ventricle. The left lateral ventricle was moderately dilated. Occasional lymphocytes lay just beneath the ependyma in its anterior part. The track in the septum showed minimal reaction in its margin, characterized by increased microglial cells in a zone about 30 to 50 μ thick; but some old cellular detritus was mixed with the fresh red cells in the track. The depth of the track in the septum extended to a horizontal plane that would lie on a level of the inferior angles of the lateral ventricles. Its most caudal extent lay in the medial septal nucleus in a frontal plane that would pass through the anterior border of the anterior commissure, but the track ended 700 μ above that structure. The track lay 400 μ lateral to the column of the fornix, and its terminal 200 μ passed through bundles of the septo-hypothalamic tract. It involved chiefly the medial rather than the lateral septal nucleus. (The photograph is of a section 180 μ caudal to the rostral-most extent of the electrode tip as seen in frontal sections.)

CD-4. The track in this animal was generally similar to that in CD-3, except that it was slightly curved and at the same time more truly vertically placed. It passed a

little more laterally through the right cingular region of the cortex, through the genu of the corpus callosum, and across the lateral part of the angle of the ventricle at its very rostral end. From here, it coursed through the top of the medial part of the head of the caudate nucleus and into the superior part of the right septum. It curved slightly through the medial part of the septum, ending in the midline just at the point where the descending columns of the fornix curve back over the superior border of the anterior commissure, just touching these structures. In its terminal 200 μ the track involved medially placed bundles of the septo-hypothalamic tract on both sides of the midline but more on the right. There was relatively little terminal hemorrhage in this track associated with the removal of the electrode, and the reaction in the wall of the track was similar to that in CD-3. Some microscopic fragments of amorphous foreign material (electrode) remained in the lower-most part of the track, but there was no recent cellular reaction to it. A few macrophages containing fine brown pigment granules were present. (The photograph is of a section 130 μ caudal to the rostral-most extent of the electrode tip as seen in frontal sections.)

Locus of Stimulation. In CD-3, major structures close

to the exposed tip of the electrode were the posterior part of the left medial septal nucleus and its associated septo-hypothalamic fiber bundles. These fibers sweep downward, then lateralward, from the medial septal region, joining the medial forebrain bundle, and ending chiefly in the lateral hypothalamic regions (Gurdjian, 1927). The left fornix and the anterior commissure were 400 and 700 μ distant, respectively, from the stimulating tip.

In CD-4, the right medial septal nucleus and its associated septo-hypothalamic fiber bundles, and some of the analogous structures on the left, were at the tip of the electrode. In addition, the anterior medial borders of the columns of the fornix and the anterior border of the anterior commissure were in contact with the electrode.

Behavioral Procedure and Results

The animals were implanted with electrodes in the septal area, and 2 days were allowed for recovery. Daily 1-hour sessions were given in the apparatus, with no deprivation of food or water (except during the session), to assess the adequacy of the ICS as a reinforcer on a CRF schedule. The drinkometer was not attached; only the lever was present. Voltage levels were selected which yielded intra- and intersession stability of response rate. For CD-3, this was 1.75 volts; for CD-4, it was 2.50 volts. The upper record in Fig. 2 shows a typical session for CD-3 (291 rf.). Each response stepped the recorder and offset the pen momentarily (report rf.) in order to reveal the grain of the record more clearly. Throughout the experiment, the response rate of CD-4

was higher; but since its performance in every way confirmed the observations on CD-3, little specific reference will be made to this set of results.

From this point on, the animals were subjected to a water-deprivation schedule which allowed, unless otherwise stated, water from the drinkometer during the daily experimental session, and another hour of water in the home cage immediately after the session. Thus, the animals typically commenced their session with 22 hours of water deprivation. Under this condition, time was divided between drinking and lever pressing, as shown in the lower record of Fig. 2. Drinkometer licking simply stepped the recorder (1100 steps per excursion), and ICS's again were indicated by reinforcement marks.

Figure 3 (upper record) shows the result of removing the ICS bar, allowing access to only the drinkometer. (The reinforcement marks have no significance, only indicating every twentieth lick.) The water intakes under this condition and where both the ICS bar and drinkometer are available (Fig. 2) are comparable. This type of ICS seems to have no direct effect on thirst, as measured by intake, either during the 1-hour experimental session or in the 1-hour postsession period when water was again available.

Since comparable water intakes occurred with and without the ICS bar, it was concluded that septal stimulation did not directly alter water intake. But could ICS be used to increase water consumption if it were made contingent upon drinkometer licking rather than bar pressing? This seemed a reasonable expectation in view of the demonstrated reinforcing value of the ICS used. The lower record of Fig. 3 shows that

Fig. 2. Top: Lever CRF (ICS) All sessions lasted 1 hour.
Bottom: Lever CRF (ICS), drinkometer (H₂O)

Fig. 3. Top: Drinkometer (H₂O)
Bottom: Drinkometer (H₂O + ICS FR 20)

this prediction was not borne out. With the bar removed, ICS was delivered on a licking schedule of FR 20. The animals drank amounts similar to those on control days. With an ICS licking schedule of FR 5, intake decreased to about one-half of control days for both animals. A 0.5-second ICS delivered on a CRF licking schedule was omitted since this would have maintained a continuous ICS in force during drinking. The decreased licking rate and intake with FR 5 indicate that either closely spaced ICS's evoked responses incompatible with licking, or that short inter-ICS times were aversive. Whichever factor explains the decreased licking on FR 5, it was evident that closely spaced ICS's resulting from licking were decreasing, not increasing, water intake. However, the failure to increase intake under FR 20 cannot be attributed to any of the above hypothesized effects or to an ICS satiation effect. With the ICS bar and the drinkometer both available, CD-3 pressed for a mean of 192 ICS's per session and CD-4 for 486; but, under the licking FR 20 schedule, only 75 and 91 ICS's were received, respectively. Obviously, ICS satiation cannot explain the FR 20 results. Inter-ICS times were typically longer on FR 20 than on bar CRF.

Increasing the ICS voltage with this FR 20 schedule to as high as 5.0 volts also failed to change water intake (Fig. 4, middle record), although licking rate was af-

fected. The retardation of licking rate remains unexplained, especially since double the original base-line ICS bar-pressing rate was observed for 5.0 volts for both animals at this time.

In order to prove that the failure to potentiate water intake was not due to a stomach-load limit, 0.9% NaCl solution was substituted for water in the drinkometer and the bar was replaced. The session fluid intake was doubled for CD-3 (Fig. 4, top record, 4.5 volts), and more than tripled for CD-4.

As a final variation in the attempt to potentiate intake, the bar was removed and each tongue contact was programmed to deliver a 30-millisecond ICS. The result was a lack of effect at low voltages, while higher voltages yielded a decrease in both session intake and licking rate (Fig. 4, bottom record, 2.5 volts).

At this time, I re-investigated the choice condition between water drinking and pressing for ICS at higher voltages than previously used. With both bar and drinkometer present, the ICS was set at 5.0 volts. Figure 5 shows four successive daily sessions. No post-session water intake was permitted during this series. On the first day, CD-3 eschewed water entirely; on the second and third days, only 0.5 milliliter was consumed. After 94 hours of almost complete water deprivation, there was a "break-through" to drinking. The animal had lost considerable weight, and it probably could not have survived another 24 hours of deprivation.

Figure 6 shows the same session series for CD-4 (6.0 volts). The break-through occurred after only 70 hours of deprivation; and, unlike CD-3, drinking was initiated after a series of ICS's. A full 6.0 volts was found necessary to produce this effect in CD-4. With 5.5 volts, pressing was increased at the expense of time spent drinking, but intake still occurred.

Fig. 4 Top: Lever CRF (ICS), drinkometer (0.9% NaCl)
Middle: Drinkometer (H_2O + ICS FR 20)
Bottom: Drinkometer (H_2O + ICS CRF)

Fig. 5. Four successive sessions: Lever CRF (ICS), drinkometer (H_2O).

CD-4

22 HR. H₂O DEPRIVATION46 HR. H₂O DEPRIVATION70 HR. H₂O DEPRIVATION

Fig. 6. Three successive sessions: Lever CRF (ICS), drinkometer (H₂O).

DISCUSSION AND CONCLUSIONS

At an adequately reinforcing level, ICS had no effect upon water consumption. Neither the concomitant availability of ICS on a CRF schedule, nor the direct

use of ICS as a reinforcer for fixed ratios of licking, was able to increase water intake. Likewise, the experimental literature does not show that septal stimulation interferes with deprivation-induced drive states, or that they are mutually inhibitory. On the contrary, deprivation has been shown to increase the response rate for septal ICS (Brady *et al.*, 1957), and ICS can potentiate consummatory behavior (Olds, 1958). Therefore, the choice of ICS over water in the last high-voltage sessions cannot be relegated to a direct effect of ICS upon deprivation-induced drive.

The two major results can be viewed as something of a paradox: Under the conditions explored, septal stimulation cannot be used to increase water intake, although it is preferred to water as a reinforcer until a high level of dehydration is attained.

REFERENCES

- Brady, J. V., Boren, J. J., Conrad, D., and Sidman, M. The effect of food and water deprivation upon intracranial self-stimulation. *J. comp. physiol. Psychol.*, 1957, **50**, 134-137.
- Falk, J. L., and Emma, C. A simple intracranial electrode carrier for the rat. *J. exp. anal. Behav.*, 1961, **4**, 44.
- Gurdjian, E. S. The diencephalon of the albino rat. *J. comp. Neurol.*, 1927, **43**, 1-114.
- Olds, J. Self-stimulation of the brain. *Science*, 1958, **127**, 315-324.
- Olds, J., and Milner, P. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J. comp. physiol. Psychol.*, 1954, **47**, 419-427.

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