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Subfornical Organ Stimulation Elicits Drinking

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ABSTRACT: The subfornical organ is a specialized central nervous system structure known to be involved in the control of drinking. We report here that electrical activation of subfornical organ neurons (100 μA, 10 Hz, 1 ms pulse width, for 5 min.) induced drinking in 67% (six of nine) of animals. This effect was site-specific as none of the animals with electrode placements (n = 7) dorsal or rostral to the subfornical organ drank in response to electrical stimulation. In contrast, activity levels were increased significantly during stimulation in both the subfornical and nonsubfornical organ stimulated groups. These results suggest that electrical stimulation of the subfornical organ can elicit site-specific drinking behaviour and provide further support for the involvement of the subfornical organ in the control of drinking behaviour.

KEY WORDS: Subfornical organ, Drinking, Motor activity, Electrical stimulation.

INTRODUCTION

The subfornical organ (SFO), as one of seven circumventricular organs (CVOs), is devoid of a normal blood brain barrier and has a dense vascular supply [6,26]. It is thus ideally suited to its primary role as a receptor site for blood borne information that would otherwise not directly access the central nervous system (CNS).

The SFO was first suggested to be an essential CNS structure involved in the control of angiotensin II (ANG) induced drinking by studies showing that microinjection of ANG directly into the SFO produced drinking [20]. SFO lesions were found to block the drinking responses elicited by peripheral ANG administration [1,21], as did transections of the efferent projections of the SFO [4,11,12], and injections of a competitive ANG antagonist directly into the SFO [19]. Electrophysiological studies demonstrating excitatory actions of ANG on SFO neurons both in vivo [5] and in vitro [9] add support to the view that SFO neurons play an essential role in mediating ANG induced drinking.

Anatomical tracing studies [15,16,25] have demonstrated efferent projections from SFO to the median preoptic area (MnPO), the organum vasculosum of the lamina terminalis, the periventricular nucleus of the anteroventral third ventricular area, and the hypothalamus. The terminal projections in the hypothalamus include the supraoptic (SON) and the paraventricular nuclei (PVN) [16]. A role for ANG as a neurotransmitter in the SFO projections to the MnPO, SON, and PVN has been indicated by anatomical [13,14] and electrophysiological studies [2,10]. A recent study by Tanaka and Nomura [23] suggests that the dipsogenic response to ANG acting at the SFO may be mediated by angiotensinergic pathways from the SFO to the MnPO. The data support a role for the SFO-MnPO pathway in mediating dipsogenic responses to ANG [11].

Extracellular single unit recordings from phasically active neurons in the PVN have also demonstrated increased activity in response to intracarotid hypertonic saline, effects that were diminished after local anaesthetic administration directly within the SFO [24]. These studies implicate the SFO directly in the osmotic activation of the PVN and subsequent VP release.

Although a previous study [18] has reported drinking in response to stimulation of the SFO, electrode placement in immediately adjacent anatomical regions also elicited drinking. In this study we have attempted to define the specific cell groups responsible for such stimulation induced drinking.

METHODS

Sodium pentobarbitol anaesthetized (65 mg/kg, IP) male Sprague–Dawley rats (150–200 g) were placed in a stereotaxic frame. An incision in the skin overlying the skull was made and a small burr hole drilled in the bone such that a concentric bipolar stimulating electrode (SNE 100, Rhodes Medical Instruments, tip exposure 250 μm) could be advanced into the region of SFO according to the coordinates of Paxinos and Watson [17]. The electrode was secured to the skull with jeweller's screws and dental cement. The animal was removed from the stereotaxic frame and allowed to recover for a minimum of 5 days.

On the days of experimentation, the animal was placed in an isolation cage in which animals could be observed through one way glass. Food and water were available ad libitum. The stimulating electrode was connected (centre pole as the cathode) to a stimulator (SD9, Grass Medical Instruments), and stimulating currents were monitored by measuring the voltage drop across a known resistance.

All experiments were performed between the hours of 7:00 and 10:00 a.m. Immediately following the 30-min habituation period, a prestimulation control period was begun during which drinking behaviour was monitored, and the animal's activity level was evaluated every minute using Ellinwood and Balster's behavioural arousal scale [3]. The prestimulation period was continued until the animal was quiescent [mean activity level in the previous 5 min was less than 3.0 (inplace activities, i.e.,

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grooming)]. The animal was then either electrically stimulated (100 μA, 10 Hz, 1 ms pulse width) or sham stimulated (the stimulator was turned on but not plugged in) for 5 min during which activity was assessed every 30 s. The latency to drink was determined, and we attempted to measure the water consumed at the termination of stimulation. After these test periods, a 15-min poststimulation observation period was conducted during which the animal's activity level was assessed every minute. Each animal underwent a single randomized electrical and sham stimulation protocol with at least 24 h between experiments.

At the completion of a series of experiments animals were overdosed with urethane or sodium pentobarbitol and the brain perfused by the administration of 0.9% saline followed by 10% formalin through the left ventricle of the heart. The brain was removed and placed in formalin for at least 24 h. Using a vibratome, 100 μm coronal sections were cut through the region of the SFO. These sections were then mounted, stained with cresyl violet, and the anatomical location of the stimulating electrode site was then verified at the light microscopic level by an observer unaware of the experimental protocol and behavioural results.

Data Analyses

Animals were grouped into SFO and non-SFO groups according to stimulating electrode placement determined histologically. An electrode was considered to be in the region of SFO when the tip of the electrode was within 200 μm of SFO without penetrating the third ventricle. Electrode placements outside of this region were considered non-SFO sites. Within each of these groups the records were further divided according to the treatment the animal received, that is, whether the animal was electrically stimulated or sham stimulated. The association between electrode placement and drinking behaviour was evaluated using a Fisher's Exact Test.

Means of activity scores were calculated for each group for the 5 min prestimulation period, 5 min stimulation period, and each of the three, 5 min poststimulation observation periods. A multifactor 2 (electrode site) $\times$ 2 (treatment condition) $\times$ 5 (time) analysis of variance with repeated measures on the treatment condition and time factors was conducted to determine the effect of each of these factors on activity levels. Significant main effects were further tested by Bonferroni Multiple Comparisons post hoc analysis.

RESULTS

A total of nine animals had SFO electrode placements, whereas the remaining seven animals had electrode placements in either the hippocampal commissure, lateral, or medial septum and were placed in the non-SFO group (Fig. 1B). The photomicrograph presented in Fig. 1A shows a typical SFO site.

Drinking Data

Drinking occurred only if the electrode was in the SFO and if this region was electrically stimulated. Six of the nine animals in the SFO group drank in response to electrical stimulation. Drinking occurred only during electrical stimulation and/or poststimulation observation periods after receiving electrical stimulation, and the mean latency to drink was 235 $\pm$ 11.8 s (mean $\pm$ SEM). The majority of the animals that drank did so during electrical stimulation ($n = 5$), whereas the remaining animal drank during the first poststimulation period. In addition, most animals (67%) drank from the bottle more than once. These animals drank during the poststimulation period as well as the electrical stimulation period. The volume of fluid consumed was too small to be measured with the equipment used (<1 ml). In contrast, no animals with nonSFO electrode placement drank during the habituation period, the prestimulation period, electrical or sham stimulation (see Fig. 2A), or poststimulation observation period. A 1-tailed Fisher's Exact Test revealed that there was a significant ($p < .02$) association between drinking and electrode placement.

Activity Levels

To ensure scale and rater reliability, an observer, aware of the rating scales but unaware of the experimental manipulation, independently observed and rated an animal's activity level. These ratings were plotted against the experimenter's ratings for the same animal over the same time periods, and the subsequent Pearson's correlation coefficient was .946.

Effects of electrical stimulation versus sham stimulation on activity levels. Electrical stimulation resulted in an increase in activity levels in most animals (78%) with SFO placement and in all animals with non-SFO electrode placement. This increased activity level lasted throughout the course of the stimulation and into the poststimulation observation period (see Fig. 2B). However, this increase in activity dissipated more rapidly in the non-SFO stimulated than in the SFO stimulated animals.

A 2 (electrode site) $\times$ 2 (treatment condition) $\times$ 5 (time) analysis of variance with repeated measures on the treatment condition and time factor revealed that there were significant main effects of treatment condition ($F_{(1,14)} = 17.643$, $p < .001$) and time ($F_{(4,56)} = 10.355$, $p < .001$), whereas significant interactions exist between treatment and time ($F_{(4,56)} = 9.235$, $p < .001$).

The Bonferroni Multiple Comparisons post hoc analysis revealed that both SFO and non-SFO electrical stimulation resulted in a significant increase in activity as compared to prestimulation levels ($p < .001$) and to the sham controls for the same period ($p < .001$). The immediate increase in activity elicited by stimulation of the SFO was not significantly different than the non-SFO stimulation induced activity level ($p > .05$) during the stimulation and first poststimulation observation period (see Fig. 2B). Poststimulation activity levels remained significantly elevated from baseline and corresponding shams at the $p < .001$ level for all three of the poststimulation periods in those animals with SFO electrode placements. Activity levels for animals with non-SFO electrode placements, on the other hand, remained significantly elevated from baseline ($p < .001$) and sham ($p < .001$) levels for the first 5-min poststimulation observation period only.

DISCUSSION

The results of this study show that electrical stimulation of the SFO causes drinking in the majority of awake, freely moving, water-satiated rats tested. In contrast, animals with non-SFO electrode placements did not drink in response to electrical stimulation. Activity levels increased in response to electrical stimulation in most animals regardless of electrode placement. Thus, it appears that a general increase in activity level is not, in itself, sufficient to cause drinking. The fact that electrical stimulation of SFO elicits drinking and that stimulation of immediately adjacent regions (medial septum, lateral septum, hippocampal commissure) does not, attests to the specificity of behavioural patterns attributable to SFO activation.

It is interesting to note that three of the animals with confirmed SFO electrode placements did not drink in response to electrical stimulation. In fact, in two of these animals, electri-

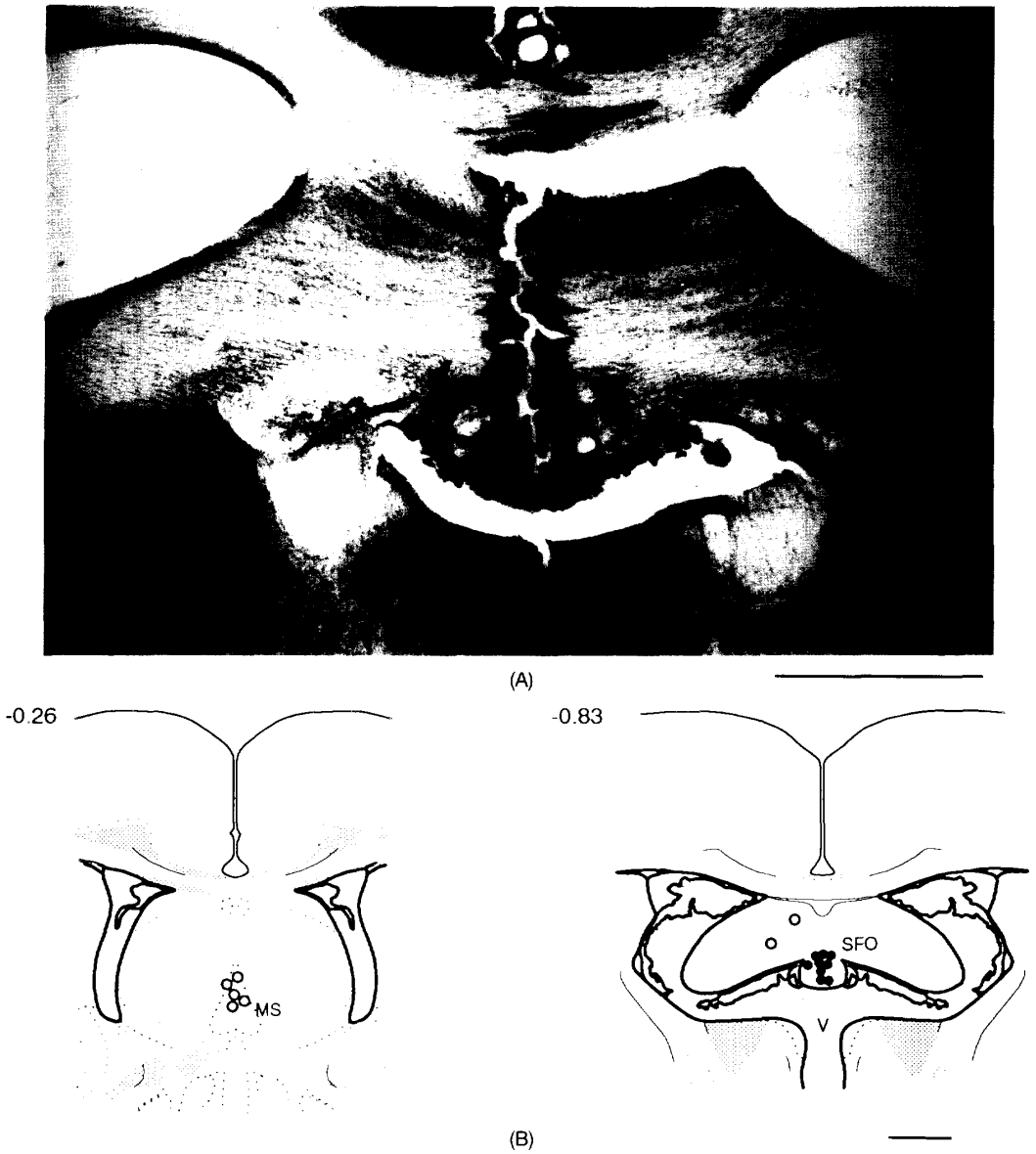

FIG. 1. (A) Photomicrograph showing a typical subfornical organ electrode placement. (B) Schematic representations of coronal sections through the region of the subfornical organ (adapted from Swanson [22]) illustrate the electrode placement in each of the 16 animals. Closed circles (n = 9) indicate subfornical organ electrode placement, whereas open circles (n = 7) indicate nonsubfornical organ electrode placements. The numbers beside each section indicate the distance posterior to Bregma. Scale bar represents 500 μm.

cal stimulation did not elicit increases in activity levels. This lack of activity and lack of drinking may support the hypothesis that increased activity may represent fluid seeking behaviour that underlies the drinking response. The other nondrinking SFO stimulated animal demonstrated increased activity levels during stimulation and throughout the poststimulation period, suggesting that, in this animal, the intensity and/or frequency of stimulation may not have been sufficient to activate drinking pathways.

Although it has been demonstrated that electrical stimulation of the SFO at $\geq$ 200 μA or $>$ 10 Hz causes increases in blood pressure in anaesthetized animals, we specifically chose an intensity (100 μA) and frequency (10 Hz) that do not induce such cardiovascular changes [6–8]. However, even if these stimula-

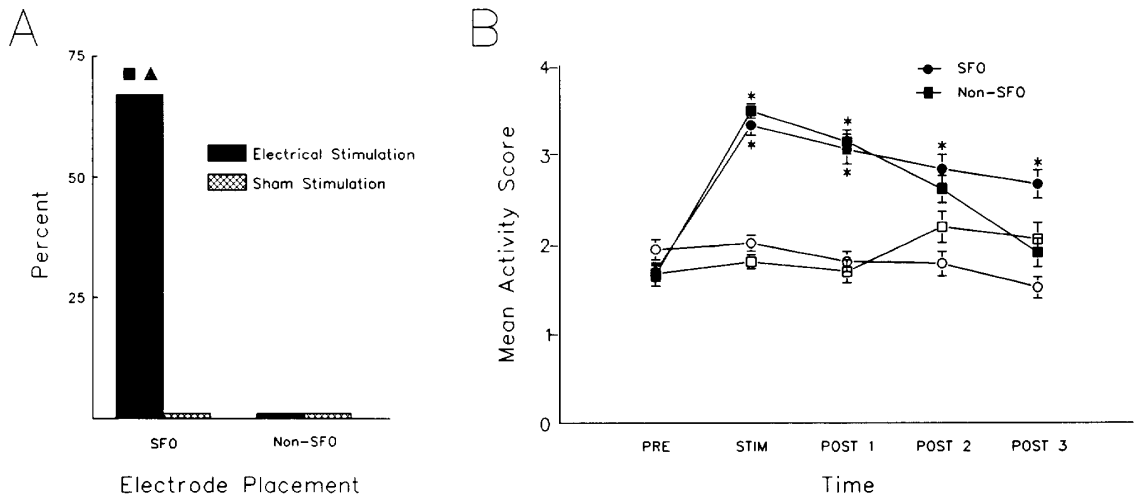

FIG. 2. (A) This histogram illustrates the percentage of animals that drank in response to electrical stimulation (solid bars) and sham stimulation (cross-hatched bars) in SFO or non-SFO sites. ■ denote $p < .01$ as compared to SFO sham stimulation, Fisher's Exact test; ▲ denote $p < .05$ as compared to non-SFO stimulated animals, Fisher's Exact test. (B) Mean activity levels of all sham stimulated (open symbols) and electrically stimulated (closed symbols) animals with SFO electrode placements (circles) and non-SFO electrode placements (squares) during the prestimulation (PRE), stimulation (STIM), and the 3 poststimulation periods (POST 1, POST 2, POST 3). The following are the behavioural descriptions underlying the activity scores: 1 = Asleep (lying down, eyes closed); 2 = Inactive (lying down, eyes open); 3 = Inplace activities (normal grooming or chewing cage litter); 4 = Normal, alert, active (moving about cage, sniffing, rearing). Error bars represent the SEM and * denote $p < .001$ as compared to sham stimulation for the same time period, Bonferroni Multiple Comparisons post hoc analysis.

tion parameters were to elicit increases in blood pressure, we would expect drinking to be inhibited by such cardiovascular changes in an integrated system.

The increase in activity demonstrated by the majority of animals regardless of electrode placement is not all that surprising. Given the size of the stimulating electrode and the intensity and pulse width of stimulation parameters, one would expect a certain amount of current spread. We would expect that hippocampal cholinergic fibres would be activated; however, we are not aware of information linking stimulation and drinking or increased motor activity. All of the non-SFO electrode placements were either rostral and dorsal, or just dorsal to the SFO, and all were within 800 μm of the SFO. Thus, it is not unexpected that some current could spread to cell bodies and/or fibres of passage within or originating from the subfornical organ. Its efferent projections have been shown to course dorsally and rostrally [16], and thus stimulation in these regions may result in activation of the same pathways that direct SFO stimulation activates. Although this non-SFO stimulation may activate some of the efferent subfornical organ pathways, it would not stimulate as many as direct stimulation of the SFO, and this may explain why the non-SFO stimulated animals returned to baseline activity levels during the second poststimulation observation period whereas the SFO stimulated animals had significantly elevated activity levels throughout the three poststimulation observation periods. Thus, SFO stimulation may indeed induce site-specific activity patterns.

In summary, this study demonstrates drinking can be elicited through electrical stimulation of the SFO. Such SFO stimulation may thus provide a chronic animal model for drinking, which may permit a clearer characterization of the various components (chemical, neural, anatomical, second messenger systems, etc.) involved in this ingestive behaviour.

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