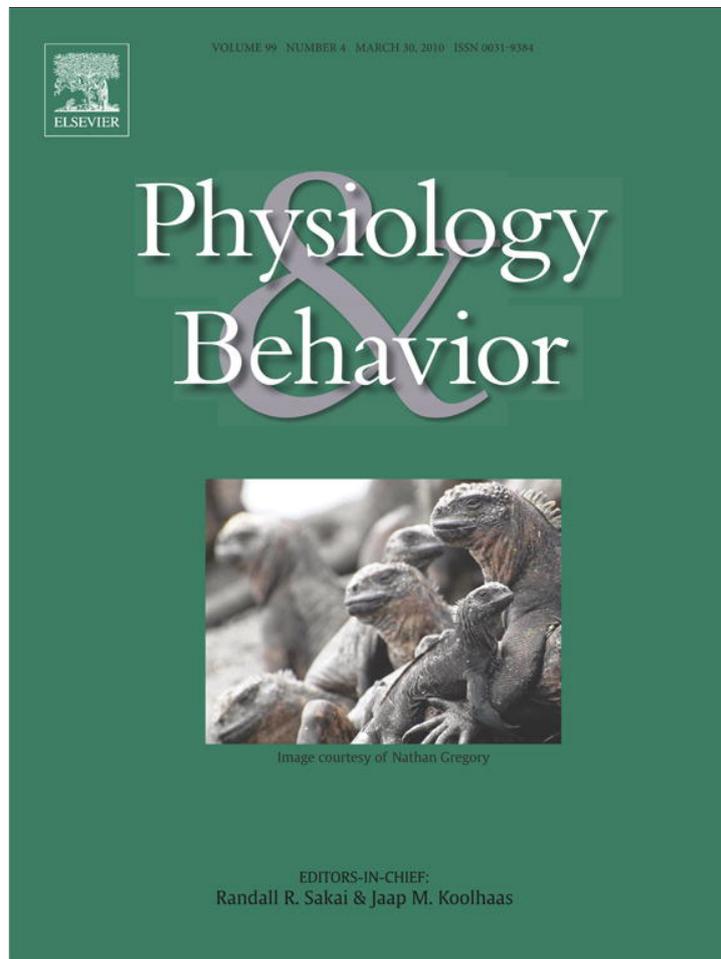


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## Acute electrical stimulation of the subfornical organ induces feeding in satiated rats

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### ABSTRACT

The SFO, a circumventricular organ (CVO) that lacks the normal blood–brain barrier, is an important site in central autonomic regulation. A role for the SFO in sensing circulating satiety signals has been suggested by electrophysiological studies demonstrating that the anorexigenic satiety signals, leptin and amylin, as well as the orexigenic satiety signal, ghrelin, influence the excitability of separate populations of SFO neurons. The present study examined whether acute, short duration, electrical stimulation of the SFO influenced feeding in satiated rats. Electrical stimulation (200  $\mu$ A) of satiated animals with subfornical organ (SFO) electrode placement ( $n=6$ ) elicited feeding in all animals tested with a mean latency to eat of  $8.0 \pm 4.0$  min after termination of SFO stimulation (mean food consumption:  $0.6 \pm 0.12$  g/100 g bw). These same rats undergoing a sham stimulation did not eat (mean food consumption:  $0.0 \pm 0.0$  g,  $n=6$ ) nor did animals receiving stimulation with non-SFO electrode placements (mean food consumption:  $0.0 \pm 0.0$  g,  $n=6$ ). SFO stimulation at this intensity elicited drinking in 5/6 animals with a mean latency to drink of  $15.2 \pm 2.6$  min. Feeding effects were specific to higher stimulation intensities as lower intensity stimulation (100  $\mu$ A,  $n=6$ ) elicited drinking (mean latency to drink:  $6.2 \pm 2.6$  min) but did not cause any animal to eat.

The results of the present study show that acute, short duration, SFO stimulation induces feeding in satiated rats, lending support for a role for the SFO as an integrator of circulating peptides that control feeding.

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### 1. Introduction

The subfornical organ (SFO), located on the midline wall of the third ventricle, belongs to a group of specialized central nervous system (CNS) structures known as the sensory circumventricular organs (CVOs). CVOs are characterized by the lack of the normal blood–brain barrier (BBB), a dense vascular supply, and the presence of a wide variety of peptidergic receptors.

Classically, the SFO has been viewed primarily as an angiotensin sensor, with roles in body fluid homeostasis [1,2] and cardiovascular regulation [3,4]. Electrical activation of SFO has been shown to cause drinking in satiated rats [5] and increase blood pressure in anesthetized rats [6]. The SFO has been shown to influence cardiovascular function through projections to the hypothalamus and other autonomic control centers [4,7]. SFO neurons send efferent projections to important hypothalamic autonomic nuclei including the paraventricular nucleus (PVN) and supraoptic nucleus of the hypothalamus (SON) [7,8], the median preoptic nucleus (MnPO) [7], the arcuate nucleus (ARC) [9] and lateral hypothalamic nuclei (LH) [10]. Although the majority of input to the SFO is sensory in nature, afferent inputs to SFO are received from the LH, MnPO, lateral parabrachial nucleus, midbrain raphe, the nucleus reuniens of the thalamus, and nucleus tractus solitarius [7,11,12].

In addition to its roles in body fluid homeostasis and cardiovascular regulation, more recently the SFO has been suggested to be involved in

the regulation of energy homeostasis. The SFO has also been shown to contain receptors or receptor mRNA for a variety of peripheral signals involved in energy homeostasis including the satiation signal, amylin [13,14], ghrelin [15], a peptide that triggers meal initiation, and the adiposity signals, adiponectin [16] and leptin [16,17]. A functional role for the SFO in sensing circulating signals involved in energy homeostasis has been suggested by electrophysiological studies from our laboratory demonstrating that the anorexigenic satiety signals, leptin [17] and amylin [15,17,18], as well as the orexigenic satiety signal, ghrelin [15], influence the excitability of separate populations of SFO neurons and is further supported by the anatomical data showing that SFO neurons send dense efferent projections to important hypothalamic metabolic control centers including the ARC [9], LH [10], and PVN [7,8,19] nuclei. In addition, electrophysiological studies have demonstrated functional projections from the SFO to the PVN and LH [20,21] and that glutamate stimulation of ARC neurons alters the firing rate of SFO neurons [22]. Together, these anatomical and functional data provide evidence for routes through which large, lipophobic, peptidergic peripheral signals which do not cross the BBB could gain access to the CNS via the SFO to influence metabolic control centers in the hypothalamus.

The present study was undertaken to determine whether electrical activation of SFO neurons influences feeding in satiated rats.

### 2. Methods

Animals were maintained on a 12:12 light:dark cycle and provided food and water ad libitum for the duration of the experiment. Sodium

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pentobarbital anesthetized (65 mg/kg, ip) male Sprague Dawley rats (175–200 g) were placed in a stereotaxic frame, an incision in the skin of the skull was made, and a small burr hole drilled such that a concentric bipolar stimulating electrode (SNE100 Rhodes Medical Instruments, tip exposure 250  $\mu\text{m}$ ) could be advanced into the region of the SFO (Bregma,  $-0.7\text{ mm}$ , midline, 4.5 mm ventral to surface) according to the coordinates of Paxinos and Watson [23]. The electrode was secured to the skull using jeweller's screws and dental cement. The animal was allowed a minimum 7 day recovery period. Food and water intake as well as body weight were recorded daily. On the days of experimentation the animal was placed in an observation cage designed for monitoring feeding and drinking 30 min after 'lights on' and allowed a minimum 30 min habituation period. Immediately following the 30 min habituation period, a pre-stimulation control period was begun during which the eating and drinking behavior was monitored and the activity level of the animal was assessed every minute using a modified Ellinwood and Balster's Behavioral Arousal Scale [24] a nine point scale where 0 indicates the animal is asleep, 5 is characterized by hyperactivity (rapid, jerky changes in position; no animal received a score this high) as previously described [5]. Once the animal was considered quiescent (mean activity was less than 3, a score represented by some in-place activities such as grooming) the 5 min stimulation period was begun during which the animal received either a 5 min electrical stimulation (10 Hz, 1 ms pulse duration, 100  $\mu\text{A}$  or 200  $\mu\text{A}$ , center pole as cathode) or a 5 min sham stimulation (electrode connected but no current passed) during which eating and drinking were monitored and activity level was assessed every 30 s. After termination of electrical or sham stimulation (post-stimulation period), the animal remained in the observation cage for 20 min (four, 5 min post-stimulation periods) during which eating, drinking and activity level was evaluated every minute. Approaches to, and time spent at, the food hopper were recorded as were latency to eat and drink. The amount of food consumed and water intake was measured at the end of the 20 min post-stimulation period.

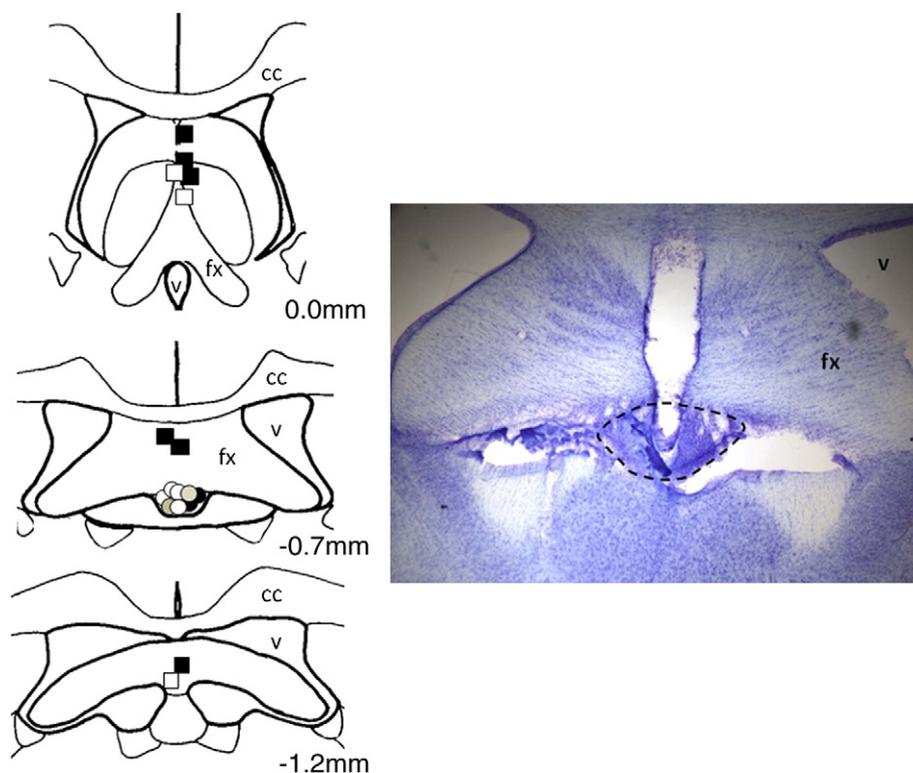
Animals received both electrical and sham stimulation protocols, separated by at least 72 h and performed in a randomized order. Following these protocols, animals were overdosed with sodium pentobarbital and perfused through the left ventricle of the heart with saline followed by 10% formalin and the brain was removed. The following day, 100  $\mu\text{m}$  coronal sections were cut through the region of SFO, mounted and cresyl violet stained. An observer, unaware of the experimental outcome, verified the anatomical location of the stimulating electrode at the light microscopic level.

Animals were grouped according to the anatomical location of the stimulation electrode (SFO or non-SFO) and stimulation intensity (100 or 200  $\mu\text{A}$ ). These groups were further divided according to stimulation protocol (electrical or sham stimulation). Latency to eat and/or drink was measured. Food and water intake were measured at the end of the last post-stimulation period (20 min after termination of stimulation). Mean activity scores were calculated for each animal in each group for the 5 min pre-stimulation time period, the 5 min stimulation period, and each of the four, 5 min post-stimulation time periods. Mean activity scores were then calculated for each group.

An ANOVA was used to determine the effect of stimulation and sham stimulation in SFO or non-SFO on these parameters. A Tukey post hoc analysis was used to determine where these differences occurred.

### 3. Results

A total of 24 rats were used in the current study of which 10 were placed in the SFO group and 8 were placed in the non-SFO group according to the anatomical location of the stimulating electrode. Animals were placed in the SFO group when the tip of the stimulating electrode was located within 400  $\mu\text{m}$  of the center of SFO without penetrating the ventricle while animals with electrode placements outside this region were placed in the non-SFO group (see Fig. 1). In the remaining 6 animals the tip of the stimulating electrode was either



**Fig. 1.** Animals were grouped according to anatomical location of the stimulating electrode. Schematic diagrams on the left show the anatomical locations of the stimulating electrode. Electrode placements in the SFO group are indicated by circles while non-SFO electrode placements are indicated by the squares. Open circles indicate stimulation intensities of 200  $\mu\text{A}$  while black closed symbols indicate stimulation intensities of 100  $\mu\text{A}$ . Closed grey circles indicate animals stimulated at both intensities. The photomicrograph on the right shows an example of a stimulating electrode site within SFO. Bregma level is indicated at the bottom of each schematic. Scale bars = 500  $\mu\text{m}$ . Abbreviations: SFO: subfornical organ, v: ventricle, fx: fornix, cc: corpus callosum.

surrounded by significant tissue damage, or it penetrated the ventricle and thus these animals were excluded from further analysis. Animals within these anatomical groups (SFO or non-SFO) were further divided according to the stimulation intensity (100 or 200  $\mu$ A) delivered.

### 3.1. SFO stimulation induced feeding in satiated rats

Electrical stimulation of the SFO at the higher intensity (200  $\mu$ A, 10 Hz, 1 ms) induced feeding in all satiated rats tested (6/6). Animals did not eat during the 30 min habituation period or the pre-stimulation period. Feeding usually occurred within 4 min of termination of SFO stimulation (5/6 animals) with a mean latency to eat of  $8.0 \pm 4.0$  min after termination of SFO stimulation. Mean food consumption in SFO stimulated animals was  $0.6 \pm 0.12$  g/100 g bw ( $n = 6, p < .01$ ). These same SFO stimulated animals did not eat during sham stimulation (electrode connected but no current passed, mean food consumption =  $0.0 \pm 0$  g/100 g bw,  $n = 6$ , see Fig. 2, Table 1). Feeding was specific to stimulation sites within the anatomical boundaries of SFO as animals with stimulation locations outside of the SFO did not eat in response to stimulation (mean food consumption =  $0.0 \pm 0$  g/100 g bw,  $n = 6$ , see Fig. 2, Table 1). SFO stimulation at this intensity also elicited drinking in 5/6 animals (mean water consumption =  $1.4 \pm 0.4$  ml,  $n = 6$ ) with a mean latency to drink of  $15.2 \pm 2.6$  min (see Table 1). These effects were also specific to SFO stimulation as these same animals undergoing sham stimulation did not drink nor did animals with stimulating electrodes outside the anatomical boundaries of SFO (non-SFO,  $n = 6$ ).

Feeding effects were specific to higher intensity stimulation (200  $\mu$ A) as lower intensity SFO stimulation (100  $\mu$ A,  $n = 6$ ) did not cause feeding in any of the animals tested (0/6), although these animals did appear to have a greater interest in food as determined by the number of times the animals approached the food hopper and time spent at the food hopper after termination of stimulation. SFO stimulated animals approached the hopper  $7.5 \pm 2.1$  times for  $42.8 \pm 20.1$  s during the first post-stimulation period while sham stimulated animals did not approach the food hopper at all nor did non-SFO stimulated animals. This lower intensity stimulation did however elicit drinking (mean latency to drink:  $6.2 \pm 2.6$  min) in 5 of 6 SFO stimulated animals, as previously reported [5].

### 3.2. Activity level was not different during stimulation

Activity levels were measured prior to (Pre), during (Stim), and after (Post) stimulation protocols. Activity levels during the pre-stimulation period (Pre) and during electrical stimulation (Stim) were not different between the SFO group (SFO) and non-SFO group (non-SFO), nor were activity levels between electrically stimulated (Stim) and sham (Sham)

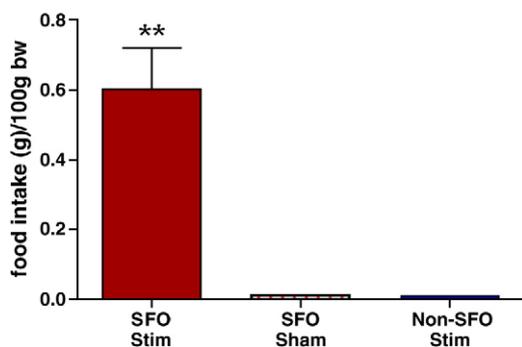


Fig. 2. SFO stimulation induced feeding. Electrical stimulation (200  $\mu$ A, 10 Hz, 1 ms) of the SFO induced feeding in satiated rats. Bar graph shows food intake as a result of SFO stimulation (SFO Stim,  $n = 6$ ), sham stimulation (SFO Sham, electrode connected but no current passed,  $n = 6$ ) and stimulation of locations outside of the SFO (non-SFO Stim,  $n = 6$ ).  $**p < .01$ .

Table 1  
Summary of the effects of electrical stimulation at 100 and 200  $\mu$ A of the SFO on eating and drinking.

Stimulation intensity	# of animals responding		Latency to response (min)	
	Drink	Eat	Drink	Eat
100 $\mu$ A ( $n = 6$ )	5 (83%)	0 (0%)	$6.2 \pm 2.6$	No eating
200 $\mu$ A ( $n = 6$ )	5 (83%)	6 (100%)	$15.2 \pm 2.6$	$8.0 \pm 4.0$

stimulated animals (see Fig. 3). There was, however, an increase in activity in SFO stimulated animals throughout the entire post-stimulation period ( $p < .005, p < .01$ ). This increase in activity was likely related to the feeding and subsequent grooming activities of these animals.

## 4. Discussion

The results of the present study show, for the first time, that electrical stimulation of the SFO elicits feeding and confirm our previous reports of drinking [5] in satiated rats.

Rats were tested at the beginning of the light cycle, a period during which these animals are typically asleep and thus do not ingest either food or water. It is during the dark cycle that rats consume the majority of their daily food and water. Although small bouts of eating and drinking are known to occur during the light cycle, these bouts typically occur several hours after the beginning of the light cycle and in the last hour of the light cycle in anticipation of lights off. In this study, electrical stimulation (200  $\mu$ A) of the SFO elicited feeding in rats that were not previously food or water deprived. Not only did the rats eat, but they ate up to 10% of their total daily food intake in less than 30 min at a time when they are typically not eating. These effects were specific to SFO stimulation as animals with stimulating electrode placement outside the anatomical boundaries of SFO did not eat in response to stimulation.

The effect of electrical stimulation on feeding was intensity dependent as stimulation at lower intensities (100  $\mu$ A) did not induce feeding. Although only qualitatively assessed, low intensity SFO stimulation appeared to increase the animals' interest in food (as determined by the amount of time spent at the food hopper), however, these animals did not eat.

In contrast to feeding responses, drinking occurred in animals at both stimulation intensities (100 and 200  $\mu$ A) as previously reported [5]. Some animals receiving low intensity SFO stimulation drank while undergoing electrical stimulation whereas no animals receiving higher intensity SFO stimulation drank during the stimulation period. In fact, latency to drink in the high intensity stimulation group was significantly longer than in those animals that received low intensity stimulation. Interestingly, although all animals ate in response to higher intensity stimulation, not all animals drank and, in most cases (5/6), eating preceded drinking in animals that exhibited both ingestive behaviors.

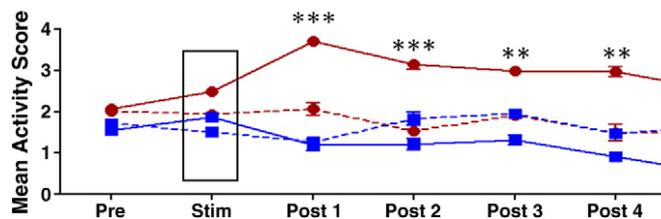


Fig. 3. Activity level was not different during stimulation. Activity levels were similar between SFO groups ( $\bullet, n = 6$ ) and Non-SFO groups ( $\blacksquare, n = 6$ ) during the pre-stimulation period (Pre) and during electrical stimulation (Stim) and in animals undergoing sham stimulation (broken lines) of SFO or non-SFO. There was an increase in activity in SFO stimulated animals in the entire post-stimulation period (Post 1–4). This increase in activity was related to the feeding and subsequent grooming activities of these animals. Each data point represents mean  $\pm$  SEM. Error bars are within the confines of the data point if not visible.  $***p < .005, **p < .01$ , one way ANOVA.

Activity levels were monitored throughout the experiment to determine whether effects seen may be attributable to a general change in activity levels (arousal) rather than a specific effect on feeding. The fact that animals in all groups (SFO and non-SFO, electrically and sham stimulated) had similar activity levels prior to and during stimulation, suggests that changes in activity did not underlie changes in feeding or drinking. The only differences seen in activity levels were during the post-stimulation period in SFO stimulated rats, presumably attributable to eating and drinking and associated grooming etc. behaviors that would be expected to accompany eating.

One interesting question that arises from these observations is why is a higher intensity stimulus required to cause feeding while lower intensity stimulation is sufficient to elicit drinking? The fact that stimulation intensities required to induce feeding were greater than those required to elicit drinking may be related to the fact that angiotensin elicits drinking by homogenous excitatory effects on approximately 60% of SFO neurons [25–27]. Thus angiotensin elicits drinking by homogenous excitatory effects on SFO neurons. This is in contrast to the effect of anorexigenic and orexigenic circulating satiety factors which have heterogeneous effects on the excitability of SFO neurons. Studies from our own laboratory have demonstrated that separate subpopulations of SFO neurons are activated by the anorexigenic peptide, amylin, or the orexigenic peptide, ghrelin [15]. Leptin has also been shown to influence the excitability of the majority of SFO neurons, however, these effects were shown to be heterogeneous as a population of SFO neurons were inhibited by leptin while a second group of cells were excited by leptin administration. Interestingly, cells excited by the anorexigenic peptide, amylin, were also excited by leptin [17]. During the light cycle, SFO neurons inhibiting food intake are likely maximally activated and, thus, in order to override this inhibitory drive to stimulate feeding, higher stimulation intensities are required to activate a sufficient proportion of SFO neurons that stimulate feeding.

The results of the present study further support a role for the SFO in the regulation of energy homeostasis. Previous *in vitro* studies have demonstrated that individual circulating satiety factors influence the activity of dissociated SFO neurons [15,17] and neurons obtained in slices [17]. In contrast to electrophysiological studies, electrical stimulation does not specifically target one particular neuronal subtype based on the receptors present, but rather activates all neurons (orexigenic and anorexigenic) in the region of the tip of the stimulating electrode. Thus, while electrical stimulation *in vivo* attempts to mimic the effect that circulating satiety signals have in activating neurons in the SFO, this approach cannot fully replicate the potential intricacies that signals such as leptin have been shown to exert by influencing different subpopulations of SFO neurons in different ways. However, the fact that such activation elicits feeding in satiated rats argues strongly that the SFO may be an important regulatory target for peripheral molecules reflecting an individual's energy status, and thus this CVO may influence integrated autonomic function through its neural projections to hypothalamic nuclei involved in energy homeostasis.

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