Behavioural Brain Research, 8 (1983) 85–108
Elsevier Biomedical Press

ACTIVITY OF BASAL FOREBRAIN NEURONS IN THE RAT DURING MOTIVATED BEHAVIORS

JONATHAN W. MINK*, HARRY M. SINNAMON** and DAVID B. ADAMS

Department of Psychology, Wesleyan University, Middletown, CT 06457, U.S.A.

(Accepted November 30th, 1982)

Key words: basal forebrain – lateral septum – preoptic area – motivation – approach – escape – neural activity – rat

SUMMARY

The activity of single neurons in the basal forebrain was recorded in the freely-moving rat with moveable fine-wire electrodes. Neural activity was observed while the water-deprived male rat was exposed to three different types of motivating stimuli that elicit locomotion in a running wheel: an estrous female rat; a drinking tube containing water; and grasping and lifting by the experimenter. The neural activity was also observed when the subject was presented with standardized sensory tests and during single pulse stimulation of other brain structures.

A majority of the 76 neurons recorded in the forebrain changed their firing rate during orienting and/or locomotion in general (23 neurons) or during behavior related to only one of the specific motivational contexts: the conspecific female (4 neurons); water (7 neurons); or grasp by the experimenter (8 neurons). Whereas the neurons related to orienting and/or locomotion in general were scattered through various brain structures, those neurons related to specific motivational contexts were concentrated in specific areas: the sexually dimorphic nucleus of the medial preoptic area (conspecific female); lateral septum (water); and lateral preoptic area (water and grasp). The present results, although based on relatively few neurons, are consonant with results of research using other techniques. This

* Present address: Division of Biology and Bio-Medical Sciences, Washington University School of Medicine, St. Louis, MO, U.S.A.

** To whom correspondence should be addressed at: Laboratory of Neuropsychology, Wesleyan University, Middletown, CT 06457, U.S.A.

0166-4328/83/0000-0000/$03.00

indicates that analyses at the level of the single neuron promise to be useful for understanding the role of the basal forebrain in motivational systems.

INTRODUCTION

The ability to elicit and direct locomotor approach and withdrawal is a key aspect of motivationally significant stimuli. In general formulations of motivation, the appetitive aspects of rewards are characterized by locomotion that tends to bring the organism in contact with the stimulus. Conversely, the aversive aspects of punishments are characterized by locomotion that tends to increase the distance between the organism and the stimulus. More specific conceptions such as Adams' [1, 2] model of social behavior in the rat and Bindra's [6] model of adaptive behavior also posit locomotion to be indicative of motivational states. In this study, locomotion was utilized as the common and objective feature of motivated behavior. A running wheel was used to facilitate and measure locomotor approach to rewarding stimuli and withdrawal from aversive stimuli. A range of motivational stimuli that provoke locomotion was selected to include social, homeostatic, and aversive stimuli.

The purpose of this study was to characterize the activity patterns of basal forebrain single neurons in a motivational context. The technique of recording single neurons in an awake, unrestrained animal has the advantage that one can study the behavioral correlates of the activity of one neuron at a time, with the result that relatively more specific information is obtained than is possible with lesion or stimulating techniques. The technique of recording single neurons during behavior has been used successfully in our laboratory in the search for brain mechanisms of motivation [29] and has proved to be useful in the study of the basal forebrain [30].

Several lines of evidence have indicated an important role for the forebrain in motivated behavior. The septal and preoptic areas of the basal forebrain are of particular interest in this regard. The medial preoptic area is involved in sexual, maternal, thermoregulatory, and exploratory behaviors [12, 23, 24, 31, 37, 38]. Gonadal hormones can act on the medial preoptic area to affect motivated behavior [5, 33, 34, 39]. The lateral preoptic area appears to be involved in the motivated behavior of osmoregulation, drinking, and defense [2, 4, 7, 26]. Gonadal hormones modify the responses of lateral preoptic neurons to olfactory stimuli [28]. The involvement of the bed nucleus of the stria terminalis and lateral septum in motivated behavior is not well defined. The septum may be involved in drinking, sexual behavior, and agonistic behavior [2, 15], whereas the bed nucleus may have a role in sexual behavior [11].

Anatomical data provide evidence that the basal forebrain is a highly interconnected region. Each basal forebrain nucleus projects to each of the other

nuclei, with the notable absence of a projection from the lateral preoptic to the medial preoptic area [10, 35, 36]. In addition, the basal forebrain nuclei have been shown to have extensive connections with the limbic system, olfactory system, hypothalamus, and midbrain tegmentum, areas that have also been shown to be involved in motivated behaviors.

Anatomical differentiation of the basal forebrain, is, in some cases, dependent upon gonadal hormones, suggesting an involvement in social behavior. Within the medial preoptic area is a group of densely packed cells that is much greater in volume in males than in females [13]. This 'sexually dimorphic nucleus' of the medial preoptic area (MPO) is dependent on perinatal androgen [20].

A standardized procedure was designed to test the properties of basal forebrain neurons. Motivational tests included locomotion in a running wheel toward a conspecific, toward a water reward, and away from aversive handling. Sensory tests included olfactory, visual, tactile, and auditory stimuli. Neuronal activity during each procedure was analyzed in relation to baseline firing rates. In addition, an attempt was made to characterize these neurons in terms of their response to electrical stimulation of certain regions known to have extensive connections with the basal forebrain regions.

METHODS

Subjects

Fischer–Dark Agouti hybrid male rats (n = 58) weighing 350–450 g were used as subjects. At 3–9 months of age, they were housed in pairs in a cage that had a central partition made of wire screen. Two female rats were housed on the other side of the center partition. Two Petri dishes were placed on the floor of each cage on which the males and females scent-marked [1]. The females were tested for behavioral estrus on each day of recording or training sessions. All animals were housed under a 12 : 12 h, light : dark cycle. Food and water were available at all times except when the subjects were placed on a 24-h water deprivation schedule. Recording occurred during the dark period.

Test apparatus

All testing was done in a 66 × 41 × 38 cm chamber equipped with a wire mesh running wheel 30 cm in diameter (Fig. 1). A partition bisected the chamber and was flush with the right side of the wheel. A window was cut in the partition so that when the subject was in the wheel, it could be exposed to the other side of the partition. An estrous female was placed in a 10 × 13 × 15 cm wire mesh box that was visible through the window from the wheel. The window was equipped with a sliding shutter that could be raised (open) and lowered (closed). A fan was placed on the right side of the cage in order to direct the odor of the female toward the subject when the shutter was open. The floor was covered with wood shavings.

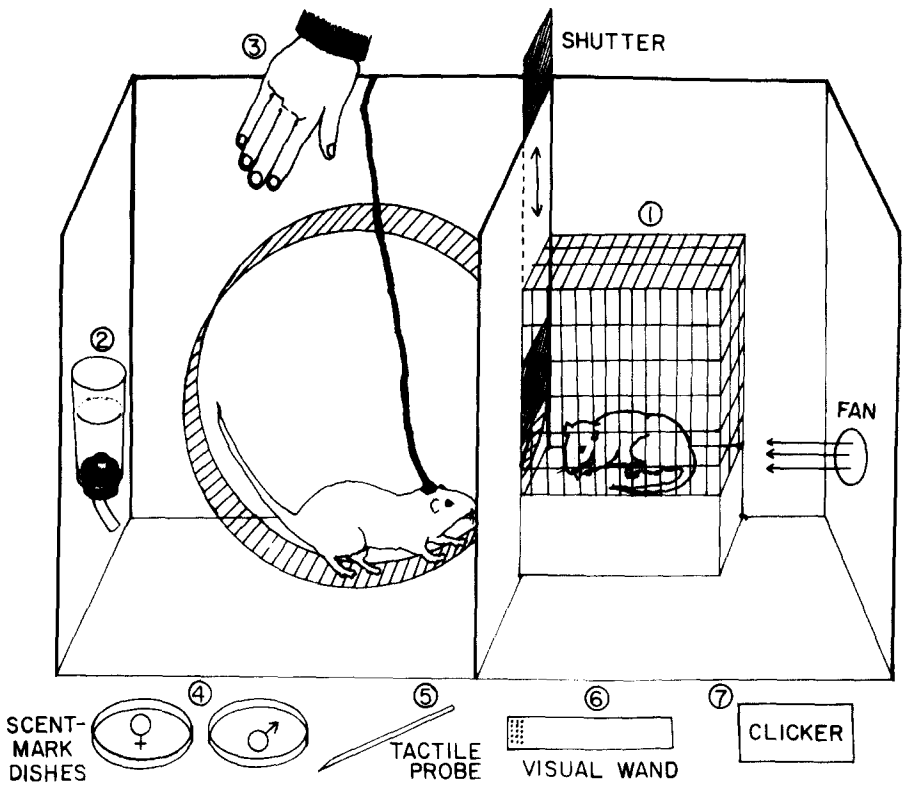

Fig. 1. Test apparatus. The male subject was placed in the side of the chamber with the running wheel. The subject was exposed to a standard series of events, as follows: (1) the shutter was raised to expose the female rat; (2) a water tube was placed in the chamber; (3) the experimenter lifted the rat and allowed it to escape; (4) scent-marked dishes from the male's and female's cages were introduced; (5) the subject's vibrissae and flanks were stimulated with the tactile probe; the visual grid was presented to right and left visual fields; (7) a click was produced as an auditory stimulus.

Electrodes

Monopolar stimulating electrodes were made from 125-μm, Teflon-insulated, stainless steel wires, cut to expose the cross-sectional area of the tip. Bipolar stimulating electrodes were made by twisting two monopolar electrodes together. The tips were cut evenly.

Two types of recording electrodes were used. Most subjects (n = 51) were implanted with a recording electrode consisting of four 25 μm, enamel-insulated, stainless steel wires that were twisted into a bundle. The electrode was cut to expose the cross-section of the tips and then tested to determine the impedance of each wire at 1 kHz. An electrode was considered adequate when all impedances were 200–400 kΩ. Amphenol pins were attached to the electrode leads, fastened together, and mounted in a custom-made microdrive. The microdrive was fashioned from a Delrin spurgear and permitted 780 μm downward travel per

revolution without rotation of the electrode wires. The remaining subjects (n = 7) were implanted with a recording electrode consisting of an untwisted bundle of 19 enamel-insulated, 12.5 μm, stainless steel wires. The bundle was coated with Epoxylite to increase stability and to prevent splaying of the electrode tips. The bundle was cut to expose the cross-sections of the tips. Amphenol pins connected to the wires were inserted into a custom-made microdrive fashioned from Amphenol connector strips and o/80 threaded stainless steel stock. This microdrive permitted 320 μm downward travel per revolution of the stock without rotation of the electrode wires. The typical impedance at 1 kHz for this electrode was 400 kΩ.

Surgery and training

Stimulating electrodes were implanted stereotaxically following anesthesia with a 3.5 ml/kg intraperitoneal injection of Chloropent. In the first 34 animals bipolar stimulating electrodes were implanted homolaterally in the medial forebrain bundle (MFB) at the level of the posterior hypothalamus and in the lateral mesencephalic central gray (CG). In an attempt to increase the number of neurons that could be activated by electrical stimulation, the subsequent 24 animals received three monopolar stimulation electrodes. They were aimed at the lateral habenula (LH), the ventral tegmental area of Tsai (VTA), and the lateral mesencephalic central gray (CG). Amphenol pins from the ground, reference, and stimulating electrodes were inserted into a plastic strip that was then secured to the skull with acrylic cement. On the second day after surgery, subjects were placed on a 24-h water deprivation schedule.

Training began on the fourth day after implantation of the stimulating electrodes. Each training session was 15–20 min long. The subject was placed in the test chamber and an estrous female was placed on the opposite side of the shutter from the subject. In a trial the shutter was raised for 30 sec and the fan was turned on. The trained subject would orient to the open window, sniff, and run toward the female. On alternate trials, the shutter was not raised, but a water tube was presented which the subject could approach by running in the wheel. The subject was allowed to drink for 5 sec after running two revolutions. The subject was considered ready for recording when he consistently ran toward the female while the shutter was open, and toward the water tube when it was presented.

Prior to implantation of the recording electrode, the subject was anesthetized with 2% halothane in oxygen and mounted in a stereotaxic device. The recording electrode was passed through a 1 mm-diameter hole in the skull above the preoptic area. The drive with the electrode was secured to the skull and the previously applied plastic connector strip with dental cement.

Recording

Extracellular potentials were recorded differentially between pairs of electrode wires which were selected through a switch on the recording cable. Preamplification was provided by a miniature, 10-gain, differential, DC-coupled amplifier (F. Haer Co.) that was mounted on the cable near the rat's head. The signal was led by Microdot cable from the miniature amplifier to two Grass Model P15B AC amplifiers that were arranged in series. Each amplifier had 1/2 amplitude filters set at 100 Hz and 10 kHz and a gain factor of 10. The amplified neural activity was displayed on a Tektronix 545A oscilloscope and simultaneously sent to a voltage and fall-time discriminator that produced a standard 5-V pulse when a neuronal spike occurred. The adequacy of discrimination was continuously monitored. The 5-V discriminator pulses were sent to a ratemeter consisting of a digital to analog converter that reset every 1 sec. The ratemeter output was recorded on a Grass Model 5DWC polygraph used with a paper speed of 2.5 mm/sec.

Neuronal activity was considered to be from a single neuron if it displayed a stable waveform, consistent amplitude, and an apparent refractory period. Cells with irregular waveforms or with extremely high discharge rates (> 100/sec) were considered to be injured and were not tested. To minimize sampling bias, all discriminable units were tested, regardless of amplitude.

Stimulation

A Grass Model S88 stimulator was used with a Model PSIU6 isolation unit to generate monophasic square wave pulses of 0.3 msec duration. The standard stimulation intensity was 500 μA. When bipolar electrodes were used, each site was stimulated with both polarities. When monopolar electrodes were used, each site was stimulated with cathodal current only.

Procedure

To locate single neurons, the recording electrode was lowered in increments of approximately 70 μm. At each recording site, baseline activity was recorded, and then single shock stimuli were delivered through each stimulating electrode in an effort to activate any units with no baseline activity. When a single neuron was isolated, a baseline determination was made during a period of at least 15 sec in which the rat was inactive. Then the testing procedure commenced. First, the shutter between the subject and the female was raised and the fan turned on. After the subject had run three revolutions, the fan was turned off and the shutter was closed. This sequence was repeated two more times. Second, a water tube was placed in the chamber, so that the subject would run, facing away from the shutter, to approach the water. When the rat had run two revolutions, he was allowed to drink for 5 sec. This procedure was repeated two more times. Third, the rat's hindquarters were lifted by the experimenter and released. Following

release, the subject would run in the wheel, away from the experimenter's grasp. This procedure was repeated one more time.

In steps 4–7 of the procedure the neuron was tested for response to sensory stimuli. The subject was presented with the Petri dishes containing scent markings from his and the female's cages to test for olfactory response. Visual responses were tested with a black and white grid at the end of a white Plexiglas wand. A pencil was used to probe the rat's vibrissae and flanks for a somatosensory test. A click from a microswitch held approximately 35 cm from the rat was used to elicit a startle response. In all cases, the behavioral response was noted on a checklist that included the following categories: passive (no change in posture), sniff only, orient and sniff, orient without sniffing, turn away, and startle. Orienting behavior was defined as a distinct head turn toward the object such that a line equidistant to the two eyes that intersected the nose would also intersect the object. After the above procedures, the neural activity was observed during spontaneous behavior in order to determine any other behavioral correlates.

Each neuron was tested for response to brain stimulation. Each stimulation site was tested with single pulses at approximately 1 Hz; several neurons were also tested for response to 0.5 sec trains of pulses at 50 Hz in the MFB. If a response was observed, the latency and threshold were noted. In addition, responsive neurons were stimulated with a train of three pulses at 200 Hz. A unit that followed three pulses at 200 Hz was considered to be antidromically activated.

Histology

To allow time for gliosis to occur around the recording tip, at least 1 day was allowed to elapse between the last recording and the perfusion. The rat was perfused transcardially with 0.9% saline, followed by 10% Formalin. The head was removed and fixed in Formalin for at least 2 days with the electrodes in place. Transverse 32 μm sections were taken on a cryostat microtome and stained with cresyl violet. Stimulation and recording electrode placements were projected onto plates adapted from König and Klippel [18]. Sites of recorded cells were located by reference to the bottom of the recording track and the notes on the movement of the microdrive.

RESULTS

A total of 28 rats were tested in the sequence of motivated behaviors and yielded 101 single cells. The neurons included 75 in the basal forebrain which were distributed anatomically as follows: 11 in the medial preoptic area (MPO), 11 in the lateral preoptic area (LPO), 19 in the bed nucleus of the stria terminalis (nST), 29 in the lateral septal nucleus (SL), 5 in the nucleus of the diagonal band of Broca (nDB). There were 26 cells recorded in various other structures.

In the first stage of analysis, the behavioral correlates were determined for each individual neuron. These correlates were determined on the bases of both observations made during the recording session and visual inspection of the annotated ratemeter record. They were judged to be present when a change in activity relative to baseline consistently occurred during repetitions of the behavior or event. Of the 101 neurons, 65 had activity with such correlates, whereas the remaining 36 had activity which displayed no apparent association with any behavioral or sensory event in the test situation.

The second stage of analysis consisted of classification of the neurons. Certain behavioral correlates tended to occur together or show a maximal or most consistent change relative to baseline under particular behavioral conditions and from these patterns the neurons were divided into major classes. Six classes were formed consisting of neurons characterized by changes in activity related to (1) the female, (2) the water, (3) the grasp of the subject by the experimenter, (4) all orienting and locomotion, (5) all locomotion, (6) orienting to most stimuli. A seventh class consisted of neurons that could not be subsumed under these rubrics. Fig. 2 illustrates the locations and classification of each neuron. It bears

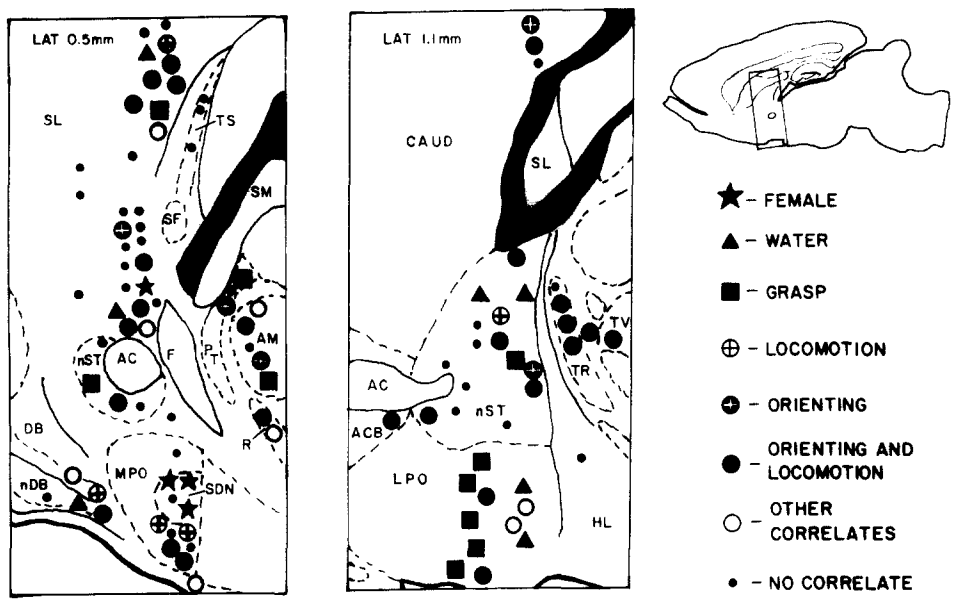

Fig. 2. Histological location of recorded neurons. Correlates are indicated by symbols as shown in the column at the right of the figure. AC, anterior commissure; ACB, accumbens nucleus; AM, anteromedial thalamic nucleus; CAUD, caudate nucleus; DB, diagonal band of Broca; F, fornix; HL, lateral hypothalamus; LPO, lateral preoptic area; MPO, medial preoptic area; nDB, nucleus of the diagonal band; nST, bed nucleus of stria terminalis; PT, parataenial nucleus; R, reuniens nucleus; SDN, sexually dimorphic nucleus; SF, septofimbria nucleus; SM, stria medullaris; SL, lateral septum; TR, reticular nucleus of thalamus; TS, triangular nucleus of septum; TV, ventral nucleus of thalamus.

emphasis that the classes are descriptive; they are operationally defined in terms of the experimental procedure and are intended to imply no specific or exclusive function of a neuron.

A critical aspect of the classification, reflecting the motivational focus of the study, was a distinction between classes of neurons with activity changes related to only one of the motivational contexts (classes 1–3) and other neurons with activity changes related to more than one motivational context (classes 4–6). This may be seen in Table I which provides a summary of the correlates displayed by 42 basal forebrain neurons with activity related to the motivational stimuli. The distinction between the different classes may be illustrated by examples from the medial preoptic area. The first three neurons listed in the medial preoptic area (31-2, 11-2 and 41-2) were classed as female-related on the basis of changes during behaviors related to the female in the absence of changes during behaviors related to other stimuli. In contrast, the last two neurons listed in the medial preoptic group (41-4 and 22-3) were classed as 'orienting-and-locomotion' on the basis of similar increases during orienting and locomotion in response to the female, water and the grasp.

Neural activity related to female stimuli

Four basal forebrain neurons were characterized by activity changes when the subject sniffed, oriented and/or locomoted toward the female. They are indicated by stars in Fig. 2. Three of the four neurons were located in the sexually dimorphic nucleus of MPO, the other was located in SL. Three of the neurons increased in activity maximally when the subject locomoted to the female upon the raising of the shutter and, to a lesser extent increased in activity during sniffing and orienting to the female in the absence of locomotion. The other neuron in the MPO decreased in activity when the shutter was raised and the subject sniffed, oriented, and locomoted toward the female. The activity of the four cells with female-related activity was uninfluenced by the scent of the Petri dish from the female's cage. Other sensory and behavioral tests failed to affect the activity of these cells. A ratemeter record of cell 31-2, illustrating the activity of this neuron during all behavioral and sensory tests is presented in Fig. 3. A comparison of the female-related activity changes of cells 31-2, 8-5, and 41-2 is presented in Fig. 4.

Neural activity related to water

Seven basal forebrain neurons were characterized by activity change related to presentation of the water tube. They are indicated by triangles in Fig. 2. Neurons with water-related activity were distributed in SL, nST, nDB and LPO. The absence of water-related unit activity in MPO is notable. Six of the seven cells with water-related activity increased in activity during orienting and locomotion

TABLE I

Basal forebrain neurons characterized by activity correlated with events related to a female, water, and an escape-provoking grasp

Changes in neural activity are indicated as follows: +, increases; + +, maximum increase; −, decrease.

	Cell	Baseline rate* (Spikes/sec)	Female			Water			Grasp			Response** or comments
			Locomote	*Orient*	*Sniff*	*Locomote*	*Orient*	*Drink*	*Locomote*	*Orient*	*Lift*	
Medial preoptic area												
★	31-2	2.2	−	−	−							
★	11-2	1.0	+	+	+							
★	41-2	1.1	+ +	+	+							
⊕	22-2	5.0	−			−			−			Synaptic excitation CG
⊕	35-2	12.7	+ +			+			+			
●	41-4	5.9	+	+	+	+	+		+ +	+	+	
●	22-3	8.6	+	+	+ +	+	+		+			
Lateral preoptic area												
▲	16-6	15.0						−				Synaptic excitation CG Synaptic inhibition MFB
▲	16-3	1.1				+ +	+	+	−			Synaptic excitation MFB
■	26-5	5.0						−	+ +		+	Synaptic inhibition MFB
■	30-4	1.9							+		+ +	
■	30-6	1.2							+ +	+	+	Head movements +
■	30-3	12.0		+			+		+ +	+	+	Synaptic excitation CG
■	30-1	18.0					+		−	−	−	
●	30-5	4.3	+	+	+	+	+		+ +	+	+	Synaptic excitation LH
●	26-6	10.0	+	+	+	+	+		+			
Nucleus of the diagonal band												
▲	J1-2	6.0				+	+	+ +				Licking +
⊕	44-4	0.8	+			+			+ +		+	
●	J1-3	19.0	+	+	+	+	+		+	+	+	Synaptic inhibition MFB Head movements +

Lateral septal nucleus												
★	8-5	0.5	+ +	+	+							
▲	3-2	0.2				+ +		+				
▲	14-12	0.5				+ +	+	+				
■	14-9	60.0							+	+		Antidromic MFB
⊖	8-1	3.3		+	+		+		+			
⊕	14-1	1.1	+			+			+		+	
●	3-3	0.5	+	+		+ +	+			+		
●	3-4	1.5	+	+	+	+	+	+				
●	8-4	0.4	+ +	+	+	+	+					
●	14-4	0.5	+	+	+	+	+		+ +	+		
●	J2-1	3.0	+	+	+	+	+		+	+		
●	14-2	14.0	+	+		+	+		+			
●	41-1	2.5	+	+		+	+		+	+	+	
Bed nucleus of stria terminalis												
▲	9-1	0.5				+		+ +				
▲	26-1	0.2						+				
■	44-3	5.9							+ +		+	
■	29-4	3.2				+			+ +	+	+	
⊖	29-1	1.8	+ +			+ +		+ +				
⊕	9-2	1.5		+	+ +		+			+		
●	9-3	4.0	+ +	+	+	+	+		+	+		Synaptic excitation CG
●	16-1	3.1	+	+	+	+	+		+	+	+ +	
●	29-2	11.8	+	+	+	+	+		+	+	+ +	
●	29-5	7.3	+	+		+	+		+ +	+	+ +	
●	J1-1	5.0		+	+	+	+	+		+		Grooming + + Synaptic excitation CG

* Baseline rates were recorded while the subject was immobile, before the test sequence was begun.

** Responses to brain stimulation are indicated as antidromic or synaptic. A response that occurred at a constant latency, and also followed three pulses at 200 Hz was considered to be antidromic. Abbreviations: CG, central gray; MFB, medial forebrain bundle; LH, lateral habenula.

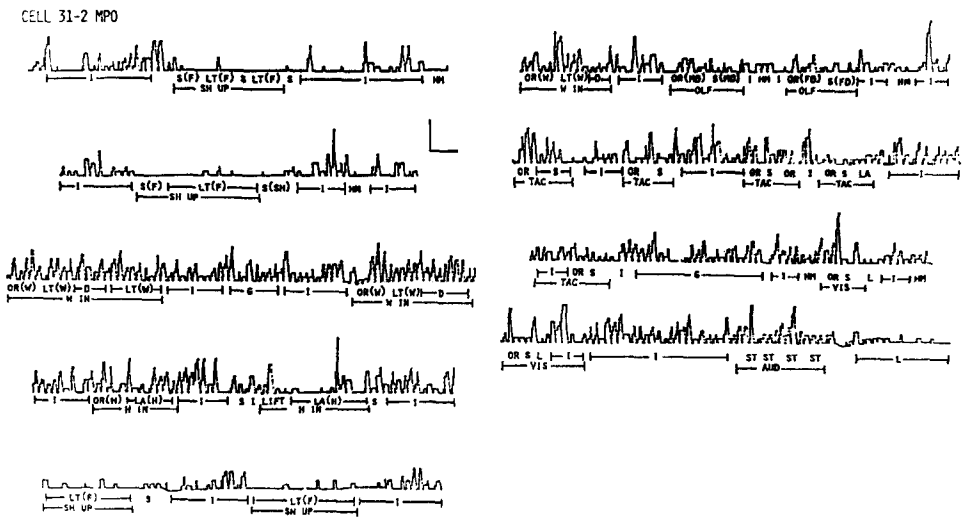

Fig. 3. Activity of cell 31-2. This is an example of a neuron that was characterized by female-related activity. The activity was decreased when the subject sniffed, oriented, and locomoted toward the female. No other consistent decreases were seen. Calibration: 5 sec and 10 spikes/sec. Abbreviations for this and subsequent figures are defined in Table II.

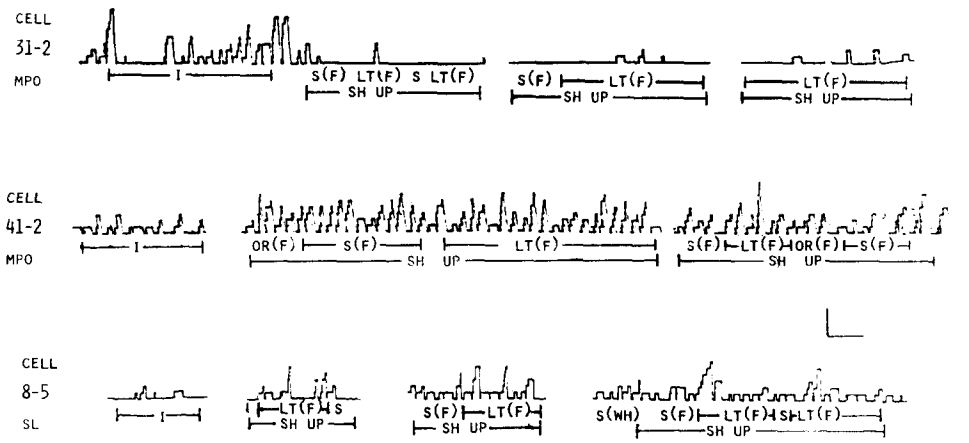

Fig. 4. Samples of female-specific activity. The ratemeters of the activity of these neurons illustrate the variability of female-related activity. No other consistent correlates were seen for these neurons. Calibration: 4 sec and 5 spikes/sec.

to the water tube and drinking. Three of these increased maximally during locomotion to water, while the others increased maximally when the subject drank from the water tube. One neuron, located in LPO, decreased in activity when the subject drank. This neuron increased in activity when the water tube was pulled away from the subject and when he tried to drink from a tube containing no water. One of these neurons decreased in activity when the subject locomoted away from the experimenter's grasp. With the exception of this neuron, all neurons with

TABLE II

Abbreviations for ratemeter figures

Behaviors	*Objects ( )*	*Procedures*
I– immobile	W– water	SH UP– shutter up (stimuli from female)
S– sniff	F– female	
OR– orient	H– hand	W IN– water in cage
R– root in chips	WH– running wheel	H IN– hand in cage
L– locomotion	CHP– chips	OLF– olfactory stimulation
LT– locomote forward	FD– female's petri dish	TAC– tactile stimulation
LA– locomote away	MD– male's petri dish	VIS– visual stimulation
UP– upright posture	SH– shutter	AUD– auditory stimulation
HA– head away		
G– groom		
C– chew		
ST– startle		
HM– head movement		
D– drink		

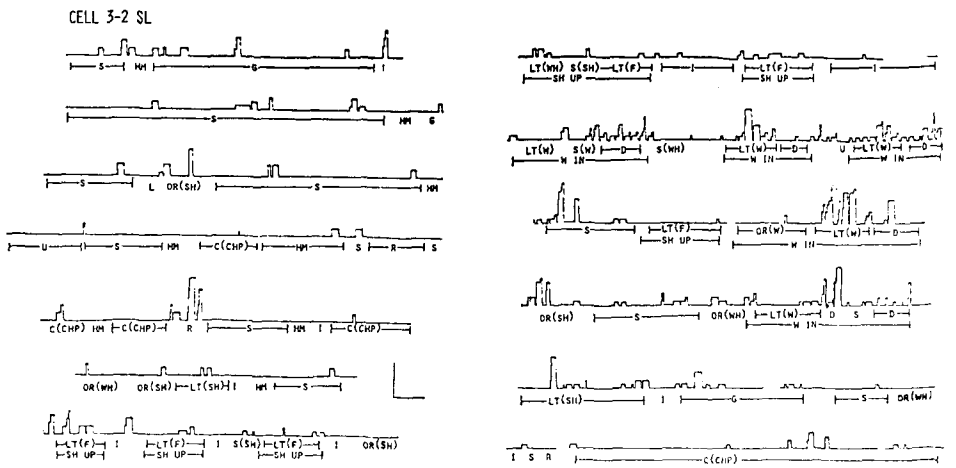

Fig. 5. Activity of cell 3-2. This cell is an example of a neuron that was characterized by water-related activity. Consistent increases were seen when the subject locomoted to the water tube and drank. Activity increased occasionally during other behaviors, but none of these changes was consistent. Calibration: 5 sec and 10 spikes/sec.

water-related activity changes were uninfluenced by other behavioral or sensory tests. A ratemeter record of the activity of cell 3-2, located in SL, is presented in Fig. 5. A comparison of the range of water-related activity changes of cells 3-2, 16-6, 16-3 and 14-2 is presented in Fig. 6.

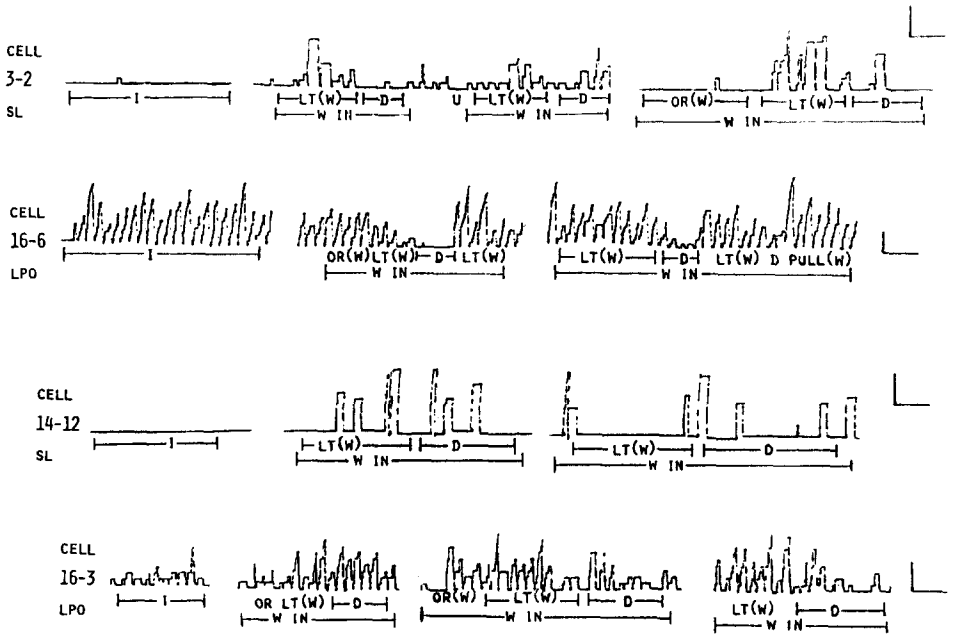

Fig. 6. Samples of water-specific activity. The above ratemeters illustrate the range of activity shown by neurons characterized as water-related. Calibration: 4 sec and 5 spikes/sec.

Neural activity related to grasp

Eight basal forebrain neurons were characterized by activity changes related to the subject escaping from the experimenter's grasp. They are indicated by squares in Fig. 2. Five of the eight neurons were located in LPO, two in nST, and one in SL. Seven of these cells increased in activity when the subject was grasped by the experimenter and allowed to escape. One neuron decreased in activity when the subject was lifted and allowed to escape, with the greatest decrease associated with the subject being grasped. Neurons with grasp correlates exhibited similar activity changes when the subject was aversively probed, and in some cases when it was startled. Three neurons with grasp-related activity increased when the subject oriented to the water tube. One neuron decreased in activity when the subject drank. A ratemeter record of cell 30-4, illustrating the activity of this cell during all behavioral and sensory tests is presented in Fig. 7. A comparison of the range of grasp-related activity changes of cells 30-4, 30-1, and 30-6 is presented in Fig. 8.

Neural activity related to orienting and locomotion

Seventeen basal forebrain neurons were characterized by activity changes during all orienting and locomotor activity. They are indicated by filled circles in

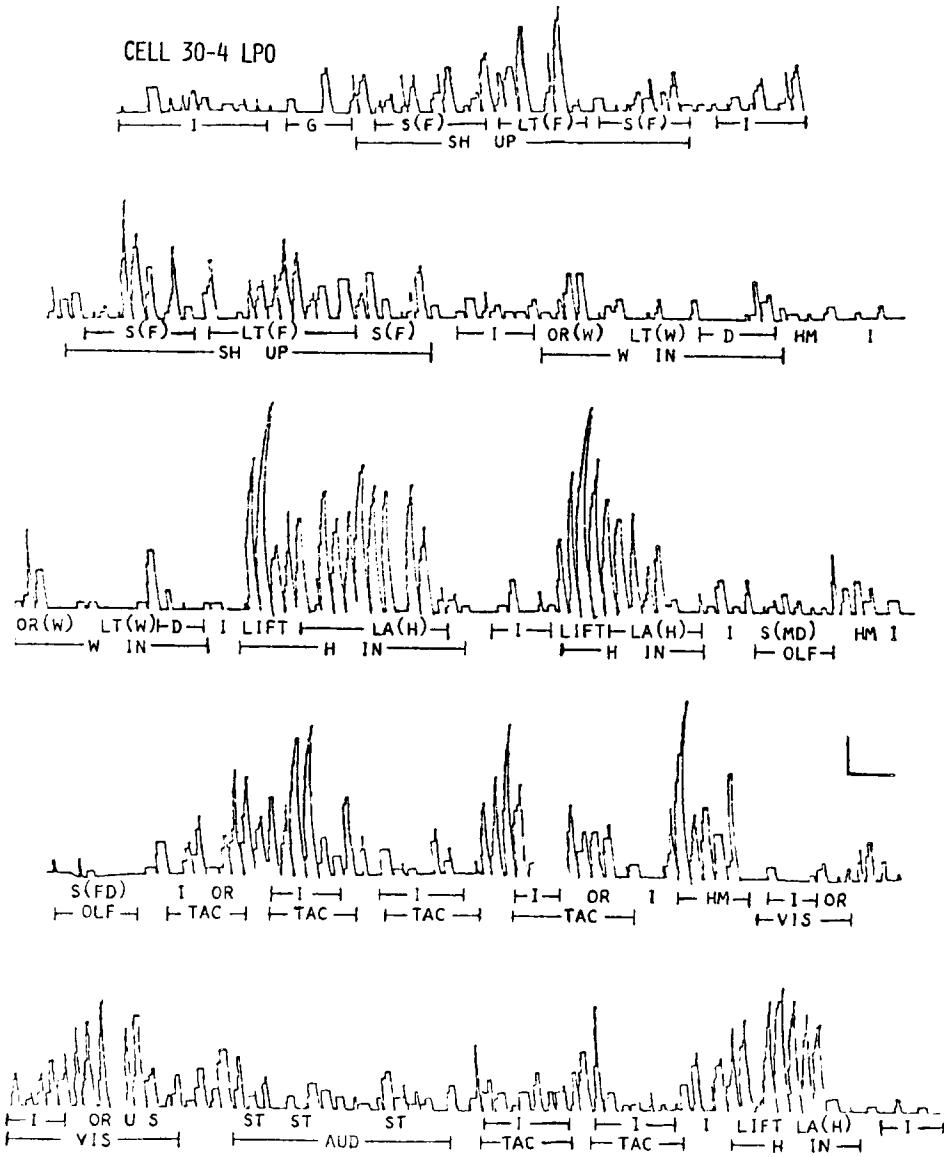

Fig. 7. Activity of cell 30-4. This cell is representative of those characterized by activity related to grasp by the experimenter. Note also the increases associated with some tactile stimulation. These increases were accompanied by aversive postures. Calibration: 4 sec and 5 spikes/sec.

Fig. 2. These neurons were distributed throughout the basal forebrain with a predominance in SL and nST. Maximal increases in activity were seen in all cells during locomotion, with sub-maximal increases during orienting, sniffing and head movements. The activity increases of these neurons were contingent on the subject's behavior and not on specific stimuli or modalities. Although these cells showed maximum increases during specific events, as indicated in Table I, the

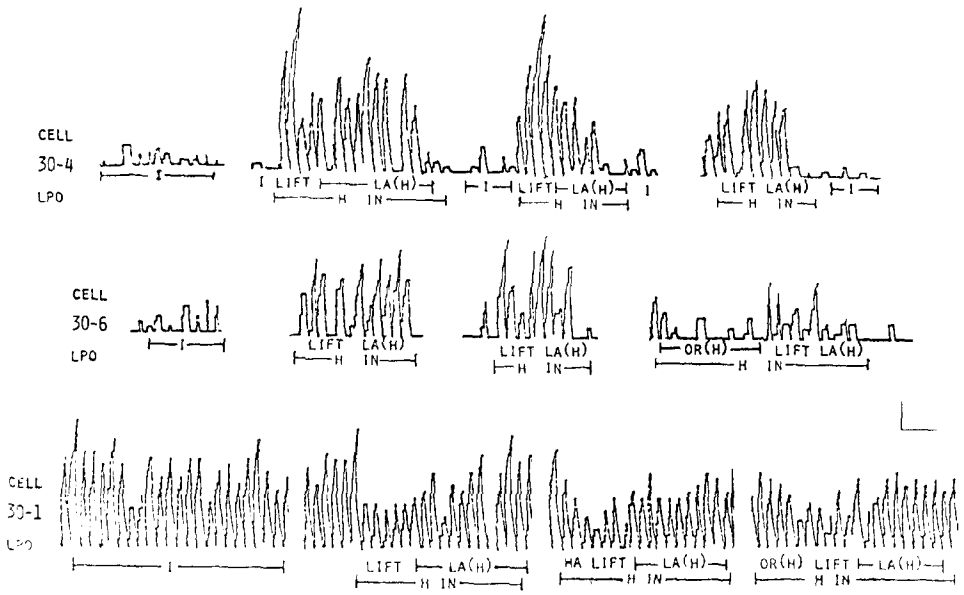

Fig. 8. Samples of grasp-specific activity. These ratemeters illustrate the variability of grasp-related activity changes. No correlates with other behaviors were seen for these cells. Calibration: 4 sec and 5 spikes/sec.

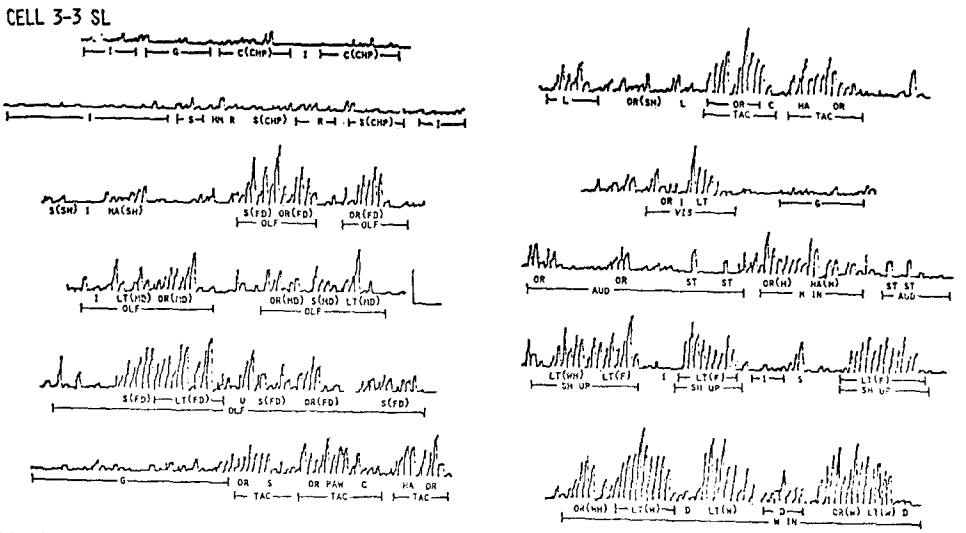

Fig. 9. Activity of cell 3-3. This cell is representative of those that increased activity during all orienting and locomotor behaviors. 'Paw' indicates paw contact. Calibration: 5 sec and 50 spikes/sec.

overall pattern of similar increases to a range of events did not seem to warrant the classification of their activity as female, water, or grasp-related. No neurons were characterized by a decrease in activity during orienting and locomotion. A ratemeter record of cell 3-3 illustrating its activity during all behavioral and sensory tests is presented in Fig. 9.

Neural activity related to locomotion

Four basal forebrain neurons were characterized by activity changes during all locomotion, but not during orienting. They are indicated by a black cross within a circle in Fig. 2. Three cells, one in MPO, one in nDB, and one in nST increased in activity when the subject locomoted toward or away from any object. One cell, located in MPO, decreased in activity during locomotion. These neurons were similar to the neurons characterized by activity change related to locomotion and orienting in that their activity was related to the subject's behavior and was not specific to a particular stimulus. They were not influenced during orienting responses; their activity change was contingent on locomotion.

Neural activity related to orienting

Two basal forebrain neurons were characterized by activity increases when the subject sniffed and oriented to stimuli. They are indicated by a white cross within a circle in Fig. 2. One of these was recorded in SL, the other in nST. These cells showed maximum increases during all orienting head movements, regardless of the object to which the subject oriented. The magnitude of orienting-related increases appeared to be correlated with the novelty of a stimulus. Repeated presentations of the same stimulus resulted in successively smaller activity increases.

Other neural activity

Five basal forebrain neurons were recorded that had activity correlates that did not fall into the above classes. These cells are indicated by open circles in Fig. 2. Cell 16-5, located in LPO, increased in activity only when the subject oriented to the visual stimulus. Cell 16-4, located in LPO, increased in activity only when tactile stimuli were presented. Cell 39-2 located in nDB, increased above baseline when the subject backed away from aversive stimuli. Escape from the experimenter's grasp did not influence the cell's activity; the activity increase was contingent on locomotion backward. Cells 8-6 and 14-6, located in SL, exhibited place-specific activity. Cell 8-6 was increased in activity when the subject oriented toward the back of the running wheel. Cell 14-6 showed only sporadic activity unless the subject was placed in the female's side of the chamber, a procedure used in few cases. A ratemeter record of cell 14-6 is presented in Fig. 10. Previous to the recording of this cell, the subject had never been placed in the female's side of the chamber.

Twenty-seven basal forebrain cells were recorded that had no detectable behavioral correlates. These uncorrelated neurons showed three types of activity: generally fast, generally slow, and variable. Generally fast cells (n = 6) had base-

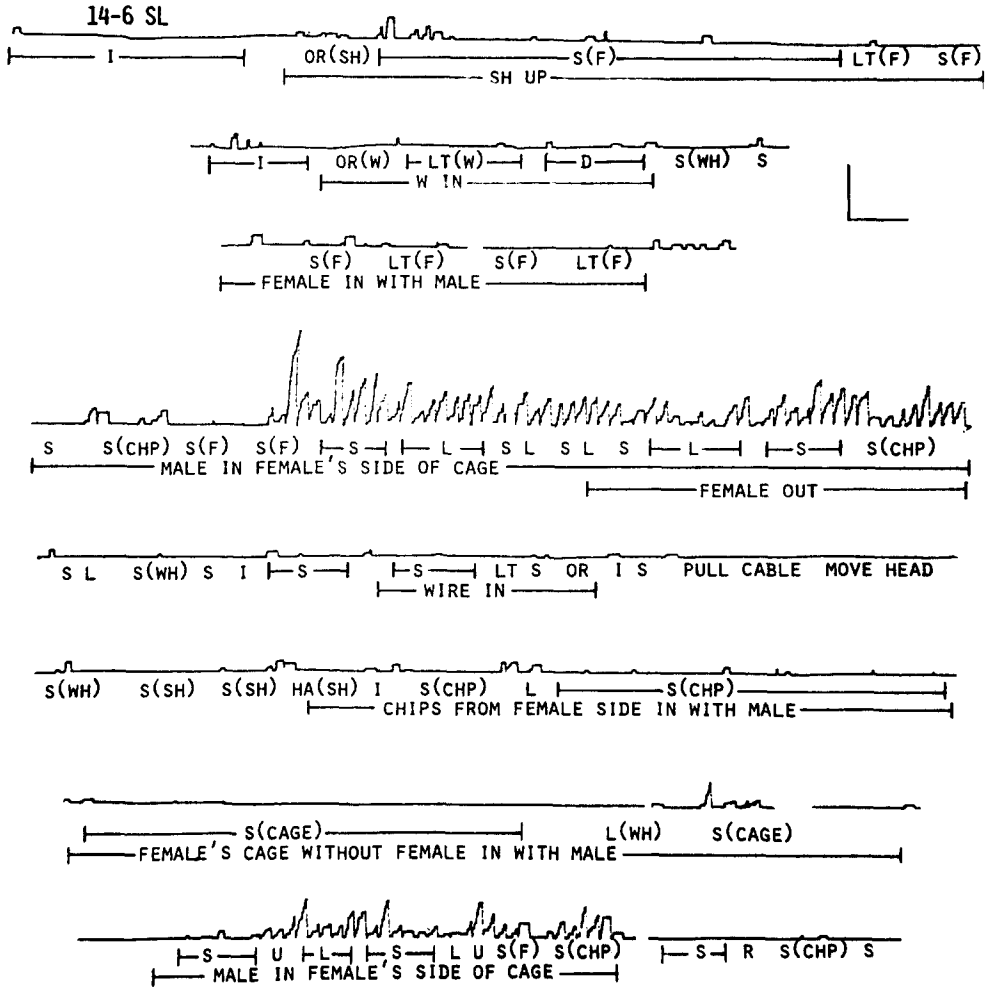

Fig. 10. Activity of cell 14-6. The activity increases of this cell were associated with the subject being placed in the female's side of the test apparatus. The fourth trace is the first time the male is placed in the female's side. The final trace is the fifth time the male was moved. 'Wire' indicates the presentation of a wire as a novel stimulus. Calibration: 5 sec and 40 spikes/sec.

line rates of 15 spikes/sec or higher and did not fluctuate more than 5% from their baseline rate. Slow uncorrelated cells (n = 11) had baseline rates of less than 2 spikes/sec and rarely fired more than 5 spikes/sec. These cells exhibited a wide range of firing rates that appeared uncorrelated with any behaviors or stimuli.

Anatomical differences in neural activity

The data in Table I indicate a differential distribution of neurons with grasp-related activity changes and neurons with female-related activity changes in MPO and LPO. A Fisher's test of exact probabilities indicates a non-random

distribution of these cell types ($P < 0.025$). The regional difference is characterized by a relative predominance of neurons with female-related activity in MPO, and grasp-related activity in LPO. Other neuron classes appear to be randomly distributed in the basal forebrain.

An analysis of the baseline rates of basal forebrain neurons revealed no anatomical differences. The baseline rates of neurons with behaviorally-correlated activity are indicated in Table I. The baseline rates of neurons with uncorrelated activity are not significantly different from those of cells with behaviorally-correlated activity in SL ($t = 1.15$, df = 27, $P > 0.1$), nST ($t = 1.80$, df = 17, $P > 0.1$), and MPO ($t = 0.73$, df = 9, $P > 0.1$). LPO and nDB did not yield a sufficiently large number of neurons to perform the appropriate statistical tests. If the baseline rates of cells with and without behaviorally-correlated activity are considered together, a one-way analysis of variance indicates no significant differences between baseline rates of SL, nST, nDB, MPO, and LPO ($F = 0.65$, df = 4,72, $P > 0.1$).

Units recorded in non-basal forebrain structures

Twenty-six neurons were recorded in structures outside the basal forebrain. These included the parafornical hypothalamus (n = 1), anterior medial thalamic nucleus (n = 5), nucleus accumbens (n = 1), reuniens nucleus (n = 2), triangular septal nucleus (n = 3), parataenial nucleus (n = 3), caudate nucleus (n = 3), reticular nucleus of the thalamus (n = 5), ventral nucleus of the thalamus (n = 1), and suprachiasmatic nucleus (n = 1). The behavioral correlates of the activity of these cells were of the same types as those found in the basal forebrain. The majority were uncorrelated (n = 9) or had orienting and locomotion correlates (n = 9). No female or water-specific correlates were found outside the basal forebrain.

Responses to brain stimulation

Responses to stimulation were seen in 13 of the 75 basal forebrain neurons that were tested for behavioral correlates. Their responses are summarized in Table I. There appears to be no clear relationship between the activity patterns during behavior and stimulation response patterns. In interpreting the responses to stimulation it should be noted that only the central gray stimulation was tested with all neurons. One possible relationship was seen in cells 16-6 and 26-5, located in LPO. Both cells decreased in activity during drinking and were also inhibited by 0.5 sec trains of 50 Hz stimulation of the medial forebrain bundle. Of the other responses to stimulation, synaptic excitation predominated. No inhibition was seen from stimulation of the central gray or lateral habenula. In addition, only one antidromic response was seen from stimulation of the medial forebrain

bundle and none were seen following stimulation of central gray or lateral habenula. Brain stimulation was not effective in evoking activity in quiescent units that were sufficiently large in amplitude to isolate for behavioral testing.

DISCUSSION

Single neuronal activity recorded during free behavior in a motivationally meaningful context presents a picture that emphasizes the role of the nervous system in the control of action. The behaviorally functional organism does not simply receive information and respond reflexively, but rather the organism actively controls the quality and magnitude of the sensory experience. The behavioral means for this modulation are provided by the head orienting and locomotor systems which determine the proximity to the sensory event of the chief receptor apparatus and the entire body, respectively. Accordingly, in the present experiment few basal forebrain neurons were found that could be classed as either sensory or motor in any simple sense. Instead, most were related to the behaviors of orienting and locomotion. Even when the pattern of behavior correlates indicated that the neuron had some degree of motivational specificity, the correlations appeared to relate to a context provided by the stimulus rather than any sensory or motoric detail.

Within the basal forebrain, at least in this experiment, two major organizational principles seemed to be operating. At one level, there was specific activation of certain neurons during orienting and locomotion related to particular motivational events such as those involving the female, water and experimenter's grasp. These neurons tend to be concentrated in particular brain areas, although this conclusion must be considered as tentative since the number of neurons sampled in each area was rather small. At another level of organization there was general activation of certain other neurons during all orienting and/or locomotion regardless of the motivational state of the animal. These neurons were found throughout the basal forebrain.

Motivational correlates

The neurons with firing patterns related only to approach toward the conspecific female were concentrated, as predicted, in the sexually dimorphic nucleus of the preoptic area. That is part of the region where lesions disrupt approach, sexual behavior, and scent-marking and where hormones affect these behaviors as well [12, 33, 39]. The activity patterns could not be completely explained by individual sensory or motor aspects of the situation, but seemed to be related to the integration of the behavior. They did not change in activity when the female's scent-marked dish was presented to the subject, and therefore they did not seem simply responsive to her odors; and their activity was similar whether the subject sniffed, oriented, or locomoted toward the female.

It could not be determined whether these 'conspecific-approach' neurons were part of a sexual motivational system or a non-sexual system such as the patrol/marking motivational system that has been postulated by Adams [3]. Although estrous females were used as targets because they are particularly effective elicitors of approach locomotion [9], male or non-estrous female targets also could have been used to elicit approach which presumably would reflect a non-sexual motivation (Lee and Adams, in preparation). Both of these motivational systems would be expected to involve neurons of the sexually dimorphic nucleus since each is characterized by behavior that is differentially directed towards conspecifics of the opposite sex.

The location of cells with water-related activity in the lateral septum and lateral preoptic area is consistent with the effects of lesions in these areas which indicate a role in the regulation of water balance [4, 15]. Those locations are also consistent with electrophysiological evidence for osmosensitive cells in the lateral septum [8] and the lateral preoptic area [7, 40]. Although the neurons reported here could be osmosensitive, this factor could not account for the short-latency activity changes that were observed. Some of the lateral preoptic neurons showed dramatic decreases in activity when the rat drank and sharp increases as the water tube was withdrawn. Similar activity patterns have been reported for lateral hypothalamic cells during feeding or drinking [14]. In the monkey, neurons of the lateral preoptic area have also been shown to change activity upon presentation or consumption of food or water [32]. The septal and bed nucleus neurons that have water-related activity are similar to those recorded by Ranck [30] in these areas that had activity related to appetitive and consummatory behaviors.

The neurons with grasp-related activity were concentrated in the lateral preoptic area, which is consistent with stimulation and lesion data on the role of that region in a defense motivational system [2]. In addition to changes during escape, these cells changed in activity when tactile stimuli were sufficiently rough to elicit aversive behavior (squeal, crouch, or withdrawal). Similar activity changes were seen when the animal was startled. These data are consistent with the finding that some cells in the lateral preoptic area of anesthetized rats respond to a tail-pinch [28]. The septal cells with escape-related activity are consistent with the findings of Ranck [30].

The activity changes of four lateral preoptic cells during drinking and escape appeared to be inverse and related to their neural connections. It is interesting that the response to stimulation of the medial forebrain bundle was the same as the response pattern during drinking and the response to stimulation of the central gray was similar to the response pattern during escape. Two of these cells which decreased in activity during drinking showed an increase above baseline when the water was removed. Stimulation of the medial forebrain bundle produced a decrease in activity in these cells which was followed by an increase. Similar types of responses in the hypothalamus have been reported by others [16, 19, 25].

General orienting and approach

The basal forebrain neurons that changed activity during all of the motivational events appear to reflect the general orienting and approach behaviors that are common to several motivational systems. These cells also changed in activity in relation to several of the sensory stimuli. The activity changes were independent of sensory modality, the sensory field to which the stimuli were presented, or the direction of the animal's response. The activity of these neurons seemed to reflect the general activity of the rat, as indicated by the frequent association of maximum firing rate with the most vigorous locomotion. This type of cell was the most common class in this study and was also found in high proportions by Ranck [30] in his study of the septum, and by Komisaruk and Olds [17], in their study of the lateral preoptic area and lateral hypothalamus. Multiple neuronal activity in the basal forebrain also correlates with general activity [22]. These data reflect a widespread and profound change in activity of basal forebrain neurons during orienting and locomotion.

Implications for future research

The method of single neuron analysis, to reach its full potential, should be carried beyond a catalog of behavioral correlates of neurons and should include characterization of the properties of their networks. We will need to know the loci of afferent and efferent projections and also the type of synaptic transactions that occur. To this end stimulation of known projection targets of the basal forebrain was used in the present study.

The infrequency of response to the stimulation was surprising in light of the extensive connections between the basal forebrain and the stimulation sites [10, 35, 36], and the high percentage of basal forebrain neuronal responses to such stimulation in anesthetized rats [16, 21, 27]. One possible explanation for the lack of responses in this study is that in an active animal, the baseline neural activity levels are higher in an anesthetized animal and thus many weak responses are masked. It may also be that in an awake animal, potential responses to stimulation are suppressed by inhibition from other neurons that are not active in acute preparations. Both possibilities could also explain the lack of olfactory responses in the LPO which is surprising in light of the sensory electrophysiological studies of Pfaff and Pfaffman [28].

Even though brain stimulation was not as productive in this experiment as had been hoped, there seems at this time no more effective way to identify a recorded neuron independent of its behavioral correlates and its location in a certain nucleus of the brain. Independent identification of neurons has proven to be of great value in the study of neural networks in invertebrates, a precedent that gives us encouragement to continue this approach in more complex vertebrate preparations.

ACKNOWLEDGEMENTS

We wish to thank Robert Russo and Robert Castillo for their assistance with surgery and recording. The work was partially supported by a Wesleyan Project Grant to H.M.S.

REFERENCES

1 Adams, D.B., The relation of scent-marking, olfactory investigation, and specific postures in the isolation-induced fighting of rats, *Behaviour*, 56 (1976) 286–297.
2 Adams, D.B., Brain mechanisms for offense, defense, and submission, *Behav. Brain Sci.*, 2 (1979) 201–241.
3 Adams, D.B., Motivational systems of agonistic behavior in muroid rodents: a comparative review and neural model, *Aggressive Behav.*, 6 (1980) 295–346.
4 Almli, C.R. and Weiss, C.S., Drinking behaviors: effects of lateral preoptic and lateral hypothalamic destruction, *Physiol. Behav.*, 13 (1974) 527–538.
5 Bean, N.J. and Conner, R., Central hormonal replacement and home-cage dominance in castrated rats, *Hormone Behav.*, 11 (1978) 100–109.
6 Bindra, D., How adaptive behavior is produced: a perceptual-motivational alternative to response-reinforcement, *Behav. Brain Sci.*, 1 (1978) 41–91.
7 Blank, D.L. and Wayner, M.J., Lateral preoptic single unit activity: effects of various solutions, *Physiol. Behav.*, 15 (1975) 723–730.
8 Bridge, J.G. and Hatton, G.I., Septal unit activity in response to alterations in blood volume and osmotic pressure, *Physiol. Behav.*, 10 (1973) 769–774.
9 Brown, R.E., Odor preference and urine marking scales in male and female rats: effects of gonadectomy and sexual experience on responses to conspecific odors, *J. comp. physiol. Psychol.*, 91 (1977) 1190–1206.
10 Conrad, L.C.A. and Pfaff, D.W., Efferents from medial basal forebrain and hypothalamus in the rat. I. An autoradiographic study of the medial preoptic area, *J. comp. Neurol.*, 169 (1976) 185–220.
11 Emery, D.E. and Sachs, B.D., Copulatory behavior of male rats with lesions in the bed nucleus of the stria terminalis, *Physiol. Behav.*, 17 (1976) 803–806.
12 Ginton, A. and Merari, A., Long range effects of MPOA lesions on mating behavior in the male rat, *Brain Res.*, 120 (1977) 158–163.
13 Gorski, R.A., Harlan, R.E., Jacobson, C.D., Shryne, J.E. and Southam, A.M., Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat, *J. comp. Neurol.*, 193 (1980) 529–539.
14 Hamburg, M.D., Hypothalamic unit activity and eating behavior, *Amer. J. Physiol.*, 220 (1971) 980–985.
15 Herrmann, T.F. and Lubar, J.F., Immediate and long-term effects of septal and frontal ablations on the species-typical behavior of the rat. In DeFrance, J.F. (Ed.), *The Septal Nuclei*, Plenum Press, New York, 1976.
16 Keene, J.J., Prolonged medial forebrain bundle unit responses to rewarding and aversive intracranial stimuli, *Brain Res. Bull.*, 1 (1976) 517–522.
17 Komisaruk, B.R. and Olds, J., Neuronal correlates of behavior in freely moving rats, *Science*, 161 (1968) 810–812.
18 König, J.F.R. and Klippel, R.A., *The Rat Brain: A Stereotaxic Atlas*, Williams and Wilkins, Baltimore, MD, 1963.

19 Ito, M., Excitability of medial forebrain bundle neurons during self-stimulating behavior, *J. Neurophysiol.*, 35 (1972) 652–664.
20 Jacobson, C.D., Shryne, J.E., Shapiro, F. and Gorski, R.A., Ontogeny of the sexually dimorphic nucleus of the preoptic area, *J. comp. Neurol.*, 193 (1980) 541–548.
21 MacLeod, N.K. and Mayer, M.L., Electrophysiological analysis of pathways connecting the medial preoptic area with the mesencephalic central grey matter in rats, *J. Physiol. (Lond.)*, 298 (1980) 53–70.
22 Malmo, H.P. and Malmo, R.B., Movement-related forebrain and midbrain multiple unit activity in rats, *Electroenceph. clin. Neurophysiol.*, 42 (1977) 501–509.
23 Malsbury, C.W., Facilitation of male rat copulatory behavior by electrical stimulation of the medial preoptic area, *Physiol. Behav.*, 7 (1971) 797–805.
24 Numan, M., Medial preoptic area and maternal behavior in the female rat, *J. comp. Physiol.*, 87 (1974) 746–759.
25 Olds, M.E., Unit responses in the medial forebrain bundle to rewarding stimulation in the hypothalamus, *Brain Res.*, 80 (1974) 479–495.
26 Panksepp, J., Aggression elicited by electrical stimulation of the hypothalamus in albino rats, *Physiol. Behav.*, 6 (1971) 321–329.
27 Perkins, M.N. and Whitehead, S.A., Neural connections between the medial forebrain bundle, the preoptic area and the basal hypothalamus in the rat: an electrophysiological study, *J. Physiol.*, 291 (1979) 443–456.
28 Pfaff, D.W. and Pfaffman, C., Olfactory and hormonal influences on the basal forebrain of the male rat, *Brain Res.*, 15 (1969) 137–156.
29 Pond, F.J., Sinnamon, H.M. and Adams, D.B., Single unit recording in the midbrain of rats during shock-elicited fighting behavior, *Brain Res.*, 120 (1977) 469–484.
30 Ranck, J.B., Jr., Behavioral correlates and firing repertoires of neurons in septal nuclei in unrestrained rats. In DeFrance, J.F. (Ed.), *The Septal Nuclei*, Plenum Publishing Corp., New York, 1976.
31 Roberts, W.W. and Martin, J.R., Effects of lesions in central thermosensitive areas on thermoregulatory responses in rat, *Physiol. Behav.*, 19 (1977) 502–511.
32 Rolls, E.T., Sanghera, M.K. and Roper-Hall, A., The latency of activation of neurones in the lateral hypothalamus and substantia innominata during feeding in the monkey, *Brain Res.*, 164 (1979) 121–135.
33 Scouten, C.W., Burrell, L., Palmer, T. and Cegavske, C.F., Lateral projections of the medial preoptic area are necessary for androgenic influence on urine marking and copulation in rats, *Physiol. Behav.*, 25 (1980) 237–243.
34 Singer, J.J., Hypothalamic control of male and female sexual behavior in female rats, *J. comp. physiol. Psychol.*, 66 (1968) 738–742.
35 Swanson, L.W., An autoradiographic study of the efferent connections of the preoptic region of the rat, *J. comp. Neurol.*, 167 (1976) 227–256.
36 Swanson, L.W. and Cowan, W.M., Connections of the septal region in the rat, *J. comp. Neurol.*, 186 (1979) 621–655.
37 Szechtman, H., Caggiula, A.R. and Wulkan, D., Preoptic knife cuts and sexual behavior in male rats, *Brain Res.*, 150 (1978) 569–591.
38 Terkel, J., Bridges, R.S. and Sawyer, C.H., Effects of transecting lateral neural connections of the medial preoptic area on maternal behavior in the rat: nest building, pup retrieval and prolactin secretion, *Brain Res.*, 169 (1979) 369–380.
39 Wade, G.N. and Zucker, I., Modulation of food intake and locomotor activity in female rats by diencephalic hormone implants, *J. comp. Physiol. Psychol.*, 72 (1970) 328–336.
40 Weiss, C.S. and Almli, C.R., Lateral preoptic and lateral hypothalamic units: in search of the osmoreceptors for thirst, *Physiol. Behav.*, 15 (1975) 713–722.