6 A Behavioral Approach to the Analysis of Reticular Formation Unit Activity

JEROME M. SIEGEL

A. INTRODUCTION

The brainstem reticular formation (RF) was one of the first structures to be investigated using single unit recording techniques. One procedure common to all the early studies was the attempt to eliminate spontaneous behavior prior to commencing unit studies. This was not done because investigators thought that behavior was unworthy of study. Rather, it was a direct result of the unit recording technology, which required the complete immobility of the animal. Immobility was most readily achieved with the use of barbiturate anesthesia. A major drawback of this anesthetic is that it almost completely eliminates spontaneous activity of cells in RF and many other regions. The subsequent use of chloralose anesthesia, which does not produce so severe a depression of spontaneous and elicited unit activity, was a great advance. More recently, techniques for recording *from* unanesthetized, head-restrained animals became available. These techniques allowed unit activity in the waking state to be observed for the first time. However, it is still necessary to eliminate most spontaneous behavior when using these procedures, since motor activity tends to produce small brain movements that cause the experimenter to "lose" the recorded cell. Thus, the behavioral expertise of researchers interested in unit activity in the RF and in many other areas has been largely devoted to teaching rats, cats, and monkeys to hold still during recording sessions. In many studies, this same goal was achieved by the use of curariform agents. The development of microwire and other recording techniques now allow unit recording in completely unrestrained, behaving animals. However, the adoption of this new methodology has been slow for several reasons. One is that many experimenters now have a considerable investment in the technology of recording from restrained animals. The apparent simplicity and lack of variability in the unit activity of the restrained preparation makes interpretation easier, although, as I will show below, this simplicity may be quite misleading. Perhaps the fundamental reason

that researchers have been slow to adopt the new unit recording methodologies is that procedures for relating unit activity to spontaneous behaviors have been slow to develop. Indeed, even most of those working with *unrestrained* animals have been content to use the methodologies developed for the restrained preparation, often presenting stimuli and observing unit response without describing or even observing the intervening behavior. Direct study of the relation between spontaneous unit activity and behavior has been rare (also see Ranck et al., Chapter 5, this volume).

The history of unit studies of the RF illustrates how divergent concepts of unit function can develop in the absence of extensive behavioral observation. The different theoretical views with which investigators approached the study of RF cells have produced widely different and apparently conflicting conclusions about the functional role of these cells. In the following discussion, I will review these conclusions. Procedures for studying these cells in unrestrained, behaving animals will then be presented. The findings in unrestrained animals suggest ways of harmonizing some of the conclusions reached in restrained animals.

B. REVIEW OF RETICULAR FORMATION UNIT LITERATURE

Most medial RF neurons respond to stimuli presented via one or more sensory modalities. Reticular formation responses to vestibular (Peterson et al., 1975), somatic (Segundo et al., 1967), vaginal (Rose, 1978), thermal (Cronin & Baker, 1977; Lee et al., 1977), auditory (Amassian & Devito, 1954; Bach-y-Rita, 1964; Ingle & Sprague, 1975), visual (Bell et al., 1964; Faingold & Caspary, 1977; Groves et al., 1973), and olfactory (Motokizawa, 1974) stimuli have been reported.

Casey (1971) studied the discharge of RF cells in cats trained to escape painful electrical shock. He found that unit discharge rates increased as stimulation intensities were raised to levels eliciting escape behavior. Procedures that reduce pain responses, such as morphine administration, are effective in reducing RF responses to noxious stimuli (Pearl & Anderson, 1977; Sun & Gatipon, 1976; Yokota & Hashimoto, 1976). The activation of RF neurons by painful stimuli has been interpreted as indicating that medial RF cells are specifically concerned with the sensory and motivational aspects of pain (Burton, 1968; Casey, 1971; Young & Gottschaldt, 1976).

Buchwald et al. (1966) observed multiple unit activity in RF and sensory and motor systems during classical conditioning of a hindlimb flexion response. They concluded that "activation of the conditioned stimulus

and reticular systems are primary events in conditioning." Most RF neurons show a progressive decrease in their response to repeated stimuli. This "habituation" process has been studied by several investigators (Bell et al., 1964; Groves et al., 1973; Peterson et al., 1976; Scheibel & Schei-bel, 1965; Segundo et al., 1967).

In 1949, Moruzzi and Magoun reported that midbrain reticular formation (MRF) stimulation in intact cats produced a long-lasting cortical desynchrony. Further work developed and refined the concept of an ascending reticular activating system contributing to both behavioral and electroencephalographic arousal. The advent of unit recording techniques allowed investigators to explore the RF for the neural substrate of these phenomena. An early study by Machne et al. (1955) reported a marked increase in the activity of most MRF units in response to arousal by either sciatic nerve or brainstem stimulation. Podvoll and Goodman (1967), Bambridge and Gijsgers (1977), and Beyer et al. (1971) reported a strong positive correlation between behavioral arousal and the level of MRF and pontine reticular formation (PRF) multiple unit activity (MUA) in the unrestrained cat. More detailed behavioral analyses have been directed at further defining the motivational variables responsible for relations between RF activity and arousal. Olds et al. (1969) found a marked acceleration in activity in every MRF unit studied as reinforcement became imminent. They conclude that the MRF activity increase is specifically related to *anticipation of reward.* This conclusion can be contrasted with the findings of Vertes and Miller (1976) and Best et al. (1973) (in rats) and Ume-moto et al. (1970) (in cats). These workers studied the activity of PRF and MRF units during a conditioned emotional response (CER). These studies reported an increased firing rate in these units during anticipation of the shock. The increased RF activity was hypothesized to relate *to fear of the shock.* Umemoto et al. reported that 68% of encountered neurons discharged *specifically in relation to the CER.*

Hobson and McCarley and their co-workers reported, in a series of studies, that cells in the "gigantocellular tegmental field" (FTG), which constitutes most of the PRF, discharge at much higher rates in REM sleep than in either waking or slow wave sleep (SWS). During waking and SWS, "most FTG neurons either showed very low discharge rates (< 1 impulse per second) or were silent," whereas during REM sleep, they discharged in rapid bursts (McCarley & Hobson, 1971). Therefore, the ratio of REM sleep rates to waking and SWS rates or "selectivity" was very high. It was concluded that because of this high selectivity "FTG units are at present the best candidates" for REM sleep generator neurons (Hobson et al., 1974).

Studies in the head-restrained monkey have demonstrated relationships between the discharge of medial RF cells and eye movement. A

variety of cell types have been reported in both midbrain and pontine regions (Buttner et al., 1977; Cohen & Henn, 1972; Fuchs & Luschei, 1972; Keller, 1974). Eye movement cell types similar to those seen in the monkey have been found in dorsomedial portions of the cat RF (Hikosaka & Kawakami, 1977). It has been concluded that discharge in PRF cells is "predominantly related to eye movement" (Cohen, 1978).

The relationship of RF unit activity to respiration has been extensively studied in acute preparations. Typically, respiratory related units are sought in cats that are paralyzed with Flaxedil and artificially ventilated. Units related to respiration are detected by visual observation of periodicities in discharge rate, or more frequently, by computer analysis of temporal relations in unit firing. Units related to respiration have been found throughout the RF. The proportion of units that are related to respiration in the cat ranges from 21% in the MRF to 36% in the PRF (Bertrand et ah, 1973).

It has been found that stimulation of a circumscribed "midbrain locomotion region" (MLR) produces rhythmic stepping behavior in a decerebrate cat placed on a treadmill (Grillner & Shik, 1973) and speeds locomotion in the intact cat (Sterman & Fairchild, 1966). Since there are no strong direct connections between the MLR and the spinal cord, and since spinal cord areas containing reticular projections show a marked activity increase during locomotion, it was hypothesized that reticulospinal pathways might form part of the system producing both spontaneous and MLR-induced locomotion (Orlovskii, 1970). Orlovskii has, therefore, studied the activity of reticulospinal neurons during locomotion. It was shown that 69% of reticulospinal neurons were activated during MLR-induced locomotion and that the "transfer phase" of limb movement was the most active point for reticulospinal cells.

When one considers the variety of different findings made in RF studies, one naturally assumes that there are several different cell populations within the RF responding either during pain, reward, sensory stimuli, REM sleep or any of the other investigated conditions. However an analysis of the locations of the cells recorded, the proportions of cells sampled in each study, and other technical issues (Siegel, 1979a) lead to the conclusion that all of these studies are reporting on different aspects of *the same cells* (Fig. 6.1; Table 6.1).

C. RETICULAR FORMATION UNIT ACTIVITY IN THE UNRESTRAINED CAT

To try to explain how the same cells can have so many apparently different roles, we have attempted to get the "big picture" of what aspects of behavior these cells were related to by observing these units in unre-

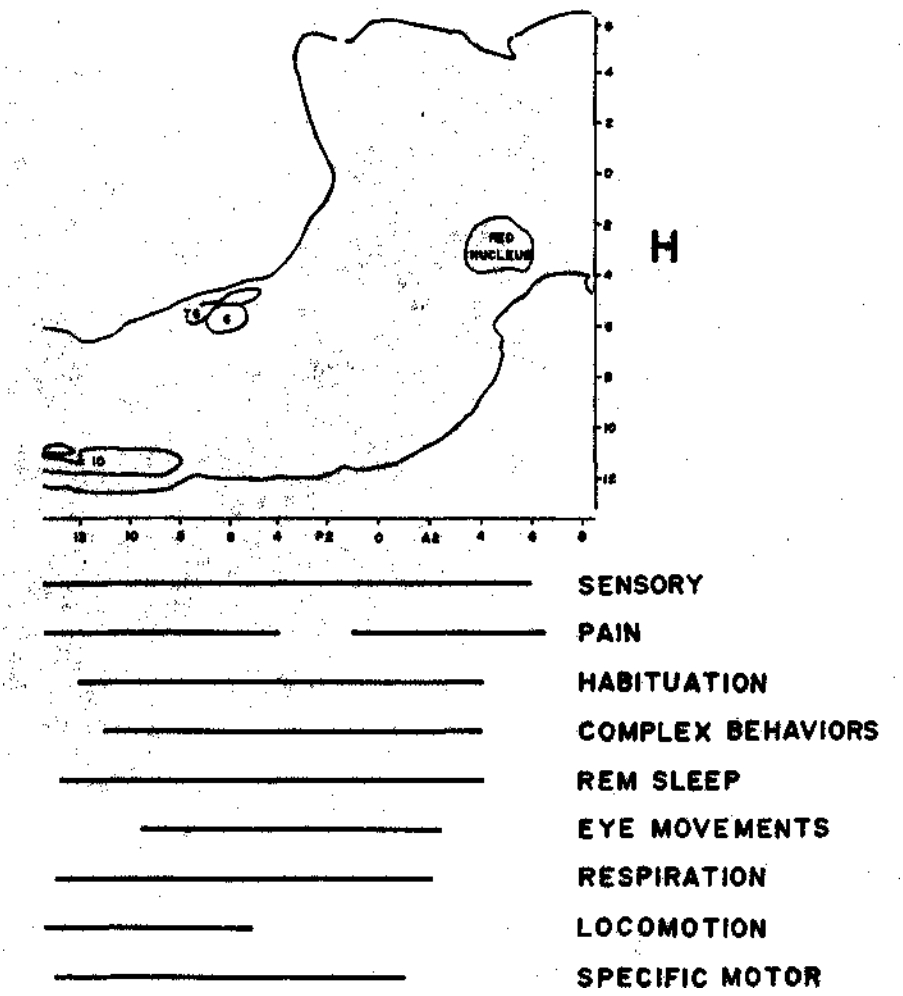


FIGURE 6.1. Anatomical distribution of cell types within the reticular formation, sagittal view of the brainstem of the cat. The bars (bottom) indicate the anterior-posterior distribution of cells identified as having specific behavioral functions. Cells with different behavioral properties are not localized to different RF areas. Key: 6, abducens nucleus; 7G genu of the facial nerve; I.O., inferior olive. From Siegel (1979a), by permission.

strained cats. It soon became obvious that simply categorizing the behavior the animal engaged in when RF units were active would be misleading. Thus, for example, certain RF cells would discharge when the cat was eating, suggesting that they be labeled "eating cells." However, closer study revealed that this was not the case. These cells would only discharge when

TABLE 6.1. A PARTIAL LIST OF THE BEHAVIORAL FUNCTIONS ATTRIBUTED TO RF CELLS

Cat stereotaxic coordinates were estimated from figures or taken from the authors' description. General anatomical terms were used In rat and monkey studies and In cat studies where precise anterior-posterior levels were not available. Third column lists percentage of encountered cells having function listed in column one.

Function or Stimulus	*CatA-P Stereotaxic Coordinate or Anatomical Designation*	*Percentage of Encountered Cells*	*Reference*
Vestibular	*P15-AO*	*75*	*Peterson et al., 1975*
So\matosensorv	*P18.5-P8.S*	*77*	*Segandoetal..1967*
	P8-P3	*71*	*Siegel & McGinty, 1977*
	A2-A4	*68*	*Bell et al 1964*
Auditorv	*P8-P3*	*40*	*Siegel & McGintv. 1977*
	BRF, MRF	*23*	*Schelbel et al.,1955*
Olfactory	*APO-A6*	*58*	*Motokizawa, 1974*
Pain	*P18.3-P12.1*	*82*	*Burton, 1968*
	P11-P6	*57*	*Casev et al..1974*
	AO-A3	*88*	*Barnes.1976*
Habitation	*BRF*	*75*	*Scheibel and Scheibel, 1968*
	A2-A4	*75*	*Bell et al.. 1964*
Reinforcement	*MRF†*	*100*	*Olds et al., 1969*
Fear	*MRF†*	*85*	*Best et al., 1973*
	MRF	*88*	*Umemoto et al.. 1970*
REM sleep	*P7-A0*	*100*	*Hobsonetal., 1974*
	P9-P3	*72*	*Siegel et al.. 1977*
Eye movements	*MRF*	*70*	*Buttner et al.,1977*
	PRF	*92*	*Luschei & Fuchs. 1972*
Locomotion	*P15-P5*	*89*	*Orlovskil. 1970*
Respiration	*PRF*	*36*	*Bertrand et al., 1973*
	MRF	*21*	*Bertrand et al., 1973*
Specific movements	*P8-P3*	*91*	*Siegel & McGinty. 1977*

From Siegel (1979a), by permission. †Rat studies. "Monkey studies.

the cat was eating food pellets placed in a bowl on the floor, not when it was eating pellets from a bowl lifted a few centimeters *off* the floor, or when it was chewing food pellets placed in its mouth. Conversely this same cell might fire when the cat was sniffing or exploring the floor and not eating, i.e., this cell type was related to the neck-extended posture, not to the behavioral category of eating. We have found that this is the case for most RF cells. Their activity is related to specific postures or movements.

In seeking to describe the underlying relation between RF unit activity and behavior, we have found that there is no substitute for the trained human observer carefully watching the behaving cat, listening to an audio

monitor of the unit signal, and systematically (Siegel & McGinty, 1976, 1977; Siegel et al., 1977) noting the behaviors that occur when the unit is firing. Although such an approach may seem crude, it can produce a much more accurate picture of the behavioral role of units than can other more quantitative techniques. To take the example mentioned above, one could precisely describe the correlation between discharge in certain RF cells and food consumption and sail completely miss the underlying postural relationship. The use of the full powers of human perception is, in reality, a very sophisticated and necessary methodology for determining the nature of the interrelations between unit activity and behavior. Only after a general understanding of the behavioral role has been achieved will the appropriate quantitative measures to correlate with unit activity become apparent (also see O'Keefe & Nadal, 1978, pp. 190-96; Ranck et al., Chapter 5, this volume).

After observing a variety of spontaneous behaviors for a minimum of 2 hours, we administered the discrete auditory, visual, and somatic stimuli that have been used in restrained animals to determine response modalities and latencies. A series of vestibular and proprioceptive stimuli, including active and passive movements of the vertebral column, EMG recording of selected muscles, and AC and DC EOG recording, were also used (Siegel & McGinty, 1976, 1977). In addition, all cells were recorded during both REM and non-REM sleep. This behavioral analysis takes a total of 4- to 6-hours per cell, far longer than the 5- to 10-minute observation common in acute studies. Therefore this technique is basically one of substituting intensive examination of a relatively small number of cells for a more superficial screening of a larger number of units.

In addition to this standard observation routine, we have developed other techniques to document behavioral relations. These include operant reinforcement of increased unit discharge and photographic analyses of the relation between unit activity and behavior. I will first describe the major behavioral subtypes we have seen in the medial RF and then outline the techniques for analyzing these relationships with photographic and operant reinforcement techniques.

1. CELL TYPES IN THE RETICULAR FORMATION

Table 6.2 lists the frequency of occurrence of the major cell types that we have found in the feline RF.

(i) Eye Movement Related Cells

These cells have previously been described by others in studies in the monkey and cat. They were found in the dorsomedial RF (Fig. 6.2) in the vicinity of the abducens and trochlear nuclei (we have not recorded exten-

sively in the RF in the region of the oculomotor nucleus). They constituted 10.0% of the total number of cells we have analyzed.

Eye movement cells do not respond to cutaneous, discrete auditory or visual stimuli or to punctate or natural somatic stimuli. They do not respond to manipulation of the limbs or axial musculature (unless these manipulations cause the specific eye movement associated with discharge). Most cells in the vicinity of the abducens nucleus (but not histologically localized to the nucleus itself) discharge in relation to ipsilateral movement of the eyes, although we have seen the previously reported omniburst and omnipause cell types. Cells in the region of the trochlear nucleus (but lateral to it) discharged maximally during ventral eye movement.

All cells were tested during passive head movements, which induce vestibular compensatory slow eye movements. All abducens cells discharged in relation to ipsilateral slow phase and saccadic eye movements. Reticular formation eye movement cells in the region of the trochlear nucleus all discharged during ventral slow phases as well as during spontaneous ventral saccadic movements. On the basis of response to passive lateral head movement, the RF cells near the trochlear nucleus cells could be further subdivided into cells responding to passive contralateral or ipsilateral head movement. The latter group (n = 8) was located 1.6 mm lateral to the midline; the former group (n= 3) was 1.2 mm from midline.

(ii) Cells Related to Pinna Movement

Sherrington first demonstrated that structures caudal to the inferior colliculus were sufficient to control a variety of protective reflex movements of the pinna. We have seen that cats with the brainstem transected

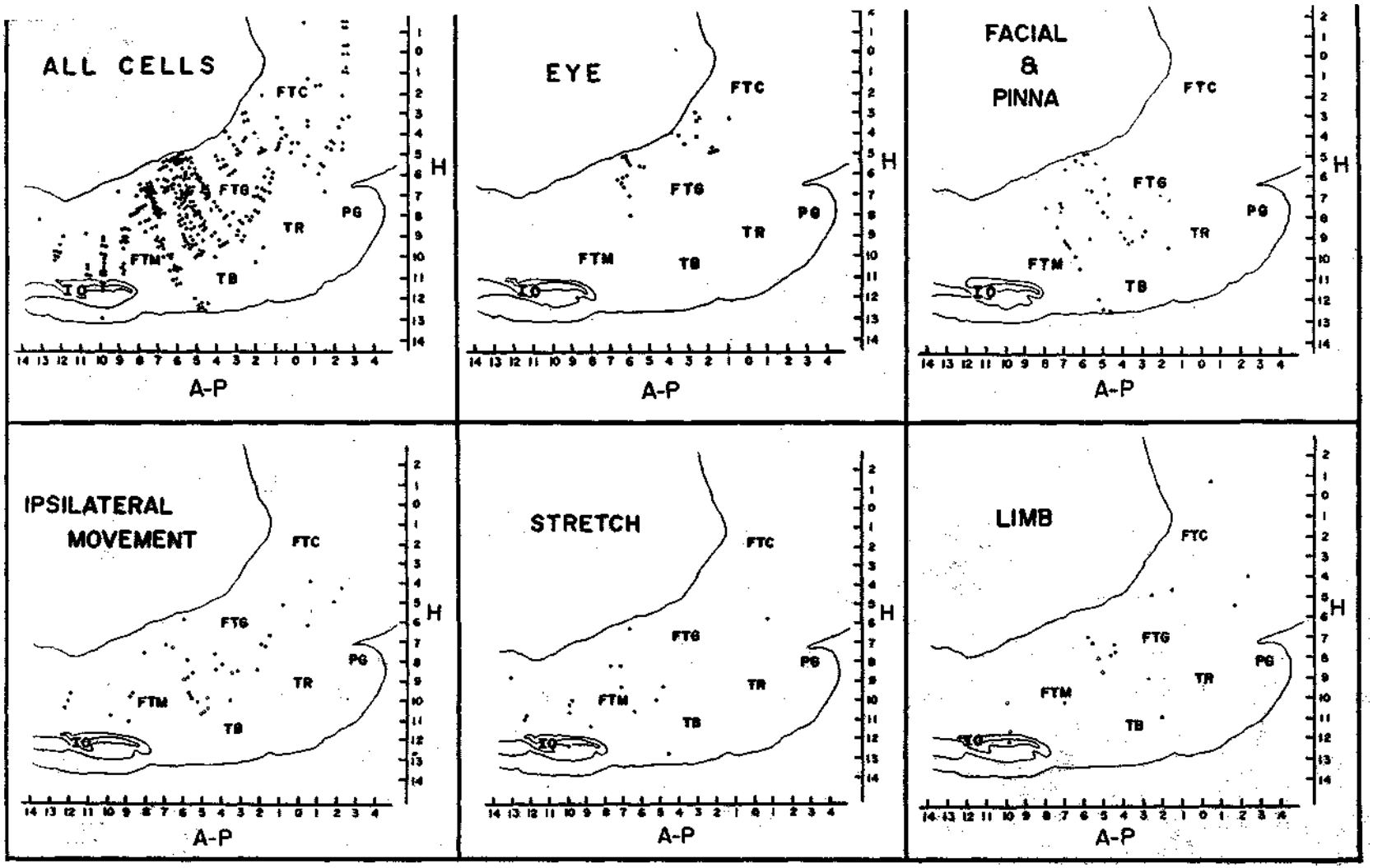

ALL CELLS
FTC
FTG
PG
TR
FTM
TB
TG
H
A-P
EYE
FTC
FTG
PG
TR
FTM
TB
TG
H
A-P
FACIAL
&
PINNA
FTC
FTG
PG
TR
FTM
TB
TG
H
A-P
IPSILATERAL
MOVEMENT
FTC
FTG
PG
TR
FTM
TB
TG
H
A-P
STRETCH
FTC
FTG
PG
TR
FTM
TB
TG
H
A-P
LIMB
FTC
FTG
PG
TR
FTM
TB
TG
H
A-P

FIGURE 6.3. Head stretch cells were maximally activated when the cat assumed posture pictured, but hot with lateral or dorsal head movements.

close to the facial nucleusor nerve. These cells resembled the pinna movement cells in their lack of sleep activity and in the absence of response to auditory, visual, and somatic stimulation.

(iv) Cells Discharging in Relation to a Variety of Specific Movements of the Head or Axial Musculature

These were the most common cell type in the medial RF, constituting 53.4% of our sample. To date we have been able to distinguish three distinct subtypes.

Ipsilateral Movement Cells. The largest subtype of cells related to movements of the vertebral column was cells related to active ipsilateral movement of the head or ipsilateral curvature of cervical and thoracic vertebrae. These cells, constituting 13.9% of our total sample, were also *the most common cell type in the RF,* more common than eye or facial movement related cells. Fifty-six percent of these cells discharged during *passive* movement of the head to the *contralateral* side, the remainder having no response to passive head movement. Brainstem cells with opposite active and passive head movement relations have also been reported in the rabbit (Duensing & Schaefer, 1960). Ipsilateral movement cells were scattered throughout the area explored. In contrast to facial, pinna, and eye movement cells, 50% of these cells responded to auditory stimulation, 60% of

those tested responded to electrical or punctate stipulation of theskin and 70% were active during sleep.

Head Extension Cells. The second largest group; 5.7% of the total sample was cells that discharged during active head extension. Figure 6.3 illus-trates the posture associated with discharge in these cells. None of these cells responded to passive head movement in any direction. None re-sponded to auditory or somatic stimuli. In contrast to adjacent lateral head movement related cells, 80% had little or no tonic activity during sleep. These cells were concentrated between 1.2 and 1.6 mm lateral to midline in the nucleus gigantocellularis and reticularis pontis caudalis (Fig. 6.2).

Head-Neck Dorsoflexion Cells. These cells constituted 3.9% of our total sample (n = 11). They were silent when the cat sat in the sphinx position with its head lowered. They increased their discharge rate when the head was raised. Highest rates were achieved when the cat was standing and rapidly dorsoflexed its head. Active head-neck movements of the same speed ventrally or to the cats left or right did not produce discharge. Two of these cells had both tonic sleep activity and auditory response, the rest had neither. These cells were restricted to between 1.2 and 1.6 mm from the midline, but were widely scattered in the A-P dimension (Fig. 6.2).

(v) Cells Related to Movement of Proximal Limb Joints

A total of 6.1% (n = 17) of the cells studied were related to active and/ or passive movement of one or more limb joints. In every case, these cells were related to active or passive movement of the proximal portion of the limb (i.e., movement that involved the scapula or pelvic girdle). We saw no RF cells related to movement of more distal portions of the limbs. The largest subgroup was related to passive flexion and active extension of the ipsilateral forelimb. All but one of the cells related to ipsilateral forelimb movement were located at or lateral to 1 .9 mm from the midline, whereas all those related to contralateral forelimb movement were located at or medial to 1.6 mm from the midline. They were widely distributed in the A-P dimension in medullary, pontine, and caudal midbrain regions. Forty-seven percent of these cells (» = 8) were type 2 cells (i.e., had high rates of tonic sleep activity). Three of the cells (18%) responded to auditory stimuli. Three of the four cells tested responded to shock stimulation of the skin in contrast to facial, eye, and head extension cells, none of which re-sponded to this same stimulus.

(vi) Cells Related to Other Behaviors

A small number of cells discharged in relation to one of a variety of specific behaviors. As a group, these cells constituted 11.8% of the total

number studied. Three cells were related to protrusion of the tongue. One of these was remarkably specific in its behavioral relations, discharging only with tongue protrusions to the ipsilateral side; the other two discharged with any tongue protrusion. These three cells were spread over a 9-mHi A-P region, but were all located less than 1.6 mm from the midline and more than 4 mm from the hypoglossal nucleus. One cell was related to swallowing. Eight were related to vestibular stimulation, discharging with certain head movements, whether active or passive, and not responding if the head was held and the body moved to produce the same neck stimulation. Twenty-seven cells had somatosensory, auditory, or visual responses, which could account for all their activity. Four cells were related to jaw movement, and three to respiration.

It is important to point out that *all* the cells that were studied with our complete testing procedure (n = 280) were tested for *all* the behaviors we have described; all were observed during tongue movements induced by manipulating the tongue with a cotton swab, all were observed during swallowing while lapping, and all were tested for response to vestibular stimuli induced by passive head movement. Thus, the low frequencies of the cells described in this section do not reflect selective testing procedures, but rather the rarity of these cell types in the RF cell population.

(vii) Cells Unrelated to Tested Behaviors

Five percent of the cells encountered (*n*= 14) did not discharge in specific relation to any of the sensory tests or the motor observations we made. Many of these cells had considerable variability of discharge rate. However, despite extended periods of observation, their discharge could not be related to any specific sensory or motor event.

2. OPERANT REINFORCEMENT OF UNIT DISCHARGE

Our finding that activity in RF cells was related to specific movements was derived from systematic sensory stimulation and behavioral observation in unrestrained cats. We sought to document these observations using a different technique. Therefore, we developed procedures for operantly reinforcing increased discharge in RF units (Breedlove et al., 1979). This procedure, in a sense, requires the cat to do the behavioral analysis, figuring out what, if any, behaviors are required to gain reinforcement. Lateral hypothalamic stimulating and pontine gigantocellular microwire recording electrodes were implanted in three cats. After the cats recovered from surgery, current levels for reinforcing brain stimulation were determined by reinforcing the absence of eye movements with 400-msec trains of 0.3-msec pulses. Increased discharge was then reinforced in a total of 22 cells. During 12 of these experiments, a second nonreinforced control

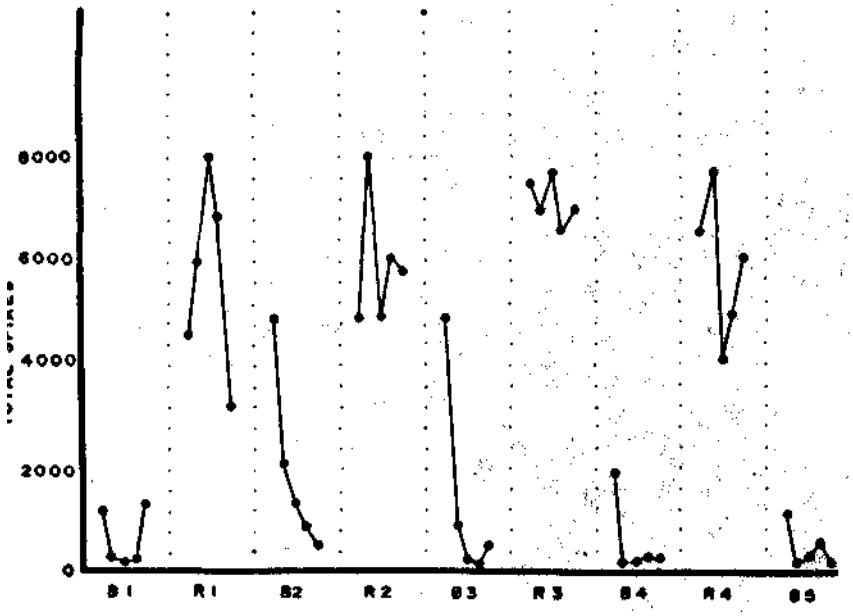


FIGURE 6.4. Unit discharge of a reinforced FTG cell. Each point represents 2 minutes. Key: B, base line (no reinforcement) periods; R, reinforcement periods. The cat was presented with a discriminative stimulus signaling reinforcement periods. Note the increased discharge during reinforcement and successive extinction curves in base line periods. From Breedlove et al. (1979) by permission.

cell was also recorded. Ten-minute reinforcing sessions alternated with ten-minute time out periods. Unit discharge was significantly increased by the procedure (Fig. 6.4) and experimental cells had a significantly greater increase in discharge during reinforcement than simultaneously recorded control cells.

The initial stages of training were accompanied by a general increase in motor activity. However, as reinforcement sessions progressed, the movement pattern became more specific. For example, during the later reinforcing sessions in one experiment, the cat sat quietly in its litter box bobbing its head up and down and receiving reinforcement. Before and during conditioning, the cell reinforced in this experiment was observed to fire whenever the animal moved its head up. The repetitive movements associated with conditioning corresponded well with the behaviorally determined correlate of discharge.

We attempted to determine if proprioceptive feedback is necessary for operant control of RF unit activity in further studies. Two cats, after having been trained to increase unit discharge as described above, were anesthetized with Halothane. We then inserted an endotracheal tube and in-

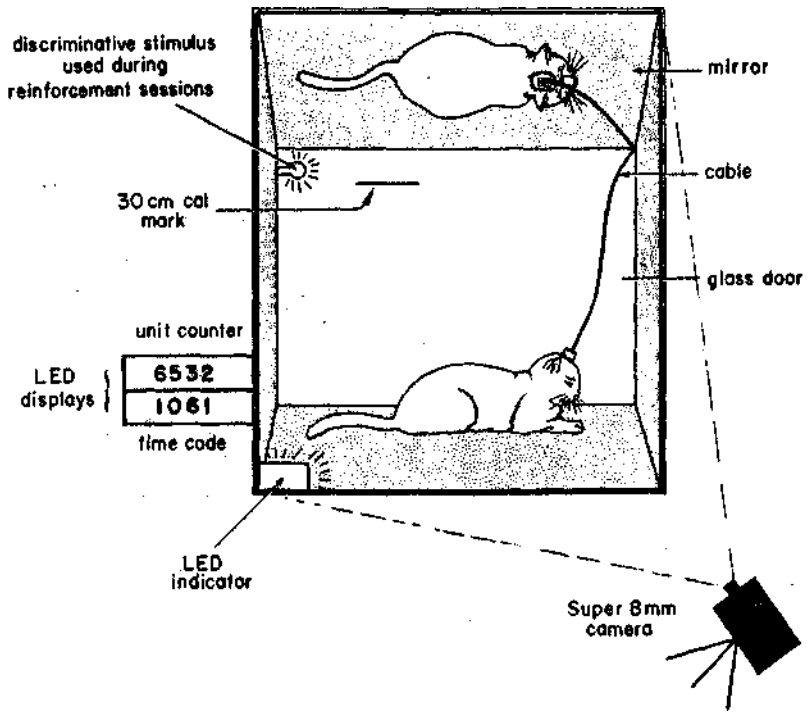


FIGURE 6.5. Arrangement of equipment used for filming unit discharge movement relations. From Siegel et al (1979a) by permission.

travenous catheter. After placing the cat in a comfortable position, we injected Flaxedil, discontinued the Halothane, and began artificial respiration, monitoring CO_2 and EEG as well as unit activity. We found that even in these conditions it was possible to reinforce RF unit activity with hypothalamic stimulation. Therefore, at least in some units, proprioceptive feedback is not essential for achieving operant control of RF activity.

3. PHOTOGRAPHIC ANALYSIS OF THE RELATION BETWEEN UNIT ACTIVITY AND MOVEMENT

We have developed a photographic procedure that allows us to more quantitatively describe the time-course and topography of RF unit activity-movement relations in unrestrained cats (Siegel et al., 1979). Two LED counters are attached to the side of an experimental chamber equipped with a mirror to allow simultaneous observation of top and side views of the cat (Fig. 6.5). One counter is set to increment whenever the unit discharges; the other displays a time code to allow correlation with a simultaneous tape recording of unit and other electrophysiological data. The

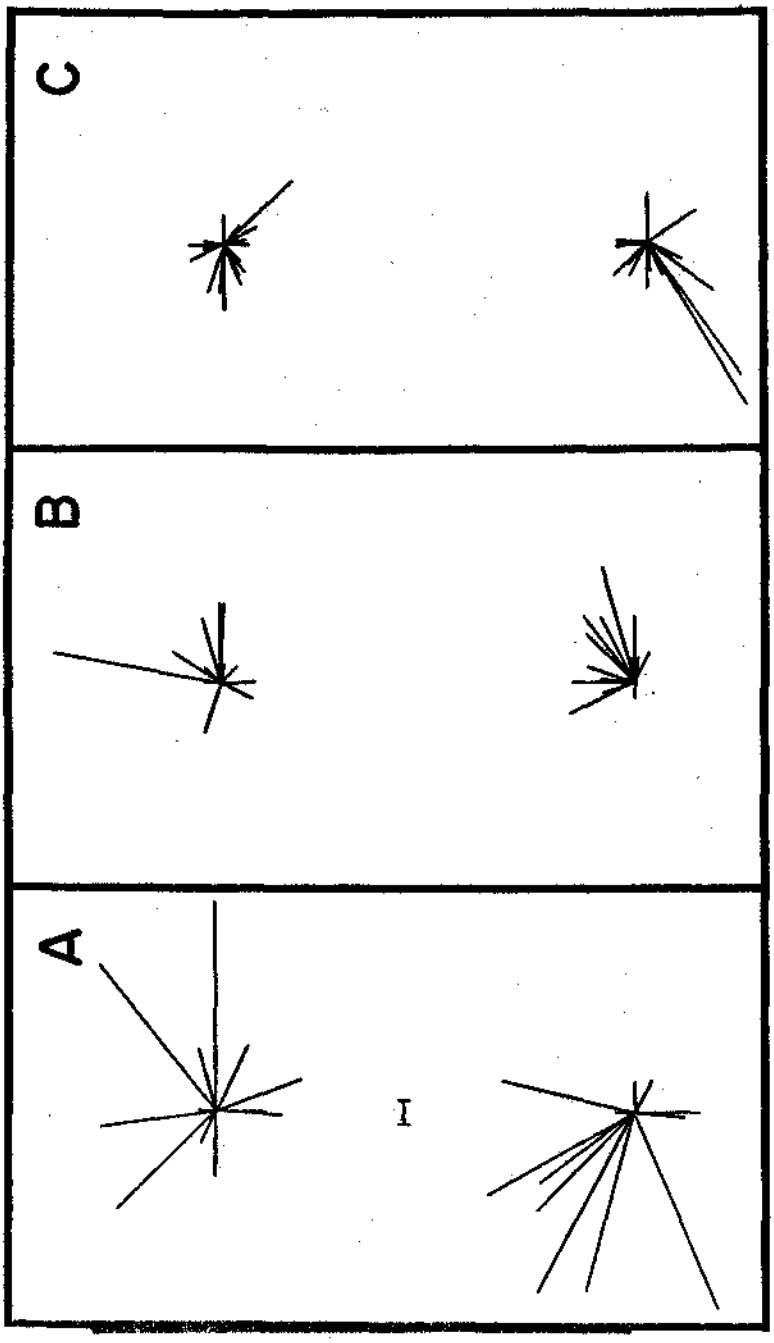
chamber and counters are then filmed with an 8-mm "existing light" jector. Frame by frame analysis on a viewer allows One to plot movement vectors accompanying unit discharge. The simultaneously recorded time code signal allows the experimenter to verify that no noise has triggered the unit counter.

We found that unit discharge was accompanied by movements of from 10 to 30 mm within the same 56-msec interframe interval (Siegel et al., 1979). The movements associated with activity in each unit shared a common directional vector, which was in close agreement with the visually observed movement relation of the cell (Fig. 6.6). In the interframe interval preceeding unit discharge, the movement vectors were randomly distributed. The differences were significant.

Both the photographic and operant reinforcement techniques independently confirm our behavioral analysis procedures. However, since our behavioral procedures can provide much of the same information in less time, we do not use photographic and reinforcement techniques on all cells we encounter. We do, however, continue to use them to document new cell types.

4. SLEEP STUDIES

We observe all the units we record during sleep. The sleep studies have two overlapping goals. The first is to identify cells that might have a role in generating one or more states of sleep. The second is to observe the sleep activity of a cell with known behavioral correlates in waking and, therefore, allow us to make inferences about the interrelations between brain activity in these two states. It is generally accepted that REM sleep is generated by brainstem mechanisms, although the exact anatomical location of the cells underlying these mechanisms is uncertain. One would expect cells involved in the control of REM sleep to have a unique pattern of discharge during this state. They might fire only or mainly during this state, they might be silent only at this time, or they might change their pattern of discharge in some way. Previous reports had indicated that virtually all cells in the medial PRF [or "FTG" in Berman's (1968) terminology] discharged selectively in REM sleep (Hobson et al., 1974). We have not found this to be the case (Siegel et al., 1977, 1979; Siegel & McGinty, 1977). We found three cell types in the FTG region. Type 1 had no spontaneous activity during quiet waking and sleep, discharging only during movements. Type 2 had high rates of tonic activity during both quiet waking and SWS, which further increased during waking movement and REM sleep. Type 3 had low activity rates during quiet waking and SWS, but discharged in bursts during both waking movements and REM sleep.


C
B
A
I

In every cell we have so far encountered with substantiatlREM sleep activity, we also observed comparable levels of waking activity. Indeed, there was a very strong positive correlation between waking and REM sleep activity rates (Fig. 6.7). The reason previous researchers reported that these cells discharge selectively in REM sleep was apparently because they used cats that were adapted to quietly tolerate head restraint. These procedures effectively eliminated most waking movement and correlated unit discharge, thus making the cells appear to be selectively active in REM sleep. These studies provide a good illustration of how the elimination of behavior from a preparation, far from clarifying the situation, can be misleading.

In studies of unit activity, experimenters will often give a value for waking discharge rate and other values for REM and non-REM sleep rates. In some cases, two waking rates are given, one for "active" and another for "quiet" waking. However, it should be obvious that because of the relation of PRF cells to specific movements, rates in "active" waking are a very crude, variable measure and may vary considerably depending on exactly what kinds of movements the animal is making (Fig. 6.8). We have tried to deal with this problem by providing maximum and minimum rates for the waking state, to give numerical expression to the range of activity visible in waking. However, ultimately there is no substitute for systematic observation and stimulation in determining the range of unit activity.

The positive correlation between RF activity rates in active waking and REM sleep can be most parsimoniously understood as a correlate of the motor activation of REM sleep. During this state, the eyes move rapidly as in active waking, the facial muscles and distal limb muscles twitch, and units throughout the brain's motor systems become active. Thus, the positive correlation between the waking and REM sleep activity of medial RF

FIGURE 6.6. A. *Upper:* Vectors describing the movement of the cat's head during the film frames spanning the 56-msec period before the onset of unit activity. Center point for each vector plot represents initial head position and terminations of lines represent head position one frame later. Calibration mark is 10 mm. *Lower:* vectors describing the movement of the cat's head during the film frames spanning 56 msec period in which the unit began to discharge. Note the nearly random distribution of vectors in the upper portion of the figure, and the preponderance of movements toward the upper left-hand quadrant accompanying the onset of unit activity in the lower portion of the figure. B. Note the shift in movement to upper right-hand quadrant during onset of unit activity. C. Note the shift in movement in the left-hand quadrants during the onset of unit activity. From Siegel et al (1979a) by permission.

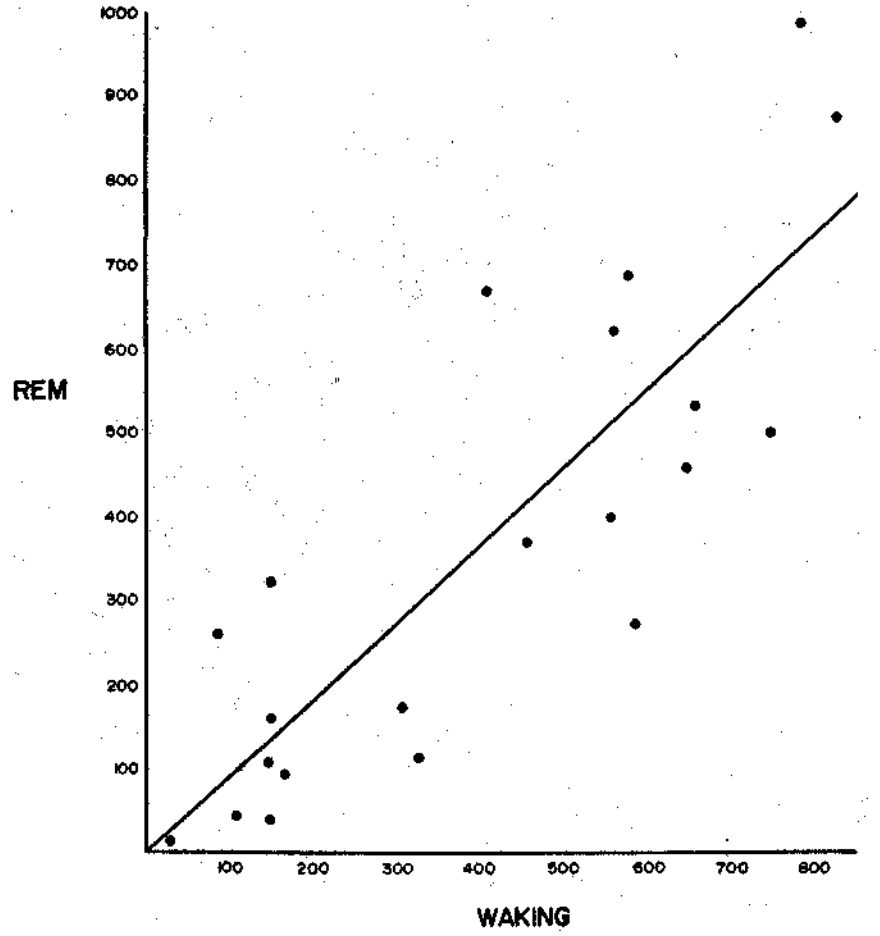


FIGURE 6.7. A scatter plot showing the positive correlation of waking and REM sleep rates in medullary cells, and the least squares best fit line. Maximum 10-sec REM sleep and waking counts are plotted. From Siegel et al (1979b) by permission.

cells provides a quantitative measure of the similarity of central motor activation in these states.

D. DISCUSSION

There is little doubt that as the mechanical and behavioral technology for the behavioral study of unit activity in unrestrained animals becomes better understood these procedures will become an indispensable starting point for the investigation of neural activity. The great advantage of the behavioral approach is that it allows one to describe the cell's relation to

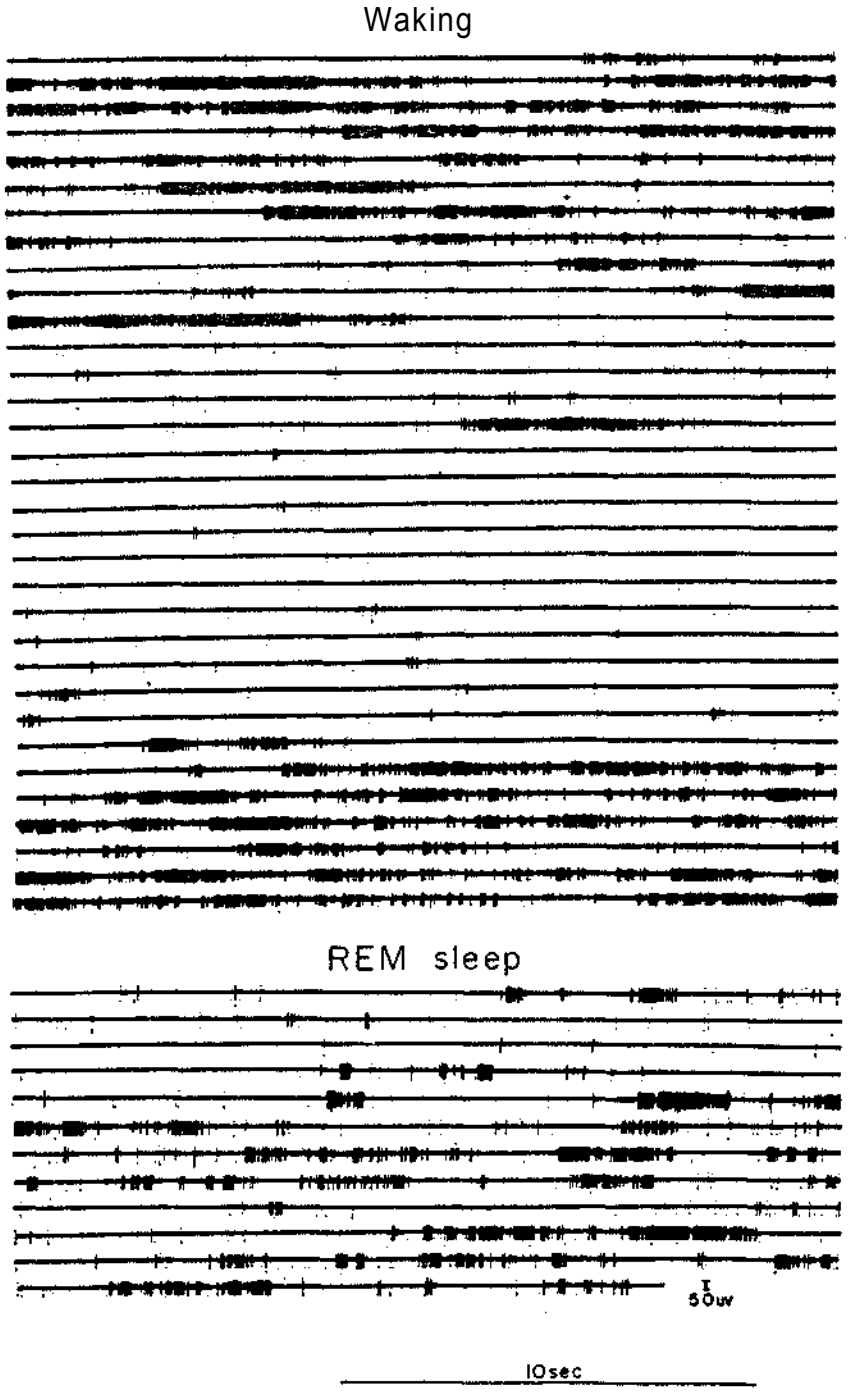


FIGURE 6.8. Two continuous samples of the amplified unit recording of an FTG cell. The upper portion is an 11-min waking sample, and the lower is a 4-min recording spanning an entire REM sleep period. Note the variability of rate. "Average" waking rates would be a function of the cat's behavior during the sample period. From Siegel et al (1977) by permission.

behavior in the broadest possible terms, on the basis of direct observation. This is preferable to the premature use of highly controlled and restricted studies (also see Ranck et al., Chapter 5, this volume). Concepts of the role of RF cells in behavior as diverse and vague as arousal, sensory integration, anxiety, and reward anticipation have advanced largely as a result of studies in restrained or motionless animals. Our observation that these cells discharge in relation to specific movements in the unrestrained cat suggest a new perspective for interpreting prior studies. The response of RF cells to sensory stimuli and pain may reflect motor responses to these stimuli. Similarly, changes in RF activity during the emotional states created in previous studies may reflect alterations in muscle tone and movement patterns caused by the experimental situations; Only careful observation of and control for the exact movements correlated with unit activity in these situations can determine if our explanation is valid. In one recent study, in which movements were quantified, it was found that midbrain RF activity changes during learning could be precisely explained by the correlation between midbrain RF unit activity and specific head movements occurring during the task (Lecas and Malmo, 1981). Observation of unit activity during behavior is the most direct way of identifying the functional relations of cells. Behavioral control, after all, is not just one of the many functions a cell has. Rather, behavioral control, defined in its broadest sense, is *the* function of brain cells (also see Sperry, Chapter 3, this volume).

In many brain regions, anatomically adjacent cells can have completely different patterns of inputs and outputs arid, presumably, completely different functional roles. Thus, the usual acute electrophysiological techniques are often difficult to interpret. Electrical stimulation not only excites fibers of passage, but may also recruit adjacent, functionally distinct, cells and may therefore create a misleading impression of diffuse control. Anridromic identification is another valuable technique for identifying cell projections. However, it is virtually impossible to gauge the relative importance of various projections or to infer the behavioral role of these cells from such studies. Often the monosynaptic effects caused by a cell's activity are minor relative to its polysynaptic effects (Peterson, 1979; Jankowska et al., 1968). However, acute neurophysiological studies are often limited to an analysis of monosynaptic effects. It therefore should not be surprising that much of the electrophysiological data, which emphasizes the diffuse, nonspecific effects of RF activation, stands in such dramatic contrast to the highly specific motor relations that can be seen in behavioral studies.

Although careful observation in the unrestrained animal can help identify the behavioral role of a cell, one must be cautious in interpreting the

data obtained. A cell that discharges whenever the animal makes a specific movement need not be causing that movement. Experimental analysis is needed to determine the cell's precise role. If a cell can also be activated when the muscles involved contract isometrically and when the animal is curarized, one can rule out an essential role of proprioceptive and tactile feedback in its activation. These sorts of experiments can considerably narrow the range of possible functions of the cell. However, one must still be cautious in inferring the synaptic mechanisms underlying the behavior. Even latency studies are not definitive. If one can show that unit firing precedes the observed movement by some constant duration, one need not conclude that this activity is causing the movement. Most body movements result from complex sequences of muscle contractions. If one is monitoring a muscle, or a movement that occurs late in some specific sequence, and records a unit that is related to a muscle contraction early in the sequence, one will see a clear, relatively invariant lead that might suggest that the unit was causing the observed movement, even if, in fact, it was *responding* to an early aspect of the movement. This is a particularly likely error if the movement is related to axial musculature, since the action of this musculature may not be obvious, even though it often contracts in conjunction with or prior to (Bizzi et al., 1971) limb and other movements. Since most RF cells are related to axial movements (Siegel, 1979a, b), one must be especially cautious in implying their synaptic mechanisms from behavioral data. One should also consider the possibility that activity early in the movement sequence may have roles other than the excitation of motorneurons. Various kinds of corollary discharge to sensory receptors and the excitation and inhibition of specific interneuron pools may occur prior to or in conjunction with motoneuron depolarization.

Thus, both behavioral and acute electrophysiological techniques used alone have serious drawbacks. Just as one cannot determine the synaptic connections of a cell by using only behavioral techniques, one cannot determine the behavioral role of a cell solely through knowledge of its synaptic connections. However, when used together, behavioral and anatomical techniques can complement each other. For example, we need to know how the projection pattern of an RF cell related to tongue movement differs (we hope) from those of an adjacent cell related to limb movement. Recent findings (e.g., Evinger et al., 1979) suggest that the apparently obvious answers to these types of questions may not be correct. Anatomical projections may not always be simply related to behavioral role. Conversely, we need to know how the behavioral correlates of RF cells with ascending projections differ from those of adjacent cells with descending projections. We need to know if behavioral correlates are pharmacologi-

cally coded, i.e., Do cholinergic cells have different behavioral relations than adjacent adrenergic cells? We must also determine if there are morphological correlates of a cell's behavioral relations. These questions can only be answered by performing behavioral, electrophysiological, pharmacological, and anatomical studies of the same cells.

ACKNOWLEDGMENT

Supported by the Medical Research Service of the Veterans Administration USPHS Grant NS14610 and NSF Grant BNS8200023. -