A STUDY OF CONTINUOUS FORTY-EIGHT HOUR SLEEP-WAKING RECORDINGS IN FIVE DOGS

By

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CHAPTER I

INTRODUCTION

All people and probably all higher animals sleep (Dement and Kleitman, 1957a; Jouvet, 1967). Kleitman (1963), in the well known classic Sleep and Wakefulness, listed all the significant articles published on the subject; there were only 4,300 citations. Over 12,000 papers have been published during the last 13 years generating much information describing sleep stages in a variety of animals, including man, and the possible role of certain brain structures and neurochemicals in controlling sleep-waking behavior. Sleep, once thought to be a passive phenomenon, is an active process having at least two major stages and involving several different interacting brain mechanisms (Jouvet, 1967). Although the application of sophisticated biochemical, histochemical, neuroanatomical, and neurophysiological methods have contributed much to our understanding of sleep mechanisms, such basic questions as why an animal must sleep, how it goes to sleep, what controls the cycling of different sleep states, and how an animal wakes up, are still largely unanswered (Jouvet, 1967; Lewis, 1974). Attempts to experimentally induce disruptions in sleep-waking cycles of animals based on present neuroanatomical and neurochemical hypotheses have generally yielded poor models for human sleep disorders (Mitler, et al., 1974; Mitler and Dement, 1974; Mitler, 1976).

Early experiments seemed to indicate that sleep was necessary for

sustained mental and physical performance (Klemm, 1970). However, there has been great difficulty substantiating unambiguous physiologic changes after sleep loss (Mitler and Dement, 1974).

There are three phases of waking: alert, quiet, and drowsy. Awake animals may have open or closed eyes, be standing, sitting, or lying down. They are usually responsive to sound and sight or are aroused very easily. Their electroencephalogram (EEG) patterns are described as desynchronous and are characterized by low voltage and frequencies of 15-30 cps with no dominant rhythm. As the animal progresses to quiet waking, then drowsiness, the EEG contains progressively more high voltage slow activity (frequency) patterns with 1-3 cps superimposed on the alert pattern (Jouvet, 1967).

Two distinct modes of sleep can be distinguished in mammals. Slow-wave or non rapid eye movement (NREM) sleep has a higher threshold of sensory stimulation than waking; the eyes are usually closed; the animal is in sleep posture with some postural tonus; the EEG pattern has high voltage and slow activity with some sleep spindles (11-16 cps, high voltage); it is usually associated with decreased heart rate, respiration, and blood pressure. After a period of time in NREM sleep (variable depending on the species), the normal adult enters rapid eye movement (REM) sleep. REM sleep (also called paradoxical, active, or deep sleep) is typified by lack of muscle tone with an occasional sudden onset of phasic motor activity such as paddling of limbs, REM, and vocalization; the arousal threshold in response to sensory stimulation is greater than in slow-wave sleep; the EEG tracing shows low voltage fast activity patterns, similar to the waking patterns; autonomic signs occur such as increased heart rate, blood pressure, and respiration, and erection of

the penis or vascular engorgement of the vagina; REM sleep is associated with dreaming in humans (Dement and Kleitman, 1957a; Jouvet, 1967.

Twenty-four hour continuous polygraphic and behavioral observations of a variety of mammals indicate that individuals within a species tend to have similar sleep-waking patterns. The average human sleeps slightly more than seven hours per night; this represents approximately 33% of a 24 hour cycle. Horses, sheep, and cows spend a majority of their 24 hour period in wakefulness or drowsiness, with only 15% in a sleep state. Pigs, and humans, spend approximately 33% of the circadian cycle in a sleep state (Ruckebusch, 1972). Adult cats spend approximately 55% of a 24 hour period in sleep. Throughout sleep periods, alternating cycles of slow-wave sleep and REM sleep may be repeated as many as three or four times; in normal animals, REM sleep is always preceded by slow-wave sleep. Adult mammals which have been studied spend from 15% (sheep) (Ruckebusch, 1972) to 33% (cats) (Jouvet, 1967) of their total sleep time in REM sleep. The amount of REM sleep in newborn animals is great (90% in kittens and human babies), decreasing to much lower amounts in adult animals (Jouvet, 1967). A major portion of the research on sleep-waking mechanisms has been conducted on cats because of the large amount of time they spend sleeping and their adaptability to laboratory conditions.

Procedures which have been successfully utilized for long term continuous polygraphic studies on mammals such as the rat and rabbit (Dement and Kleitman, 1957a), horse, cow, pig (Ruckebusch, 1972), and cat (Jouvet, 1967; Jouvet, 1974), are not easily adaptable to the surviving dog. They have included implantation techniques which would preclude their use on survival or clinical animals, or they have not had adequate electrode and lead fixation to accommodate the anatomy and behavior of

the dog. Although each of the sleep stages has been recorded in the dog (Mitler, et al., 1974; Stanton and Fox, 1967), few long term continuous polygraphic studies have been published which describe the sleep-waking cycle in normal healthy dogs under controlled cage conditions.

The dog is presently being used as a model for human sleep disorders such as narcolepsy-cataplexy (Jouvet, 1974; Koe and Weissman, 1966). Information from these models is limited without normal values for the dog. The primary purpose of this study was to describe the sleep-waking patterns of the normal dog. It was also the purpose of this study to develop a noninvasive method for recording sleep-waking patterns of dogs that could be used on private veterinary patients.

CHAPTER II

LITERATURE REVIEW

General History of Sleep-Waking Research

Prior to the early 1900's only one state of sleep was assumed. Derbyshire, et al. (1936), the first to use the electroencephalogram (EEG) to study sleep, described two states of sleep in cats. In one state the animal was laying down, with nictitating membranes relaxed and neck muscles exhibited less tone than in waking. The EEG associated with this state consisted of 1-4 cps high voltage slow waves and 11-16 cps sleep spindles of large amplitude. They called this state slow-wave sleep because of the prevalence of slow EEG activity. The second, a "less quiet state of sleep" was associated with occasional twitching of the limbs. It had a low voltage fast cortical EEG activity pattern and between twitches, the animal exhibited very little postural tone. They called it fast sleep and considered it to be similar to the alert state.

Unfortunately Derbyshire's discovery of two states was forgotten until the early 1950's when Aserinsky and Kleitman (1953) noticed a period of increased activity in sleeping human babies. They observed this activity in adults and found that subjects awakened during this period of activity and rapid eye movement (REM) reported dreaming more frequently than when awakened during slow-wave sleep. This was confirmed when Dement and Kleitman (1957b) in their studies in man and Dement (1958) in his work with cats demonstrated that the periodic recurrence of

activated sleep was related to REM's resulting in the name REM sleep. At this time, however, it was interpreted as an intermediate phase between slow sleep and arousal. Dement and Kleitman (1957a) also described cycles of EEG activity which could be expected during the course of a typical night in a normal subject. Dement (1958) summarized sleep as the electrical brain activity of two recurring patterns. The first, called slow-wave sleep, manifests itself in the presence of synchronized cortical activity and sleep spindles and/or high voltage slow waves. The other reveals itself by a low voltage fast cortical rhythm similar to arousal activity and is called activated, REM, or paradoxical sleep.

Jouvet (1962) found that the threshold for behavioral arousal by electrical stimulation of the reticular formation was much greater (up to 300%) in REM sleep than in slow sleep. This indicated that although REM sleep has EEG behavioral patterns similar to waking, it is "deeper" than slow sleep in the sense of arousal threshold. This is approximately the present day concept; however, slow sleep is frequently called nonREM (NREM) sleep for convenience.

Structural Influences on Sleep-Waking

The experiments of Moruzzi and Magoun (1949) indicated that the brainstem reticular formation is important for cortical and behavioral arousal via the ascending reticular activating system (ARAS). Utilizing electrical stimulation experiments which induced arousal, they found the ARAS extended from the medulla to the posterior diencephalon when delimited.

Hodes and Dement (1964) and Pompeiano (1970) observed an active and forceful inhibition of motor output at the spinal level exclusively in

REM sleep. There was an abolition of tonic electromyographic (EMG) potentials that were present in waking and NREM sleep. Jouvet (1961) found that destruction of the medial and descending vestibular nuclei suppressed REM bursts but not isolated eye movements during paradoxical sleep. These nuclei, the unit activity of which is increased during REM sleep, apparently control most of the phasic phenomenon during that stage.

Jouvet (1972) found that pontine lesions inhibited REM sleep while electrical stimulation of that area increased the amount of REM sleep. He was the first to describe the "spontaneous phasic potentials" during and just prior to REM sleep from the pontine reticular formation and the geniculate and occipital areas (PGO spikes). These spikes, and the evidence from the lesion and electrical stimulation studies, implicated the pontine brainstem structures in the generation of REM sleep.

Jouvet (1972) felt that NREM sleep was dependent on the functional integrity of serotonergic neurons of the raphe nucleus and REM reflected the activity of catecholamine cells of the locus coeruleus. Traditionally these parts have been considered part of the ARAS which is associated with maintaining arousal and facilitation of motor activity. This contradiction may be resolved by the results of Chase and Babb (1973). They concluded that certain brain areas can exert opposite functions depending on the behavioral state of the animal. They found that stimulation of the reticular tegmentum facilitated masseteric muscle reflex activity in waking and NREM sleep while it inhibited the reflex in REM sleep.

McCarley and Hobson (1971) and Hobson, et al. (1975) found that neurons in the gigantocellular tegmental field (FTG) of the cat's pontine brainstem had a dramatic increase in discharge rates during REM sleep.

The ratios of discharge rates in REM sleep to those of waking and NREM were 5-10 times higher than those of neurons in adjacent tegmental fields and 25-30 times higher than in other brain sites. This indicated to these investigators that the gigantocellular neurons have an active role in generating the REM phase. Neurons localized in the posteriolateral pole of the nucleus locus coeruleus and nucleus subcoeruleus had firing rates that decreased as the animal entered NREM sleep from waking (Hobson, et al., 1975).

Biochemical Influences on Sleep-Waking

The major neurotransmitters that are considered to be involved in sleep-waking are serotonin (5-HT) and norepinephrine (NE). One technique for selectively eliminating 5-HT was developed by Koe and Weissman (1966). They found that p-chlorophenylalanine (PCPA) blocked the first step in 5-HT metabolism. The studies by Delorme, et al. (1966) and Mouret, et al. (1967) on cats demonstrated (1) PCPA had no direct pharmacological action on the brain as indicated by an unchanged sleep pattern during the first 18-24 hrs. after dosing; (2) an abrupt decrease of both NREM and REM sleep occurred after 24 hrs. with almost total insomnia (maximum at 40 hrs.) after 30 hrs.; recovery began after 40 hrs. and the animal was normal at about 200 hrs.; and (3) the normal sleep pattern was restored within 6-8 hours when 5-hydroxytryptophan (a 5-HT precursor) was given when insomnia was at its maximum. Similar studies indicated NE depletion leads to NREM sleep while adrenergic activity leads to arousal (Jouvet, 1972).

Pin, et al. (1968) developed a histofluorescent technique allowing the perikarya containing 5-HT and NE to be mapped. He found cells

containing 5-HT concentrated in the raphe system. Destruction of this system led to insomnia which correlated with a decrease of cerebral 5-HT.

NE perikarya appear concentrated in the dorsolateral locus coeruleus with rostral projections primarily via a neurochemically homogeneous fascicle designated as the dorsal noradrenergic bundle. The pathway runs in the dorsal tegmentum, ventrolateral to the medial longitudinal fascicle, and is diffusely distributed via the medial forebrain bundle throughout the cerebral cortex and hippocampus (Olson and Fuxe, 1972). Hypersomnia occurs when the dorsal NE bundle is destroyed (Panksepp, et al., 1973). A neuroanatomically distinct ventral pathway that ascends through the mesencephalic tegmentum has an unknown function in sleep-waking (Olson and Fuxe, 1972; Ursin, 1976).

Monoamine-oxidase (MAO) inhibitors suppress REM sleep and usually increase NREM sleep, suggesting that MAO is required for the transition between NREM and REM sleep (Jouvet, 1972). Reserpine, which releases monoamines at the monoaminergic terminals, can selectively trigger phasic EEG components of REM sleep (PGO spikes). The atonic component of REM sleep appears to be dependent on a cholinergic mechanism because it is suppressed by atropine. It also appears to need a catecholaminergic mechanism as DOPA is able to induce normal REM sleep after reserpine has been administered (Jouvet, 1972).

Based on many of the above observations, Jouvet (1974) proposed a biochemical model for the regulation of sleep by two antagonistic ascending systems of neurons. He theorizes that 5-HT neurons of the nucleus raphe induce slow sleep and prime REM sleep while the NE neurons of the locus coeruleus are responsible for waking and REM sleep.

Ontogenesis of Sleep-Waking

Newborn animals have a different sleep pattern from adults. Kittens and neonatal rats have REM patterns immediately after birth while NREM sleep is almost nonexistent. REM sleep comprises 80-90% of this behavioral sleep and occurs immediately after waking with no intervening NREM phase. The phasic phenomena in REM sleep at that time overshadows the tonic phenomena. During cortical maturation NREM sleep appears and increases progressively while REM sleep decreases. Behavioral sleep in the adult cat has about 70% NREM and 20-25% REM sleep (Jouvet, 1967).

Stanton and Fox (1967) did extensive observational studies in developing puppies. They observed a rapid decline in the percent of REM sleep during the early postnatal period. NREM sleep first emerged after two weeks. There was a gradual development of ability to maintain wakefulness (or attentiveness) in a novel environment. This ability was fully developed at about four weeks, which was also the onset of a relatively mature EEG. This indirectly suggested to the authors that reticular activation attains adult functional integration with thalamocortical structure at that time.

Sleep-Waking Studies in Various Animals

Dement, et al. (1966) suggested that the dichotomous nature of sleep was common to all mammals. REM and NREM sleep have been observed in the cat (Dement, 1958; Derbyshire, et al., 1936), dog (Stanton and Fox, 1967), rat (VanTwyver, 1969), monkey (Weitzman, 1961), sheep, (Ruckebusch, 1972), and most other mammals studied except possibly the Echidna (spiny anteater) (Allison and Goff, 1968). Chickens, hens and pigeons have a sleep stage that resembles NREM in mammals but birds' REM

periods are very short, about 10 sec. Only NREM has been recorded from the tortoise (Ruckebusch, 1972).

There have been many sleep-waking studies on the cat. Lucus and Sterman (1974) reported values for waking, NREM, and REM sleep as percents of a 24 hr. period as 45%, 39%, and 15%, respectively. These values are similar to other studies (Jouvet, 1961; Jouvet, 1967; Jouvet, 1974; Lucas, et al., 1976). Lucas and Sterman (1974) noted that cats had two periods of increased sleep in a 24 hr. day, one around 1:00 P.M., the second around midnight.

VanTwyver (1969) studied the sleep patterns of five rodent species using implanted electrodes (rats, squirrels, hamsters, mice, and chinchillas). He found (1) well defined stages of NREM and REM sleep in all five species; (2) sleep occupied from 52-60% of each day for all species; (3) the percent of REM sleep differed for each species varying from 10% in mice to 24% in hamsters and squirrels; and (4) hibernators (hamsters and squirrels) slept for longer periods, had a higher percent of REM sleep and total sleep, and slept deeper than nonhibernators (rats, mice, and chinchillas). Sleep spindles, which are prominent in primate and cat EEG's, were less frequent in the rodent EEGs.

Ruckebusch (1972) studied the sleep-waking patterns of four farm animal species (horses, cows, sheep, and pigs) using implanted electrodes. Horses, cows and sheep spent about 83-88% of the day awake while pigs spent only 67% of the time awake. Drowsiness was considered as a state apart from alert waking. It was defined as an intermediary state between alert waking and NREM sleep characterized by a mixture of low voltage fast activity and high voltage slow activity patterns in the cortical EEG concurrent with a small decrease in muscular tone. The percent of waking

spent in drosiness varies from 37% for the cow to 9% for the horse. One general conclusion by Ruckebusch was that herbivores had a lower percent of REM sleep in a 24 hr. period than carnivores.

The EEG sleep patterns recorded from farm animals by Ruckebusch were qualitatively similar to those in the cat; however, there were minor peculiarities in each species. Horses were able to sleep while standing, probably due to the stay-apparatus of their legs. During waking, pigs had an alpha rhythm (8-13 cps) while horses had a theta rhythm (4-7 cps). Sheep had sleep spindles associated with drowsiness while cows had a delta rhythm (1-3 cps), especially when rumenating. Pigs had a very rapid decrease in postural tone that often disappeared before the onset of NREM sleep. Sheep, however, lost postural tone slowly and some a activity persisted into REM sleep. Horses also lost muscular tone gradually till the middle of the NREM period when it became negligible. In cows the abrupt loss of postural tone only occurred at REM onset. The eyes were completely closed during NREM sleep in the pigs and partially closed in the sheep, cows and horses. All the species had closed eyes during REM sleep.

There are few 24 hr. studies in the literature using adult dogs. Most of them involve sleep disorders in dogs (Mitler, et al., 1974). Frequently normal feline values are applied to the dog but experimental evidence indicates this assumption is incorrect. Lucas, et al. (1976), using cats and dogs with implanted cortical electrodes, indicated that dog values were different from cat values. He reported 65%, 23%, 13% for waking, NREM sleep, and REM sleep, respectively, in a 24 hr. period for the dog, and 49%, 37%, 14% for waking, NREM sleep, and REM sleep, respectively, in the cat. He noted a distinct circadian rhythm in the

dog's sleep pattern with maximum sleep occurring around 10:00 P.M.

Narcolepsy - A Disorder of the Sleep-Waking Mechanism

Several major types of sleep disturbances such as sleep apnea, insomnia and narcolepsy have been described in the human being (Lewis, 1974). The syndrome of narcolepsy is one of the most dramatic. Mitler and Dement (1974) state that this syndrome is a "life-long debilitating illness which affects between 100,000 and 150,000 Americans" annually. The most characteristic feature of narcolepsy, according to Sours (1963), is the occurrence of relatively sudden, irresistible attacks of sleep. About 80% of these patients also exhibit cataplexy, which is usually precipitated by a strong, emotional experience. Cataplexy is characterized by sudden transient loss of muscle tone, so that the patient falls to the ground without loss of consciousness. Sleep paralysis (transient loss of muscle tone just prior to or following sleep) and hypnogogic hallucinations may occasionally be associated with sleep episodes in narcoleptic patients (Lewis, 1974; Sours, 1963).

The pathophysiology of this syndrome (narcolepsy-cataplexy) is presently unknown (Zarcone, 1973). It has been suggested that REM sleep mechanisms may be important in its appearance and development. The daytime sleep attacks could be an inappropriate intrusion of REM sleep into wakefulness. Although cataplexy is not a state of sleep in the conventional sense, it could be a sleep process since REM sleep is the only state in which tonic electromyographic (EMG) activity normally disappears in control subjects (Rechtschaffen, et al., 1963; Wharton, et al., 1971). Long term polygraphic studies on human narcoleptic patients have

demonstrated the abnormal presence of sleep onset REM periods and wake-like low voltage fast activity EEG patterns during cataplexy (Rechtschaffen, et al., 1963; Sours, 1963; Zarcone, 1973).

Attempts to produce animal models of narcolepsy using drugs, electrical stimulation, or lesions based on Jouvet's theories of sleep control, have had limited success (Mitler and Dement, 1974).

A syndrome believed to be analogous to narcolepsy in the human has been described in dogs. Oliver, et al. (1973) described a dog which suddenly developed a "bobbing gait" (manifestation of cataplexy) or collapsed and appeared to sleep for a few seconds. This occurred with emotional excitement such as eating and could be reversed with arousing stimulation such as sound. Mitler, et al. (1974) and Mitler and Dement (1974) have reported two dogs which demonstrated narcolepsy-cataplexy. Both dogs collapsed when given food, when called, or in response to other emotional stimuli. Recent polygraphic studies on one of these dogs demonstrated sleep onset REM periods and a normal low voltage high frequency EEG; no information was obtained regarding total time spent in each of the sleep stages. Two dogs have been reported as narcoleptic at the Oklahoma State University College of Veterinary Medicine (Rubin, 1975).

Accurate polygraphic and behavioral descriptions of sleep-waking cycles and seizure activity in a dog exhibiting behavioral symptoms of narcolepsy-cataplexy can establish the suitability of this animal as a model for studying the mechanisms, management, and possible therapy of this condition in the human (Chase and Dement, 1973).

CHAPTER III

MATERIALS AND METHODS

Preparation of the Dogs

Five mature, mixed, 15-20 kg, male and female dogs were used. A physical examination, hemogram, blood glucose, blood urea nitrogen and urinalysis were done to establish that all the experimental animals were clinically normal; they all had current rabies and distemper vaccinations. The dogs were recorded at different times over a period of two months.

The Dog's Environment

The dogs were acclimated to the cage, room and regimen during a two week preparatory period. The dogs had contact with humans (8:00 A.M. and 5:00 P.M.) only when they were fed, watered, walked outside and the cage cleaned; they were isolated from sight and sound the remainder of the time. The room light was kept on from 6:00 A.M. to 10:00 P.M. The cage faced away from the entrance to the room and the physiograph to minimize visual and auditory stimuli (Figure 1). An observation window on the cage top (4 cm x 6 cm) and the cage door were the only areas light could enter the cage (cage description, Appendix A). The floor of the room was covered with a rubber pad to minimize noise; a small lamp on the machine aided the observer while the room light was off.

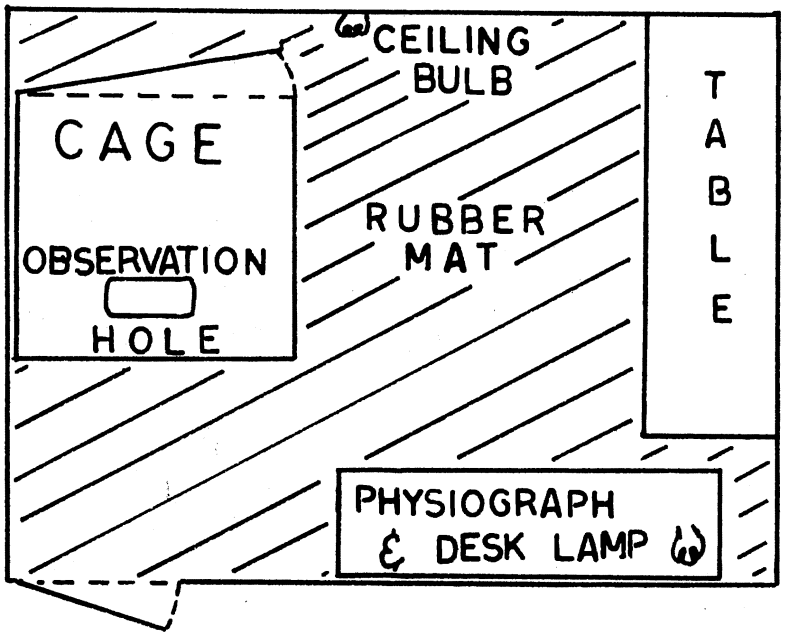

Figure 1. Layout of Recording Chamber

Electrode Attachment

The electrodes were attached to the dog's skin under general anesthesia. Food was withheld 12 hrs. prior to surgery. An intramuscular injection of acetylpromazine (Acepromazine^{R}) at a dose of .11 mg/kg (maximum 3 mg) was administered 30 minutes before an intravenous injection of thiamylal Na (Surital^{R}) at a dose of 17.6 mg/kg was administered for general anesthetic. The following ten skin areas were prepared for electrode attachment by shaving circles one inch in diameter and wiping them with 70% alcohol to remove excess dirt and oil (Table I).

TABLE I

ELECTRODE ATTACHMENT SITES

Electrode Lead Number	Anatomic Area (Figure 2)
1	right frontal area of head
2	left frontal area of head
3	right occipital area of head
4	left occipital area of head
5	just above left upper eyelid
6	at lateral canthus of left eye
7	right dorsal mid neck
8	left dorsal mid neck
9	over frontal sinus
10	left mid thorax at fourth rib

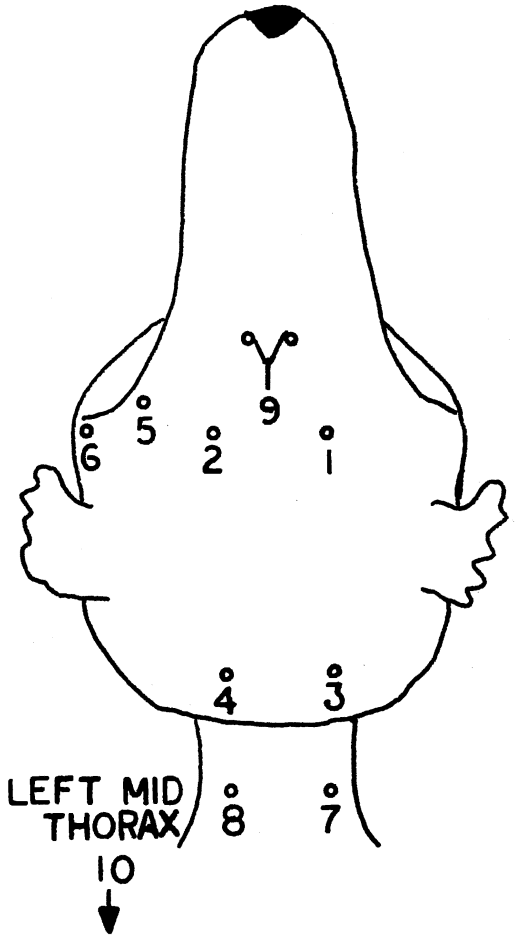

Figure 2. Position of Electrodes on the Dog

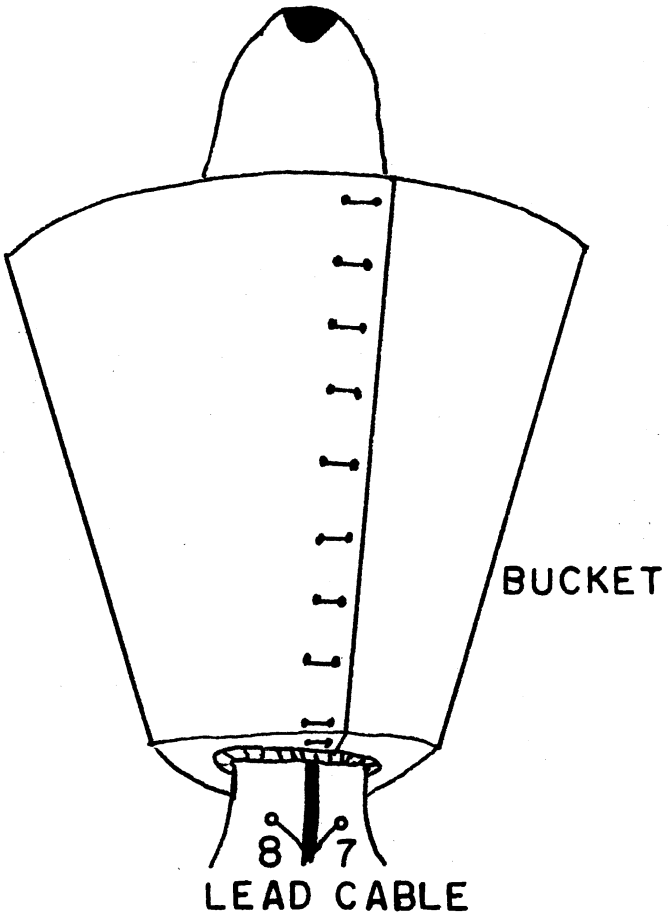

Figure 3. Position of the Bucket on the Dog

The electrode leads were constructed from teflon coated wires with Michelle wound clips soldered to one end and a plug to the other (Appendix A). The clips were then attached to the animal at the areas listed in Table I.

The seven electrode leads from the head were taped together at the occipital crest forming a cable. The two neck leads were taped to the cable at the dorsal mid neck region while the chest lead was added posterior to the shoulder.

Elastic bandages were used to immobilize the cable and permit easy access to the plug. They were wrapped around the chest and neck securing the cable over the back. The plug was covered with one strip of bandage to allow ready access when attaching the recording system to the dog. A plastic bucket (bucket preparation, Appendix A) was placed around the dog's neck with the top toward the nose and the laces were tightened (Figure 3). The combination of the elastic bnadage and bucket gave the dog freedom to move, see, eat and drink without disturbing the electrode leads.

Recording and Behavioral Observation Procedures

Each dog was monitored behaviorally and polygraphically for 48 consecutive hours except for the 15-20 minutes twice a day when being walked. The electroencephalogram (EEG), electroocculogram (EOG), electromyogram (EMG) and electrocardiogram (EKG) were recorded on a Grass Model II EEG machine using the lead systems in Table II.

The paper speed was 7.5 mm/sec and the recording channels (for the various lead systems) were calibrated as shown in Table III. A low frequency pass filter was used with the EEG and EKG lead systems to

TABLE II

LEAD SYSTEMS

Lead System	Description	Electrode Lead Pairs
I	transfrontal EEG	1-2
II	transoccipital EEG	3-4
III	combination EEG	1-3
IV	combination EEG	1-4
V	EOG	5-6
VI	EMG	7-8
VII	EKG	10-4

TABLE III

RECORDING CHANNELS

Lead	Amplitude
EEG	10 mm/ 50 μV
EOG	5 mm/100 μV
EMG	10 mm/ 20 μV
EKG	5 mm/100 μV

eliminate some of the muscle artifact.

Following each 48 hr. recording period a second physical examination, hemogram, blood glucose, blood urea nitrogen and urinalysis ensured that all dogs were still clinically normal. The electrodes were then removed and the dogs returned to the research colony.

Analysis of Data

There were 48 hr. recordings and behavioral observations for five dogs. Each session was split into 96 half hour periods which were in turn divided into 60, 30 second epics. Each epic was classified by the predominant state of consciousness. The number of epics per period spent in waking, non rapid eye movement (NREM), and rapid eye movement (REM) sleep was determined by behavioral observations and polygraphic recordings using the criteria summarized by Jouvet (1967). Sample recordings are in Figure 4.

Waking

Observations were most valuable in determining the waking state. The animal was often standing, sitting or lying down with eyes open. Frequent movement could be heard. There was a large amount of muscle tone on the EMG and when the EEG was not hidden by muscle artifact it had a low voltage fast frequency pattern. Eye movement was present in the EOG but not of the same amplitude or pattern that was present in REM sleep. The EKG exhibited normal sinus arrhythmia.

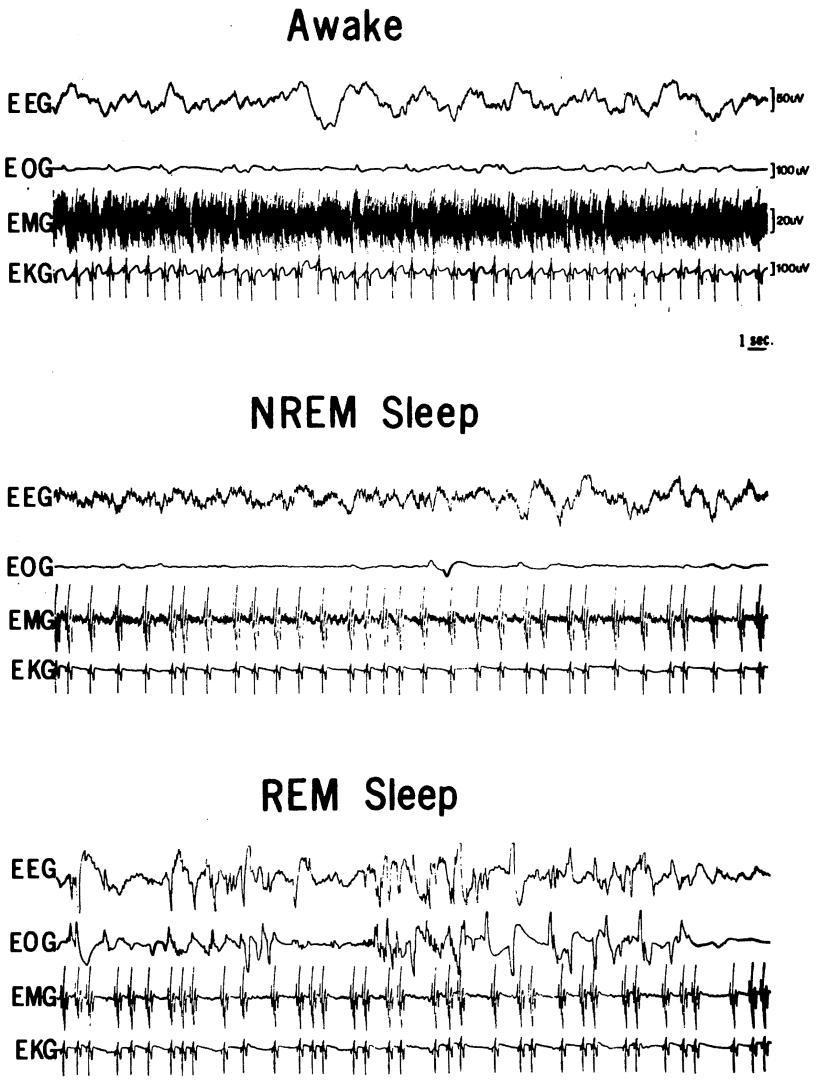

Figure 4. Sample Recordings of Dogs During Waking, NREM Sleep, and REM Sleep

NREM Sleep

Dogs in NREM sleep were lying down with their eyes closed. They occasionally shifted positions but were otherwise still and quiet. The EEG was of higher voltage and slower frequency than in waking and fewer muscle artifacts were present. The EMG indicated some tone but less than when awake. The EOG showed little eye activity and the heart rate was slower and had less sinus arrhythmia than waking.

REM Sleep

This stage usually followed NREM sleep and was characterized by a lack of muscle tone with occasional phasic motor activity such as paddling of limbs, sudden bursts of REM and vocalization. These phenomena were seen with EOG, EMG and visual observation. The EEG tracings had low voltage fast frequency patterns similar to waking patterns. The heart rate was more irregular and rapid than in NREM sleep.

Interpretation of Data

The percent of 24 hours spent in each state was calculated for each dog as well as the ratio between REM sleep and total sleep. These values were averaged for all five dogs. The number of epics for each state in 30 minutes were plotted against time for each dog as was the average of waking for all the dogs. The graphs indicated any possible relationships between sleep and waking during a 24 hour day. The percents and graphs were used to analyze the sleep-waking patterns of normal dogs.

CHAPTER IV

RESULTS AND DISCUSSION

The technique used in this study was adequate for determining sleep-waking patterns of dogs. The electrode fixation was relatively noninvasive (Michelle clips) and had no adverse effects on the dogs except for minor skin irritations at their contact sites. Sleep spindles, observed in dogs with implanted cortical electrodes (Lucas, 1976), are not observed when using the surface recording technique because it is less sensitive. With the Michelle clips, movement artifacts of high voltage are a problem when the dog is awake but they do not interfere with the recording during sleep. The EMG lead system picks up EKG as well as EMG potentials.

The polygraphic recordings of the electroencephalogram (EEG), electromyogram (EMG), electroocculogram (EOG), electrocardiogram (EKG), and behavioral observations were used to determine the percent of waking, non rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep in a 24 hr. day for each dog (Table IV). Average values for the five dogs are in Table V. These values were obtained using a 16 hr. light, 8 hr. dark cycle, surface electrodes, and dogs isolated from extraneous auditory and visual stimuli. A similar dog study by Lucas, et al. (1976), using a 12 hr. light, 12 hr. dark cycle, implanted cortical electrodes and non-isolated dogs, demonstrated similar results for waking, NREM sleep and REM sleep (65%, 23%, and 12%, respectively). The results of both studies

TABLE IV

PERCENT OF RECORDING TIME SPENT IN WAKEFULNESS, NREM SLEEP, AND REM SLEEP FOR EACH DOG

Dog #	Wakefulness	NREM Sleep	REM Sleep
1	64.46	25.24	10.19
2	63.34	25.20	9.71
3	76.84	19.74	6.42
4	68.70	21.93	9.38
5	68.54	23.24	8.21

TABLE V

MEAN PERCENT (± STANDARD ERROR) OF RECORDING TIME SPENT IN WAKEFULNESS, NREM SLEEP, AND REM SLEEP FOR FIVE DOGS

Wakefulness	NREM Sleep	REM Sleep
67.80 ± .91	23.07 ± .68	8.78 ± .55

indicated a significant difference between dog and previously reported cat sleep-waking patterns (Lucas and Sternman, 1974; Lucas, et al., 1976). The cat values obtained by Lucas, et al. (1976) were 49%, 37%, and 14% for wakefulness, NREM sleep, and REM sleep, respectively. The ratio of REM sleep to total sleep time for the dogs in this study was .276 which is similar to the same ratio previously reported for the cat, .274 (Lucas, et al., 1976).

The graphs of waking, NREM sleep and REM sleep for the 48 hr. period for each animal indicated a general increase in sleep related to the eight hour dark period (Appendix B, Figures 7-21). This relationship observed on the graphs of the individual animals was more obvious on the graph averaging the waking values for all five animals (Figure 5). It was characterized by a gradual increase of sleep that began about one hour prior to lights off and a gradual decrease that began about one hour prior to lights on. This suggests that the dogs may be entrained to the light cycle which was similar to the natural light cycle to which they were previously accustomed. Lucas, et al. (1976), using a slightly different light cycle in dogs than in this study, also reported an increase in sleep during the dark period. The fact that total sleep times were similar in both studies suggests that while the light-dark cycle modulates sleep patterns, it does not quantitatively alter total sleep-waking time in the dog.

The dog's sleep-waking patterns reported in this study and by Lucas, et al. (1976), differ from those reported in cats by Lucas, et al. (1976). Cats have two peaks of increased sleep (one in the afternoon and one at night) while dogs have only one (occurring at night). As

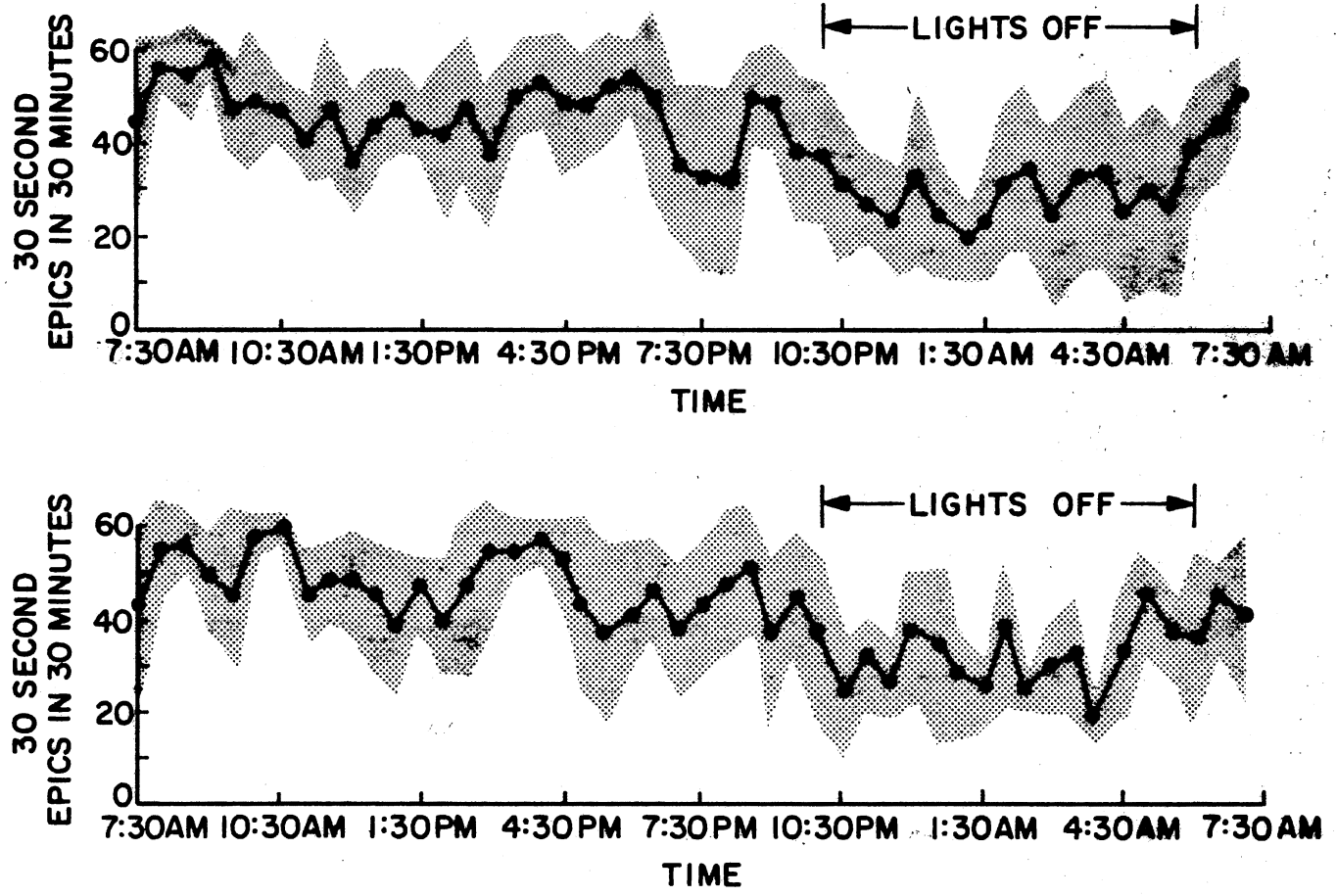

Figure 5. The Number of 30 Second Epics in 30 Minutes Spent in Waking - Average for the Five Dogs. Gray areas describe the standard deviation from the mean.

discussed earlier, the percent time spent in waking, NREM sleep, and REM sleep also differs between dogs and cats.

CHAPTER V

SUMMARY AND CONCLUSIONS

Twenty-four hour polygraphic and behavioral observations have been reported for a variety of mammals, amphibians, and birds; however, there are few 24 hr. studies of the domestic canine. We felt such data on the dog would be valuable because at least one sleep disorder, narcolepsy-cataplexy, has already been described in the dog. A noninvasive technique was developed to allow 48 hr. continuous polygraphic and behavioral observations of dogs; their sleep-waking patterns were determined.

The procedure using surface clips for electrodes provides a noninvasive, safe method for chronic studies on normal dogs. This method may be useful in studies of dogs with sleep-waking disorders. The EEG, EOG, EMG, and EKG polygraphic parameters, along with behavioral observations, were adequate to determine the state of sleep-waking.

In this study the dogs spent 67.8% of the 24 hr. period awake, 23.07% in NREM sleep and 8.78% in REM sleep; the ratio of REM sleep to total sleep was .276. The dogs had a 24 hr. trend of increased sleep during the dark hours. A dog study by Lucas, et al. (1976) using a different light-dark cycle also demonstrated an increase of sleep during the dark period as well as similar percents for waking, NREM sleep, and REM sleep in a 24 hr. period. Possibly the light-dark cycle modulates sleep patterns but does not quantitatively alter total sleep-waking times in the dog.

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APPENDIX A

SPECIALLY CONSTRUCTED EQUIPMENT

Electrode Leads

The electrode leads were made by soldering stainless steel Michelle wound clips (Clay Adams #B-2335-11mm) to one end of ten teflon coated multistrand 22 gauge wires. The wires were cut to approximately 75 cm. One of the wires had an extra two cm length and clip soldered near the end making a "Y". This wire was electrode lead #9 for the animal ground. Female copper "Relia-tac" contacts (Amphinol #220-SO2) were soldered to the other end of each lead. These contacts were inserted in a male "Tiny Tim" plug (Amphinol #223-1109). The female "Tiny Tim" plug (Amphinol #223-2109) was attached to a ten lead color coded plastic cable using male copper guide pins (Amphinol #220-PO2). EEG electrode jacks were attached to the other end of this color coded cable for insertion into the lead box of the Grass model II EEG machine (Figure 6).

Cage

A wooden cage with a false bottom for easy cleaning was constructed. The cage (36 in. high, 31 in. wide, and 32 in. deep) was painted with green epoxy paint and the edges were sealed with silicone sealer to make it waterproof. The sides and top were solid except for a small hole for the electrode lead cable and an observation hole (2 in. x 3 in.) covered with plexiglass. The front had a metal door with food and water pan clips.

Bucket

A plastic bucket about one foot in diameter and one foot deep was sliced on one side and half the bottom. A circle big enough to fit around the dog's neck was removed from the bottom. The neck edge was

lined with foam and adhesive tape to protect the dog from chafing. Holes were punched along the cut edge (using a soldering iron) to lace the bucket securely to the dog's head.

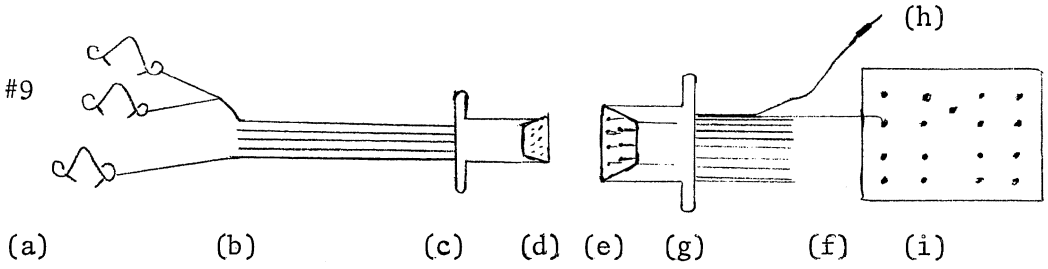

Figure 6. Electrode System for Polygraphic Recording on Dogs. (a) wound clips, (b) mutistrand wire, (c) female copper contacts, (d) male plug, (e) female plug, (f) ten lead cable, (g) male copper guide pins, (h) EEG electrode jacks, (i) lead box.

APPENDIX B

GRAPHS OF THE NUMBER OF 30 SECOND EPICS IN 30 MINUTES SPENT IN WAKING, NREM SLEEP, AND REM SLEEP FOR EACH DOG

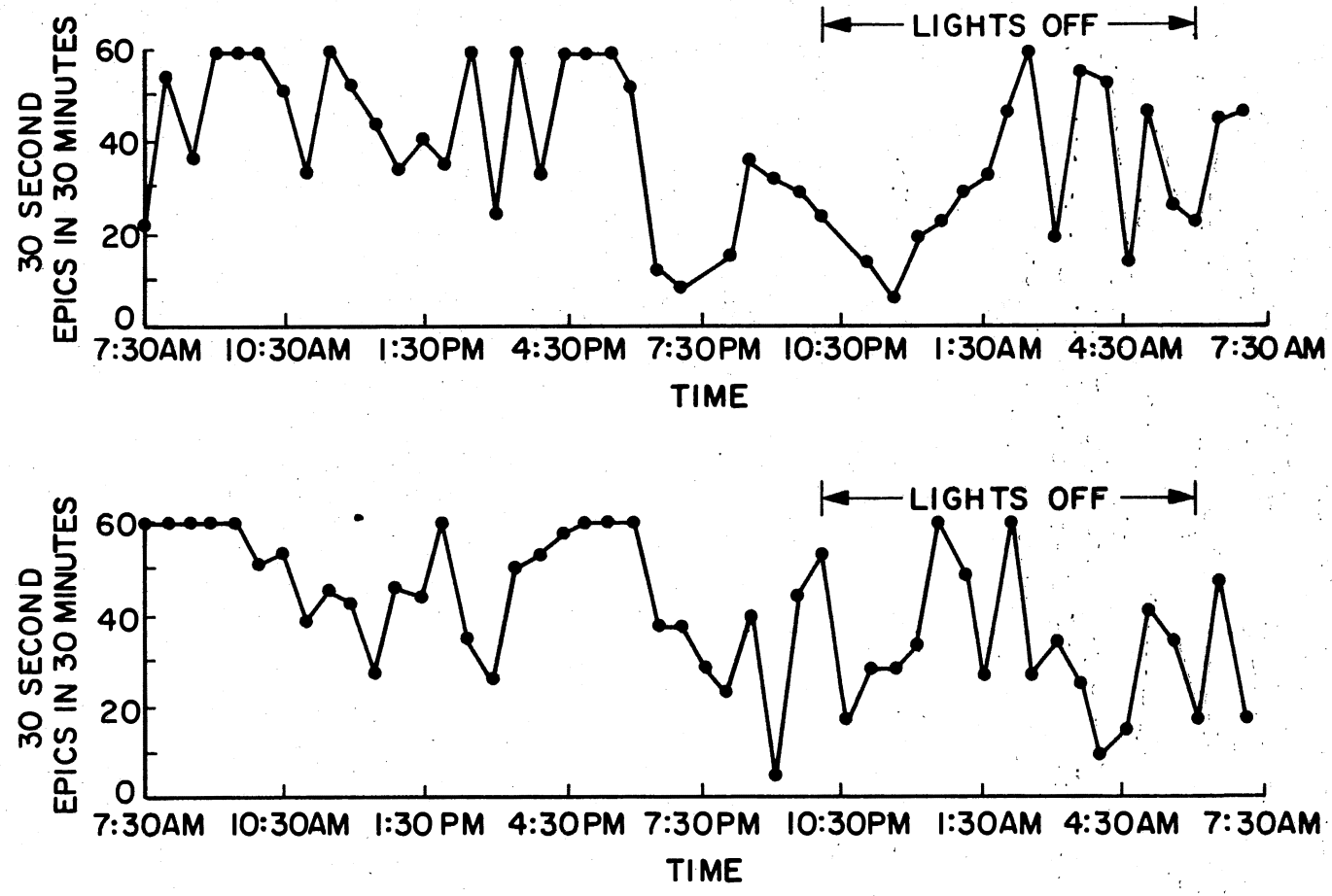

Figure 7. The Number of 30 Second Epics in 30 Minutes Spent in Waking - Dog #1, Forty-Eight Hours

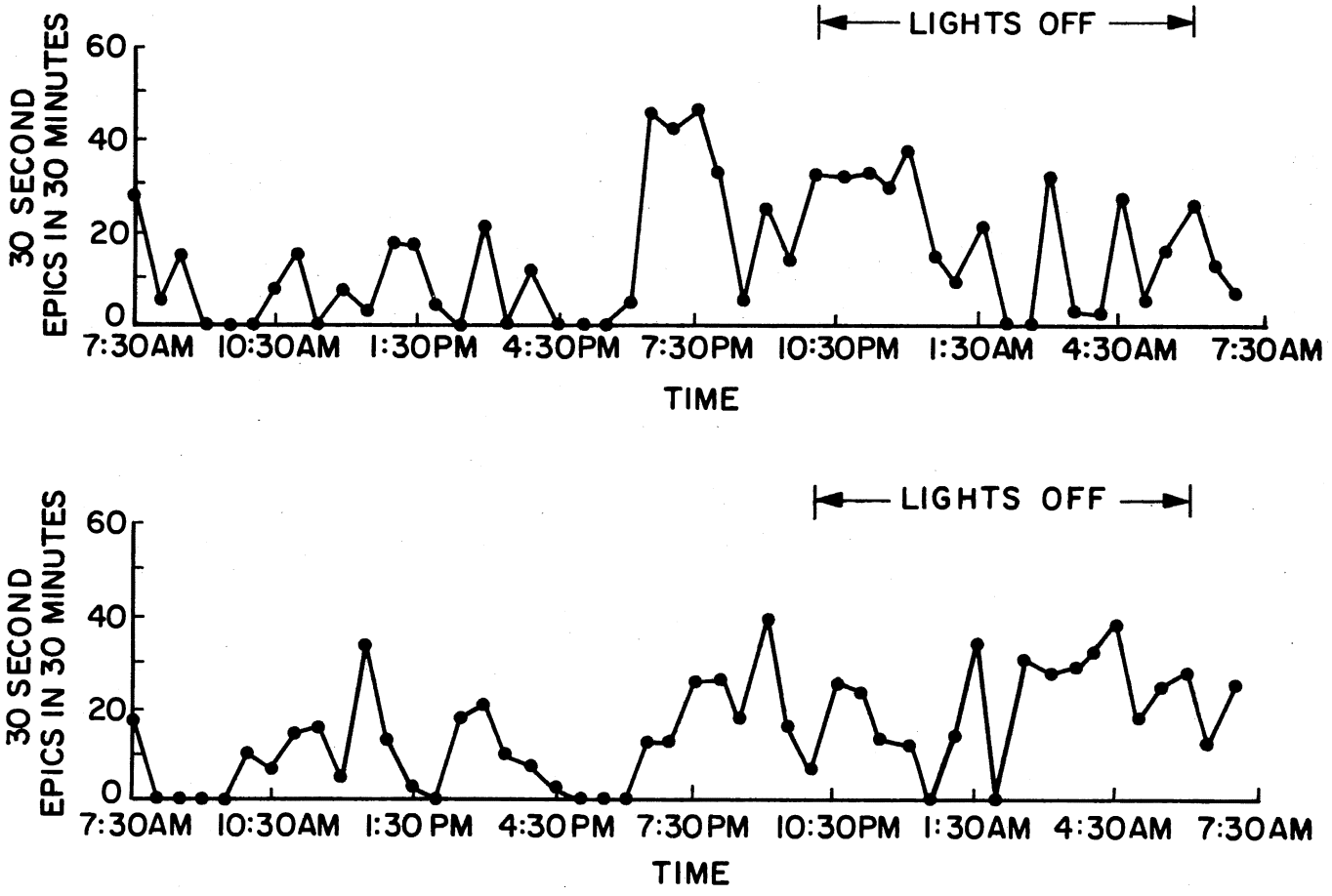

Figure 8. The Number of 30 Second Epics in 30 Minutes Spent in NREM Sleep - Dog #1, Forty-Eight Hours

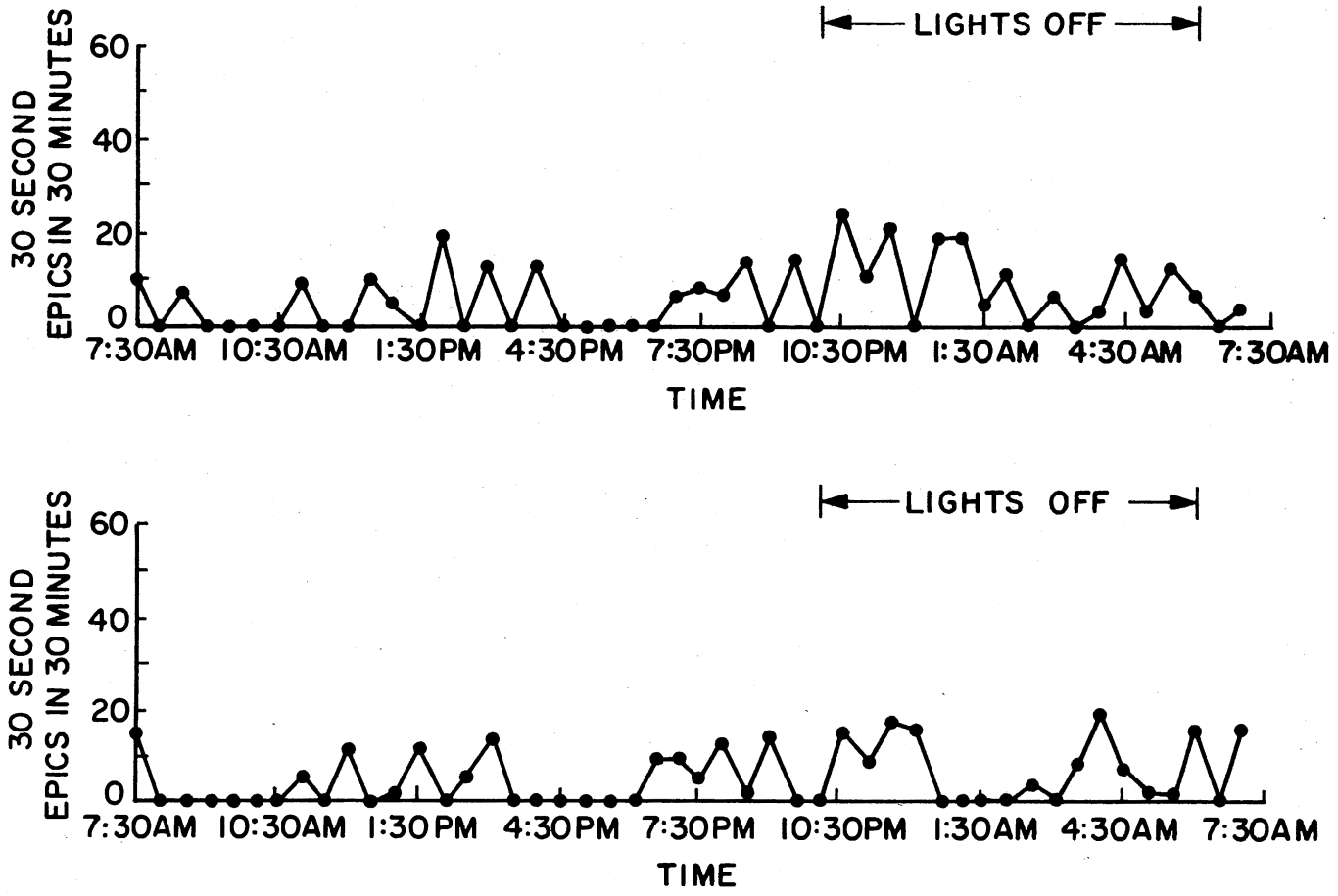

Figure 9. The Number of 30 Second Epics in 30 Minutes Spent in REM Sleep - Dog #1, Forty-Eight Hours

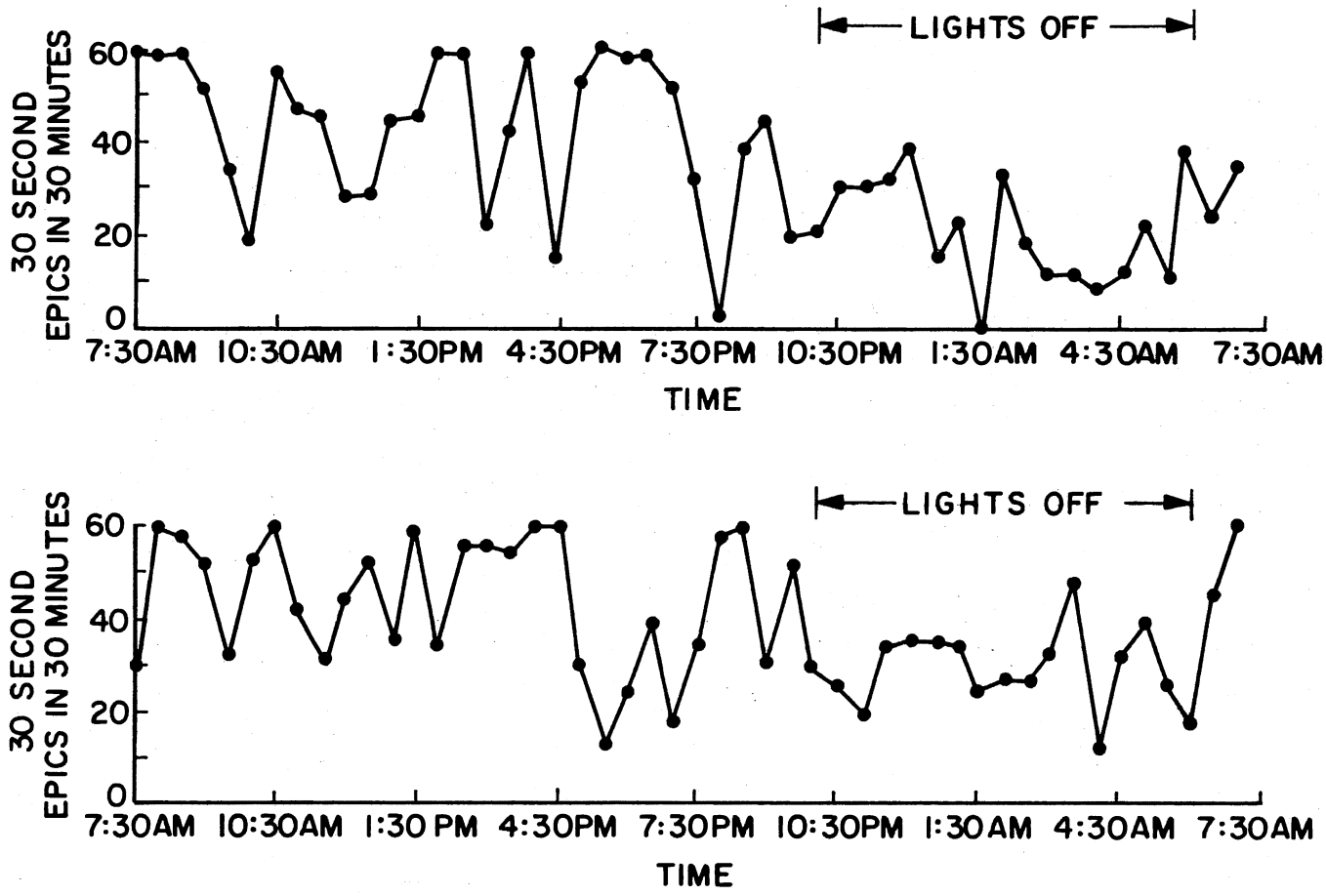

Figure 10. The Number of 30 Second Epics in 30 Minutes Spent in Waking - Dog #2, Forty-Eight Hours

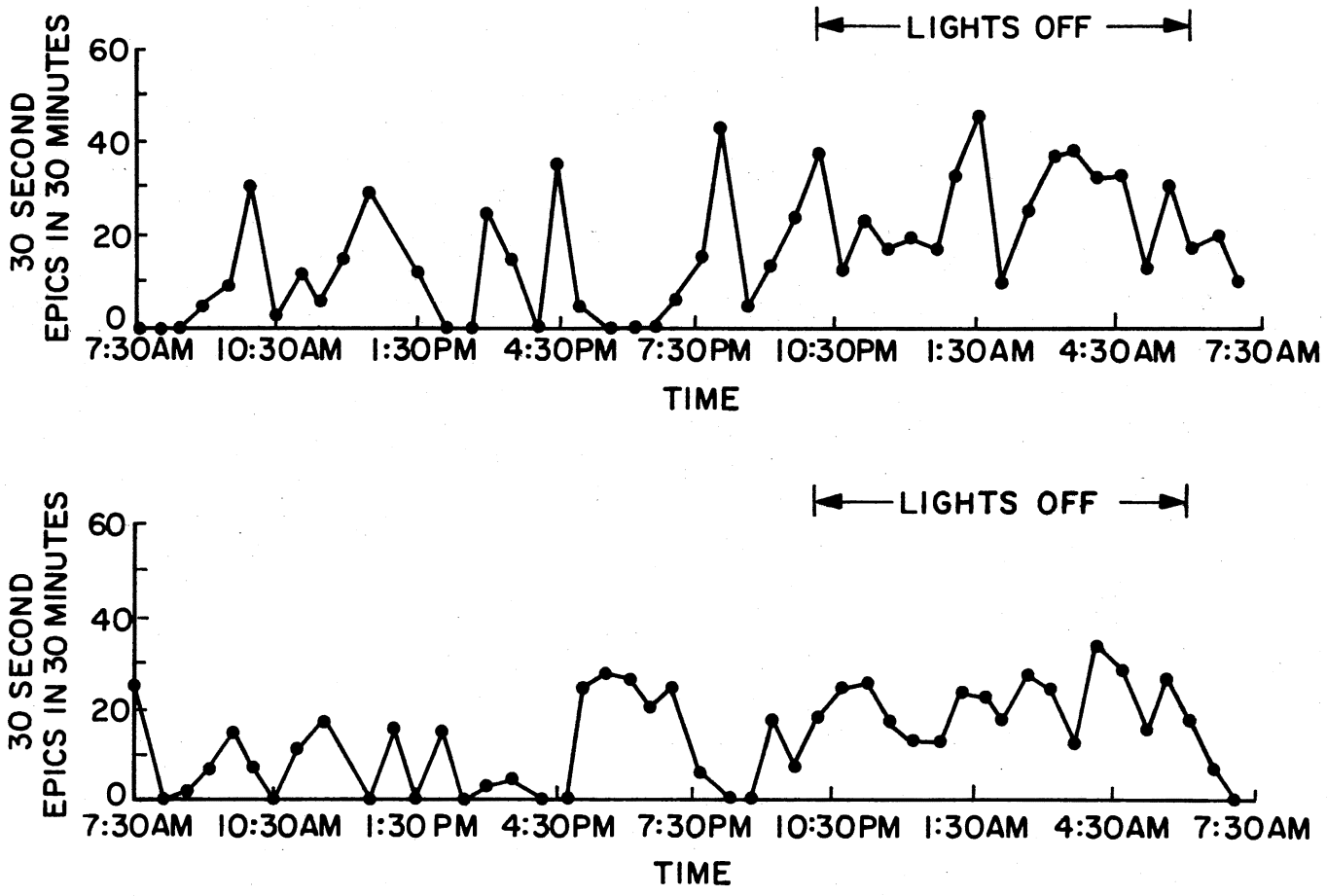

Figure 11. The Number of 30 Second Epics in 30 Minutes Spent in NREM Sleep - Dog #2, Forty-Eight Hours

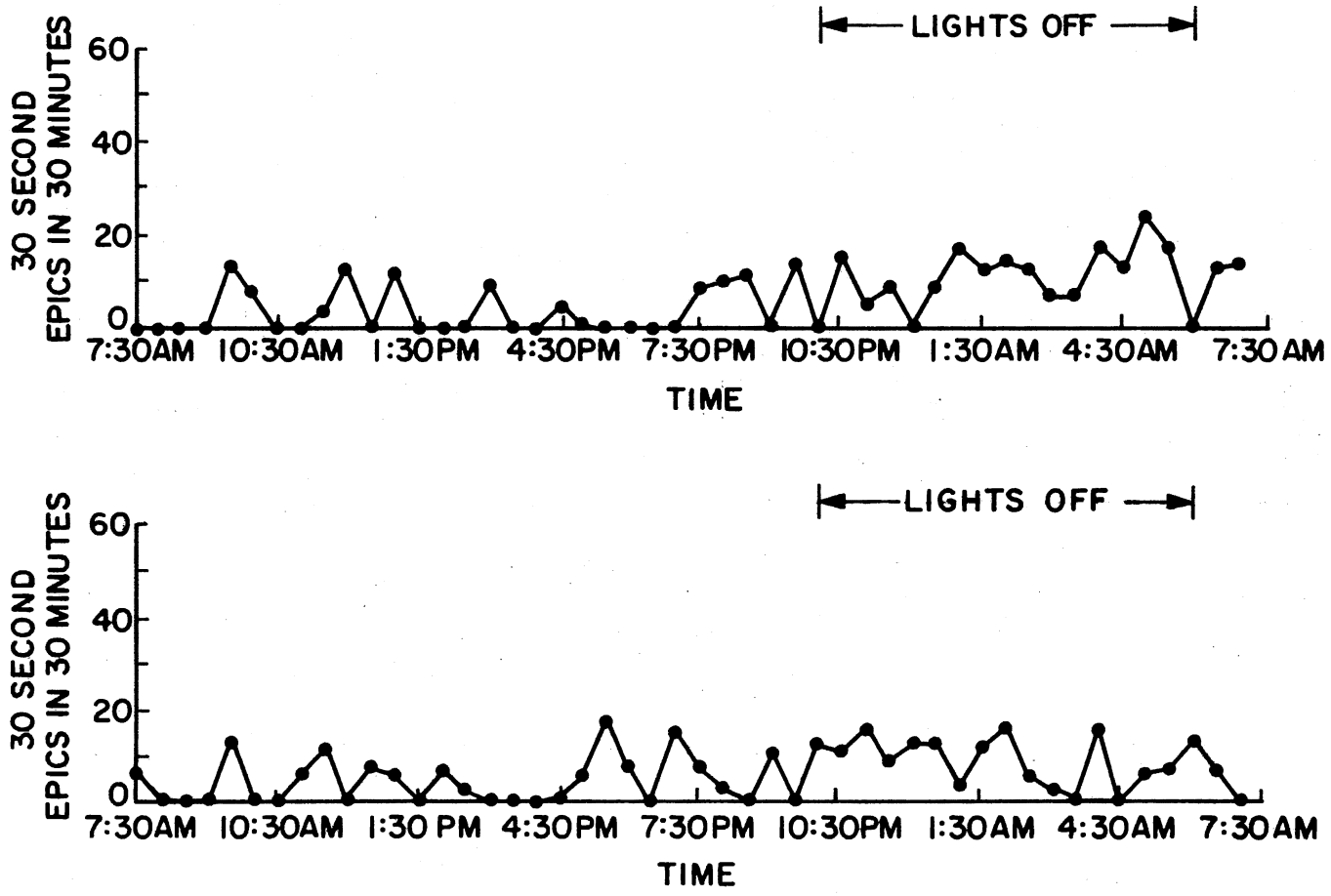

Figure 12. The Number of 30 Second Epics in 30 Minutes Spent in REM Sleep - Dog #2, Forty-Eight Hours

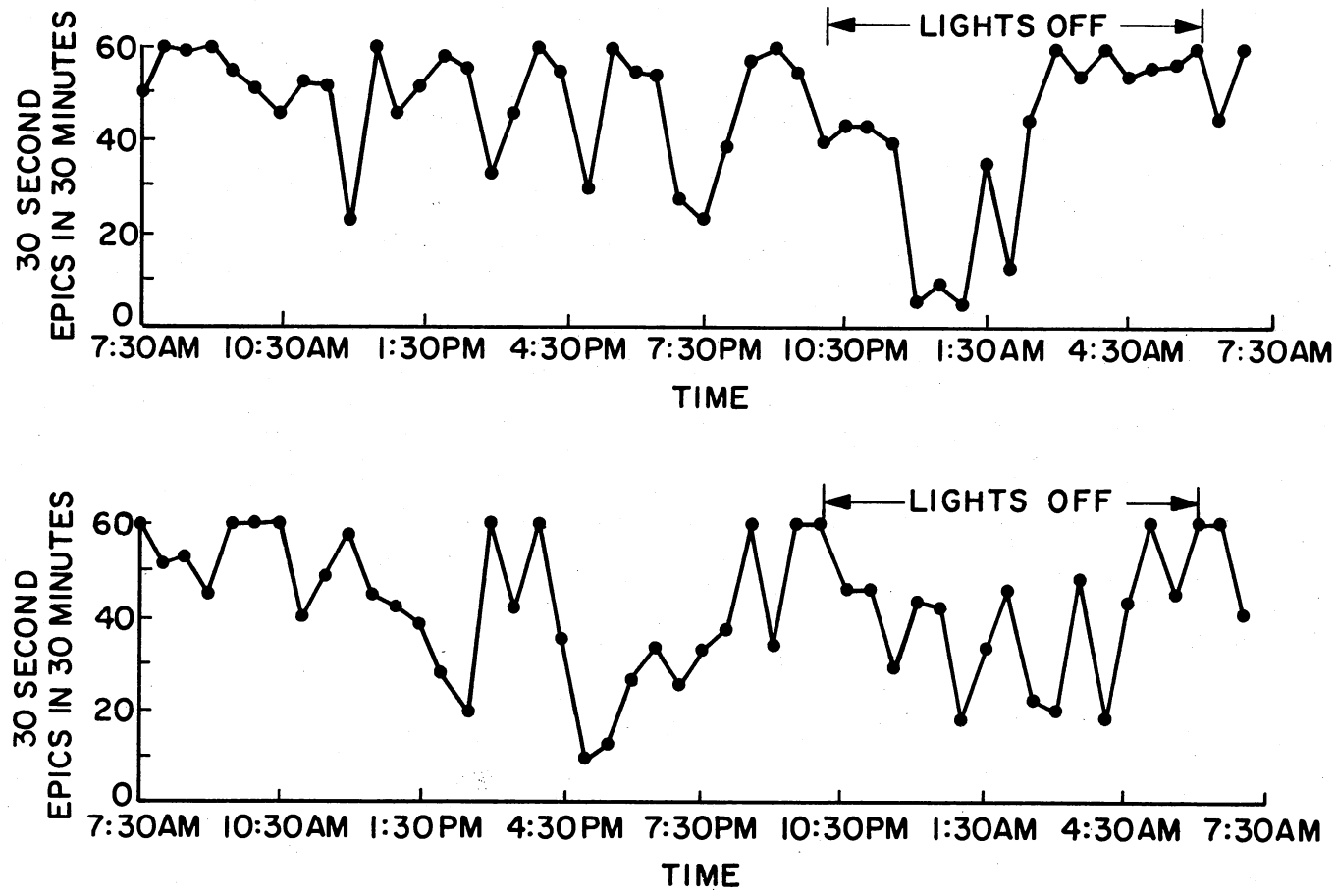

Figure 13. The Number of 30 Second Epics in 30 Minutes Spent in Waking - Dog #3, Forty-Eight Hours

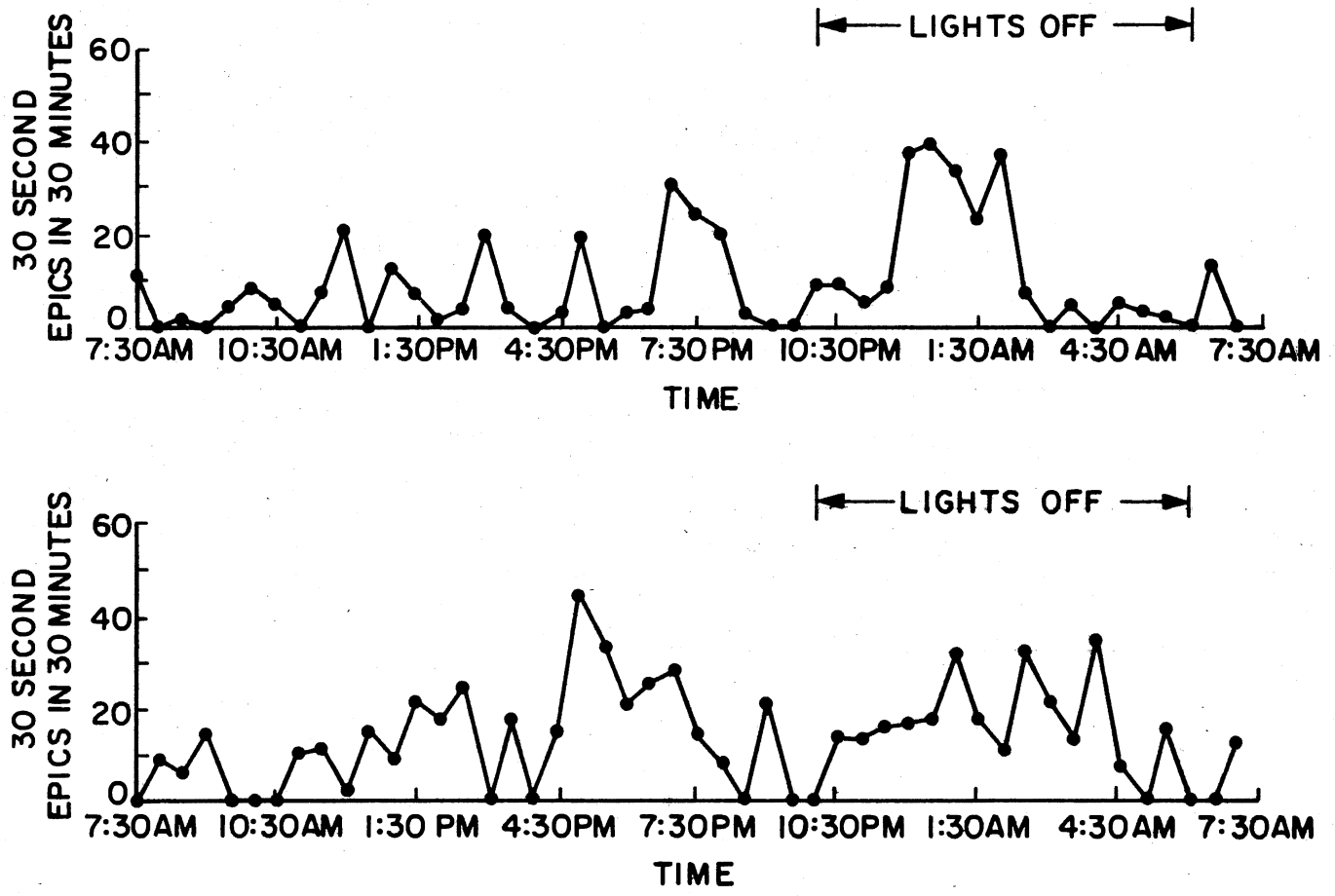

Figure 14. The Number of 30 Second Epics in 30 Minutes Spent in NREM Sleep - Dog #3, Forty-Eight Hours

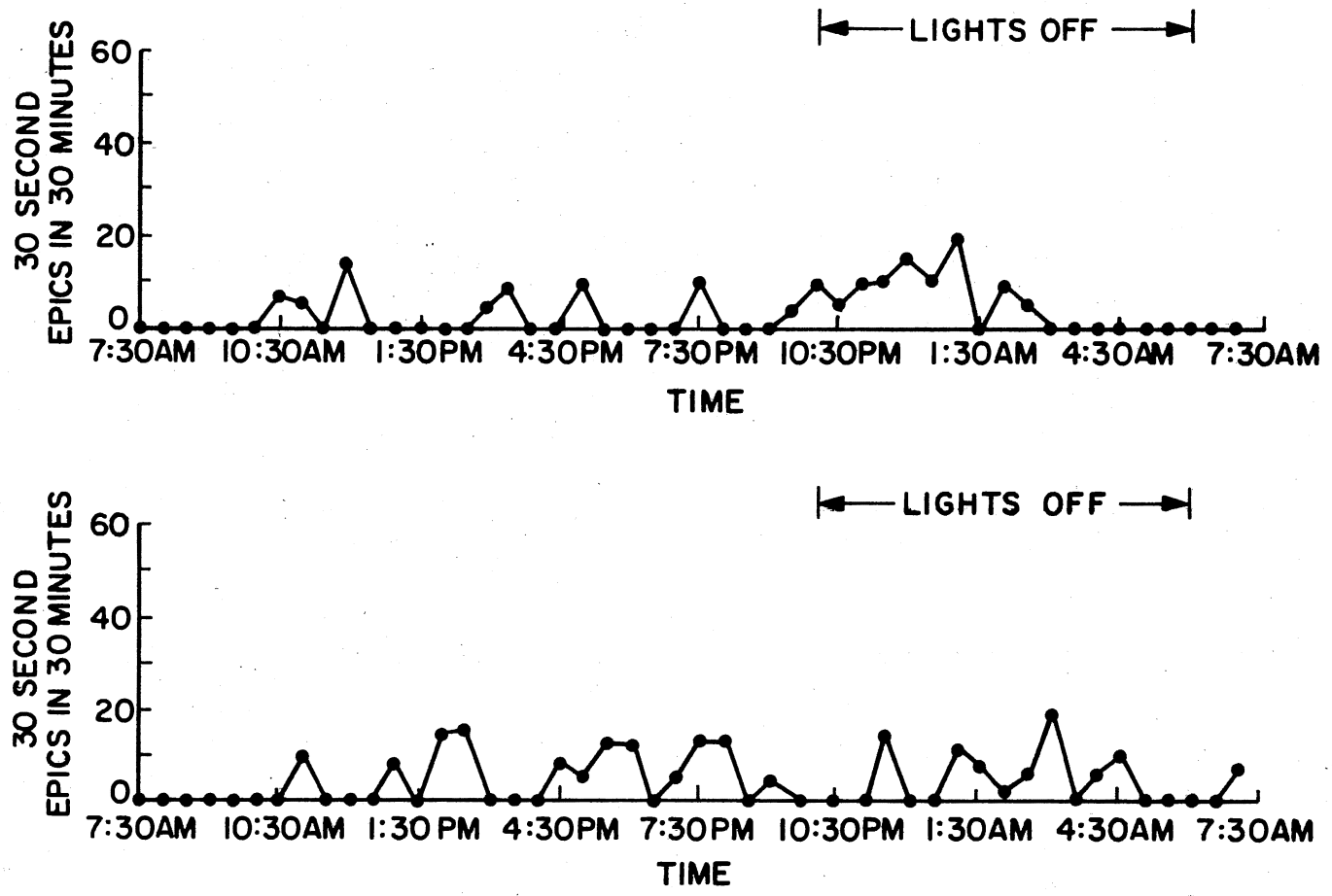

Figure 15. The Number of 30 Second Epics in 30 Minutes Spent in REM Sleep - Dog #3, Forty-Eight Hours

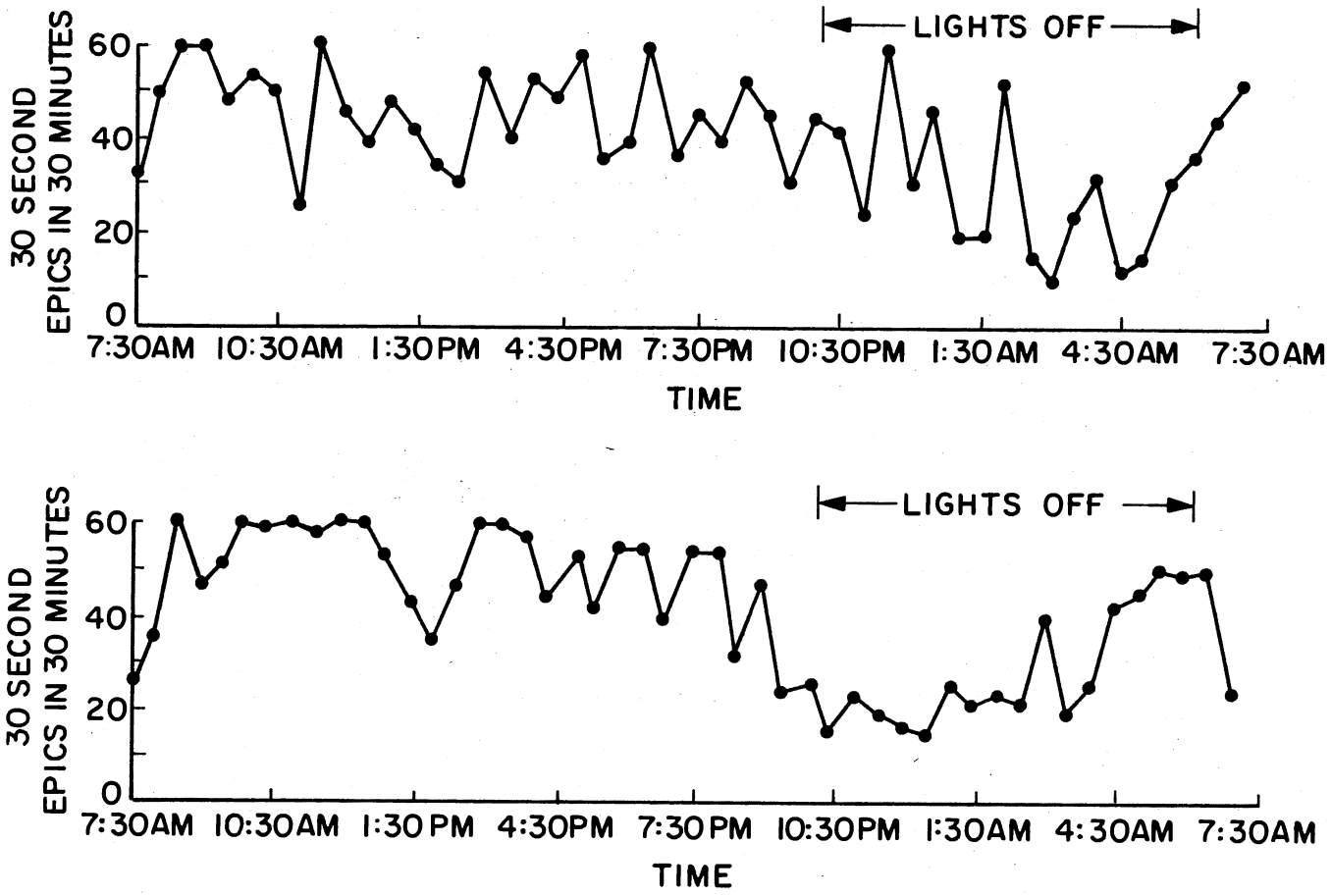

Figure 16. The Number of 30 Second Epics in 30 Minutes Spent in Waking - Dog #4, Forty-Eight Hours

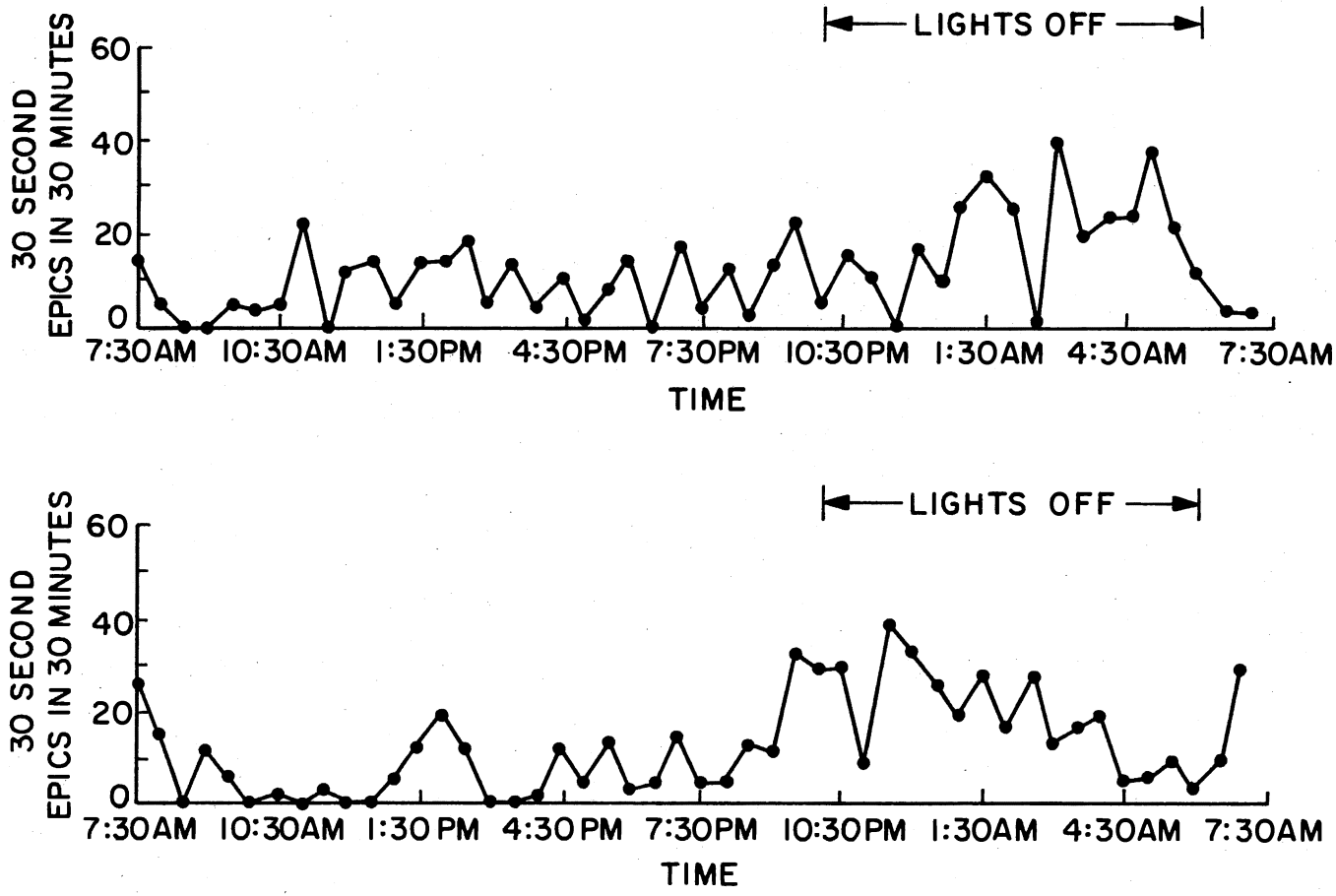

Figure 17. The Number of 30 Second Epics in 30 Minutes Spent in NREM Sleep - Dog #4, Forty-Eight Hours

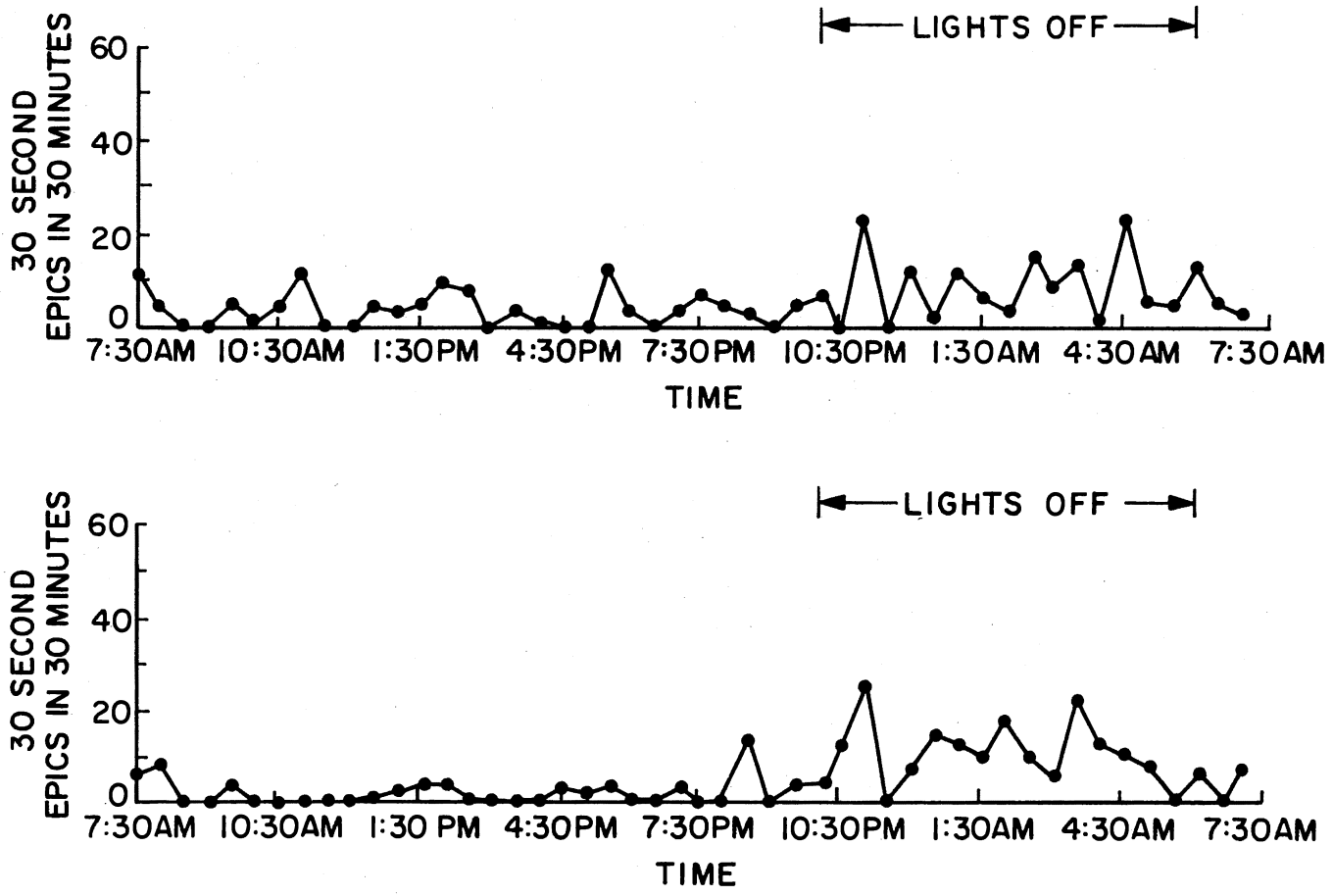

Figure 18. The Number of 30 Second Epics in 30 Minutes Spent in REM Sleep - Dog #4, Forty-Eight Hours

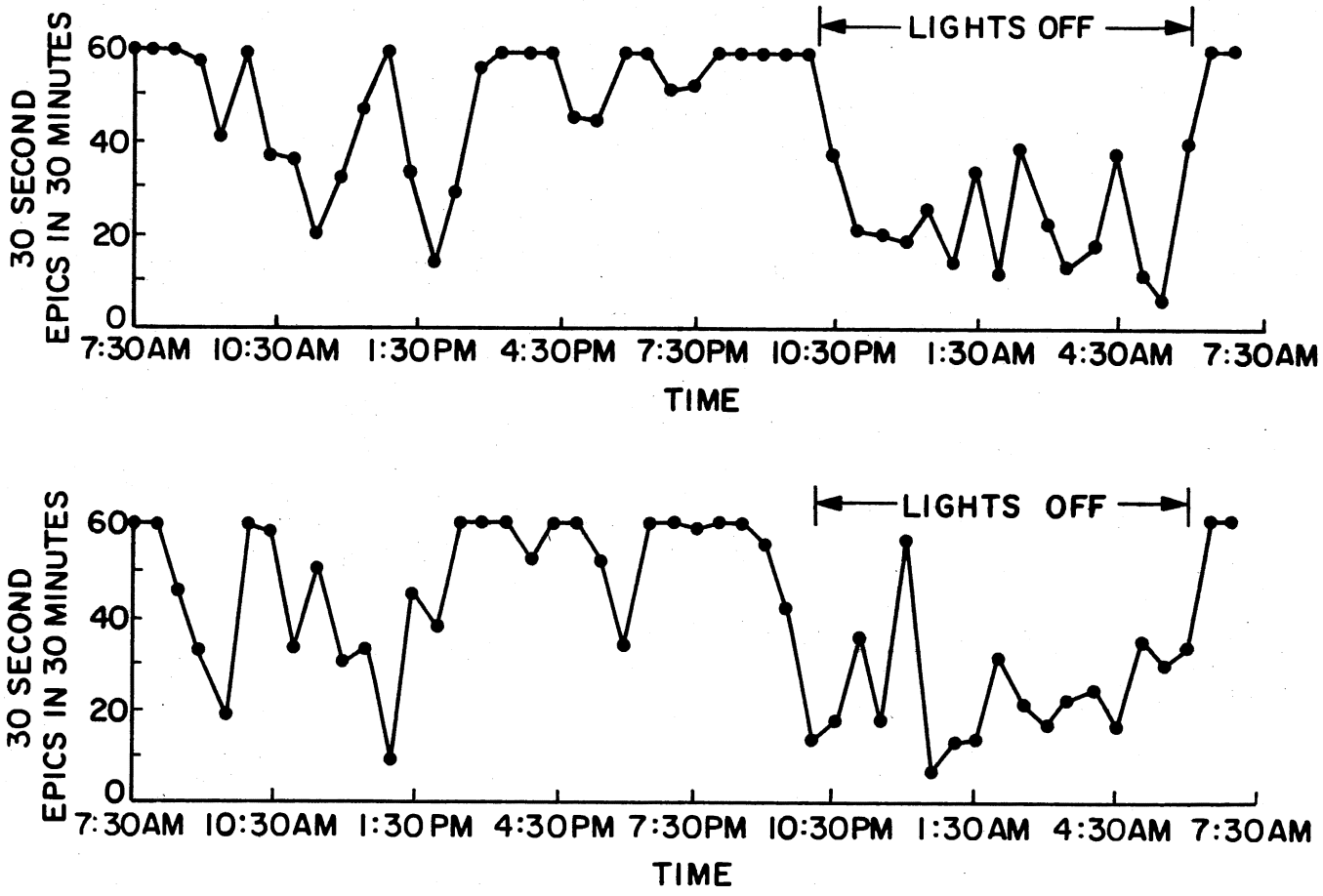

Figure 19. The Number of 30 Second Epics in 30 Minutes Spent in Waking - Dog #5, Forty-Eight Hours

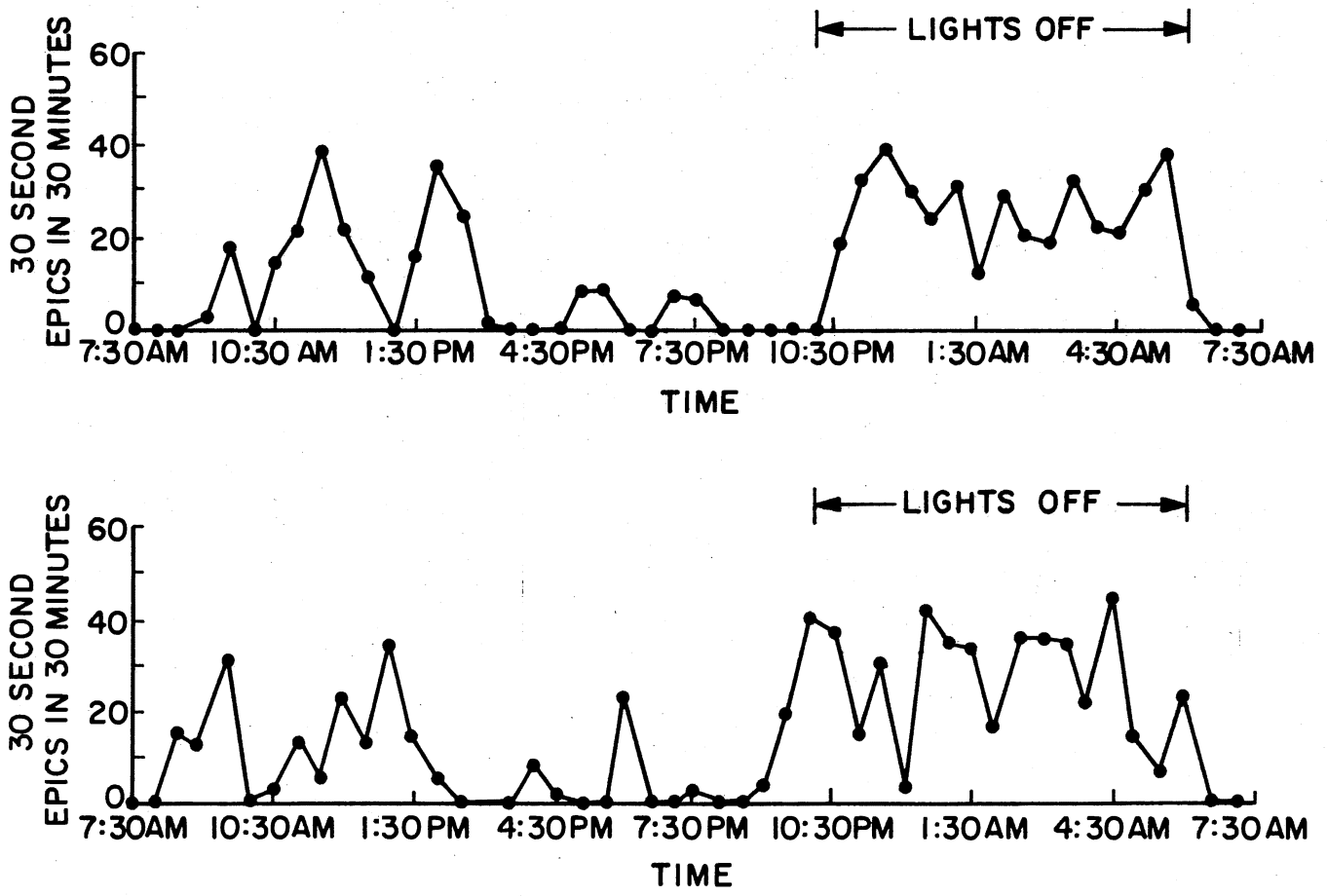

Figure 20. The Number of 30 Second Epics in 30 Minutes Spent in NREM Sleep - Dog #5, Forty-Eight Hours

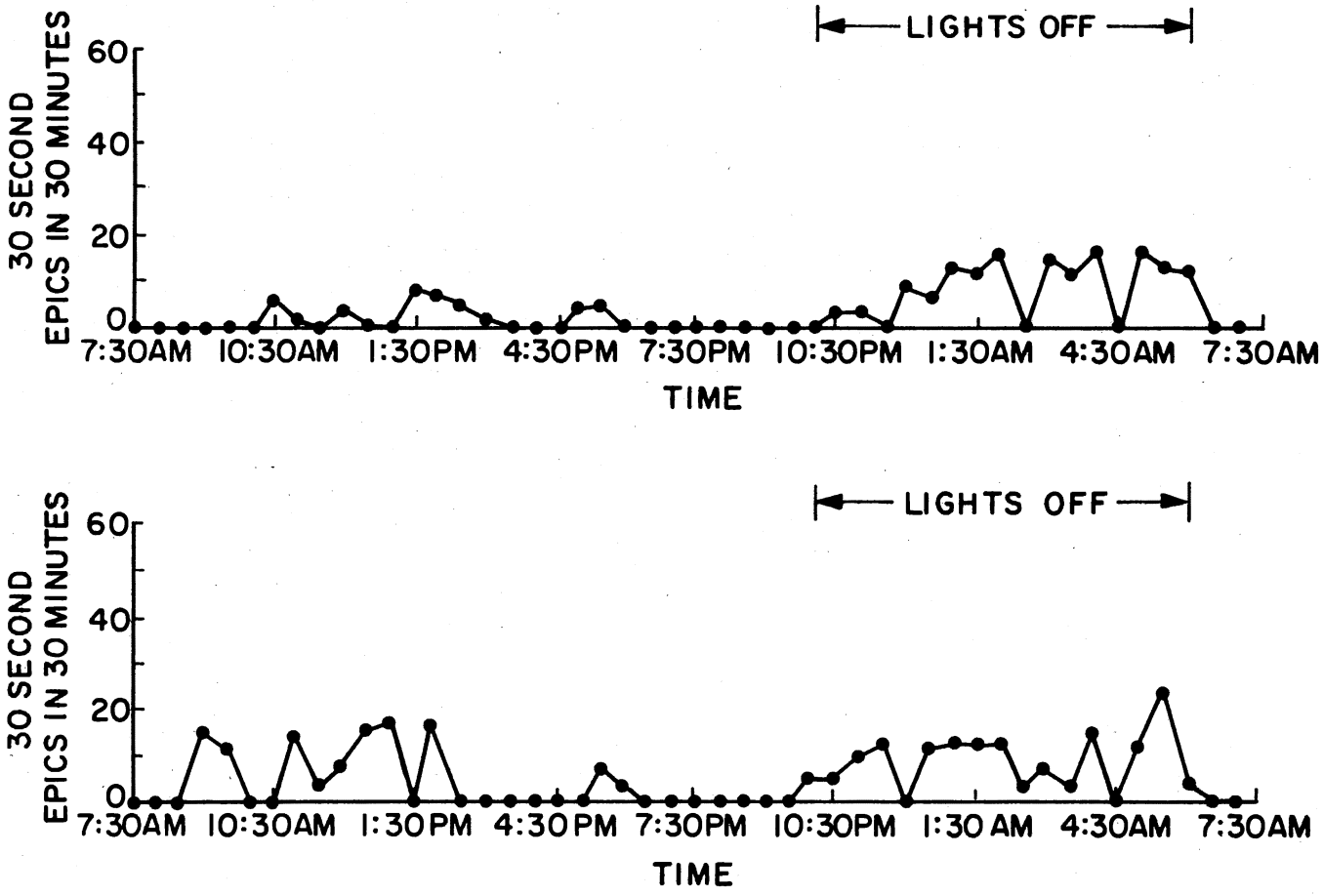

Figure 21. The Number of 30 Second Epics in 30 Minutes Spent in REM Sleep - Dog #5, Forty-Eight

VITA

Marion Pamela Copley

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Master of Science

Thesis: A STUDY OF CONTINUOUS FORTY-EIGHT HOUR SLEEP-WAKING RECORDINGS IN FIVE DOGS

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