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Opioid-induced changes in spectral densities of the rat electroencephalograph

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Opioid-Induced Changes in Spectral Densities of the Rat Electroencephalograph

by

Stacey Young-McCaughan

DISSERTATION

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ABSTRACT

OPIOID-INDUCED CHANGES IN SPECTRAL DENSITIES OF THE RAT ELECTROENCEPHALOGRAPH

Stacey Young-McCaughan RN, PhD, Lieutenant Colonel, U. S. Army Nurse Corps
University of California, San Francisco, 1997

Opioids are routinely used for relief of moderate to severe pain. While effective in relieving pain, these drugs are not without side effects. One of the most common and troublesome side effects associated with these drugs is an altered level of consciousness, commonly referred to as sedation.

The purpose of this study was to describe the effects of two doses of three relatively-selective opioid agonists (i. e., morphine 0.5 μg/kg & 500 μg/kg, pentazocine 0.5 μg/kg & 500 μg/kg, and naloxone 0.5 μg/kg & 500 μg/kg) on selected electroencephalograph (EEG) parameters of conscious rats.

Adult, male Sprague-Dawley rats weighing between 240 and 260 grams were surgically implanted with cortical EEG recording electrodes. On the day of the experiment, two hours of baseline EEG recordings were collected before each rat received a subcutaneous injection of normal saline or one of the doses of drug. EEG recordings continued for another four hours for a total recording time of six hours.

For EEG frequencies between 1 and 30 hertz (Hz), time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, edge frequency & percent of absolute power

attributable to individual one Hz frequency bands) were assessed. From three-dimensional graphs of the percent of absolute power attributable to individual one Hz frequency bands for each minute of the 360 minute experiment, cyclic fluctuations in the percent of absolute power at 7 and 8 Hz were observed. When the baseline recording period was compared with the two, two hour recording periods following drug administration using an area-under-the-curve analysis, the lower doses of all three opioid agonists significantly increased ($p < .05$) the magnitude of the percent of absolute power at 7 and 8 Hz.

The results of this study suggest that changes in the cyclic fluctuations of the percent of absolute power at 7 and 8 Hz might be a parameter indicative of alterations in central nervous system functioning following opioid administration and a focus of future research investigating the phenomenon of opioid-induced sedation.

Christine Miaskowski

CHRISTINE MIASKOWSKI RN, PhD, FAAN,
Professor, Chairperson Dissertation Committee

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CHAPTER 1 - THE STUDY PROBLEM

Introduction

Opioids are routinely used to manage moderate to severe pain (Jacox et al., 1994; Reisine & Pasternak, 1996). While effective in relieving pain, this class of analgesics produces significant side effects. One of the most common and troublesome side effects associated with opioid analgesics is a depressed level of consciousness (LOC) or sedation (Cherny & Portenoy, 1994; Jacox et al., 1994; Way, Way, & Fields, 1995).

Significance

Understanding the mechanisms underlying the sedative effects of opioids is becoming a more urgent concern in light of ongoing changes in the health care environment. With increasing frequency, patients are discharged home immediately following surgical procedures. The sedative effects of opioids prescribed to control acute surgical pain can impair patients' abilities to perform requisite self-care activities. This situation can be particularly problematic for older patients who do not metabolize drugs as quickly and are more likely to experience opioid-induced side effects.

While the pain and the need for opioids is usually short-lived in postoperative patients, sedation can be much more problematic for patients taking opioids for chronic pain. Clinical experience suggests that many patients find sedation so difficult to manage that they choose to suffer with the pain rather than feel sedated from the drugs. For patients prescribed opioids for relief of either acute or chronic pain, sedation can adversely affect the quality of their lives (Ahles & Whedon, 1993).

Even though sedation is acknowledged as one of the most common side effects of

opioid analgesics, the characteristics and mechanisms of this phenomenon remain elusive. Although no common definition of sedation exists, neither lay nor professional people express difficulty understanding the concept. Words used by patients to describe sedation include feeling drowsy, sleepy, groggy, dizzy, dreamy, cloudy, mentally foggy, or lethargic. Signs of sedation may include cognitive impairment, lack of coordination, slowed reaction time, or performance deficits. Sedation is problematic for patients to describe and practitioners to assess because although patients may relate feelings of sedation, when tested they can perform successfully both motor and cognitive tasks. However, when patients are not engaged actively in doing a specific task, they drift back into a sedated state.

Only a few studies have attempted to determine the incidence of opioid-induced sedation. In one study of postoperative patients who received either codeine, oxycodone, pentazocine, or morphine (Kantor, Hopper, & Laska, 1981), the incidence of adverse effects ranged between 22% and 28%. Dizziness and sleepiness were the most common adverse effects reported for all four of these opioids. Another study of 30 patients with end-stage cancer receiving opioids for pain control (Bruera, Macmillan, Hanson, & MacDonald, 1989) reported that 23% of the patients were "severely sedated," which the researchers defined as "an inability to establish a dialog with the patient" (p. 787). In addition, 53% of the patients experienced delirium, defined as a confusional state with or without hallucinations and hyperactivity. Whether these changes in mental status were due to opioid use, a metabolic process associated with the disease, or some other cause was not discussed by the investigators.

Recent reports indicate that once patients are on a stable dose of opioid for two to three days, tolerance to the sedative effects of the drug develops with a return of normal cognitive functioning (Cherny & Portenoy, 1994; Jacox et al., 1994; Levy, 1994; Way, Way, & Fields, 1995). However, clinical experience suggests that many patients on a stable dose of an opioid continue to experience unpleasant sedative effects.

Bruera and his colleagues reported improved cognitive functioning in patients who took methylphenidate with their opioid analgesics (Bruera, Brenneis, Paterson, & MacDonald, 1989; Bruera, Chadwick, Brenneis, Hanson, & MacDonald, 1987; Bruera, Fainsinger, MacEachern, & Hanson, 1992; Bruera, Miller, Macmillan, & Kuehn, 1992). The authors do not describe a mechanism for opioid-induced impairments in cognitive functioning or how methylphenidate improves cognitive functioning. Although Bruera and his colleagues did not define opioid-induced cognitive impairment as sedation, based on these studies many references recommend methylphenidate to counteract the sedative effects of opioids (Cherny & Portenoy, 1994; Jacox et al., 1994; Levy, 1994; Way, Way, & Fields, 1995).

In addition to a lack of a common definition, and a known mechanism, the study of opioid-induced sedation has been hindered by the lack of a direct means of measuring the phenomenon. As previously discussed, although patients may describe symptoms of sedation and practitioners may observe signs of sedation, existing measures of sedation are not sensitive to the central nervous system (CNS) effects of opioids in patients concentrating on performing a test. Non-invasive measures of sedation, that can assess continuously a patient's level of sedation without having to rely on repeatedly stimulating

the patient to perform a certain task, need to be investigated. Anesthesiologists have used electroencephalograph (EEG) recordings to assess a patient's level of anesthesia (Black, Mahla, & Cucchiara, 1994; Rampil, 1992). Potentially, EEG recordings could also be used to assess sedation in patients taking opioids to control pain.

Specific Aims

Because very little research has been done on opioid-induced changes in LOC, this study investigated the effects of two antinociceptive doses of three subcutaneously administered relatively-selective opioid receptor agonists (i. e., morphine, pentazocine & naloxone) on selected EEG parameters of conscious rats. The specific aims for each of the experiments conducted as part of this study are outlined below.

Normal Saline Experiment

1. To describe the EEG time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) over six hours in conscious rats who received a subcutaneous injection of normal saline.
2. To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of normal saline.
3. To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one hertz (Hz)

frequencies of the EEG in conscious rats who received a subcutaneous injection of normal saline.

4. To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of normal saline.

5. To describe the cyclic fluctuations in the percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of normal saline.

6. To determine if there are differences, over time, in the cyclic fluctuations in the percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of normal saline.

Morphine Experiment

7. To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of morphine 5 µg/kg.

8. To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of morphine 5 µg/kg.

9. To determine if there are differences, over time, in the magnitude of percent of

absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of morphine 5 μg/kg.

10. To determine if there are differences, over time, in the cyclic fluctuations in the percent of absolute power of specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of morphine 5 μg/kg.

11. To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of morphine 500 μg/kg.

12. To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of morphine 500 μg/kg.

13. To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of morphine 500 μg/kg.

14. To determine if there are differences, over time, in the cyclic fluctuations in percent of absolute power of specific EEG frequencies in conscious rats who received a subcutaneous injection of morphine 500 μg/kg.

Pentazocine Experiment

15. To determine if there are differences, over time, in any of the time domain

parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg.

16. To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg.

17. To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg.

18. To determine if there are differences, over time, in the cyclic fluctuations in the percent of absolute power of specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg.

19. To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg.

20. To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of pentazocine 5

mg/kg.

21. To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg.

22. To determine if there are differences, over time, in the cyclic fluctuations in percent of absolute power of specific EEG frequencies in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg.

Naloxone Experiment

23. To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of naloxone 5 μg/kg.

24. To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of naloxone 5 μg/kg.

25. To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of naloxone 5 μg/kg.

26. To determine if there are differences, over time, in the cyclic fluctuations in the percent of absolute power of specific EEG frequencies of interest in conscious rats

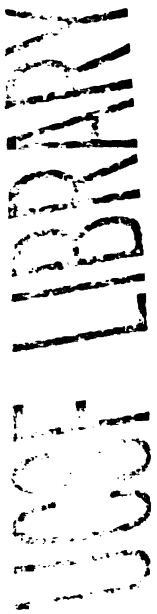
who received a subcutaneous injection of naloxone 5 μg/kg.

27. To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of naloxone 500 μg/kg.

28. To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of naloxone 500 μg/kg.

29. To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of naloxone 500 μg/kg.

30. To determine if there are differences, over time, in the cyclic fluctuations in percent of absolute power of specific EEG frequencies in conscious rats who received a subcutaneous injection of naloxone 500 μg/kg.



CHAPTER 2 - LITERATURE REVIEW

Introduction

Although little research exists that specifically addresses the mechanism of opioid-induced sedation, a large body of research exists on the mechanisms of action of opioid analgesics within the CNS. Additionally, a great deal of research has been done on the phenomenon of consciousness. Therefore, the purpose of this chapter is to attempt to meld these two bodies of research and propose a model of how opioids may act in the CNS to produce sedation. The domain of consciousness will be reviewed and integrated into a discussion of how opioids might induce sedation. Part of this discussion will be to differentiate between what opioid-induced sedation is and what it is not. Based upon this discussion, a definition of opioid-induced sedation is proposed that sets the stage for an analysis of EEG measurement techniques and a review of the literature describing what is currently known about the effects of systemically administered opioids on EEG recordings in experimental animal models.

Consciousness

Consciousness is a complex physiologic process accomplished by all mammals through the coordinated actions of the CNS. Plum and Posner (1980) proposed that consciousness is composed of two interrelated domains: arousal and content. Many authors have adopted this approach to explore the phenomena of consciousness (Ackerman, 1993; Alcorn, 1983; Crigger & Strickland, 1985; Grant & Kinney, 1990; Summers, 1992; Turner & Knapp, 1995). According to Plum and Posner (1980), arousal refers to the organism's state of awakeness, while content addresses how the organism

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Arousal

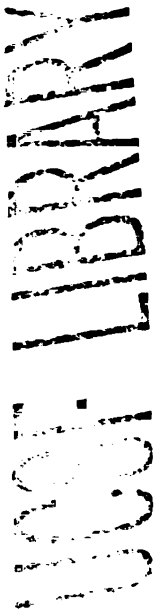
Arousal is the organism's state of responsiveness to sensory stimuli. Throughout a 24-hour day, arousal normally fluctuates between awake and sleep states. Arousal is believed to be mediated primarily by a large group of neurons that are known as the reticular formation, distributed throughout the medulla, pons, and midbrain (Kelly & Dodd, 1991; Moruzzi & Magoun, 1948; Role & Kelly, 1991). The reticular formation is not a distinct anatomic site per se, but rather a network of neurons whose axons branch both caudally and rostrally.

Work by Moruzzi and Magoun (1949) identified the role of the ascending reticular activating system (ARAS) in arousal. In experiments using cats, when transections of the brain stem were made at the junction of the spinal cord and medulla, a chronic wake state was produced. When transections were made more rostrally in the brain stem, at the level of the midbrain, a chronic sleep state was produced in the cats. Prior to these studies, wake states were thought to be dependent upon sensory input (Neylan, 1995), but the work of Moruzzi and Magoun (1949) suggest that wake states are themselves intrinsic to the CNS, not simply a state induced with sensory bombardment.

The thalamus also plays an integral role in arousal (Newman, 1995). Neurons of the ARAS project to neurons in the thalamus. These thalamic neurons, which are reciprocally linked with neurons in the cerebral cortex, have two distinct settings which correspond to awake and sleep states and are under the control of aminergic and

cholinergic neurotransmitters (Hobson, 1990). The depolarized, or awake setting responds to sensory input while the hyperpolarized, or sleep setting is relatively unresponsive to sensory input (Steriade, McCormick, & Sejnowski, 1993). The mechanism that triggers the switch from one setting to the other is not understood (Hobson, 1990).

Within a 24-hour day, mammals normally fluctuate between sleep and wake states. These cycles will be discussed in more detail later in this chapter. As depicted in Figure 1, various pathologic or drug-induced conditions can disrupt arousal and be manifested in the extreme as either hyperactive delirium or unconsciousness. However, the gradations among the various arousal states between these two extremes have not been well defined using explicit clinical criteria. Disrupted arousal states are best considered in conjunction with content processing and will be discussed in more detail later in this chapter.



Content Processing

Content processing is what adds quality to consciousness by means of sensation, thought, speech, imagination, and interpretation of somatic modalities (Crigger & Strickland, 1985). Content is primarily a function of the cerebral cortex which integrates sensory and motor information from the thalamus thus coordinating appropriate responses to various inputs (Kelly & Dodd, 1991). While arousal can occur without content processing, content processing cannot occur without some level of arousal (Plum & Posner, 1980).

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content processing can be manifested in the extreme as either hallucinations or the absence of content processing. Figure 1 attempts to integrate the two domains of consciousness into a box-shaped model with seven columns representing gradations of arousal and seven rows representing gradations of content processing. Terms commonly used in the literature to describe alterations in consciousness have been placed within the model as points of reference. The placement of these conditions along the continuums of arousal and content is certainly subject to debate because of the lack of clarity in the definitions of these conditions as well as a lack of knowledge about the physiologic mechanisms underlying them. In Figure 1, only delirium (American Psychiatric Association, 1994) and coma (Plum & Posner, 1980) have agreed upon diagnostic criteria. As indicated by the shaded boxes in the figure, sedation can occur when *either* arousal *or* content processing are depressed, or when *both* arousal and content processing are depressed. This conceptualization is very similar to how Plum and Posner (1980) defined clouding of consciousness. They referred to a clouding of consciousness as a state of reduced wakefulness or awareness, while making note that it has neither been described nor investigated in any detail (Plum & Posner, 1980).

Sleep-Wake Cycles

Medical literature often uses the terms sedation and sleepiness interchangeably. Although not well studied, distinct differences exist between naturally occurring sleepiness followed by sleep, medical conditions producing sedation such as head trauma or CNS lesions, and pharmacologically-induced sedation.

Sleep is a naturally occurring circadian behavior that is believed to be a

restorative process essential for normal metabolic, thermoregulatory, and information-processing functions (Adams & Victor, 1993; Hobson, 1990). There are five distinct stages of sleep. Stages I, II, III, and IV are collectively called non-rapid eye movement (NREM) sleep. The fifth stage is labeled rapid eye movement or REM sleep. All stages can be identified by EEG, electrooculogram (EOG), and electromyogram (EMG) recordings. Table 1 describes the characteristics of the EEG waveforms for both sleep and wake states.

In humans, each of the sleep stages is experienced several times during normal sleep in cycles lasting approximately 90 minutes (Kelly, 1991a). During NREM sleep, the EEG becomes progressively more synchronized. Temperature drops, as does heart rate, blood pressure, and respiratory rate. REM sleep is characterized by the sudden onset of an asynchronous EEG pattern. Very little muscle movement occurs during REM sleep, except for muscle twitches of the face and the eyes. Sleepers awakened from REM sleep typically report dreaming.

While at one time the awake and sleep states were thought to be two extremes on the continuum of consciousness, the characteristics of REM sleep contradict this notion. During REM sleep, thalamic neurons continue to be relatively unresponsive to sensory input, and yet the cerebral cortex is very active (Adams & Victor, 1993; Hobson, 1990; Kelly, 1991a). Because of this, sleep was placed into Figure 1 as two vectors normally fluctuating along the continuums of arousal and content processing. States of disordered consciousness and sleep may appear to be very similar to the casual observer. However, while awake and sleep states normally exhibit a circadian rhythm, states of disordered

consciousness do not fluctuate within predictable parameters of degree or time.

Sleep deprivation can have profound negative consequences. Humans deprived of sleep for more than two days experience increasing levels of fatigue and irritability. They have increased difficulty concentrating, and their motor coordination deteriorates. When sleep-deprived individuals are finally able to sleep, they initially increase their Stage IV NREM sleep time at the expense of Stage II NREM and REM sleep. Subsequently, REM sleep time rebounds in proportion to the amount of time REM sleep was curtailed (Adams & Victor, 1993; Kelly, 1991a).

Various drugs, particularly those active in the CNS, can change the proportion of time an individual spends in the different sleep stages (Vogel, Buffenstein, Minter, & Hennessey, 1990). Benzodiazepines, the most commonly prescribed sleeping medication, reduce the number of awakenings during sleep so that people sleep more continuously (Kelly, 1991b). However, benzodiazepines depress the amount of Stage III and IV NREM sleep which are considered the most restful stages of sleep. Yet, people taking benzodiazepines for sleeplessness report that the quality of their sleep is improved with the short-term use of drug (Kales & Kales, 1984; Vgontzas, Kales, & Bixler, 1995).

Like benzodiazepines, opioids change the proportion of time spent in the different stages of sleep. However, the effects of opioids on sleep have not been as well studied as they have been for benzodiazepines. Kay, Eisenstein, and Jasinski (1969) reported that morphine depressed REM sleep, but this study was done with only eight men, all of whom were addicted previously to opioids.

Opioid Analgesics

The opioids are a large class of drugs that have been employed for centuries to relieve pain (Bailey & Stanley, 1994; Reisine & Pasternak, 1996; Way, Way, & Fields, 1995). Endogenous opioids presumably function as the body's own analgesics. Morphine and codeine are commonly used naturally occurring opioids and many synthetic opioids have been manufactured.

Mechanism of Action of Opioid Analgesics

Nociceptive information is conveyed to the brain through at least five ascending pathways (i. e., spinothalamic tract, spinoreticular tract, spinomesencephalic tract, spinocervical tract, and the dorsal column of the spinal column). En route to the brain, these signals can be modified by input from various afferent fibers and by central control mechanisms that make use of opioids, as well as other neurotransmitters and peptides (Basbaum & Fields, 1984; Jessell & Kelly, 1991; Melzack & Wall, 1965).

Both endogenous and exogenous opioids exert their antinociceptive effects within the CNS in at least three ways. In the brain stem, opioids activate descending pain modulatory pathways that originate within neurons of the periaqueductal gray matter (PAG). PAG neurons project to the nucleus raphe magnus located in the medulla which in turn send projections to the spinal cord inhibiting dorsal horn neurons in laminae I, II, and V (Basbaum & Fields, 1984). In the spinal cord, opioids directly inhibit the firing of dorsal horn neurons that carry nociceptive signals to the brain (Sabbe & Yaksh, 1990; Yaksh, 1981). Peripherally, opioids modulate the primary afferent synapse depressing both presynaptic and postsynaptic membrane potentials (Stein, 1993).

Opioids inhibit the firing of individual neurons through interactions with cell surface opioid receptors and second messenger systems. Three major classes of opioid receptors have been described, mu (μ), kappa (κ), and delta (δ), as well as subtypes of each class. μ- and δ-opioid receptors gate potassium ions hyperpolarizing the cell membrane and making it resistant to excitation (Williams, Egan, & North, 1982). κ-opioid receptors have a different mechanism of action that blocks calcium flux across the cell membrane thereby decreasing neurotransmitter release (Macdonald & Werz, 1986). Table 2 lists the three major classes of opioid receptors, their location in the CNS, and the proposed effects that can occur when the receptor is occupied with their specific endogenous or exogenous ligand.

Sedative Effects of Opioid Analgesics

In addition to their antinociceptive effects, opioids can produce sedation. As previously discussed, there is a paucity of information about the mechanism of opioid-induced sedation. It has been postulated that opioids cause sedation by decreasing sensory input thereby increasing the probability of sleep (Martin, 1984). There is limited evidence that opioids disrupt sleep patterns by decreasing REM sleep in both humans (Kay, Eisenstein, & Jasinski, 1969) and animals (Furst, 1990; King, Masserano, Codd, & Byrne, 1981; Landis, 1988). However, based on the work of Moruzzi and Magoun (1949), awake and sleep states are not determined by the amount of sensory input but rather are dependent upon intact systems that are intrinsic to the CNS.

Using spectral analysis, EEG studies of both humans and animals given opioids have reported changes in power to the lower frequencies between 1 and 10 Hz.

Quantitative changes in spectral densities of EEG recordings of humans (Bowdle & Ward, 1989; Chi, Sommer, & Jasaitis, 1991; Scott, Cooke, & Stanski, 1991; Scott, Ponganis, & Stanski, 1985; Smith et al., 1984; Wauquier, Bovill, & Sebel, 1984) and animals (Bronzino et al., 1982; Campi & Clarke, 1995; Hong, Young, & Khazan, 1988; Paquette & Young, 1991; Stamidis & Young, 1992, 1993; Young, Hudson, Stamidis, & Steinfels, 1993a, 1993b; Young & Khazan, 1984) given opioids reported that spectral power had shifted to lower frequencies between 1 and 10 Hz. The significance of these power shifts in explaining the mechanism of opioid-induced sedation has not been explored thoroughly (Avramov & White, 1995).

There is some evidence from animal studies that the sedative effects of opioids can be disassociated from their antinociceptive effects. μ-receptor agonists have been observed to produce sedation when given subcutaneously at doses 3 to 34 times higher than their antinociceptive dose, and κ-receptor agonists produce sedation at doses 29 to 2,500 times higher than their subcutaneous antinociceptive doses (Hayes & Tyers, 1983). These researchers postulated that high doses of κ-agonists produced sedation through drug interaction at μ-receptors, rather than at κ-receptors.

Use of Opioids with Benzodiazepines

It has been difficult to study the sedative effects of opioids in the clinical setting because patients taking these drugs for pain relief are often taking many other drugs that also depress LOC. Commonly, opioids are combined with benzodiazepines to control pain and reduce anxiety in critically ill patients (Avramov & White, 1995; Levine, 1994), patients undergoing diagnostic or surgical procedures (Stevens & White, 1994; Willenkin

& Polk, 1994), and patients experiencing chronic pain (Hendler, Cimini, Long, & Long, 1980).

Biochemically, benzodiazepines and opioids have different mechanisms of action. Benzodiazepines interact with the gamma-aminobutyric acid (GABA) receptor to potentiate the actions of the inhibitory neurotransmitter, GABA (Becker, 1991; Möhler & Richards, 1988). When occupied with GABA, the GABA receptor gates chloride which hyperpolarizes the cell membrane thus making the cell resistant to firing. GABA receptors are located throughout the CNS, the highest densities being measured in the cerebral cortex, hypothalamus, cerebellum, brain stem, and spinal cord (Möhler & Okada, 1977). GABA is widely used throughout the CNS for both feedback inhibition and modulation of excitatory neurotransmitter release (Möhler & Okada, 1977; Möhler & Richards, 1988). The GABA receptor complex has three functional domains: 1) a GABA binding site, 2) a benzodiazepine binding site, and 3) a barbiturate binding site. Although benzodiazepines cannot open the chloride ion channel independently, the actions of GABA can be potentiated in the presence of either benzodiazepines or barbiturates (Becker, 1991; Möhler & Richards, 1988).

In addition to interacting with a different receptor, the EEG profiles of patients taking a benzodiazepine are completely different from EEG profiles of patients taking an opioid. Patients taking opioids are more likely to have increased power in the lower frequencies between 1 and 10 Hz (Bowdle & Ward, 1989; Chi, Sommer, & Jasaitis, 1991; Scott, Ponganis, & Stanski, 1985; Smith et al., 1984; Wauquier, Bovill, & Sebel, 1984), whereas patients taking benzodiazepines are more likely to have increased power

in the higher frequencies between 12 and 35 Hz (Hendler, Cimini, Long, & Long, 1980; Seifert, Blouin, Conard, & Gross, 1993).

The behavioral correlates associated with these EEG changes are not well understood. One study evaluated both EEG recordings and tests of cognitive function in patients admitted to a chronic pain treatment center (Hendler, Cimini, Long, & Long, 1980). Of the 106 consecutively admitted patients, 74 were taking both benzodiazepines and opioids, 13 were taking benzodiazepines alone, 13 were taking opioids alone, and 6 were not taking any drugs. In addition to the diffuse, high frequency waves observed on EEG recordings, the 13 patients taking benzodiazepines had significant decreases in cognitive function when compared with the 13 patients taking only opioids. The patients taking opioids alone did not have any EEG changes. However, the researchers did not report either the type or doses of drugs being taken. Neither was the duration of drug therapy reported. In addition to the differences in cognitive functioning, this study points out that many patients experiencing chronic pain are treated with both benzodiazepines and opioids. Without a better understanding of the unique sedative effects of both opioids and benzodiazepines, a reasoned approach to maximizing therapy and minimizing side effects is impossible.

Conscious Sedation

Different from sedation as a side effect of opioids, conscious sedation is an anesthetic technique that uses combinations of opioids and benzodiazepines to reduce the pain and anxiety associated with short diagnostic, endoscopic, and surgical procedures (Stevens & White, 1994). Drugs are chosen for their rapid onset of action, predictable

dose-response relationship, minimal respiratory and cardiovascular effects, and rapid recovery to normal consciousness. Mention is made of conscious sedation here because the definition and classification of this type of intentional sedation for a specific procedure using multiple drugs is a different phenomenon than sedation as a side effect of opioids.

The definition and classification of sedation induced with general anesthesia is, likewise, a different phenomenon than opioid-induced sedation. General anesthesia is a process where combinations of anesthetics, barbiturates, sedative hypnotics, analgesics, and muscle relaxants are administered to a patient in preparation for a surgical procedure. Anesthesia aims to induce unconsciousness, analgesia, muscle relaxation, and amnesia while maintaining adequate tissue oxygenation and perfusion (Rampil, 1992). Induction of general anesthesia ideally accomplishes a rapid loss of consciousness and progression to a light surgical plane of anesthesia characterized by decreased ventilation, decreased blood pressure, and a minimal response to stimuli (Willenkin & Polk, 1994). As with conscious sedation, the sedative hypnotics are administered for their sedative effects and analgesics, such as opioids, are given for their antinociceptive effects. Currently, the sedative effects unique to opioids cannot be identified or described when used in combination with other drugs that also cause sedation.

Proposed Definition of Opioid-Induced Sedation

Based on the preceding review of the anatomy of the CNS, the physiology of consciousness, and opioid pharmacology, the proposition is made that opioid-induced sedation represents a disordered LOC where both arousal mechanisms and content

processing are functional but attenuated because of the action of opioids at receptors within the CNS. Attenuated arousal is manifested as decreased wakefulness and attenuated content is manifested as a slowed or faulty interpretation of the environment. This depressed LOC is associated in humans with EEG spectral power shifts to lower frequencies between 1 and 10 Hz. However, the clinical implications of these EEG changes in the study of opioid-induced sedation has not been explored fully.

Electroencephalograph Recordings

Communication within the CNS and between the CNS and the rest of the body is accomplished through an integration of electrical and chemical signals (Kandel, Siegelbaum, & Schwartz, 1991). In neurons, the flux of sodium and potassium ions across a cell membrane generates an action potential that travels the length of the cell releasing various neurotransmitters that act upon other neurons and influence their ability to generate an action potential. Activation of a receptor, present in the cell membrane can either facilitate or block ion movement across the membrane. Various drugs, including opioids, act on cell membrane receptors to influence ion movements and thus cell signalling and communication.

The amount of neurotransmitter released and the action potential generated by a single cell can only be measured in vitro under experimental conditions. Measuring the electrical activity of organized groups of cells, such as in the cerebral cortex, is possible in vivo and is an important clinical tool for the diagnosis and management of various neurologic conditions (Martin, 1991). The cerebral cortex is organized in vertical columns of neurons and supporting glial cells that extend radially from the center of the

brain outward toward the skull. Because these columns of cells operate in concert, summed changes in the electrical potential across many cell membranes can be measured between two electrodes.

An electroencephalograph is an instrument that detects and displays a difference in electrical potential between two electrodes placed on the external surface of the scalp or in the CNS. Electrode placement determines which portion of the CNS is being monitored. Electrical activity of cortical neurons can be recorded from electrodes affixed to the scalp (Rampil, 1987). To record the electrical activity of structures located deeper within the CNS, electrodes must be implanted surgically into or near the structure of interest. While tracings from scalp electrodes are attenuated by cerebral spinal fluid, the skull, and the scalp, electrodes placed surgically into the scalp or skull can improve the quality of the recordings. In the research setting, differences in skull size, scalp diameter, volume of cerebral spinal fluid, and individual variations in neuron columns must be considered when comparing EEG tracing between subjects.

The EEG voltage signal is processed through a series of filters and amplifiers to filter out wave artifact and amplify specific wave frequencies of interest (Black, Mahla, & Cucchiara, 1994; Rampil, 1987, 1992). EEG wave frequencies of interest range between 1 and 30 Hz, and so frequencies above 30 Hz are filtered out of the signal while frequencies between 1 and 30 Hz are amplified. This filtered, amplified signal is still subject to electrical artifact occurring between 1 and 30 Hz. Skeletal muscle activity, cardiac contractions, and environmental electrical interference can inadvertently contribute to the recorded EEG signal and influence the interpretation of the signals.

EEG recordings can be analyzed either qualitatively, by a trained electrophysiologist, or quantitatively, using computer analysis. Both methods describe the EEG waveform by its frequency and amplitude. Delta (δ), theta (θ), alpha (α), sigma (σ), and beta (β) are Greek letters that have been arbitrarily assigned to describe waveforms of specific frequencies and amplitudes. In addition to these underlying waveforms of a particular frequency and amplitude, discrete wave complexes can be identified at various times throughout the wake and sleep cycles.

Experienced electrophysiologists learn to recognize many different waveforms and can correlate changes in waveforms, or the occurrence of a specific waveform, with the patient's neurologic state. Qualitative analysis of the EEG remains the gold standard for describing sleep stages and sleep stage transition, as well as for describing seizure activity (Black, Mahla, & Cucchiara, 1994; Gasser & Molinari, 1996).

Computerized quantitative assessment of the EEG is another approach to analyzing EEG recordings (Donegan & Rampil, 1990; Rampil, 1987, 1992). The EEG waveform is digitalized by converting the analog signal into a series of discrete numbers that a computer can mathematically manipulate. Once digitalized, the computer can analyze the EEG signal as a function of time or as a function of wave frequency.

Time domain analysis evaluates the wave voltage as a function of time (Donegan & Rampil, 1990; Rampil, 1987, 1992). Activity, mobility, and complexity are three time domain analyses. Activity is the variance of the wave amplitude (Hjorth, 1970). Mobility is the relative number of zero crossings of the signal (Haberny & Young, 1994; Hjorth, 1970). Complexity is the measure of the deviation of the observed wave from a

sine wave (Haberny & Young, 1994; Hjorth, 1970). Hjorth (1973) described an algorithm to determine sleep and awake states in the rat by evaluating the time domain parameters of activity and complexity. According to Hjorth, periods of decreased activity and increased complexity were associated with both awake states and REM sleep. Periods during which complexity and activity both returned to baseline were associated with NREM sleep.

Frequency domain analysis makes use of fast Fourier-transformations (FFTs) to decompose a digitalized EEG waveform into the frequencies of the component waves, thereby evaluating wave voltage as a function of frequency (Donegan & Rampil, 1990; Gottman, 1980; Rampil, 1987, 1992). Just as a prism breaks down white light into its component colored light frequencies, so too does spectral analysis break down a waveform into its component wave frequencies. This method is particularly useful in EEG analysis where thousands of neuron units contribute to the observed waveform as a means of determining the prominent brain wave frequency at any given time. The spectral density, or variance, is plotted according to wave frequency. The greater the spectral density at a particular frequency, the more prominent that wave is in the original EEG recording. The spectral density below 1 Hz is the variance in the waveform that is not attributable to a sinusoidal wave (Gasser & Molinari, 1996; Thomas, 1990). This variance is unexplained and may be related to artifact within the frequencies of interest that cannot be filtered or to measurement error that needs to be considered when analyzing EEG data.

Treating the frequency spectrum as a statistical distribution, four frequency

domain descriptors of the EEG waveform are absolute power, peak frequency, median frequency, and edge frequency. Absolute power is the summation of spectral densities over the frequency range of interest and is measured in μV^2 (Hudson, Marquis, Stamidis, & Young, 1992). Peak frequency is the frequency at which the greatest power exists and is analogous to the mode of the spectrum (Donegan & Rampil, 1990; Hudson, Marquis, Stamidis, & Young, 1992; Rampil, 1987, 1992). Median frequency is the frequency at which one-half of the power lies above and one-half of the power lies below in the spectrum (Rampil, 1987, 1992). And, edge frequency is the frequency below which 97% of the power lies (Hudson, Marquis, Stamidis, & Young, 1992; Rampil, 1987, 1992; Rampil et al., 1980).

Quantitative assessment of the EEG that allows for an objective assessment of the EEG waveform has been most useful in studying the neurologic effects of drugs, including opioids, that act upon the CNS (Wauquier, 1993; Young, Hong, & Khazan, 1987). Quantified pharmaco-EEG, or QPEEG, has been used to define certain drug classes, to assess both therapeutic and toxic drug levels, to assess the duration of drug action, and to identify CNS effects of drugs not intended to target the brain or nervous system (Gasser & Molinari, 1996; Wauquier, 1993).

Effects of Opioid Agonists on the Electroencephalograph

Drugs acting on receptors present in neuronal cell membranes can either facilitate or block ion movements across the membrane, thereby influencing the electrical activity within cell columns and the CNS. These changes can be observed with EEG recordings (Wauquier, 1993). Opioid agonists produce specific, quantitative changes in EEG

recordings.

μ-Opioid Agonists

In rats, μ-opioid agonists produce dose-dependent, high-voltage, slow-frequency waves and increase spectral power in the lower frequencies of 0 to 10 Hz. Bronzino and colleagues (1982) described EEG spindling within one minute of an intraperitoneal injection of morphine (2.5 mg/kg or 5 mg/kg) which lasted for 30 to 60 minutes. At higher doses of morphine (15 mg/kg or 30 mg/kg) they described more intense spindling that lasted up to 100 minutes, as well as the occurrence of short epochs of synchronized high-voltage, slow-frequency activity. With increasing doses of morphine, EEG spectral power progressively shifted from the higher frequencies to the lower frequencies of 5 to 7 Hz and spectral power shifts persisted for longer periods of time. At a lower dose of morphine (5 mg/kg), the percent of absolute power at 5 to 7 Hz was elevated for 60 minutes, while at a higher dose of morphine (30 mg/kg), the percent of absolute power at 5 to 7 Hz was elevated for 120 minutes. After every dose of morphine, the researchers reported that, "the animals became immobile and rigid remaining in one corner of the recording chamber until the drug effect abated" (Bronzino et al., 1982, p. 18). The latency to the onset of this behavior and the duration of the behavior corresponded with the measured EEG effects, specifically latency to peak spindle effect and duration of spindle effect.

Stamidis and Young (1993) reported an increase in EEG spectral power between 1 and 10 Hz associated with "behavioral stupor" (p. 512) when 3 mg/kg of morphine was given intravenously to rats. These EEG and behavioral changes persisted for 60 minutes

and were followed by a period of "EEG and behavioral excitation" (p. 512-513) which lasted another 60 minutes. Neither stupor nor excitation was defined using either EEG or behavioral characteristics. Young and Khazan (1984) reported a similar increase in spectral power between 1 and 10 Hz associated with stupor, exophthalmos and respiratory depression lasting 30 to 60 minutes when another μ-opioid agonist, methadone (1.0 mg/kg), was given intravenously to rats.

κ-Opioid Agonists

The κ-opioid agonists, like the μ-opioid agonists, administered systemically to rats produce high-voltage, slow-frequency bursts and increased power in the lower frequencies between 2 and 8 Hz (Campi & Clarke, 1995; Young, Hudson, Stamidis, & Steinfels, 1993a, 1993b; Young & Khazan, 1984). Similar findings have been reported for seven different κ-opioid agonists including ketocyclazocine, enadoline, spiradoline, DuP 747, U-50,488H, BRL 52656, and BRL 53001. In each of these studies, researchers observed sedative behaviors (Campi & Clarke, 1995) and stupor (Young, Hudson, Stamidis, & Steinfels, 1993a, 1993b; Young & Khazan, 1984) associated with the EEG changes. Neither sedation nor stupor was defined.

δ-Opioid Agonists

Naloxone, a drug commonly considered an universal opioid antagonist, has been identified as a δ-receptor agonist at low doses between 5 and 500 ng administered intrathecally (Taiwo, Basbaum, Perry, & Levine, 1989). Doses of naloxone between 50 ng/kg and 5 μg/kg administered subcutaneously have been observed to be analgesic in pain-free rats (Levine, Gordon, Taiwo, & Coderre, 1988), while doses between 10 and 30

μg/kg administered intravenously were observed to be analgesic in arthritic rats but not in pain-free rats (Kayser & Guilbaud, 1981). Higher doses of naloxone (1 mg/kg) administered intravenously, increased EEG spectral power in the frequency bands between 1 and 3.5 Hz (Grasing & Szeto, 1990, 1991). Naloxone was presumably acting as an opioid antagonist at this dose however, not as a δ-agonist.

Conclusions

Opioid-induced sedation is a clinical phenomenon that has received little scientific investigation to date, although it is a commonly reported side effect of these drugs (Cherny & Portenoy, 1994; Jacox et al., 1994; Way, Way, & Fields, 1995). Based on a review of the anatomy of the CNS and the physiology of consciousness, this chapter has proposed that opioid-induced sedation is a disordered level of consciousness where both arousal mechanisms and content processing are functional but attenuated because of the actions of opioid at receptors within the CNS. The acquisition, analysis, and interpretation of EEG recordings as a measure of CNS functioning have been described. As reviewed in this chapter, EEG recordings have been used previously to glean information about the CNS effects of opioids. This study aims to expand the body of knowledge of CNS effects of opioids by describing the effects of two antinociceptive doses of three subcutaneously administered relatively-selective opioid receptor agonists (i. e., morphine, pentazocine & naloxone) on selected EEG parameters of conscious rats.

CHAPTER 3 - METHODS

Sample

Experiments were done using male Sprague-Dawley rats (Bantin Kingman, Fremont, CA) weighing between 240 and 260 grams. Throughout the study, the rats were maintained on a 12 hour light-dark schedule with lights on at 0700 and off at 1900. Rats were housed in individual cages to maintain the integrity of the implanted EEG electrodes. They were fed standard rat chow (Purina Corporation) and water ad libitum.

Animal Preparation

One week prior to the experiments, rats were anesthetized with sodium pentobarbital (50 mg/kg) and four stainless steel wire electrodes were placed in the dura through holes drilled in the skull. To permit comparison of EEG measurements among rats and to the findings of previous studies, electrodes were stereotactically implanted over both frontal (2 mm anterior and 2 mm lateral to bregma) and both parietal (3 mm posterior and 2 mm lateral to bregma) cortices (Khazan, 1975). The electrodes were seated in a receptacle strip connector (D. A. Grahn, PhD, Stanford University, personal communication, January 18, 1996; Khazan, 1975), and the strip connector was secured to the skull with dental acrylic cement. Rats were allowed five days to recover before any testing was done.

Opioid Agonists

Three relatively-selective opioid agonists believed to act at the μ-opioid receptor (morphine), the κ-opioid receptor (pentazocine), and the δ-opioid receptor (low-dose naloxone) were chosen for this study (Reisine & Pasternak, 1996; Taiwo, Basbaum, Perry

& Levine, 1989). Antinociceptive doses of these opioids (Levine, Gordon, Taiwo, & Coderre, 1988) were administered to provide information about the effects of these drugs on the EEG not previously reported, and to more closely approximate clinical situations where opioids are used to manage pain.

Electroencephalogram

EEG signals were acquired, filtered, amplified, displayed, and stored using a Model 7H polygraph, 7P511L amplifiers (Astromed-Grass, West Warwick, RI) and a personal computer. Each day, prior to data collection, the amplifiers and the computer were calibrated to each other. During the calibration procedure, the 50 microvolt (μV) test signal produced by each amplifier set the standard by which the computer measured the voltage of the recorded EEG. Analog signals above 100 Hz and below 1 Hz were filtered and the remaining signals were amplified at 7.5 microvolts per millimeter (μV/mm) by the polygraph. The analog signal was then digitalized at a sampling rate of 250 per second and stored on the computer hard drive in binary files.

A computer program written by Chad E. Kennedy (Software Engineer, VI Technology, Sunnyvale, CA) using LabVIEW® software (National Instruments Corporation, Austin, TX) analyzed the data files computing various time domain and frequency domain parameters of the EEG.

Time domain parameters calculated by the computer program included activity, mobility, and complexity. Activity is the variance of the wave amplitude and is calculated by squaring the standard deviations of the amplitude of the EEG wave (Hjorth, 1970). Mobility is the relative number of zero crossings of the signal and is calculated by

taking the square root of the ratio between the wave variance of the first derivative (the slope of the curve) and amplitude (Hjorth, 1970). Complexity is the measure of the deviation of the observed wave from a sine wave and is calculated by taking the ratio between the mobility of the first derivative of the EEG and the mobility of the EEG itself (Haberny & Young, 1994; Hjorth, 1970).

Frequency domain parameters calculated by the computer program included absolute power, peak frequency, median frequency, edge frequency, and percent of absolute power attributable to individual one Hz frequencies. To calculate the frequency domain parameters, the computer program applied a fast Fourier transform (FFT) to consecutive 60 second epochs of digitalized data. The computer program calculated frequency bands in hundredths of a Hz. For this study, 1 Hz was defined as the frequencies between 0.50 and 1.00 Hz; 2 Hz was defined as the frequencies between 1.01 and 2.00 Hz; 3 Hz was defined as the frequencies between 2.01 and 3.00 Hz. This pattern continued through the frequency bands ending with 30 Hz which was defined as the frequencies between 29.01 and 30.00 Hz. Absolute power is the summation of spectral densities over the 1 to 30 Hz range measured in μV^2 (Hudson, Marquis, Stamidis, & Young, 1992). Peak frequency is the frequency at which the greatest power exists and is analogous to the mode of the spectrum (Donegan & Rampil, 1990; Hudson, Marquis, Stamidis, & Young, 1992; Rampil, 1987, 1992). Median frequency is the frequency at which one-half of the power lies above and one-half of the power lies below in the spectrum (Donegan & Rampil, 1990; Rampil, 1987, 1992). And, edge frequency is the frequency below which 97% of the power lies (Hudson, Marquis, Stamidis, & Young,

1992; Rampil, 1987, 1992; Rampil et al., 1980).

Procedures

On testing days, animals were weighed and then placed in individual acrylic recording cages with their implanted recording electrodes connected to a flexible recording cable. The recording cable was in turn connected to the polygraph through a commutator (Airflyte Electronics, Bayonne, NJ) which permitted free movement of the animals in the recording cages. Overhead lights in the room were turned off and gray shades drawn over the windows to minimize bright light. After animals acclimated to the recording environment for at least 30 minutes, continuous EEG recordings were begun between 0800 and 0830 and continued for six hours.

Upon the completion of the experiments, the rats were euthanized with an overdose of 200 mg/kg of sodium pentobarbital (J. Wyrick, DVM, University of California San Francisco, personal communication, February 16, 1996) which is a method consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (American Veterinary Medical Association, 1993).

Normal Saline Experiment

Procedure for Normal Saline Experiment. For the normal saline experiments, 24 animals were given a subcutaneous injection of 0.2 milliliters (mls) of normal saline just prior to beginning the EEG recordings. This injection stimulated the animals so that the EEG recording were begun with all of the animals at a similar LOC. Two hours after the EEG recordings were begun, animals were given another subcutaneous injection of normal saline (1 ml/kg). EEG recordings continued for another four hours for a total

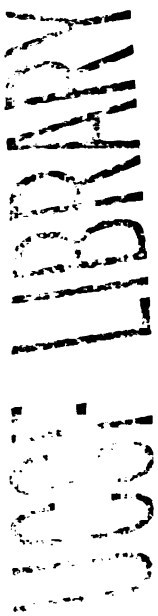
recording time of six hours. The first two hours of EEG recording, between 0800 and 1000, were considered the baseline recording period; the second two hours of EEG recording, between 1000 and 1200, were considered the saline recording period; and the final two hours of EEG recording, between 1200 and 1400, were considered the post-saline recording period.

Data Analysis Plan for Normal Saline Experiment. To describe the EEG parameters, means (± standard error of the mean or SEM) were calculated for each of the time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency), for each animal for the entire six hour experiment. To determine if there were differences over time in any of the EEG parameters, means (± SEM) were calculated for each of the time and frequency domain parameters for each animal for each of the three, two hour recording periods, and a one-way repeated measures analysis-of-variance (RMANOVA) was performed. When a significant difference was identified in any of the parameters, pairwise comparisons were made using the Dunnett's post-hoc test to determine differences from the baseline recording period.

To assess the percent of absolute power attributable to individual one Hz frequencies over time, data from the 24 animals were meaned and three-dimensional graphs were created for each of the three, two hour recording periods displaying the percent of absolute power of individual one Hz frequency bands at each minute of the 360 minute experiment. Visual assessments of these graphs were done and frequency bands with fluctuations in percent of absolute power over time were identified for further

evaluation. The raw data were evaluated to confirm that the frequency bands with the greatest variability in percent of absolute power over time were identified correctly from the visual assessment.

To assess the magnitude of absolute power attributable to specific EEG frequencies of interest, area-under-the-curve (AUC) (Max & Laska, 1991) was calculated for the frequencies of interest identified from the three dimensional graphs for each of the three, two hour recording periods. To determine if there were differences over time in the magnitude of absolute power attributable to specific frequencies of interest, a one-way RMANOVA was performed. When a significant difference was identified, pairwise comparisons were made using the Dunnett's post-hoc test to determine differences from the baseline recording period.



To describe cyclic fluctuations in the percent of absolute power attributable to specific EEG frequencies of interest, the data were subjected to a time-series analysis (Abraham & Neundorfer, 1990; Gottman, 1981, Taylor, 1990, 1994). For this analysis, the data from the first five minutes of the baseline recording period and the first five minutes of the saline recording period were deleted because the animals had just received an injection. Therefore, for the six hour recording period, 350 data points were available for each animal's time-series analysis. Data from individual animals were analyzed for shape, structure, and statistical significance using an autocorrelation function (ACF). This statistical technique measures the correlation between observations in a time-series using a predetermined time lag. The ACF can be defined as the shared variance of one observation with successive observations and assesses the extent to which observations at

one point in time are predictive of observations at a future point in time (Taylor, 1990). Using each animal's time-series data of percent of absolute power, 60 autocorrelation coefficients for time lags of 1 through 60 minutes were plotted as a correlogram. From this correlogram, the shape of the time-series data could be visualized and recurring patterns identified from highly correlated observations. If a recurring pattern was identified, the period length (i. e., the time lag at which observations were most highly correlated) was used to describe the shape of these time-series data. Then, another correlogram using a time lag just a few minutes longer than the observed period length was done to determine the structure and statistical significance of existing patterns. The structure of the data was described by the strength of the autocorrelation coefficient for the observed period length. The statistical significance ($p < .05$) of the pattern was established when the correlation coefficient of the period length was greater than two times the standard deviation of the autocorrelation falling outside the so-called Bartlett band of statistical significance (Gottman, 1984; Taylor, 1990). Descriptive statistics were used to describe the percent of animals establishing a pattern, the period length, and the strength of that pattern.

To determine if there were differences, over time, in the cyclic fluctuations in percent of absolute power attributable to specific EEG frequencies of interest, the data were again subjected to a time-series analysis. For this analysis, data from the first five minutes of the baseline recording period and the first five minutes of the saline recording period were deleted because the animals had just received an injection. Therefore, for the baseline recording period and the saline recording period, 115 data points were available

for each animal's time-series analysis. And for the post-saline recording period, 120 data points were available for each animal's time-series analysis. Again, data from individual animals were analyzed for shape, structure, and statistical significance using an autocorrelation function (ACF). This statistical technique measures the correlation between observations in a time-series using a predetermined time lag. Using each animal's time-series data of percent of absolute power, 60 autocorrelation coefficients for time lags of 1 through 60 minutes were plotted as a correlogram. From this correlogram, the shape of the time-series data could be visualized and recurring patterns identified from highly correlated observations. If a recurring pattern was identified, the period length (i. e., the time lag at which observations were most highly correlated) was used to describe the shape of this time-series data. Then, another correlogram using a time lag just a few minutes longer than the observed period length was done to determine the structure and statistical significance of existing patterns. The structure of the data was described by the strength of the autocorrelation coefficient for the observed period length. The statistical significance ($p < .05$) of the pattern was established when the correlation coefficient of the period length was greater than two times the standard deviation of the autocorrelation falling outside the so-called Bartlett band of statistical significance (Gottman, 1984; Taylor, 1990). Descriptive statistics were used to describe the percent of animals establishing a pattern, the period length, and the strength of that pattern.

Morphine Experiment

Procedure for Morphine Experiment. For the morphine experiments, eight animals were given a subcutaneous injection of 0.2 mls of normal saline just prior to

beginning the EEG recordings. This injection stimulated the animals so that the EEG recording were begun with all of the animals at a similar LOC. Two hours after the EEG recordings were begun, animals were given a subcutaneous injection of morphine (5 μg/kg). EEG recordings continued for another four hours for a total recording time of six hours. The first two hours of EEG recording, between 0800 and 1000, were considered the baseline recording period; the second two hours of EEG recording, between 1000 and 1200, were considered the drug recording period; and the final two hours of EEG recording, between 1200 and 1400, were considered the post-drug recording period. At a minimum of 24 hours later, this procedure was repeated giving the animals a higher dose of morphine (500 μg/kg).

Data Analysis Plan for Morphine Experiment. To determine if there were differences over time in any of the EEG parameters, means (± SEM) were calculated for each of the time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency), for each animal for each of the three, two hour recording periods, and a one-way RMANOVA was performed. When a significant difference was identified in any of the parameters, pairwise comparisons were made using the Dunnett's post-hoc test to determine differences from the baseline recording period.

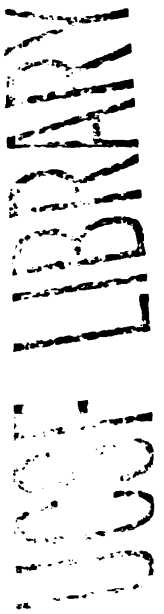
To assess the percent of absolute power attributable to individual one Hz frequencies over time, data from the eight animals were meaned and three-dimensional graphs were created for each of the three, two hour recording periods displaying the percent of absolute power of individual one Hz frequency bands each minute of the three,

two hour recording periods. Visual assessments of these graphs were done comparing the baseline, drug, and post-drug recording periods. Frequency bands with fluctuations in percent of absolute power over time were identified for further evaluation. The raw data were evaluated to confirm that the frequency bands with the greatest variability in percent of absolute power over time were correctly identified from the visual assessment.

To assess the magnitude of absolute power attributable to specific EEG frequencies of interest, AUC was calculated for the frequencies of interest identified from the three dimensional graphs for each of the three, two hour recording periods. To determine if there were differences over time in the magnitude of absolute power attributable to specific frequencies of interest, a one-way RMANOVA was performed. When a significant difference was identified, pairwise comparisons were made using the Dunnett's post-hoc test to determine differences from the baseline recording period.

To determine if there were differences, over time, in the cyclic fluctuations in percent of absolute power attributable to the specific frequency bands of interest the data were subjected to a time-series analysis (Abraham & Neundorfer, 1990; Gottman, 1981, Taylor, 1990, 1994). For this analysis, the data from the first five minutes of the baseline recording period and the first five minutes of the drug recording period were deleted because the animals had just received an injection. Therefore, for the baseline recording period and the drug recording period, 115 data points were available for each animal's time-series analysis. And for the post-drug recording period, 120 data points were available for each animal's time-series analysis. Again, data from individual animals were analyzed for shape, structure, and statistical significance using an ACF. Using each

animal's time-series data of percent of absolute power, 60 autocorrelation coefficients for time lags of 1 through 60 minutes were plotted as a correlogram. From this correlogram, the shape of the time-series data could be visualized and recurring patterns identified from highly correlated observations. If a recurring pattern was identified, the period length (i. e., the time lag at which observations were most highly correlated) was used to describe the shape of this time-series data. Then, another correlogram using a time lag just a few minutes longer than the observed period length was done to determine the structure and statistical significance of existing patterns. The structure of the data was described by the strength of the autocorrelation coefficient for the observed period length. The statistical significance ($p < .05$) of the pattern was established when the correlation coefficient of the period length was greater than two times the standard deviation of the autocorrelation falling outside of the Bartlett band of statistical significance (Gottman, 1984; Taylor, 1990, 1994). Descriptive statistics were used to describe the percent of animals establishing a pattern, the period length, and the strength of that pattern.



Pentazocine Experiment

Procedure for Pentazocine Experiment. For the pentazocine experiments, eight animals were given a subcutaneous injection of 0.2 mls of normal saline just prior to beginning the EEG recordings. This injection stimulated the animals so that the EEG recording were begun with all of the animals at a similar LOC. Two hours after the EEG recordings were begun, animals were given a subcutaneous injection of pentazocine (50 µg/kg). EEG recordings continued for another four hours for a total recording time of six hours. The first two hours of EEG recording, between 0800 and 1000, were considered

the baseline recording period; the second two hours of EEG recording, between 1000 and 1200, were considered the drug recording period; and the final two hours of EEG recording, between 1200 and 1400, were considered the post-drug recording period. At a minimum of 24 hours later, this procedure was repeated giving the animals a higher dose of pentazocine (5 mg/kg).

Data Analysis Plan for Pentazocine Experiment. To determine if there were differences over time in any of the EEG parameters, means (± SEM) were calculated for each of the time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency), for each animal for each of the three, two hour recording periods, and a one-way RMANOVA was performed. When a significant difference was identified in any of the parameters, pairwise comparisons were made using the Dunnett's post-hoc test to determine differences from the baseline recording period.

To assess the percent of absolute power attributable to individual one Hz frequencies over time, data from the eight animals were meaned and three-dimensional graphs were created for each of the three, two hour recording periods displaying the percent of absolute power of individual one Hz frequency bands each minute of the three, two hour recording periods. Visual assessments of these graphs were done comparing the baseline, drug, and post-drug recording periods. Frequency bands with fluctuations in percent of absolute power over time were identified for further evaluation. The raw data were evaluated to confirm that the frequency bands with the greatest variability in percent of absolute power over time were correctly identified from the visual assessment.

To assess the magnitude of absolute power attributable to specific EEG frequencies of interest, AUC was calculated for the frequencies of interest identified from the three dimensional graphs for each of the three, two hour recording periods. To determine if there were differences over time in the magnitude of absolute power attributable to specific frequencies of interest, a one-way RMANOVA was performed. When a significant difference was identified, pairwise comparisons were made using Dunnett's post-hoc tests to determine differences from the baseline recording period.

To determine if there were differences, over time, in the cyclic fluctuations in percent of absolute power attributable to the specific frequency bands of interest the data were subjected to a time-series analysis (Abraham & Neundorfer, 1990; Gottman, 1981, Taylor, 1990, 1994). For this analysis, the data from the first five minutes of the baseline recording period and the first five minutes of the drug recording period were deleted because the animals had just received an injection. Therefore, for the baseline recording period and the drug recording period, 115 data points were available for each animal's time-series analysis. And for the post-drug recording period, 120 data points were available for each animal's time-series analysis. Again, data from individual animals were analyzed for shape, structure, and statistical significance using an ACF. Using each animal's time-series data of percent of absolute power, 60 autocorrelation coefficients for time lags of 1 through 60 minutes were plotted as a correlogram. From this correlogram, the shape of the time-series data could be visualized and recurring patterns identified from highly correlated observations. If a recurring pattern was identified, the period length (i. e., the time lag at which observations were most highly correlated) was used to

describe the shape of this time-series data. Then, another correlogram using a time lag just a few minutes longer than the observed period length was done to determine the structure and statistical significance of existing patterns. The structure of the data was described by the strength of the autocorrelation coefficient for the observed period length. The statistical significance ($p < .05$) of the pattern was established when the correlation coefficient of the period length was greater than two times the standard deviation of the autocorrelation falling outside of the Bartlett band of statistical significance (Gottman, 1984; Taylor, 1990, 1994). Descriptive statistics were used to describe the percent of animals establishing a pattern, the period length, and the strength of that pattern.

Naloxone Experiment

Procedure for Naloxone Experiment. For the naloxone experiments, eight animals were given a subcutaneous injection of 0.2 mls of normal saline just prior to beginning the EEG recordings. This injection stimulated the animals so that the EEG recording were begun with all of the animals at a similar LOC. Two hours after the EEG recordings were begun, animals were given a subcutaneous injection of naloxone (5 μg/kg). EEG recordings continued for another four hours for a total recording time of six hours. The first two hours of EEG recording, between 0800 and 1000, were considered the baseline recording period; the second two hours of EEG recording, between 1000 and 1200, were considered the drug recording period; and the final two hours of EEG recording, between 1200 and 1400, were considered the post-drug recording period. At a minimum of 24 hours later, this procedure was repeated giving the animals a higher dose of naloxone (500 μg/kg).

Data Analysis Plan for Naloxone Experiment. To determine if there were differences over time in any of the EEG parameters, means (± SEM) were calculated for each of the time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency), for each animal for each of the three, two hour recording periods, and a one-way RMANOVA was performed. When a significant difference was identified in any of the parameters, pairwise comparisons were made using the Dunnett's post-hoc test to determine differences from the baseline recording period.

To assess the percent of absolute power attributable to individual one Hz frequencies over time, data from the eight animals were meaned and three-dimensional graphs were created for each of the three, two hour recording periods displaying the percent of absolute power of individual one Hz frequency bands each minute of the three, two hour recording periods. Visual assessments of these graphs were done comparing the baseline, drug, and post-drug recording periods. Frequency bands with fluctuations in percent of absolute power over time were identified for further evaluation. The raw data were evaluated to confirm that the frequency bands with the greatest variability in percent of absolute power over time were correctly identified from the visual assessment.

To assess the magnitude of absolute power attributable to specific EEG frequencies of interest, AUC was calculated for the frequencies of interest identified from the three dimensional graphs for each of the three, two hour recording periods. To determine if there were differences over time in the magnitude of absolute power attributable to specific frequencies of interest, a one-way RMANOVA was performed.

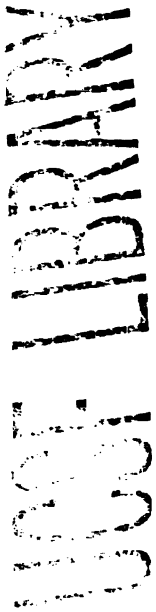
When a significant difference was identified, pairwise comparisons were made using Dunnett's post-hoc tests to determine differences from the baseline recording period.

To determine if there were differences, over time, in the cyclic fluctuations in percent of absolute power attributable to the specific frequency bands of interest the data were subjected to a time-series analysis (Abraham & Neundorfer, 1990; Gottman, 1981, Taylor, 1990, 1994). For this analysis, the data from the first five minutes of the baseline recording period and the first five minutes of the drug recording period were deleted because the animals had just received an injection. Therefore, for the baseline recording period and the drug recording period, 115 data points were available for each animal's time-series analysis. And for the post-drug recording period, 120 data points were available for each animal's time-series analysis. Again, data from individual animals were analyzed for shape, structure, and statistical significance using an ACF. Using each animal's time-series data of percent of absolute power, 60 autocorrelation coefficients for time lags of 1 through 60 minutes were plotted as a correlogram. From this correlogram, the shape of the time-series data could be visualized and recurring patterns identified from highly correlated observations. If a recurring pattern was identified, the period length (i. e., the time lag at which observations were most highly correlated) was used to describe the shape of this time-series data. Then, another correlogram using a time lag just a few minutes longer than the observed period length was done to determine the structure and statistical significance of existing patterns. The structure of the data was described by the strength of the autocorrelation coefficient for the observed period length. The statistical significance ($p < .05$) of the pattern was established when the correlation

coefficient of the period length was greater than two times the standard deviation of the autocorrelation falling outside of the Bartlett band of statistical significance (Gottman, 1984; Taylor, 1990, 1994). Descriptive statistics were used to describe the percent of animals establishing a pattern, the period length, and the strength of that pattern.

Committee on Animal Research Approval

This study was approved by the Committee on Animal Research (CAR) at the University of California San Francisco (A7025-11249-02). Appendix A includes a copy of the CAR approval letter.



CHAPTER 4 - RESULTS

Introduction

This chapter is divided into four sections describing the results of each of the four experiments conducted as part of this study. All 24 of the rats used in this study received normal saline and the results of that experiment are discussed in the section entitled, Normal Saline Experiment. Following the normal saline experiment, groups of eight rats each received one of the three study drugs (i. e., morphine, pentazocine, or naloxone). A minimum of 24 hours later, the same eight rats received a higher dose of the same study drug. The results of those experiments are discussed in the sections entitled, Morphine Experiment, Pentazocine Experiment, and Naloxone Experiment.

Normal Saline Experiment

Aim #1: To describe the EEG time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) over six hours in conscious rats who received a subcutaneous injection of normal saline.

Figures 2 - 6 show the various EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency) over the entire six hour recording period for the 24 conscious rats who received a subcutaneous injection of normal saline. Table 3 shows the mean values of the EEG parameters.

Aim #2: To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain

parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of normal saline.

Figures 2 - 6 show the various EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency) over the entire six hour recording period for the 24 conscious rats who received a subcutaneous injection of normal saline. Table 4 provides a statistical analysis of the EEG parameters using a one-way RMANOVA to compare the three, two hour recording periods. Statistically significant differences between the three recording periods were identified for mobility ($F_{2,46} = 3.87$, $p = .02$), complexity ($F_{2,46} = 3.73$, $p = .03$), and median frequency ($F_{2,46} = 5.25$, $p = .007$). Testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons revealed that the median frequency for the post-saline recording period was significantly higher than for the baseline recording period ($p = .01$). No significant differences from baseline ($p < .05$) were identified for either mobility or complexity. The statistical differences in mobility, complexity, and median frequency following the injection of normal saline can be attributed to the low variance of these measures rather than to physiologically significant differences in these EEG parameters.

Aim #3: To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of normal saline.

The percent of absolute power of individual one Hz frequencies of the right and

left EEG recordings were meaned for the 24 animals who received a subcutaneous injection of normal saline. Results are shown in Figures 7 (0800-1000), 8 (1000-1200), and 9 (1200-1400). The highest density of spectral power occurred in the frequencies below 5 Hz and persisted over the six hour recording period. Spectral power in the very low frequencies is usually considered to be artifact (Gasser & Molinari, 1996). The second highest density of spectral power occurred at 7 and 8 Hz and also persisted over the six hour recording period. Approximately every 10 to 15 minutes recurring fluctuations in power at 7 and 8 Hz were evident. The raw data were evaluated to confirm that 7 and 8 Hz were the frequency bands where these fluctuations occurred. Above 9 Hz, the percent of absolute power for individual one Hz frequency bands remained below 5% of the absolute power.

Aim #4: To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of normal saline.

From the visual analysis of the three-dimensional plots (Figures 7 - 9) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of normal saline, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to further analyze these data.

Figure 10 shows the percent of absolute power at 7 and 8 Hz over the entire six hour recording period for the 24 rats who received a subcutaneous injection of normal

saline. Figure 11 shows the AUC for percent of absolute power at 7 and 8 Hz for each of the three, two hour recording periods. Table 4 provides a statistical analysis of AUC data using a one-way RMANOVA to compare the three, two hour recording periods. No statistically significant differences ($p < .05$) between the three recording periods were identified.

Aim #5: To describe the cyclic fluctuations in the percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of normal saline.

From the visual analysis of the three-dimensional plots (Figures 7 - 9) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of normal saline, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to do a time-series analysis of these data.

Over the six hour recording period (0800-1400), 350 observations of percent of absolute power at 7 and 8 Hz were available for the time-series analysis. Correlograms plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 8 and 39 minutes in 23 (96%) of the 24 animals. For the 23 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, 18 of the animals established a statistically significant cyclic pattern ($0.12 <$

$r < 0.26$, $p < .05$). Data for each of the animals are summarized in Table 5.

Typical of the 23 animals who established a cyclic pattern, Figure 12 is a graph of the percent of absolute power at 7 Hz and 8 Hz over the entire six hour experiment for one animal. Figure 13 is the correlogram with a lag of 60 minutes showing positive correlations at 11, 21, 34, 45, and 58 minutes. And, Figure 14 is the correlogram with a lag of 15 minutes showing a statistically significant autocorrelation ($r = 0.26$, $p < .05$) at 11 minutes that falls outside the Bartlett band.

In contrast, Figure 15 is a graph of the percent of absolute power at 7 and 8 Hz over the entire six hour experiment for an animal who did not establish a cyclic pattern. Figure 16 is the correlogram with a 60 minute lag for these data demonstrating that this animal never established a regular pattern.

Aim #6: To determine if there are differences, over time, in the cyclic fluctuations in the percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of normal saline.

From the visual analysis of the three-dimensional plots (Figures 7 - 9) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of normal saline, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to do a time-series analysis of these data.

Over the baseline recording period (0800-1000), 115 observations of percent of

absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 6 and 24 minutes in 20 (83%) of the 24 animals. For the 20 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 10 of the animals established a statistically significant cyclic pattern ($0.24 < r < 0.49$, $p < .05$). Data for each of the animals are summarized in Table 6.

Over the saline recording period (i. e., the first two hours following normal saline administration, 1000-1200), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 6 and 22 minutes in 19 (79%) of the 24 animals. For the 19 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 7 of the animals established a statistically significant cyclic pattern ($0.25 < r < 0.45$, $p < .05$). Data for each of the animals are summarized in Table 6.

Over the post-saline recording period (i. e., third and fourth hours following normal saline administration, 1200-1400), 120 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes

revealed recurring patterns with a period length between 7 and 27 minutes in 20 (83%) of the 24 animals. For the 20 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 7 of the animals established a statistically significant cyclic pattern ($0.24 < r < 0.38$, $p < .05$). Data for each of the animals are summarized in Table 6.

Morphine Experiment

Aim #7: To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of morphine 5 µg/kg.

Figures 17 - 21 show the various EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency) over the entire six hour recording period for the 8 conscious rats who received a subcutaneous injection of morphine 5 µg/kg. Table 7 provides a statistical analysis of the EEG parameters using a one-way RMANOVA to compare the three, two hour recording periods. Statistically significant differences between the three recording periods were identified for mobility ($F_{2,30} = 6.13$, $p = .006$), complexity ($F_{2,30} = 5.12$, $p = .01$), and edge frequency ($F_{2,30} = 7.27$, $p = .003$). Testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons revealed that the complexity for the post-drug recording period was

significantly higher than for the baseline recording period ($p = .05$) and that the edge frequency for the post-drug recording period was significantly lower than for the baseline recording period ($p = .01$). No statistically significant differences ($p < .05$) from baseline were identified for mobility. The statistical differences in mobility, complexity, and edge frequency following the injection of morphine 5 μg/kg can be attributed to the low variance of these measures rather than to physiologically significant differences in these EEG parameters.

Aim #8: To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of morphine 5 μg/kg.

The percent of absolute power of individual one Hz frequencies of the right and left EEG recordings were meaned for the eight animals who received a subcutaneous injection of morphine 5 μg/kg. Results are shown in Figures 22 (0800-1000), 23 (1000-1200), and 24 (1200-1400). The highest density of spectral power occurred in the frequencies below 5 Hz and persisted over the six hour recording period. Spectral power in the very low frequencies is usually considered to be artifact (Gasser & Molinari, 1996). The second highest density of spectral power occurred at 7 and 8 Hz and also persisted over the six hour recording period. Approximately every 10 to 15 minutes recurring fluctuations in power at 7 and 8 Hz were evident. The raw data were evaluated to confirm that 7 and 8 Hz were the frequency bands where these fluctuations occurred. Above 9 Hz, the percent of absolute power for individual one Hz frequency bands

remained below 5% of the absolute power.

Aim #9: To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of morphine 5 µg/kg.

From the visual analysis of the three-dimensional plots (Figures 22 - 24) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of morphine 5 µg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to further analyze these data.

Figure 25 shows the percent of absolute power at 7 and 8 Hz over the entire six hour recording period for the eight rats who received a subcutaneous injection of morphine 5 µg/kg. Figure 26 shows the AUC for percent of absolute power at 7 and 8 Hz for each of the three, two hour recording periods. Table 7 provides a statistical analysis of AUC data using a one-way RMANOVA to compare the three, two hour recording periods. A statistically significant difference in AUC between the three recording periods was identified ($F_{2,30} = 4.15$, $p = .03$). Testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons revealed that the AUC for the drug recording period was significantly higher than for the baseline recording period ($p = .05$). Although Dunnett's post hoc comparisons did not show a statistically significant difference between the baseline recording period and the post-drug recording period, a paired t-test revealed that the AUC for the post-drug recording period was significantly

higher than for the baseline recording period ($p = .04$).

Aim #10: To determine if there are differences, over time, in the cyclic fluctuations in the percent of absolute power of specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of morphine 5 µg/kg.

From the visual analysis of the three-dimensional plots (Figures 22 - 24) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of morphine 5 µg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to do a time-series analysis of these data.

Over the baseline recording period (0800-1000), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 4 and 23 minutes in 6 (75%) of the 8 animals. For the 6 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 2 of the animals established a statistically significant cyclic pattern ($0.17 < r < 0.26$, $p < .05$). Data for each of the animals are summarized in Table 8.

Over the drug recording period (i. e., the first two hours following morphine 5 µg/kg administration, 1000-1200), 115 observations of percent of absolute power at 7 and

8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 10 and 25 minutes in 7 (88%) of the 8 animals. For the 7 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 3 of the animals established a statistically significant cyclic pattern ($0.28 < r < 0.54$, $p < .05$). Data for each of the animals are summarized in Table 8.

Over the post-drug recording period (i. e., third and fourth hours following morphine 5 µg/kg administration, 1200-1400), 120 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 8 and 24 minutes in 7 (88%) of the 8 animals. For the 7 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 4 of the animals established a statistically significant cyclic pattern ($0.21 < r < 0.30$, $p < .05$). Data for each of the animals are summarized in Table 8.

Aim #11: To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of morphine 500

µg/kg.

Figures 27 - 31 show the various EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency) over the entire six hour recording period for the eight conscious rats who received a subcutaneous injection of morphine 500 µg/kg. Table 9 provides a statistical analysis of the EEG parameters using a one-way RMANOVA to compare the three, two hour recording periods. No statistically significant differences ($p < .05$) between the three recording periods were identified for any of the EEG parameters.

Aim #12: To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of morphine 500 µg/kg.

The percent of absolute power of individual one Hz frequencies of the right and left EEG recordings were meaned for the eight animals who received a subcutaneous injection of morphine 500 µg/kg. Results are shown in Figures 32 (0800-1000), 33 (1000-1200), and 34 (1200-1400). The highest density of spectral power occurred in the frequencies below 5 Hz and persisted over the six hour recording period. Spectral power in the very low frequencies is usually considered to be artifact (Gasser & Molinari, 1996). The second highest density of spectral power occurred at 7 and 8 Hz and also persisted over the six hour recording period. Approximately every 10 to 15 minutes recurring fluctuations in power at 7 and 8 Hz were evident. The raw data were evaluated to

confirm that 7 and 8 Hz were the frequency bands where these fluctuations occurred. Above 9 Hz, the percent of absolute power for individual one Hz frequency bands remained below 5% of the absolute power.

Aim #13: To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of morphine 500 µg/kg.

From the visual analysis of the three-dimensional plots (Figures 32 - 34) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of morphine 500 µg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to further analyze these data.

Figure 35 shows the percent of absolute power at 7 and 8 Hz over the entire six hour recording period for the eight rats who received a subcutaneous injection of morphine 500 µg/kg. Figure 36 shows the AUC for percent of absolute power at 7 and 8 Hz for each of the three, two hour recording periods. Table 9 provides a statistical analysis of AUC data using a one-way RMANOVA to compare the three, two hour recording periods. No statistically significant differences ($p < .05$) between the three recording periods were identified.

Aim #14: To determine if there are differences, over time, in the cyclic fluctuations in percent of absolute power of specific EEG frequencies in conscious rats who received a subcutaneous injection of morphine 500 µg/kg.

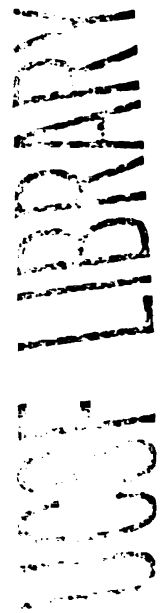
From the visual analysis of the three-dimensional plots (Figures 32 - 34) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of morphine 500 μg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to do a time-series analysis of these data.

Over the baseline recording period (0800-1000), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 5 and 11 minutes in 4 (50%) of the 8 animals. For the 4 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 3 of the animals established a statistically significant cyclic pattern ($0.22 < r < 0.27$, $p < .05$). Data for each of the animals are summarized in Table 10.

Over the drug recording period (i. e., the first two hours following morphine 500 μg/kg administration, 1000-1200), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 12 and 18 minutes in 4 (50%) of the 8 animals. For the 4 animals who established a recurring pattern, another correlogram

was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 2 of the animals established a statistically significant cyclic pattern ($0.27 < r < 0.28$, $p < .05$). Data for each of the animals are summarized in Table 10.

Over the post-drug recording period (i. e., third and fourth hours following morphine 500 µg/kg administration, 1200-1400), 120 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 6 and 37 minutes in 7 (88%) of the 8 animals. For the 7 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 2 of the animals established a statistically significant cyclic pattern ($0.25 < r < 0.48$, $p < .05$). Data for each of the animals are summarized in Table 10.



Pentazocine Experiment

Aim #15: To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of pentazocine 50 µg/kg.

Figures 37 - 41 show the various EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak

frequency, median frequency & edge frequency) over the entire six hour recording period for the eight conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg. Table 11 provides a statistical analysis of the EEG parameters using a one-way RMANOVA to compare the three, two hour recording periods. Statistically significant differences between the three recording periods were identified for mobility ($F_{2,30} = 3.23$, $p = .05$), peak frequency ($F_{2,30} = 8.95$, $p = .001$), median frequency ($F_{2,30} = 6.87$, $p = .004$), and edge frequency ($F_{2,30} = 10.86$, $p = .000$). Testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons revealed that the peak frequency and median frequency for the post-drug recording period were both significantly higher than for the baseline recording period ($p = .01$). The peak frequency for the drug recording period was also significantly higher than for the baseline recording period ($p = .01$). The edge frequency for the post-drug recording period was significantly lower than for the baseline recording period ($p = .01$). No significant differences ($p < .05$) from baseline were identified for mobility. The statistical differences in mobility, peak frequency, median frequency, and edge frequency following the injection of pentazocine 50 μg/kg can be attributed to the low variance of these measures rather than to physiologically significant differences in these EEG parameters.

Aim #16: To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg.

The percent of absolute power of individual one Hz frequencies of the right and

left EEG recordings were meaned for the eight animals who received a subcutaneous injection of pentazocine 50 μg/kg. Results are shown in Figures 42 (0800-1000), 43 (1000-1200), and 44 (1200-1400). The highest density of spectral power occurred in the frequencies below 5 Hz and persisted over the six hour recording period. Spectral power in the very low frequencies is usually considered to be artifact (Gasser & Molinari, 1996). The second highest density of spectral power occurred at 7 and 8 Hz and also persisted over the six hour recording period. Approximately every 10 to 15 minutes recurring fluctuations in power at 7 and 8 Hz were evident. The raw data were evaluated to confirm that 7 and 8 Hz were the frequency bands where these fluctuations occurred. Above 9 Hz, the percent of absolute power for individual one Hz frequency bands remained below 5% of the absolute power.

Aim #17: To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg.

From the visual analysis of the three-dimensional plots (Figures 42 - 44) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to further analyze these data.

Figure 45 shows the percent of absolute power at 7 and 8 Hz over the entire six hour recording period for the eight rats who received a subcutaneous injection of

pentazocine 50 μg/kg. Figure 46 shows the AUC for percent of absolute power at 7 and 8 Hz for each of the three, two hour recording periods. Table 11 provides a statistical analysis of AUC data using a one-way RMANOVA to compare the three, two hour recording periods. A statistically significant difference between the three recording periods were identified ($F_{2,30} = 24.50$, $p = .000$). Testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons revealed that the AUC for both the drug recording period and the post-drug recording period were significantly higher than for the baseline recording period ($p = .01$).

Aim #18: To determine if there are differences, over time, in the cyclic fluctuations in the percent of absolute power of specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg.

From the visual analysis of the three-dimensional plots (Figures 42 - 44) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of pentazocine 50 μg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to do a time-series analysis of these data.

Over the baseline recording period (0800-1000), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 8 and 24

minutes in 6 (75%) of the 8 animals. For the 6 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 1 of the animals established a statistically significant cyclic pattern ($r = 0.30$, $p < .05$). Data for each of the animals are summarized in Table 12.

Over the drug recording period (i. e., the first two hours following pentazocine 50 µg/kg administration, 1000-1200), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 8 and 17 minutes in 5 (63%) of the 8 animals. For the 5 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 2 of the animals established a statistically significant cyclic pattern ($0.24 < r < 0.29$, $p < .05$). Data for each of the animals are summarized in Table 12.

Over the post-drug recording period (i. e., third and fourth hours following pentazocine 50 µg/kg administration, 1200-1400), 120 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 7 and 19 minutes in all (100%) of the animals. For each of the animals, another correlogram was done using a time lag a few minutes longer than the observed period length. From this

second correlogram, only 3 of the animals established a statistically significant cyclic pattern ($0.25 < r < 0.36$, $p < .05$). Data for each of the animals are summarized in Table 12.

Aim #19: To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg.

Figures 47 - 51 show the various EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency) over the entire six hour recording period for the eight conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg. Table 13 provides a statistical analysis of the EEG parameters using a one-way RMANOVA to compare the three, two hour recording periods. A statistically significant difference between the three recording periods was identified for edge frequency ($F_{2,30} = 5.41$, $p = .01$). Testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons revealed that the edge frequency for the drug recording period was significantly higher than for the baseline recording period ($p = .01$). The statistical difference in edge frequency following the injection of pentazocine 5 mg/kg can be attributed to the low variance of the measure rather than to a physiologically significant difference this EEG parameter.

Aim #20: To determine if there are visual differences, over time, in the three-

dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg.

The percent of absolute power of individual one Hz frequencies of the right and left EEG recordings were meaned for the eight animals who received a subcutaneous injection of pentazocine 5 mg/kg. Results are shown in Figures 52 (0800-1000), 53 (1000-1200), and 54 (1200-1400). The highest density of spectral power occurred in the frequencies below 5 Hz and persisted over the six hour recording period. Spectral power in the very low frequencies is usually considered to be artifact (Gasser & Molinari, 1996). The second highest density of spectral power occurred at 7 and 8 Hz and also persisted over the six hour recording period. Approximately every 10 to 15 minutes recurring fluctuations in power at 7 and 8 Hz were evident. During the drug recording period (1000-1200), this pattern appeared to be interrupted after pentazocine 5 mg/kg was administered, reappearing during the post-drug recording period (1200-1400). The raw data were evaluated to confirm that 7 and 8 Hz were the frequency bands where these fluctuations occurred. Above 9 Hz, the percent of absolute power for individual one Hz frequency bands remained below 5% of the absolute power.

Aim #21: To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg.

From the visual analysis of the three-dimensional plots (Figures 52 - 54) of percent of absolute power attributable to individual one Hz frequencies over the six hour

recording period in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to further analyze these data.

Figure 55 shows the percent of absolute power at 7 and 8 Hz over the entire six hour recording period for the eight rats who received a subcutaneous injection of pentazocine 5 mg/kg. Figure 56 shows the AUC for percent of absolute power at 7 and 8 Hz for each of the three, two hour recording periods. Table 13 provides a statistical analysis of AUC data using a one-way RMANOVA to compare the three, two hour recording periods. A statistically significant difference between the three recording periods was identified ($F_{2,30} = 5.17$, $p = .01$). However, testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons failed to demonstrate significant differences ($p < .05$) from the baseline recording period.

Aim #22: To determine if there are differences, over time, in the cyclic fluctuations in percent of absolute power of specific EEG frequencies in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg.

From the visual analysis of the three-dimensional plots (Figures 52 - 54) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of pentazocine 5 mg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to do a time-series analysis of these

data.

Over the baseline recording period (0800-1000), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 7 and 36 minutes in 6 (75%) of the 8 animals. For the 6 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 1 of the animals established a statistically significant cyclic pattern ($r = 0.31$, $p < .05$). Data for each of the animals are summarized in Table 14.

Over the drug recording period (i. e., the first two hours following pentazocine 5 mg/kg administration, 1000-1200), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed a recurring pattern in only one (13%) of the 8 animals. For the animal who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. This second correlogram was not statistically significant ($p < .05$). Data for each of the animals are summarized in Table 14.

Over the post-drug recording period (i. e., third and fourth hours following pentazocine 5 mg/kg administration, 1200-1400), 120 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis.

Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 7 and 33 minutes in all (100%) of the animals. For each of the animals, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 2 of the animals established a statistically significant cyclic pattern ($0.25 < r < 0.26$, $p < .05$). Data for each of the animals are summarized in Table 14.

Naloxone Experiment

Aim #23: To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of naloxone 5 µg/kg.

Figures 57 - 61 show the various EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency) over the entire six hour recording period for the eight conscious rats who received a subcutaneous injection of naloxone 5 µg/kg. Table 15 provides a statistical analysis of the EEG parameters using a one-way RMANOVA to compare the three, two hour recording periods. Statistically significant differences between the three recording periods were identified for mobility ($F_{2,30} = 7.21$, $p = .003$), peak frequency ($F_{2,30} = 3.97$, $p = .03$), and median frequency ($F_{2,30} = 5.85$, $p = .007$). Testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons revealed that the mobility for the post-drug recording period was

significantly lower than for the baseline recording period (p = .01). The peak frequency for the drug recording period was significantly higher than for the baseline recording period (p = .05). The median frequency for the drug recording period was significantly higher than for the baseline recording period (p = .05). This effect persisted into the post-drug recording period where the median frequency was again significantly higher than for the baseline recording period (p = .01). The statistical differences in mobility, peak frequency, and median frequency following the injection of naloxone 5 µg/kg can be attributed to the low variance of these measures rather than to physiologically significant differences in these EEG parameters.

Aim #24: To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of naloxone 5 µg/kg.

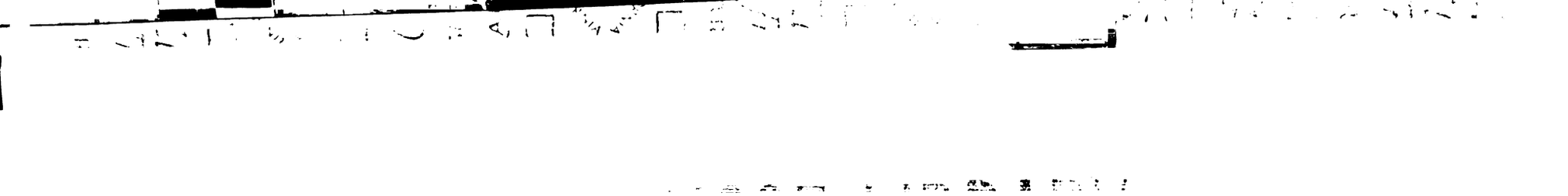
The percent of absolute power of individual one Hz frequencies of the right and left EEG recordings were meaned for the eight animals who received a subcutaneous injection of naloxone 5 µg/kg. Results are shown in Figures 62 (0800-1000), 63 (1000-1200), and 64 (1200-1400). The highest density of spectral power occurred in the frequencies below 5 Hz and persisted over the six hour recording period. Spectral power in the very low frequencies is usually considered to be artifact (Gasser & Molinari, 1996). The second highest density of spectral power occurred at 7 and 8 Hz and also persisted over the six hour recording period. Approximately every 10 to 15 minutes recurring fluctuations in power at 7 and 8 Hz were evident. The raw data were evaluated to

confirm that 7 and 8 Hz were the frequency bands where these fluctuations occurred. Above 9 Hz, the percent of absolute power for individual one Hz frequency bands remained below 5% of the absolute power.

Aim #25: To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of naloxone 5 µg/kg.

From the visual analysis of the three-dimensional plots (Figures 62 - 64) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of naloxone 5 µg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to further analyze these data.

Figure 65 shows the percent of absolute power at 7 and 8 Hz over the entire six hour recording period for the eight rats who received a subcutaneous injection of naloxone 5 µg/kg. Figure 66 shows the AUC for percent of absolute power at 7 and 8 Hz for each of the three, two hour recording periods. Table 15 provides a statistical analysis of AUC data using a one-way RMANOVA to compare the three, two hour recording periods. A statistically significant difference between the three recording periods was identified ($F_{2,30} = 5.23$, $p = .01$). Testing for pairwise differences between the recording periods, Dunnett's post hoc comparisons revealed that the AUC for both the drug recording period and the post-drug recording period were significantly higher than the baseline recording period ($p = .05$).



Aim #26: To determine if there are differences, over time, in the cyclic fluctuations in the percent of absolute power of specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of naloxone 5 µg/kg.

From the visual analysis of the three-dimensional plots (Figures 62 - 64) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of naloxone 5 µg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to do a time-series analysis of these data.

Over the baseline recording period (0800-1000), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 6 and 20 minutes in 7 (88%) of the 8 animals. For the 7 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 2 of the animals established a statistically significant cyclic pattern ($0.22 < r < 0.33$, $p < .05$). Data for each of the animals are summarized in Table 16.

Over the drug recording period (i. e., the first two hours following naloxone 5 µg/kg administration, 1000-1200), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for

individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 8 and 17 minutes in 7 (88%) of the 8 animals. For the 7 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 3 of the animals established a statistically significant cyclic pattern ($0.25 < r < 0.29$, $p < .05$). Data for each of the animals are summarized in Table 16.

Over the post-drug recording period (i. e., third and fourth hours following naloxone 5 µg/kg administration, 1200-1400), 120 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 7 and 23 minutes in all (100%) of the animals. For each of the animals, another correlogram was done using a time lag a few minutes longer than the observed period length. However none of the patterns were statistically significant ($p < .05$). Data for each of the animals are summarized in Table 16.

Aim #27: To determine if there are differences, over time, in any of the time domain parameters (i. e., activity, mobility, & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, & edge frequency) in conscious rats who received a subcutaneous injection of naloxone 500 µg/kg.

Figures 67 - 71 show the various EEG time domain parameters (i. e., activity,

mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & edge frequency) over the entire six hour recording period for the eight conscious rats who received a subcutaneous injection of naloxone 500 μg/kg. Table 17 provides a statistical analysis of the EEG parameters using a one-way RMANOVA to compare the three, two hour recording periods. No statistically significant differences ($p < .05$) between the three recording periods were identified for any of the EEG parameters.

Aim #28: To determine if there are visual differences, over time, in the three-dimensional plot of percent of absolute power attributable to individual one Hz frequencies of the EEG in conscious rats who received a subcutaneous injection of naloxone 500 μg/kg.

The percent of absolute power of individual one Hz frequencies of the right and left EEG recordings were meaned for the eight animals who received a subcutaneous injection of naloxone 500 μg/kg. Results are shown in Figures 72 (0800-1000), 73 (1000-1200), and 74 (1200-1400). The highest density of spectral power occurred in the frequencies below 5 Hz and persisted over the six hour recording period. Spectral power in the very low frequencies is usually considered to be artifact (Gasser & Molinari, 1996). The second highest density of spectral power occurred at 7 and 8 Hz and also persisted over the six hour recording period. Approximately every 10 to 15 minutes recurring fluctuations in power at 7 and 8 Hz were evident. The raw data were evaluated to confirm that 7 and 8 Hz were the frequency bands where these fluctuations occurred. Above 9 Hz, the percent of absolute power for individual one Hz frequency bands

remained below 5% of the absolute power.

Aim #29: To determine if there are differences, over time, in the magnitude of percent of absolute power attributable to specific EEG frequencies of interest in conscious rats who received a subcutaneous injection of naloxone 500 μg/kg.

From the visual analysis of the three-dimensional plots (Figures 72 - 74) of percent of absolute power attributable to individual one Hz frequencies over the six hour recording period in conscious rats who received a subcutaneous injection of naloxone 500 μg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to further analyze these data.

Figure 75 shows the percent of absolute power at 7 and 8 Hz over the entire six hour recording period for the eight rats who received a subcutaneous injection of naloxone 500 μg/kg. Figure 76 shows the AUC for percent of absolute power at 7 and 8 Hz for each of the three, two hour recording periods. Table 17 provides a statistical analysis of AUC data using a one-way RMANOVA to compare the three, two hour recording periods. No statistically significant differences ($p < .05$) between the three recording periods were identified.

Aim #30: To determine if there are differences, over time, in the cyclic fluctuations in percent of absolute power of specific EEG frequencies in conscious rats who received a subcutaneous injection of naloxone 500 μg/kg.

From the visual analysis of the three-dimensional plots (Figures 72 - 74) of percent of absolute power attributable to individual one Hz frequencies over the six hour

recording period in conscious rats who received a subcutaneous injection of naloxone 500 µg/kg, the percent of absolute power of the EEG at both 7 and 8 Hz were identified as having cyclic fluctuations over time. For each animal, the percentages of absolute power attributable to these two frequencies were combined to do a time-series analysis of these data.

Over the baseline recording period (0800-1000), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 5 and 30 minutes in all (100%) of the animals. Another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 4 of the animals established a statistically significant cyclic pattern ($0.24 < r < 0.29$, $p < .05$). Data for each of the animals are summarized in Table 18.

Over the drug recording period (i. e., the first two hours following naloxone 500 µg/kg administration, 1000-1200), 115 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 7 and 16 minutes in 7 (88%) of the 8 animals. For the 7 animals who established a recurring pattern, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 2 of the animals established a statistically significant cyclic pattern ($0.21 < r < 0.24$, $p < .05$). Data for each of the animals are summarized in Table

18.

Over the post-drug recording period (i. e., third and fourth hours following naloxone 500 µg/kg administration, 1200-1400), 120 observations of percent of absolute power at 7 and 8 Hz from each animal were available for the time-series analysis. Correlograms for individual animals plotting autocorrelation coefficients with a time lag of 60 minutes revealed recurring patterns with a period length between 11 and 32 minutes in all (100%) of the animals. For each of the animals, another correlogram was done using a time lag a few minutes longer than the observed period length. From this second correlogram, only 2 of the animals established a statistically significant cyclic pattern ($0.27 < r < 0.34$, $p < .05$). Data for each of the animals are summarized in Table 18.

CHAPTER 5 - DISCUSSION

Introduction

The purpose of this research was to describe the effects of two antinociceptive doses of three subcutaneously administered relatively-selective opioid receptor agonists (i. e., morphine, pentazocine & naloxone) on selected EEG parameters of conscious rats. While previous research used higher doses of opioid agonists, presumably to elicit a maximum effect on the EEG, this study used lower, antinociceptive doses of the opioid agonists. The results of this study demonstrate the feasibility of using EEG recordings to document opioid-induced changes in CNS functioning.

This study benefitted from the recent revolution in computer technology enabling the acquisition, analysis, and storage of large volumes of data as well as the capacity to display the data in a user-friendly environment. While previous research evaluated only short, selected segments of EEG recordings, this study evaluated six continuous hours of EEG recordings providing a more comprehensive assessment of the effects of opioid analgesics on various EEG parameters. Furthermore, this study characterized both time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency, mode frequency & percent of absolute power attributable to individual one Hz frequencies) of the EEG recordings. This contrasts with previous research that used only qualitative descriptions of voltage and frequency to describe the effects of opioids on EEG recordings (Arankowsky-Sandoval & Gold, 1995; Bronzino et al., 1982; Hong, Young, & Khazan, 1988; Stamidis & Young, 1993; Tortella, Moreton, & Khazan, 1978; Young, Hudson,

Stamidis, & Steinfels, 1993a).

Normal Saline Experiment

The normal saline experiment provided information about the typical EEG recordings for these animals during the morning and early afternoon, the same time of day the drug experiments were conducted. Additionally, the possibility of exaggerated or prolonged EEG effects from the subcutaneous injection were ruled out. From the graphs of each of the EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & mode frequency) over the six hour experiment (Figures 2 - 6), no changes were evident in any of the EEG parameters. RMANOVA did demonstrate some statistical differences between each of the three, two hour recording periods, but as discussed in the previous chapter, there was so little variability in the measures that small differences in the measures themselves became statistically significant. The physiological significance of these very small, yet statistically significant differences is currently unknown.

The normal sleep-wake cycle of the rat lasts between 10 and 30 minutes depending upon the time of day (Ambrosini et al., 1994; Van Twyver, 1969). During the course of the six hour experiment casual observations of the animals' behavior over time indicated that they experienced multiple episodes of both sleep and awake episodes. During awake periods the animals would explore the recording cage or groom themselves, while during sleep periods, the animals would be curled into a ball with their eyes closed. Sleep-wake cycles were not assessed using polygraph recordings of the EEG.

Hjorth (1973) described an algorithm to determine sleep and awake states in the rat by evaluating the time domain parameters of activity and complexity. According to Hjorth, periods of decreased activity and increased complexity were associated with both awake states and REM sleep. Periods during which complexity and activity both returned to baseline were associated with NREM sleep. In the current study, activity and complexity did not fluctuate in a discernable pattern as described by Hjorth (Figures 2 & 4).

Another method of determining sleep and wake periods in the rat has been described by Young, Steinfels, and Khazan (1978) who determined from a spectral analysis of 41-second epochs of EEG, spectral characteristics of awake, sleep, and REM sleep. Awake states are associated with less absolute power than either sleep or REM sleep. Sleep states are associated with a predominance of spectral power in the lower frequency range (i. e., between 0 and 5 Hz). NREM sleep is associated with a predominant peak of spectral power in the 6 to 9 Hz range. In the current study, cyclic fluctuations in the percent of absolute power at 7 and 8 Hz were observed and, based upon the work of Young, Steinfels, and Khazan (1978), was believed to be associated with the animals' REM sleep cycle.

These cyclic fluctuations in percent of absolute power at 7 and 8 Hz were further characterized with a time-series analysis. When evaluating the entire six hour recording period, recurring patterns with a period length between 8 and 39 minutes in 96% of the animals supported the observation that these cyclic fluctuations occurred at the same frequency as normal sleep-wake cycles of rats.

When the three, two hour recording periods were analyzed with a time-series analysis, intra-individual differences in the shape, structure, and statistical significance of the correlograms were observed. Even though animals were allowed to acclimate to the recording cages for at least 30 minutes each day before beginning the experiment, the changes in the cyclic fluctuations of percent of absolute power at 7 and 8 Hz, both throughout the day and between animals, suggest that the animals need to be conditioned to the EEG recording cables and cages more frequently and for longer periods of time before experimental data are collected. The sleep-wake cycle of the rat is so brief that disruptions of even a few minutes significantly alter the time-series analysis. The ability to increase the sensitivity of the study measures by training rats in the experimental paradigm has been previously reported by Taiwo, Coderre, and Levine (1989) who noted that familiarity with the experimental environment influences laboratory animal behavior. By training rats for three days in the Randall-Selitto paw-withdrawal test, these researchers were able to increase the sensitivity of this test for detecting the hyperalgesic effects of intradermal injections of the inflammatory mediator, bradykinin. In the current study, conditioning the animals to the recording cables and cages, thereby reducing the novelty of the experimental environment, could have reduced both the inter- and intra-individual variability.

AUC, a measure of the magnitude of the percent of absolute power at 7 and 8 Hz, was used as another way of characterizing these cyclic fluctuations over time. No differences in this measure were detected comparing the three, two hour recording period.

Opioid Experiments

From the graphs of each of the EEG time domain parameters (i. e., activity, mobility & complexity) and frequency domain parameters (i. e., absolute power, peak frequency, median frequency & mode frequency) over the six hour experiment (Figures 17 - 21, 27 - 31, 37 - 41, 47 - 51, 57 - 61, and 67 - 71), no changes were evident in any of the EEG parameters. RMANOVA did demonstrate some statistical differences between each of the three, two hour recording periods, but as discussed in the previous chapter and with the normal saline experiment, there was so little variability in the measures that small differences in the measures themselves became statistically significant. The physiological significance of these very small, yet statistically significant differences is currently unknown.

Cyclic fluctuations in the percent of absolute power at 7 and 8 Hz were observed during the opioid experiments as they were during the normal saline experiment. When the three, two hour recording periods were analyzed with a time-series analysis, inter- and intra-individual differences in the shape, structure, and statistical significance of the correlograms were observed. As with the normal saline experiment, it is difficult to interpret this time-series analysis without conditioning the animals to the recording environment, thus reducing the variability of the cyclic fluctuations in the percent of absolute power at 7 and 8 Hz.

One observation of the graph of percent of absolute power at 7 and 8 Hz over time for the higher dose of pentazocine (5 mg/kg) (Figure 55) is that the cyclic fluctuations disappear for approximately an hour following drug administration. This disappearance

of the cyclic fluctuations in percent of absolute power at 7 and 8 Hz was not observed with the lower dose of pentazocine or with either of the other drugs at either dose. If these cyclic fluctuations in the percent of absolute power at 7 and 8 Hz do represent REM sleep cycles, the absence of the fluctuations following a dose of pentazocine (5 mg/kg) supports observations made by other researchers that opioids suppress REM sleep (Furst, 1990; King, Masserano, Codd, & Byrne, 1981; Landis, 1988). Verification that REM sleep cycles are occurring when there are increases in the percent of absolute power at 7 and 8 Hz is warranted before this observation can be used as further evidence that opioids do indeed disrupt sleep patterns.

Significant differences were observed in the measure of magnitude, AUC, of the percent of absolute power at 7 and 8 Hz. As summarized on Table 19, the lower doses of the opioid agonists increased the AUC of the percent of absolute power at 7 and 8 Hz. Compared to the baseline recording period, the AUC was elevated for both the drug recording period and the post-drug recording period indicating that this effect was sustained for the four hours following drug administration. Curiously, the higher doses of opioid agonists did not change the AUC of the percent of absolute power at 7 and 8 Hz. In particular, the disappearance of cyclic fluctuations in the percent of absolute power at 7 and 8 Hz following the administration of the highest dose of pentazocine (5 mg/kg) was not manifested as a change in AUC.

The increase in AUC for the percent of absolute power at 7 and 8 Hz following the lower doses of all three opioid agonists, is not readily explainable. One possibility is that the animals experienced a tachyphylaxis reaction when first exposed to the opioid.

However, this hypothesis was tested on another group of animals given the same dose of opioid on two consecutive days. No significant differences ($p < .05$) in any of the EEG parameters were observed.

The effects of low, antinociceptive doses of opioid agonists on the EEG have not been reported previously. Higher doses of the μ-opioid agonist morphine (2.5 mg/kg to 30 mg/kg) administered systemically to rats produce dose-dependent, high-voltage, slow frequency waves and increase spectral power in the lower frequencies between 0 and 10 Hz (Bronzino et al., 1982; Hong, Young, & Khazan, 1988; Paquette & Young, 1991; Stamidis & Young, 1993; Tortella, Moreton, & Khazan, 1978; Young & Khazan, 1984). Like the μ-opioid agonists, κ-opioid agonists administered systemically to rats produce high-voltage, slow frequency waves and increase spectral power in the lower frequencies between 2 and 8 Hz (Campi & Clarke, 1995; Young, Hudson, Stamidis, & Steinfels, 1993a, 1993b; Young & Khazan, 1984). The EEG effects of systemically administered δ-opioid agonists have not been described.

Paradoxical effects of low doses of the opioid-agonists on behavior have been reported. μ-opioid agonists produce both a behavioral excitation and a behavioral depression in rodents that was both dose and time dependent. Small doses of morphine (0.01 mg/kg to 5 mg/kg) given systemically have been observed to initially increase locomotor activity (Babbini & Davis, 1972; Fog, 1970; Iwamoto, 1981, 1984; Oka & Hosoya, 1976; Ostrowski & Caggiula, 1991; Vasko & Domino, 1978). At doses of morphine greater than 3 mg/kg, a biphasic behavioral pattern has been described where activity initially decreased and then increased (Babbini & Davis, 1972; Oka & Hosoya,

1976; Sloan, Brooks, Eisenman, & Martin, 1962; Vasko & Domino. 1978). With large doses of morphine (between 60 mg/kg and 80 mg/kg), only depressed behavior has been observed that persisted for as long as four hours following drug administration (De Ryck, Schallert, & Teitelbaum, 1980; Sloan, Brooks, Eisenman, & Martin, 1962). The behavioral effects of low doses of either κ- and δ-opioid agonists given systemically have not been described. If opioid-induced changes in EEG recordings mirror behavioral changes following the administration of low doses of morphine, the increase in the measures of magnitude of the percent of absolute power at 7 and 8 Hz may correspond to the increase in locomotor activity observed by other researchers. However, why a similar increase in the measures of magnitude was not observed in the current study when a higher dose of opioid was given cannot be explained. Even the higher doses of drug administered in the current study were below the doses that produced biphasic behavioral patterns in previous studies. It was not until doses of morphine greater than 3 mg/kg were given that locomotor activity initially decreased and then increased (Babbini & Davis, 1972; Oka & Hosoya, 1976; Sloan, Brooks, Eisenman, & Martin, 1962; Vasko & Domino. 1978).

Another possible explanation might be that different areas of the CNS were activated by different thresholds of opioid receptor saturation. However, without administering the agonists to specific CNS sites it is impossible to test this hypothesis. Furthermore, although the effects of the lower doses of these three relatively-selective opioid agonists were very consistent, the receptor specific effects cannot be determined without using highly-selective agonists.

Conclusions & Directions for Future Research

This research has described the effects of opioid agonists on selected parameters of the rat EEG finding significant increases from baseline in AUC for the percent of absolute power at 7 and 8 Hz over the four hours following an injection of low doses of three relatively-selective opioid agonists. However, changes in the spectral parameters of the EEG remain to be correlated with behavior. Automated activity boxes that record both forward and horizontal locomotion, as well as inactivity could provide invaluable information about the behavior of the animal during EEG recordings under various experimental conditions.

Cyclic fluctuations in the percent of absolute power at 7 and 8 Hz were identified that may represent the animals' REM sleep pattern. However, the inter-and intra-individual variability in these patterns suggest the need to condition the animals to the experimental environment in future studies.

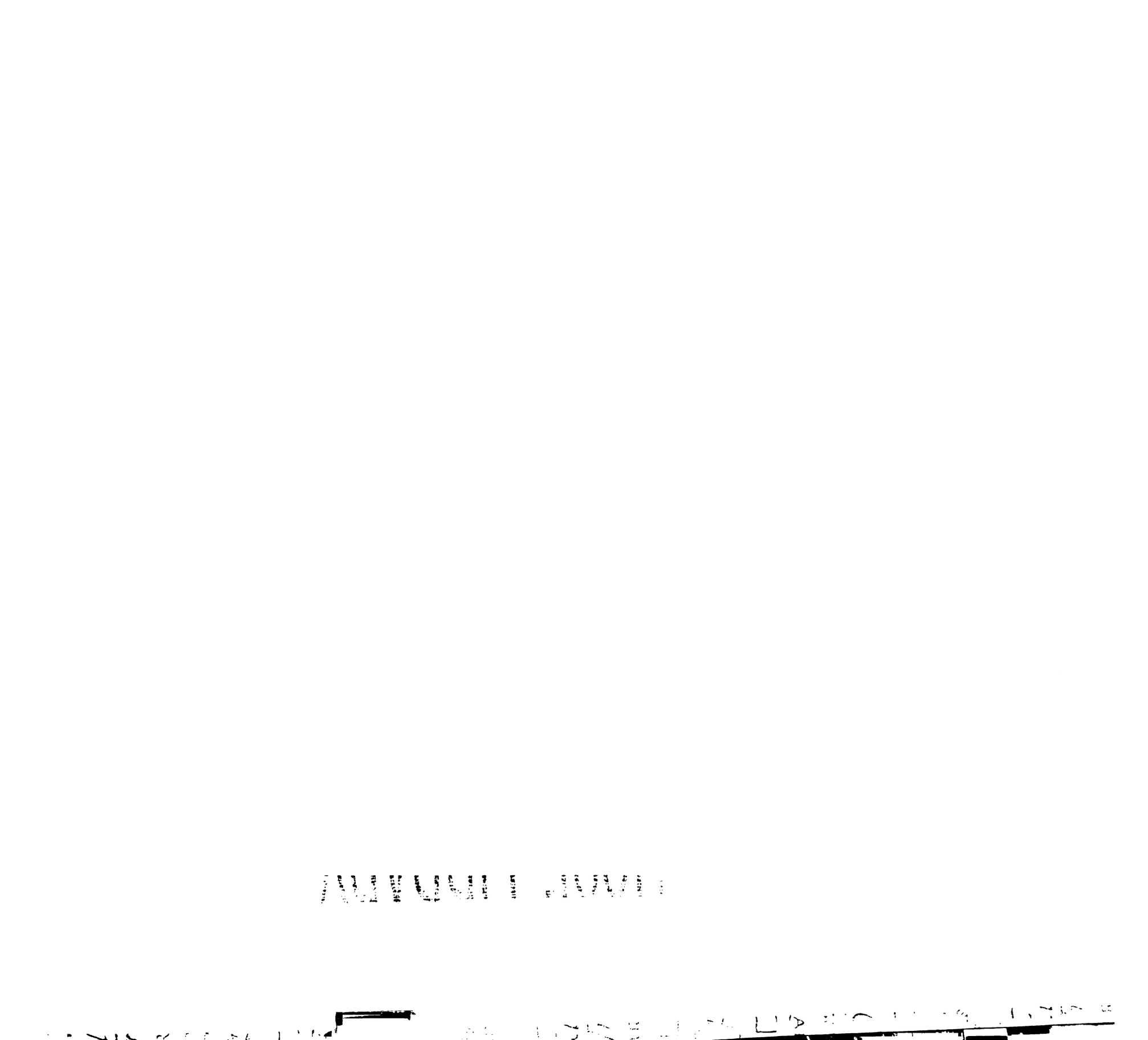
The objective of this program of research is to better understand the sedative side effects of opioids being used to manage painful conditions. These experiments were all done with pain-free animals, leaving the EEG effects of opioids in animals exposed to a chronic nociceptive stimulus yet to be described. When compared with pain-free animals, rats with a chronic, painful arthritis have a lower nociceptive threshold (Kayser & Guilbaud, 1983), disrupted sleep patterns (Landis, Levine, & Robinson, 1989; Landis, Robinson, & Levine, 1988), and more pronounced response to both non-steroidal anti-inflammatory drugs (Landis, Robinson, Helms, & Levine, 1989) and morphine (Kayser & Guilbaud, 1983, 1990). To date, no studies have compared opioid effects in pain-free

animals with animals exposed to a chronic nociceptive stimulus using spectral analysis of the EEG. And so, these experiments should be repeated using animals exposed to a nociceptive stimulus.

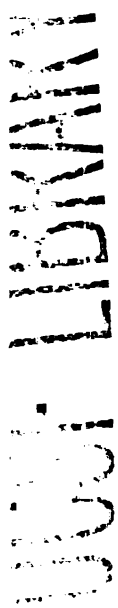
And finally, relatively-receptor selective opioid agonists were used for these experiments to develop the experimental paradigm and to demonstrate differences in spectral parameters of the EEG following opioid administration. To further the study of opioid receptor physiology and to determine how receptor agonists induce the sedative effects observed with opioids, highly-selective agonists need to be administered directly into specific sites within the CNS.

This research has begun the exploration into opioid-induced changes in CNS functioning by describing the effects of two antinociceptive doses of three subcutaneously administered relatively-selective opioid receptor agonists (i. e., morphine, pentazocine & naloxone) on selected EEG parameters of conscious rats. Cyclic fluctuations in the percent of absolute power at 7 and 8 Hz were identified that may represent the REM sleep cycle of the animals. When compared to the baseline recording period, the lower doses of all three opioid agonists significantly increased the magnitude of the percent of absolute power at 7 and 8 Hz as measured by AUC. This effect persisted for the four hours following drug administration.

Consciousness is normally expressed as a time-sequenced process that fluctuates between wakefulness and sleep. If opioids change the normal expression of consciousness by acting at receptors located throughout the CNS, then intuitively these



drugs change the continuum of consciousness expressed over time. Identifying parameters indicative of change in a nonstationary system such as the CNS is a challenge. The results of this study suggest that changes in the cyclic fluctuations of the percent of absolute power at 7 and 8 Hz might be one such parameter and could be a focus of future research investigating the phenomenon of opioid-induced sedation.



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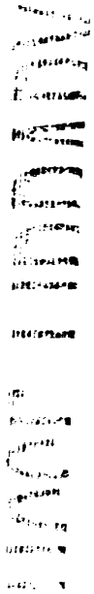

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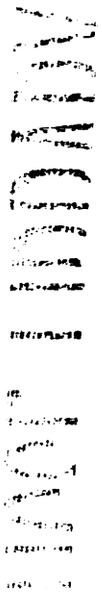

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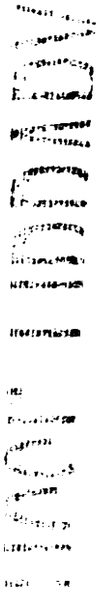

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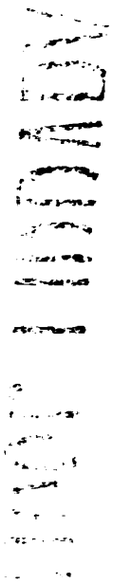


Table 1: Awake & Sleep Electroencephalograph Waveforms

Arousal State	**Waveform**	**Frequency** (range 1-30 Hertz)	**Amplitude** (range 1-100 μV)
Awake			
relaxed	alpha	9-11 Hz	10-50 μV
active	beta	13-30 Hz	low voltage
Sleep			
Stage I NREM	loss of alpha waves	mixed	low voltage
Stage II NREM	.5-2 second bursts of sleep spindles K complexes	12-16 Hz	high voltage
Stage III NREM	delta	1-2 Hz	high voltage
Stage IV NREM	delta	1-2 Hz	high voltage
REM	rare alpha	high frequency	low voltage

Information from: Adams & Victor, 1993; Alcorn, 1983; Niedermeyer, 1993a.

alpha dropout - alpha waves in the range of 10 per second are replaced by low voltage slow activity in the range of 2-7 cycles per second (Niedermeyer, 1993a, 1993b)

vertex waves - a compounded potential, a small spike discharge of positive polarity followed by a large negative wave, another small positive spiky discharge usually follows (Niedermeyer, 1993b)

spindles - a group of rhythmic waves in the range of 13-15 cycles per second characterized by progressively increasing, then gradually decreasing amplitude, also known as sigma activity or sigma waves (Niedermeyer, 1993b)

K complex - an initial sharp component, followed by a slow component that fuses with a superimposed fast component, the shape may resemble that of an isolated vertex wave, but the sharp component of the K complex shows greater complexity and greater variation (Niedermeyer, 1993b)

Table 2: Location and Proposed Effects of Opioid Receptors Within the Central Nervous System

Opioid Receptor	Locations in Central Nervous System	Proposed Effects
mu (μ)	medulla - nucleus raphe magnus; trigeminal nucleus; midbrain - PAG; substantia nigra, superior & inferior colliculi), thalamus	supraspinal analgesia
	dorsal horn of spinal cord	spinal analgesia
	solitary nucleus; nucleus ambiguous	respiratory depression
	area postrema	nausea & vomiting
	ventral tegmental area; nucleus accumbens; lateral hypothalamus	physical dependence
	cerebral cortex, cingulate cortex, amygdala, hippocampus, dentate gyrus	sedation; euphoria
	caudate putamen	
kappa (κ)	thalamus amygdala, nucleus accumbens, caudate putamen medial pre-optic area hypothalamus	spinal analgesia supraspinal analgesia respiratory depression sedation dysphoria miosis
delta (δ)	dorsal horn of the spinal cord cingulate cortex, cerebral cortex, amygdala caudate putamen, nucleus accumbens	spinal analgesia supraspinal analgesia

Information from: Brownstein, 1993; Delfs et al., 1994; Goodman, Snyder, Kuhar & Young, 1980; Kuhar, Pert & Snyder, 1973; Ling, Spiegel, Lockhart & Pasternak, 1985; Mansour, Khachaturian, Lewis, Akil & Watson, 1987; Pasternak, 1993; Reisine & Pasternak, 1996

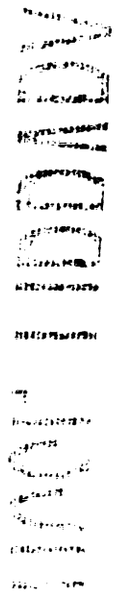


Table 3: Means for Selected EEG Parameters (±SEM) Over the Six Hour Recording Period in Conscious Rats Who Received a Subcutaneous Injection of Normal Saline (n = 48 for 24 animals)

	Six Hour Recording Period (0800-1400)
Time Domain Parameters of the EEG	
Activity	320.80 (84.78)
Mobility (crossings/minute)	124.68 (1.00)
Complexity	1.64 (0.01)
Frequency Domain Parameters of the EEG	
Absolute Power (μV^2)	101.40 (25.62)
Peak Frequency (Hz)	2.64 (0.13)
Median Frequency (Hz)	5.93 (0.14)
Edge Frequency (Hz)	27.00 (0.07)

Table 4: Means for Selected EEG Parameters (±SEM) and Magnitude of Effect for Percent of Absolute Power at 7 and 8 Hz (±SEM) Over Three, Two Hour Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Normal Saline (n = 48 for 24 animals)

	Baseline Recording Period (0800-1000)	Saline Injection Recording Period (1000-1200)	Post-Saline Recording Period (1200-1400)	Significance of RMANOVA F Statistic
Time Domain Parameters of EEG				
Activity	334.26 (± 82.46)	257.20 (± 47.22)	370.92 (± 112.67)	NS
Mobility (crossings/minute)	124.68 (± 1.08)	125.49 (± 1.02)	124.53 (± 0.91)	p = .02
Complexity	1.64 (± 0.01)	1.63 (± 0.01)	1.64 (± 0.01)	p = .03
Frequency Domain Parameters of EEG				
Absolute Power (μV^2)	106.01 (± 25.61)	82.94 (± 15.13)	115.27 (± 33.18)	NS
Peak Frequency (Hz)	2.60 (± 0.14)	2.64 (± 0.12)	2.66 (± 0.13)	NS
Median Frequency (Hz)	5.82 (± 0.14)	5.93 (± 0.14)	6.04 (± 0.14)**	p = .007
Edge Frequency (Hz)	27.05 (± 0.06)	27.00 (± 0.07)	27.00 (± 0.09)	NS
Magnitude of Effect for Percent of Absolute Power at 7 & 8 Hz				
AUC	1281.52 (± 55.49)	1305.50 (± 46.50)	1346.85 (± 51.42)	NS

NS indicates not significant, ** indicates p = .01

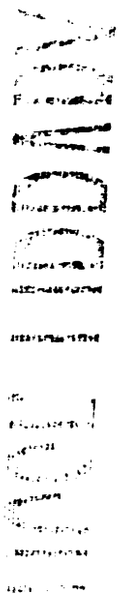
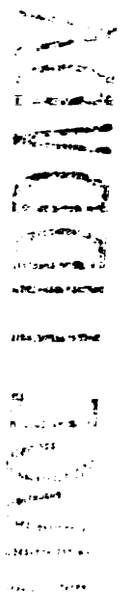


Table 5: Period Lengths and Significant Autocorrelation Functions for Conscious Rats Who Received a Subcutaneous Injection of Normal Saline (n = 24)

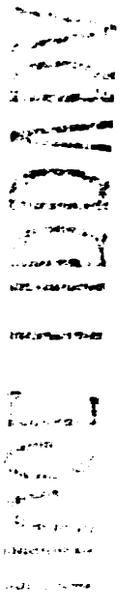
Animal Identification Number	Six Hour Recording Period (0800-1400)	
	Period Length (minutes)	ACF ($p < .05$)
59	11	0.15
60	11	0.14
61	15	
62	11	
63	12	0.21
64	11	0.12
65		
66	13	0.21
67	9	0.26
68	11	
69	9	0.13
70	13	0.26
71	10	
72	11	0.20



73	12	0.14
74	14	0.12
75	14	0.19
76	11	0.26
77	16	0.17
78	8	0.18
79	39	0.23
80	27	0.17
81	14	
82	8	0.17

Table 6: Period Lengths and Significant Autocorrelation Functions During the Baseline, Saline, and Post-Saline Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Normal Saline (n = 24)

Animal Identification Number	Baseline Recording Period (0800-1000)		Saline Recording Period (1000-1200)		Post-Saline Recording Period (1200-1400)	
	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)
59	16		22		10	0.38
60	11	0.25	11		10	
61	14		19		15	
62			12	0.27	10	
63	9		13	0.27	12	
64	14	0.24	10		27	0.27
65	9		8			
66	8		11		13	0.31
67	9	0.28	6	0.28	9	0.24
68	7	0.3	19		11	
69			17	0.25	13	
70	15	0.49	21			



71	10		10		10	
72	10	0.28	13	0.45	21	0.27
73	8	0.3	12			
74	9				14	0.37
75					14	
76	18		13	0.26	11	
77	10	0.36	16		16	
78	6	0.35			10	
79	24				24	
80	20	0.26	8	0.35	27	0.32
81						
82	8		8		7	

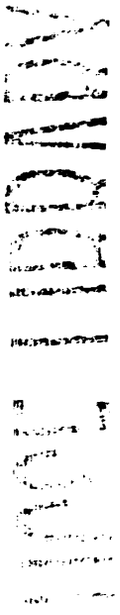


Table 7: Means for Selected EEG Parameters (±SEM) and Magnitude of Effect for Percent of Absolute Power at 7 and 8 Hz (±SEM) Over Three, Two Hour Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Morphine 5 μg/kg (n = 16 for 8 animals)

	Baseline Recording Period (0800-1000)	**Drug Recording Period (1000-1200)**	**Post-Drug Recording Period (1200-1400)**	**Significance of RMANOVA F Statistic**
Time Domain Parameters				
Activity	189.31 (± 40.26)	190.12 (± 37.85)	213.78 (± 41.80)	NS
Mobility (crossings/minute)	125.05 (± 1.60)	126.08 (± 1.71)	123.25 (± 1.67)	p = .006
Complexity	1.63 (± 0.01)	1.62 (± 0.01)	1.66 (± 0.02) *	p = .01
Frequency Domain Parameters				
Absolute Power (μV^2)	62.00 (± 13.33)	62.24 (± 12.69)	71.92 (± 14.31)	NS
Peak Frequency (Hz)	2.69 (± 0.23)	2.88 (± 0.24)	2.83 (± 0.24)	NS
Median Frequency (Hz)	6.26 (± 0.19)	6.34 (± 0.19)	6.37 (± 0.18)	NS
Edge Frequency (Hz)	26.98 (± 0.12)	26.91 (± 0.11)	26.82 (± 0.12) **	p = .003
Magnitude of Drug Effect for Percent of Absolute Power at 7 & 8 Hz				
AUC	1343.94 (± 78.46)	1489.69 (± 93.55) *	1463.69 (± 77.12)***	p = .03

NS indicates not significant, * indicates p = .05, ** indicates p = .01, *** indicates p = .04 for paired t-test comparing post-drug recording period to baseline recording period

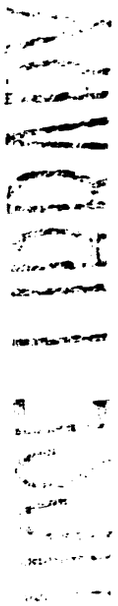


Table 8: Period Lengths and Significant Autocorrelation Functions During the Baseline, Drug, and Post-Drug Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Morphine 5 μg/kg

Animal Identification Number	Baseline Recording Period (0800-1000)		Drug Recording Period (1000-1200)		Post-Drug Recording Period (1200-1400)	
	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)
67	18		12		24	
68			25	0.28	8	0.23
69	23		10		11	
70					8	0.29
71	9		18		21	0.21
72	6		13		16	
73	5	0.17	12	0.54	15	0.30
74	4	0.26	11	0.48		

Table 9: Means for Selected EEG Parameters (±SEM) and Magnitude of Effect for Percent of Absolute Power at 7 and 8 Hz (±SEM) Over Three, Two Hour Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Morphine 500 µg/kg (n = 16 for 8 animals)

	Baseline Recording Period (0800-1000)	Drug Recording Period (1000-1200)	Post-Drug Recording Period (1200-1400)	Significance of RMANOVA F Statistic
Time Domain Parameters				
Activity	379.69 (± 141.63)	334.27 (± 78.32)	412.62 (± 103.16)	NS
Mobility (crossings/minute)	125.25 (± 2.05)	125.96 (± 2.05)	124.67 (± 1.69)	NS
Complexity	1.64 (± 0.02)	1.62 (± 0.02)	1.64 (± 0.01)	NS
Frequency Domain Parameters				
Absolute Power (μV^2)	120.84 (± 42.90)	106.03 (± 25.28)	132.26 (± 37.27)	NS
Peak Frequency (Hz)	2.44 (± 0.23)	2.53 (± 0.28)	2.44 (± 0.22)	NS
Median Frequency (Hz)	5.82 (± 0.23)	6.07 (± 0.30)	6.13 (± 0.16)	NS
Edge Frequency (Hz)	27.05 (± 0.12)	27.16 (± 0.13)	27.05 (± 0.12)	NS
Magnitude of Drug Effect for Percent of Absolute Power at 7 & 8 Hz				
AUC	1262.56 (± 98.22)	1243.69 (± 94.76)	1254.75 (± 75.84)	NS

NS indicates not significant

Table 10: Period Lengths and Significant Autocorrelation Functions During the Baseline, Drug, and Post-Drug Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Morphine 500 μg/kg

Animal Identification Number	Baseline Recording Period (0800-1000)		Drug Recording Period (1000-1200)		Post-Drug Recording Period (1200-1400)	
	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)
67			12		14	
68					9	
69					6	0.48
70	11	0.27			37	
71	6	0.22				
72	5	0.24	15	0.28	22	0.25
73			12	0.27	30	
74	8		18		10	

Table 11: Means for Selected EEG Parameters (±SEM) and Magnitude of Effect for Percent of Absolute Power at 7 and 8 Hz (±SEM) Over Three, Two Hour Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Pentazocine 50 µg/kg (n = 16 for 8 animals)

	Baseline Recording Period (0800-1000)	**Drug Recording Period (1000-1200)**	**Post-Drug Recording Period (1200-1400)**	**Significance of RMANOVA F Statistic**
Time Domain Parameters				
Activity	221.04 (± 54.16)	201.08 (± 46.67)	196.42 (± 51.87)	NS
Mobility (crossings/minute)	126.25 (± 1.86)	126.58 (± 1.65)	123.30 (± 1.87)	p = .05
Complexity	1.61 (± 0.01)	1.61 (± 0.01)	1.64 (± 0.02)	NS
Frequency Domain Parameters				
Absolute Power (μV^2)	74.36 (± 18.70)	67.08 (± 16.02)	66.92 (± 17.87)	NS
Peak Frequency (Hz)	2.83 (± 0.11)	3.24 (± 0.18)**	3.34 (± 0.16)**	p = .001
Median Frequency (Hz)	6.42 (± 0.25)	6.50 (± 0.13)	6.70 (± 0.16)**	p = .004
Edge Frequency (Hz)	27.03 (± 0.07)	26.94 (± 0.10)	26.82 (± 0.12)**	p = .000
Magnitude of Drug Effect for Percent of Absolute Power at 7 & 8 Hz				
AUC	1412.00 (± 45.25)	1560.44 (± 65.67) **	1657.38 (± 61.10) **	p = .000

NS indicates not significant, ** indicates p = .01

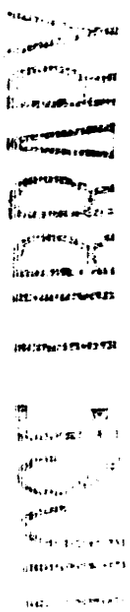


Table 12: Period Lengths and Significant Autocorrelation Functions During the Baseline, Drug, and Post-Drug Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Pentazocine 50 μg/kg

Animal Identification Number	Baseline Recording Period (0800-1000)		Drug Recording Period (1000-1200)		Post-Drug Recording Period (1200-1400)	
	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)
59			16		7	
60	8	0.30	12	0.24	15	0.25
61	18				15	
62					16	
63	24		15		11	
64	22		17	0.29	12	
65	11				19	0.30
66	11		8		15	0.36

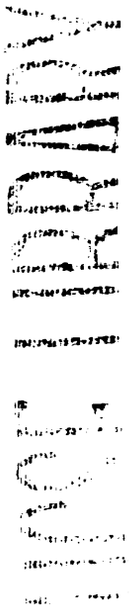


Table 13: Means for Selected EEG Parameters (±SEM) and Magnitude of Effect for Percent of Absolute Power at 7 and 8 Hz (±SEM) Over Three, Two Hour Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Pentazocine 5 mg/kg (n = 16 for 8 animals)

	Baseline Recording Period (0800-1000)	**Drug Recording Period (1000-1200)**	**Post-Drug Recording Period (1200-1400)**	**Significance of RMANOVA F Statistic**
Time Domain Parameters				
Activity	404.61 (± 138.37)	629.43 (± 345.96)	565.20 (± 307.57)	NS
Mobility (crossings/minute)	119.41 (± 1.02)	121.09 (± 1.66)	121.74 (± 2.04)	NS
Complexity	1.69 (± 0.02)	1.68 (± 0.03)	1.67 (± 0.03)	NS
Frequency Domain Parameters				
Absolute Power (μV^2)	135.34 (± 46.09)	207.74 (± 113.26)	184.83 (± 98.00)	NS
Peak Frequency (Hz)	2.97 (± 0.18)	3.12 (± 0.23)	3.38 (± 0.24)	NS
Median Frequency (Hz)	6.08 (± 0.24)	6.43 (± 0.24)	6.42 (± 0.25)	NS
Edge Frequency (Hz)	26.40 (± 0.25)	26.79 (± 0.21)**	26.63 (± 0.22)	p = .01
Magnitude of Drug Effect for Percent of Absolute Power at 7 & 8 Hz				
AUC	1501.00 (± 74.25)	1442.13 (± 76.61)	1644.94 (± 97.31)	p = .01

NS indicates not significant, ** indicates p = .01

Table 14: Period Lengths and Significant Autocorrelation Functions During the Baseline, Drug, and Post-Drug Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Pentazocine 5 mg/kg

Animal Identification Number	Baseline Recording Period (0800-1000)		Drug Recording Period (1000-1200)		Post-Drug Recording Period (1200-1400)	
	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)
59	13				9	
60	13				15	0.25
61	13				7	
62					18	
63	36		13		14	
64					11	
65	7	0.31			8	0.26
66	9				33	

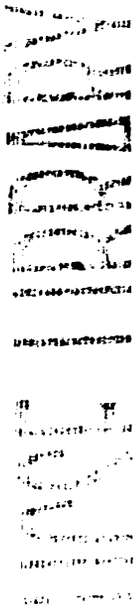


Table 15: Means for Selected EEG Parameters (±SEM) and Magnitude of Effect for Percent of Absolute Power at 7 and 8 Hz (±SEM) Over Three, Two Hour Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Naloxone 5 μg/kg (n = 16 for 8 animals)

	Baseline Recording Period (0800-1000)	**Drug Recording Period (1000-1200)**	**Post-Drug Recording Period (1200-1400)**	**Significance of RMANOVA F Statistic**
Time Domain Parameters				
Activity	361.81 (± 159.42)	355.39 (± 181.00)	349.12 (± 121.18)	NS
Mobility (crossings/minute)	125.46 (± 1.96)	125.65 (± 1.88)	123.94 (± 1.54) **	p = .003
Complexity	1.63 (± 0.02)	1.63 (± 0.01)	1.64 (± 0.01)	NS
Frequency Domain Parameters				
Absolute Power (μV^2)	114.40 (± 48.76)	114.02 (± 57.15)	110.10 (± 36.28)	NS
Peak Frequency (Hz)	2.50 (± 0.23)	2.92 (± 0.23) *	2.76 (± 0.20)	p = .03
Median Frequency (Hz)	5.92 (± 0.18)	6.21 (± 0.12) *	6.32 (± 0.14)**	p = .007
Edge Frequency (Hz)	27.10 (± 0.13)	27.07 (± 0.12)	27.03 (± 0.13)	NS
Magnitude of Drug Effect for Percent of Absolute Power at 7 & 8 Hz				
AUC	1248.63 (± 84.20)	1400.19 (± 80.77) *	1412.56 (± 79.52) *	p = .01

NS indicates not significant, * indicates p = .05, ** indicates p = .01

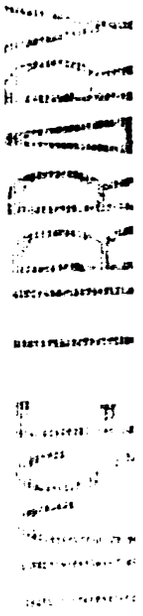


Table 16: Period Lengths and Significant Autocorrelation Functions During the Baseline, Drug, and Post-Drug Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Naloxone 5 μg/kg

Animal Identification Number	Baseline Recording Period (0800-1000)		Drug Recording Period (1000-1200)		Post-Drug Recording Period (1200-1400)	
	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)
75	20		8		20	
76	12	0.22			23	
77	16		12		10	
78	15	0.33	8	0.29	7	
79	7		11	0.28	9	
80	8		14		11	
81			17	0.25	18	
82	6		17		17	

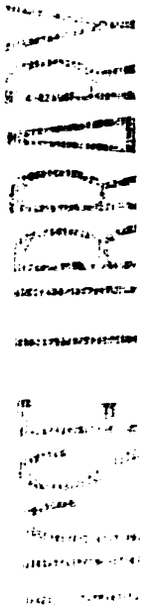


Table 17: Means for Selected EEG Parameters (±SEM) and Magnitude of Effect for Percent of Absolute Power at 7 and 8 Hz (±SEM) Over Three, Two Hour Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Naloxone 500 μg/kg (n = 16 for 8 animals)

	Baseline Recording Period (0800-1000)	**Drug Recording Period (1000-1200)**	**Post-Drug Recording Period (1200-1400)**	**Significance of RMANOVA F Statistic**
Time Domain Parameters				
Activity	280.94 (± 96.84)	269.35 (± 87.86)	236.26 (± 50.86)	NS
Mobility (crossings/minute)	121.66 (± 1.95)	120.82 (± 1.94)	120.84 (± 2.13)	NS
Complexity	1.67 (± 0.02)	1.68 (± 0.02)	1.68 (± 0.02)	NS
Frequency Domain Parameters				
Absolute Power (μV^2)	89.48 (± 29.52)	88.97 (± 27.74)	79.44 (± 19.86)	NS
Peak Frequency (Hz)	2.86 (± 0.25)	2.96 (± 0.23)	2.85 (± 0.18)	NS
Median Frequency (Hz)	6.12 (± 0.22)	6.20 (± 0.22)	6.36 (± 0.16)	NS
Edge Frequency (Hz)	26.87 (± 0.19)	26.74 (± 0.21)	26.73 (± 0.18)	NS
Magnitude of Drug Effect for Percent of Absolute Power at 7 & 8 Hz				
AUC	1401.56 (± 77.69)	1436 (± 88.06)	1432.88 (± 82.02)	NS

NS indicates not significant

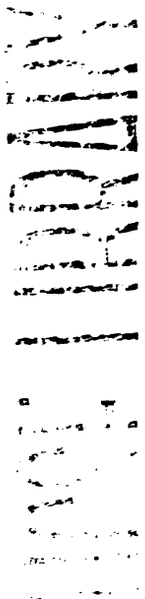


Table 18: Period Lengths and Significant Autocorrelation Functions During the Baseline, Drug, and Post-Drug Recording Periods in Conscious Rats Who Received a Subcutaneous Injection of Naloxone 500 µg/kg

Animal Identification Number	Baseline Recording Period (0800-1000)		Drug Recording Period (1000-1200)		Post-Drug Recording Period (1200-1400)	
	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)	Period Length (minutes)	ACF ($p < .05$)
75	9	0.24			15	
76	6		16		32	
77	13		9	0.24	12	
78	7	0.26	9	0.21	28	
79	30	0.29	7		17	0.34
80	5		15		14	
81	17		14		11	0.27
82	10	0.25	8		14	

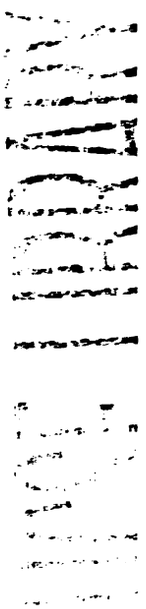


Table 19: Summary Table of the Magnitude of Drug Effect (i. e., AUC) on the Percent of Absolute Power at 7 and 8 Hz

Experiment	Compared to Baseline	
	Drug Recording Period (1000-1200)	Post-Drug Recording Period (1200-1400)
Normal Saline	no change	no change
Morphine 5 μg/kg	increase	increase (by paired t-test)
Morphine 500 μg/kg	no change	no change
Pentazocine 50 μg/kg	increase	increase
Pentazocine 5 mg/kg	no change	no change
Naloxone 5 μg/kg	increase	increase
Naloxone 500 μg/kg	no change	no change

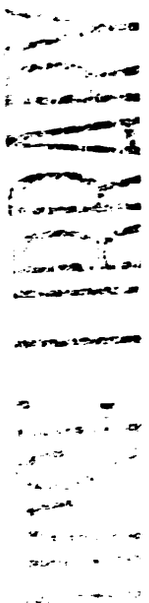
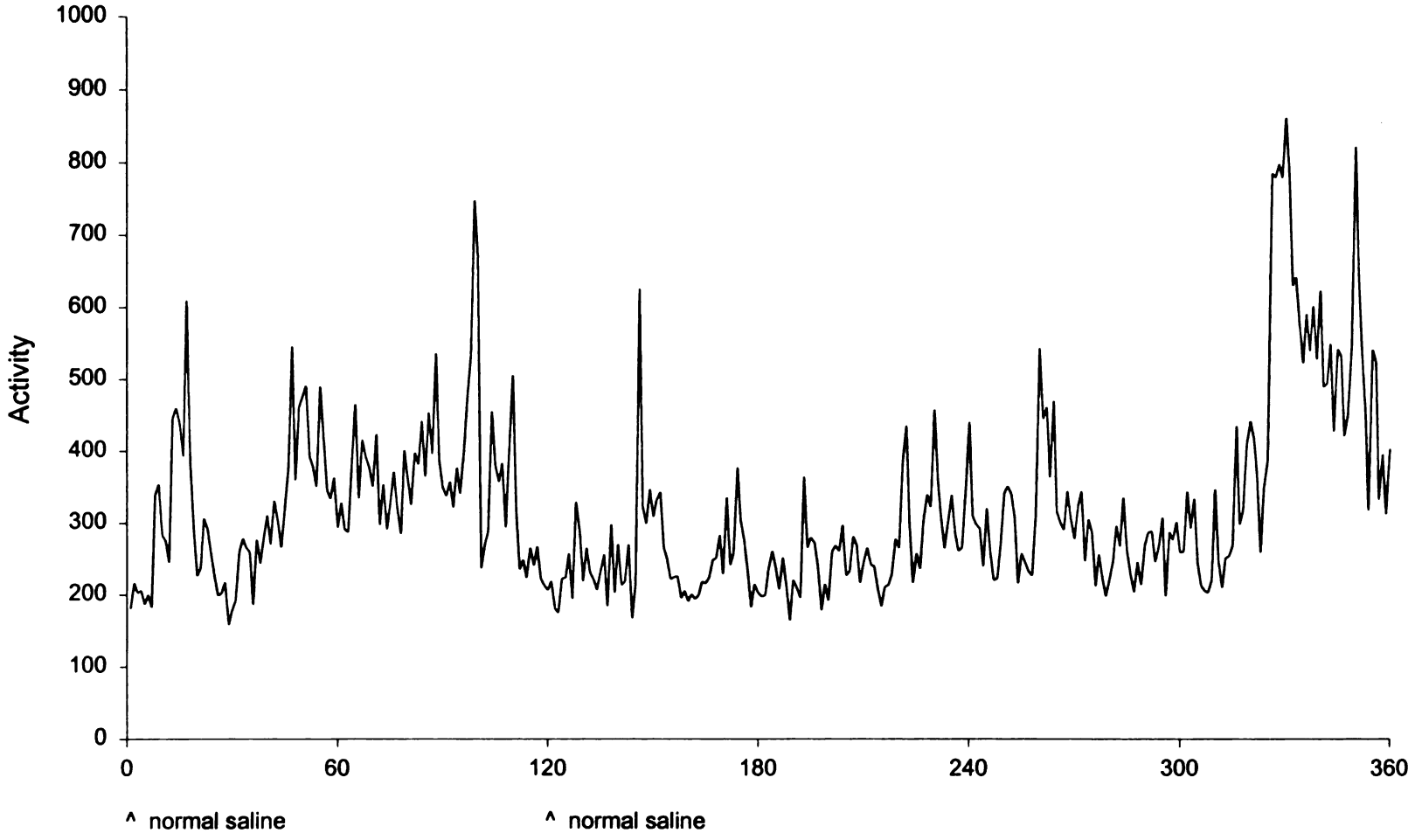


Figure 1: States of Disordered Consciousness: Arousal Mechanisms and Content Processing

	AROUSAL								
CONTENT		**hyperactivity**		**awake**		**decreased arousal**		**unresponsive**	
	hallucination	delirium (hyperactive)					delirium (hypoactive)		
	appropriate			alert		**sedation**			sleep
					clouding of consciousness				
	slowed			**sedation**		**sedation**			
					disorientation, confusion	stupor			
	absent processing							coma, unconscious	
				sleep					

Figure 2: Effect of Normal Saline on Activity (n=48 for 24 animals)



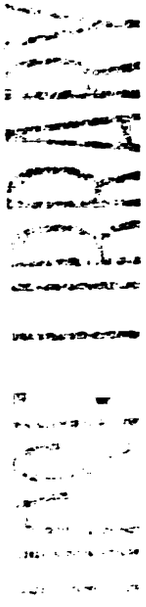
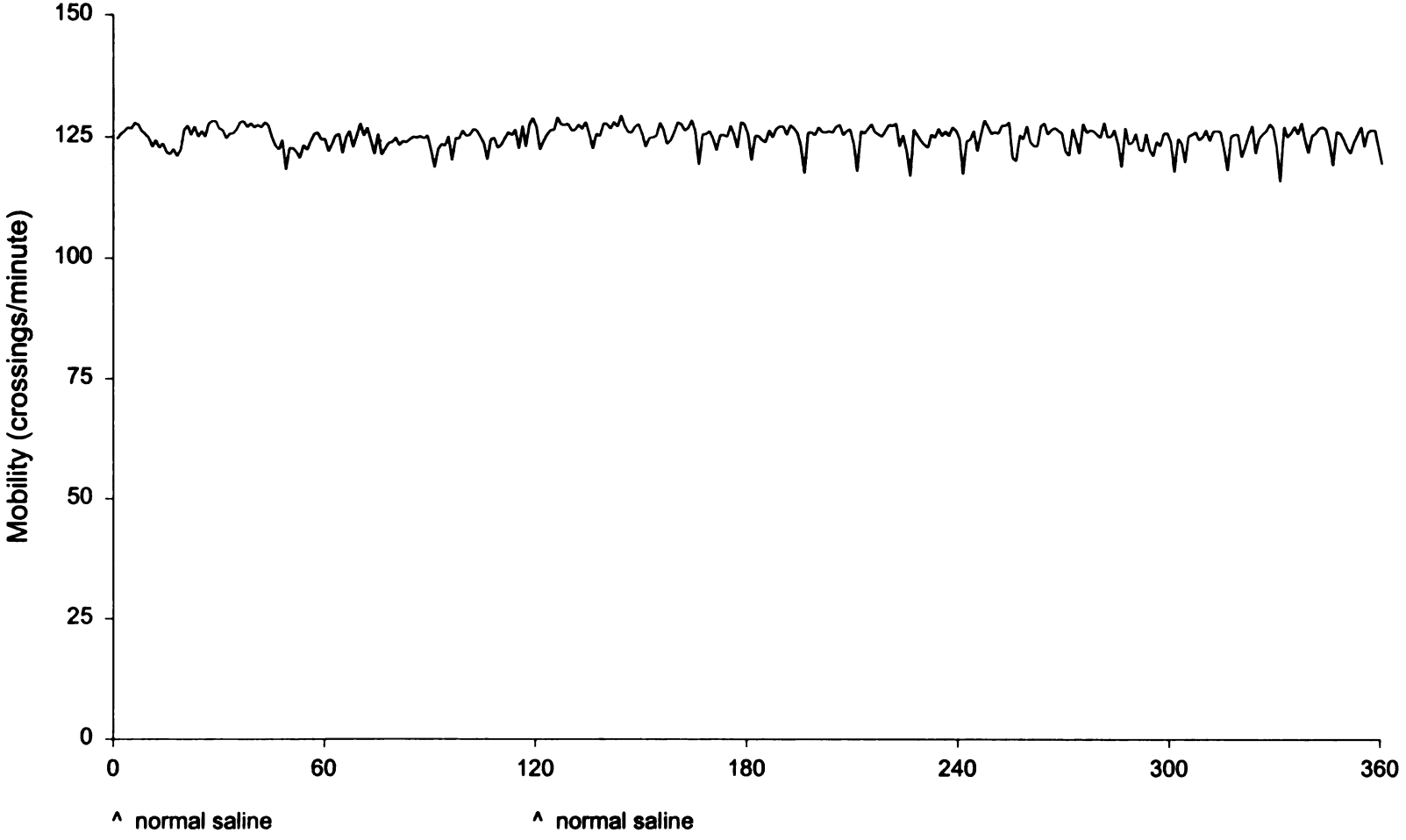


Figure 3: Effect of Normal Saline on Mobility (n=48 for 24 animals)



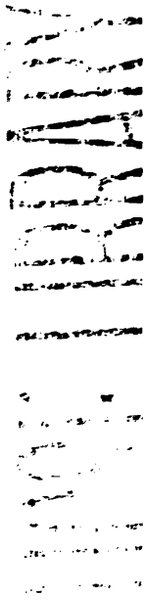
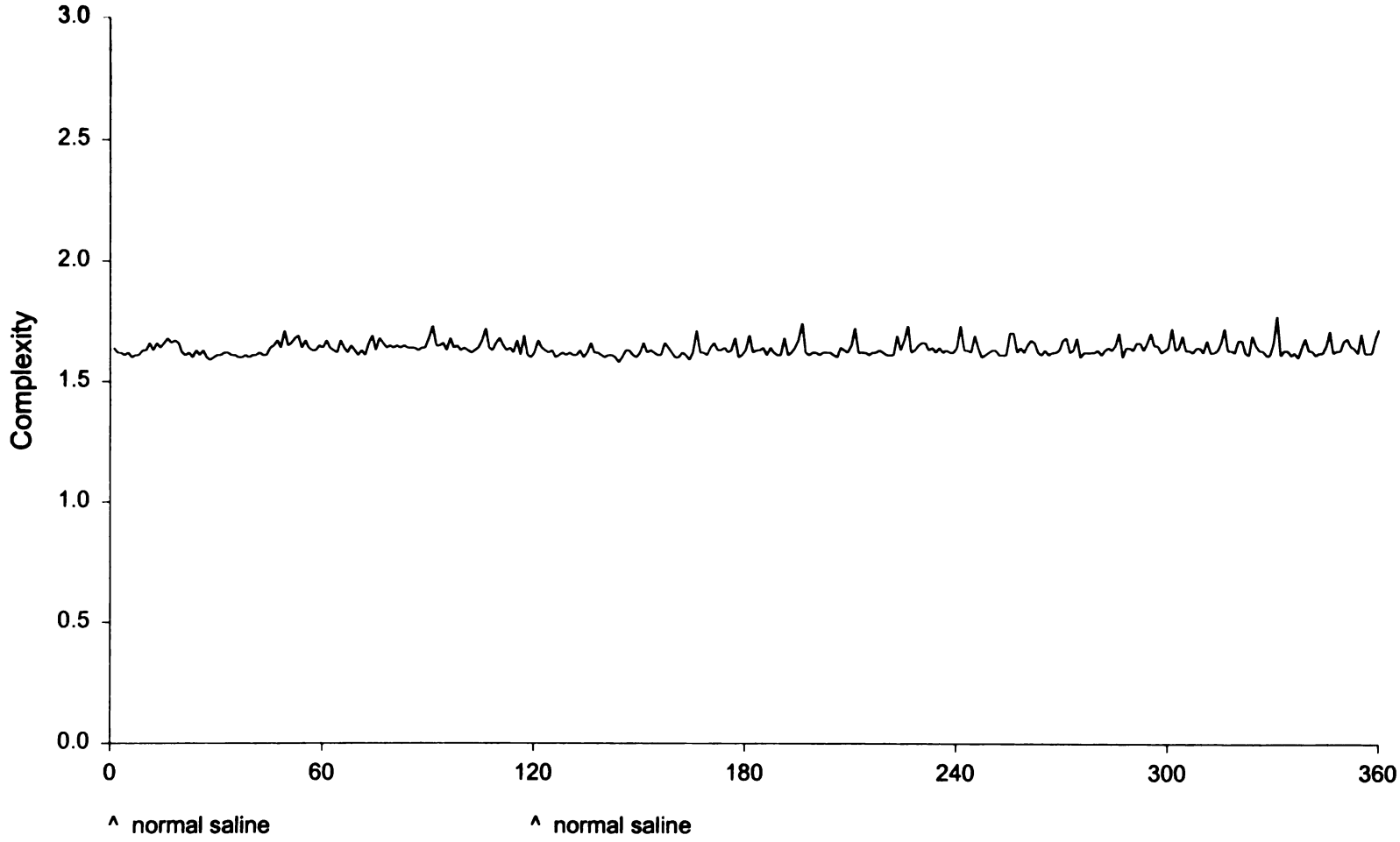


Figure 4: Effect of Normal Saline on Complexity (n=48 for 24 animals)



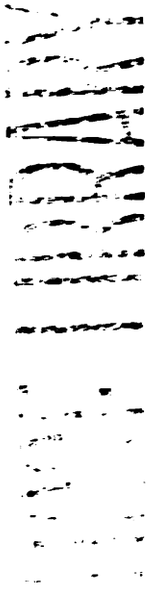
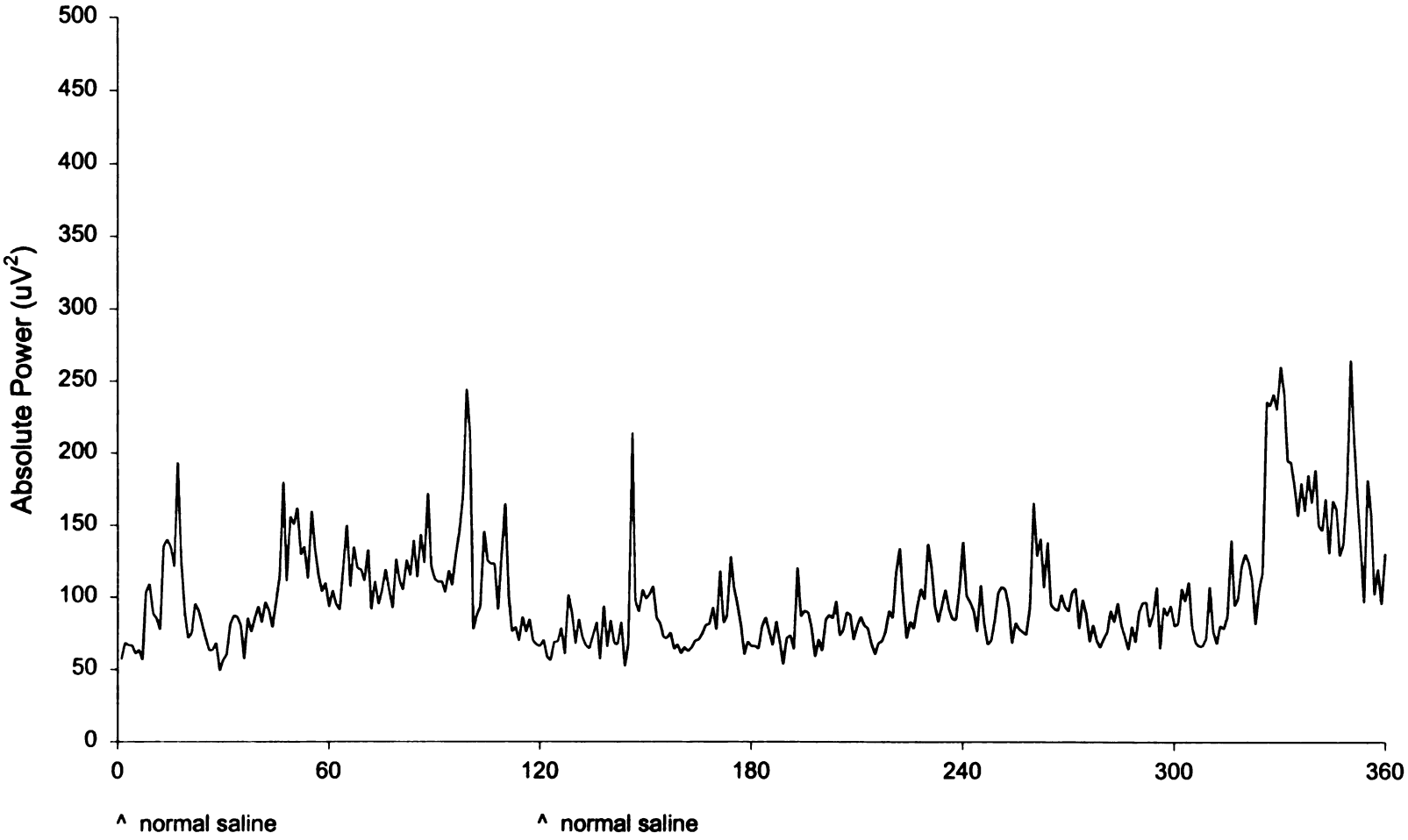


Figure 5: Effect of Normal Saline on Absolute Power (n=48 for 24 animals)



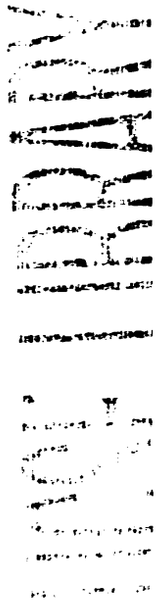
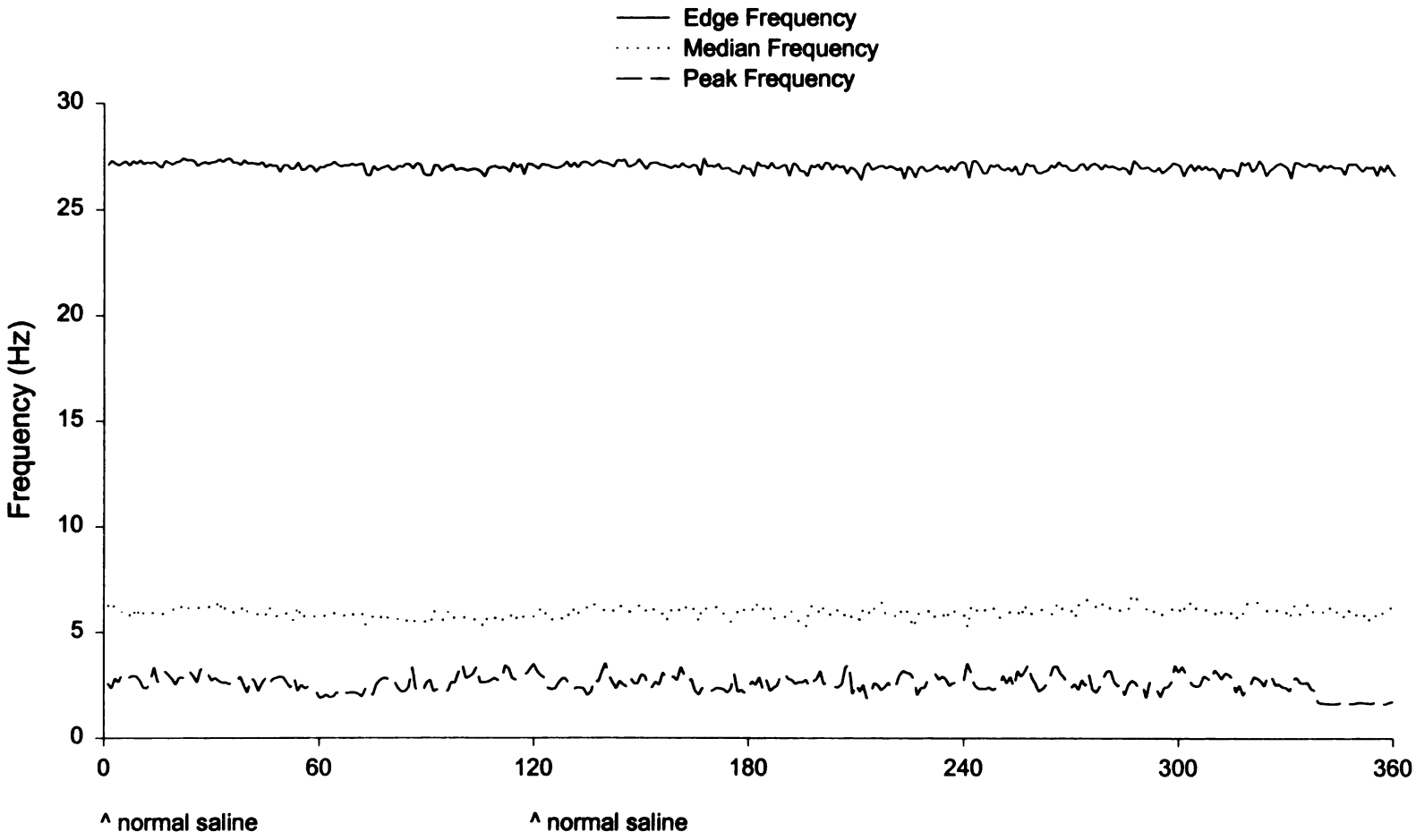


Figure 6: Effect of Normal Saline on Peak, Median & Edge Frequencies (n=48 for 24 animals)



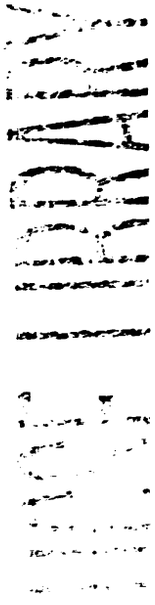
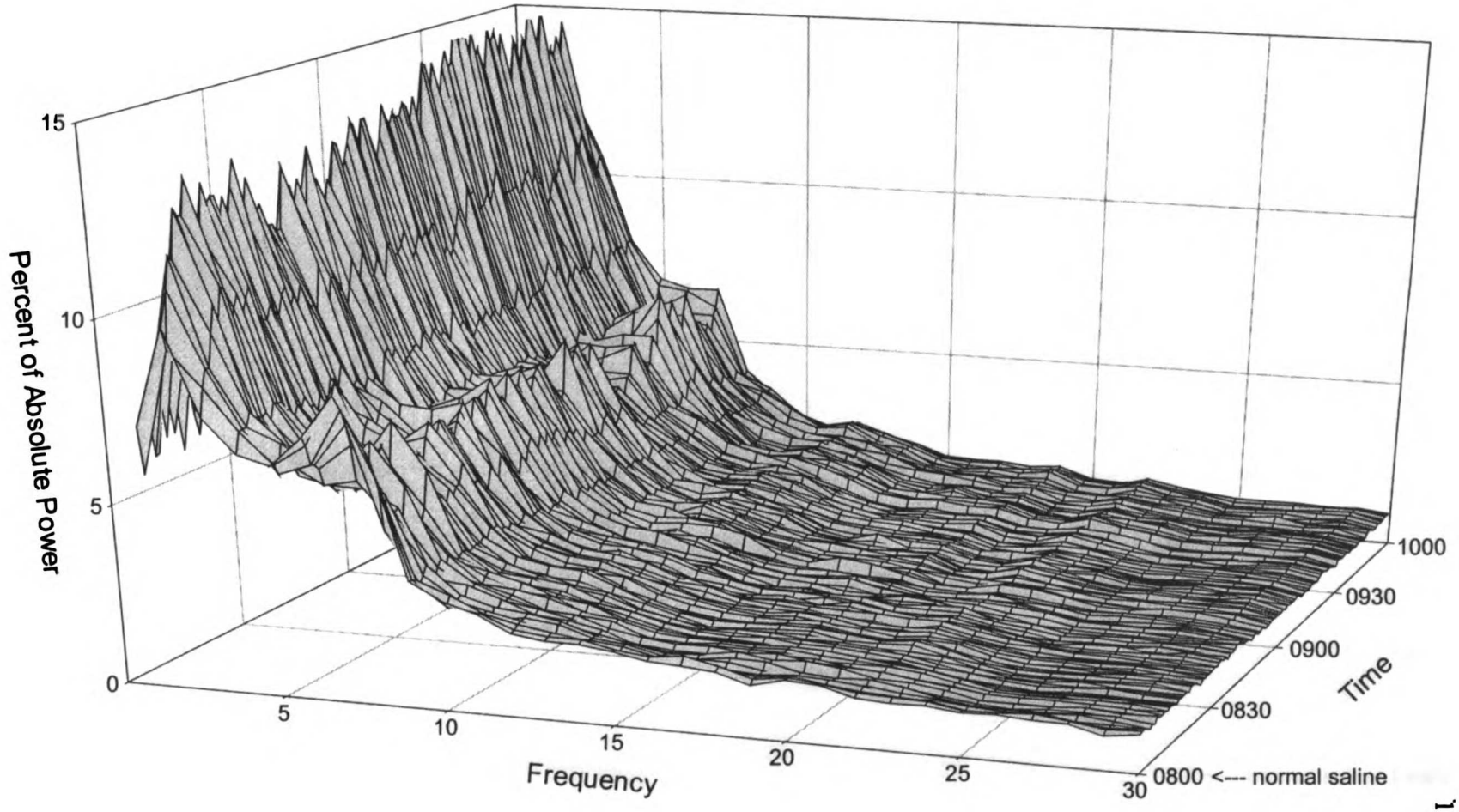


Figure 7: Effect of Normal Saline on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 0800-1000 (n=48 for 24 animals)



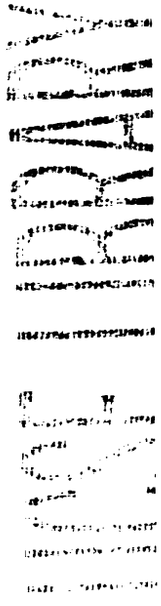
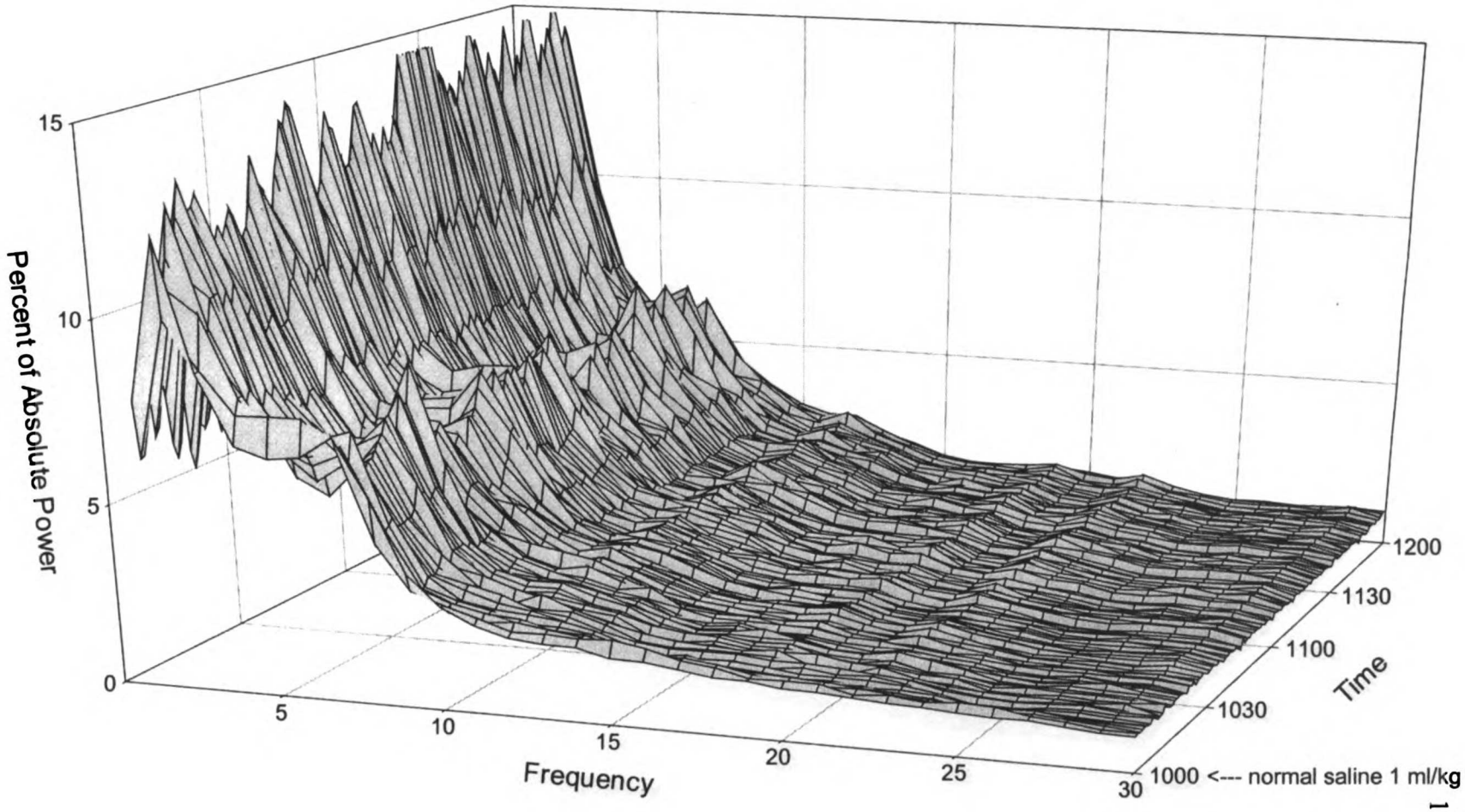


Figure 8: Effect of Normal Saline on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1000-1200 (n=48 for 24 animals)



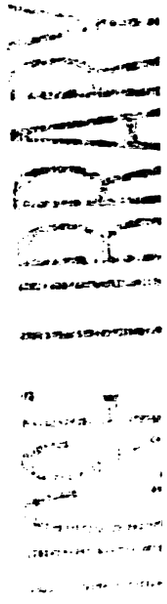
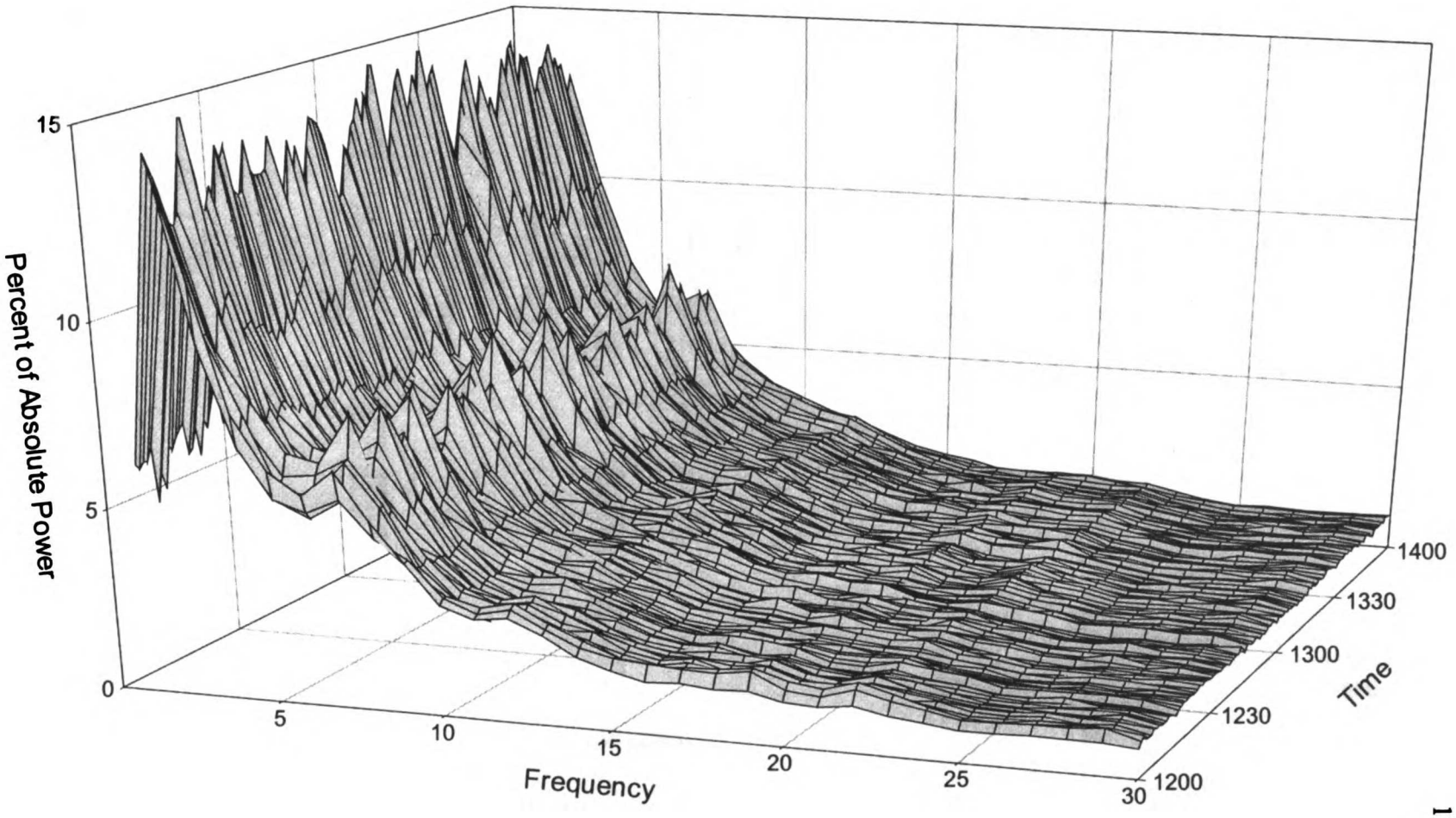


Figure 9: Effect of Normal Saline on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1200-1400 (n=48 for 24 animals)



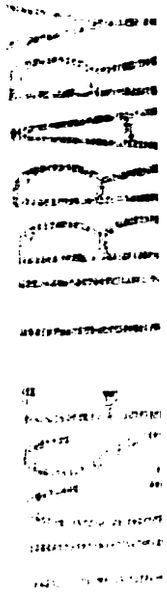
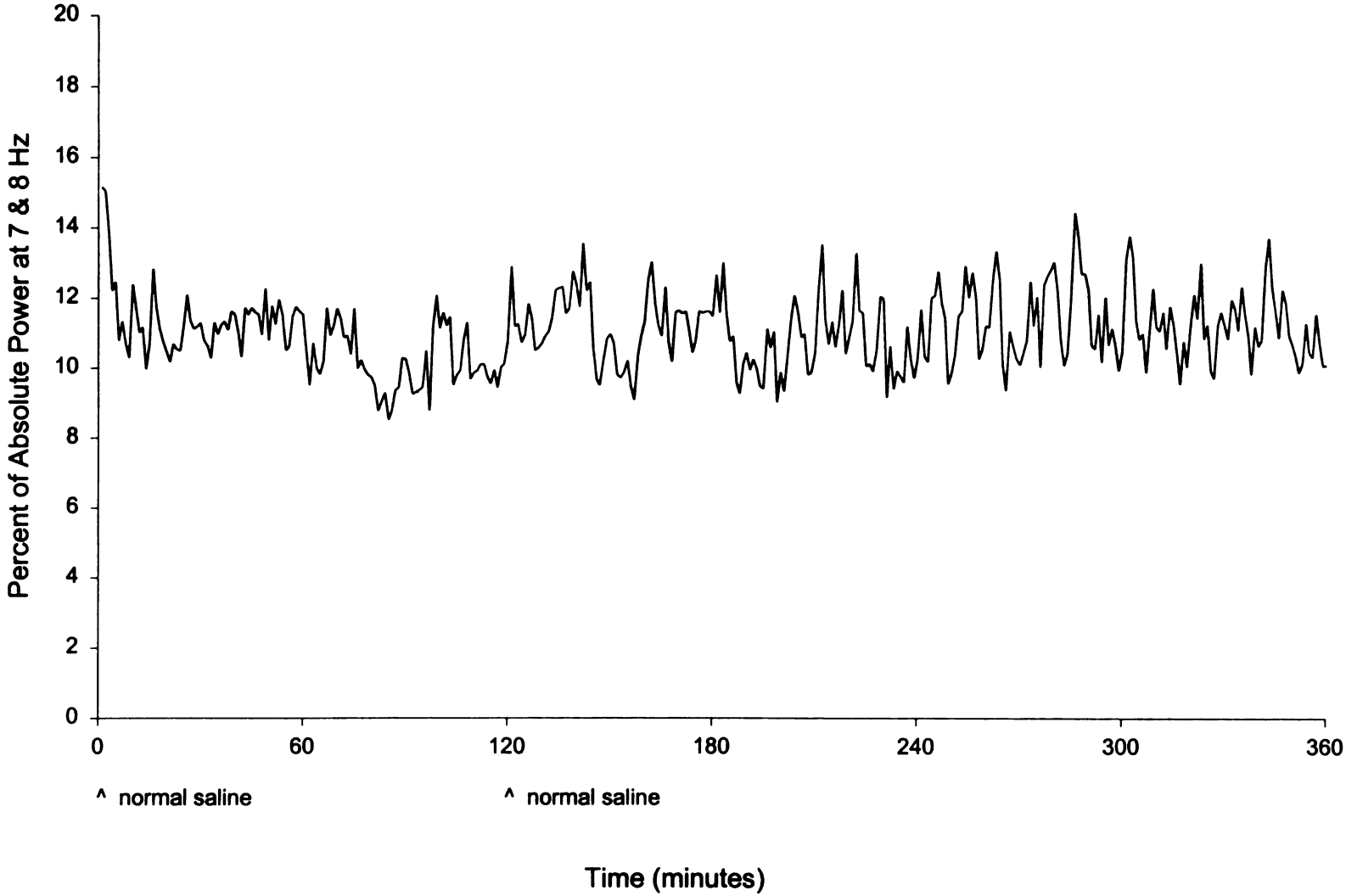


Figure 10: Effect of Normal Saline on Percent of Absolute Power at 7 & 8 Hz (n=48 for 24 animals)



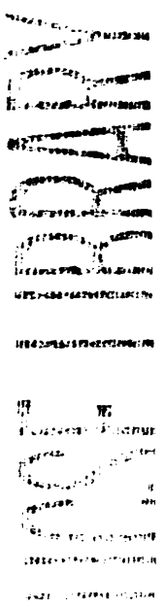


Figure 11: Effect of Normal Saline on Area-Under-the-Curve for Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)

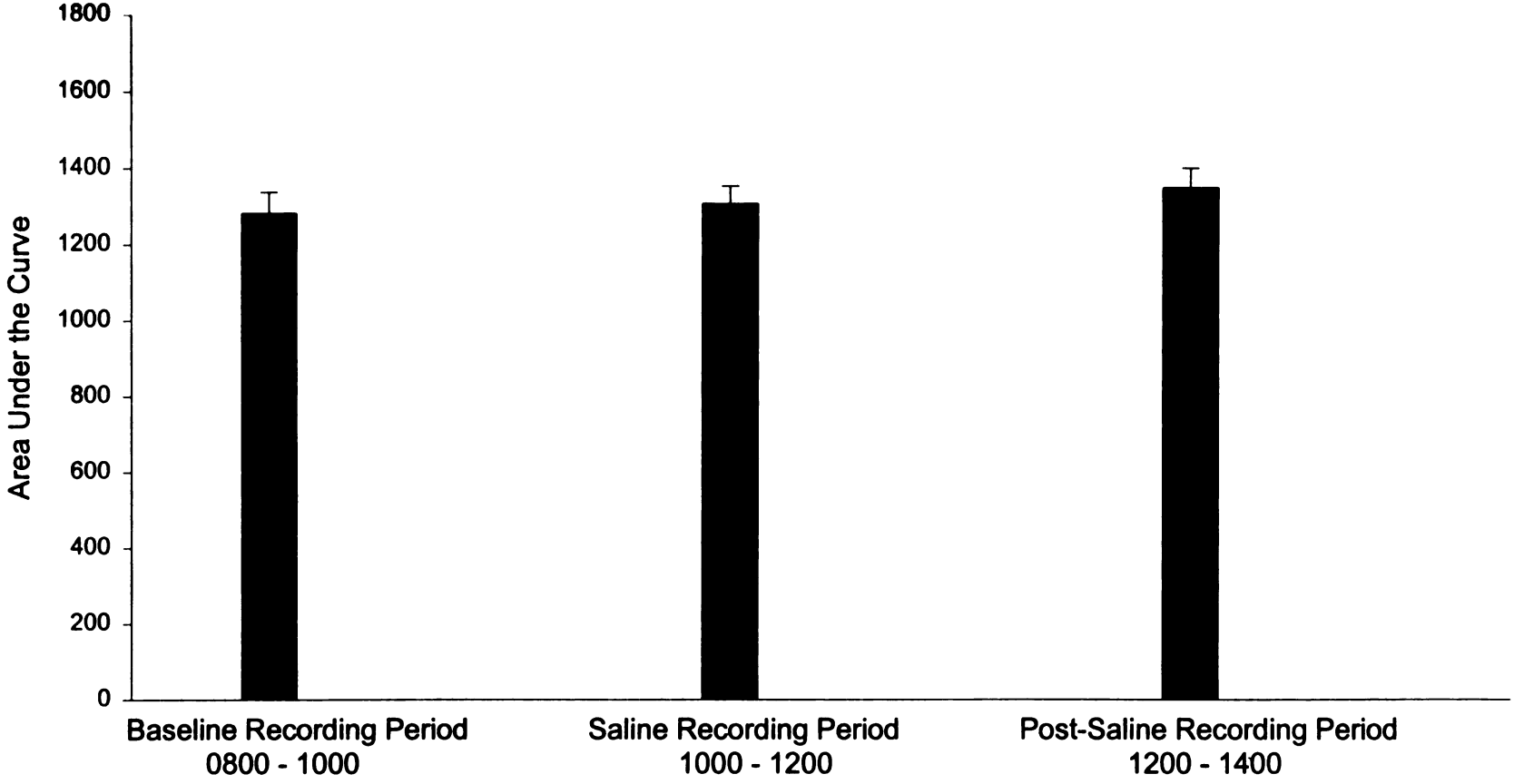
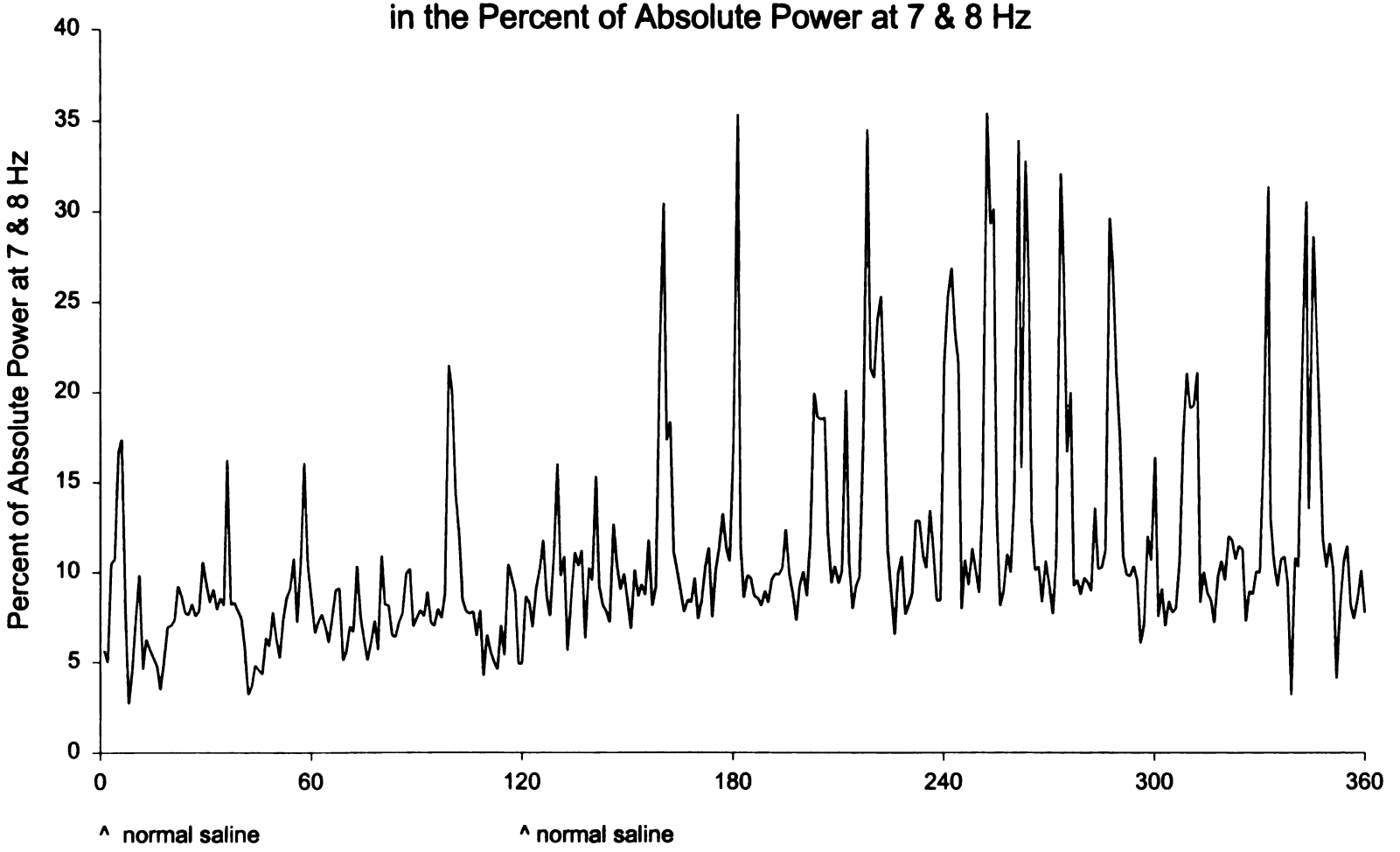


Figure 12: Example of an Animal Who Did Establish a Cyclic Pattern in the Percent of Absolute Power at 7 & 8 Hz



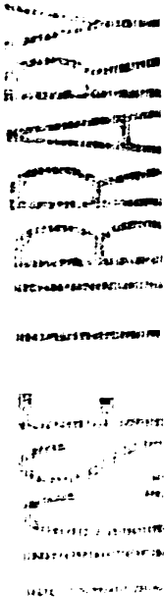
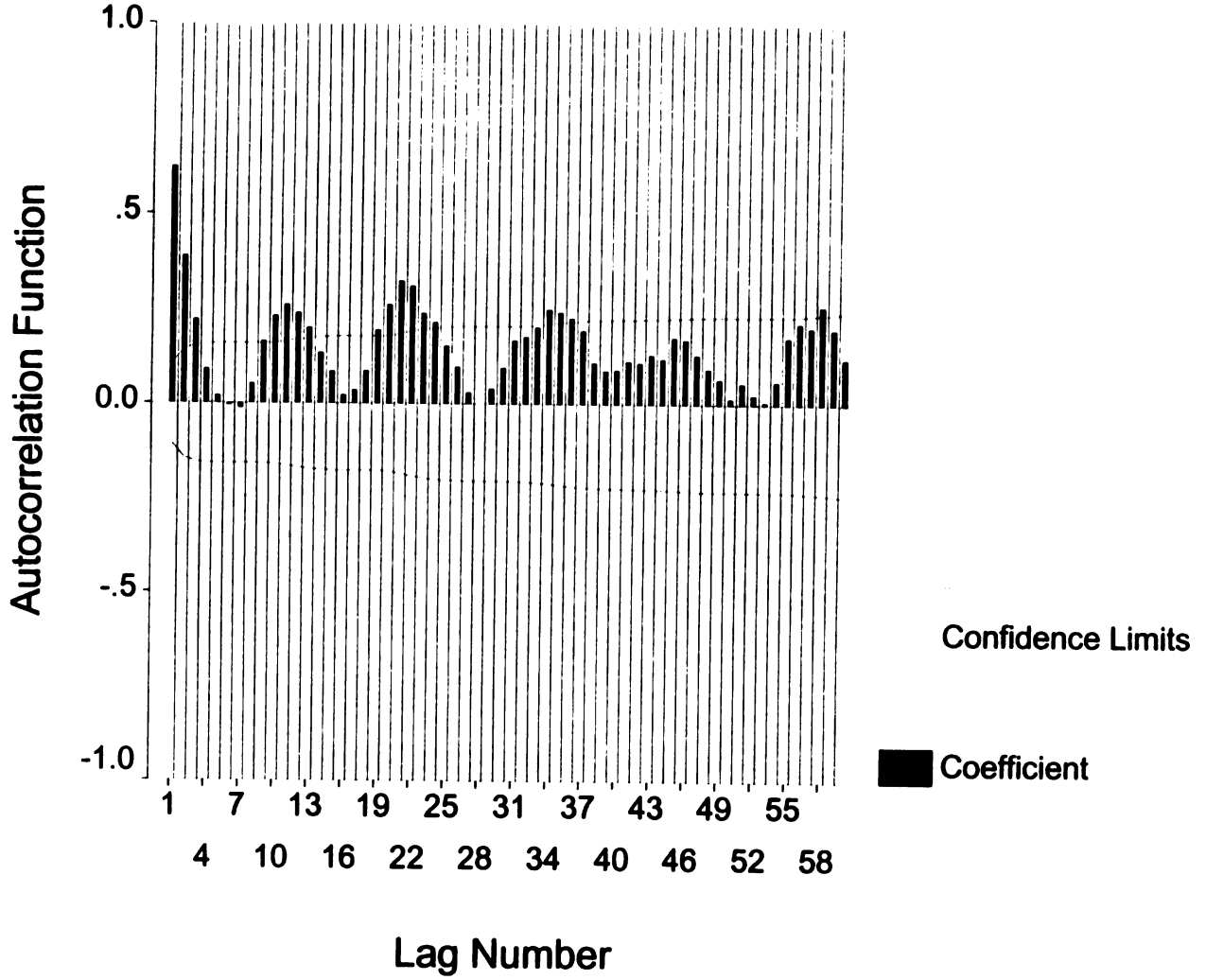


Figure 13: Correlogram with a Lag of 60 Minutes of an Animal Who Did Establish a Cyclic Pattern in the Percent of Absolute Power at 7 & 8 Hz



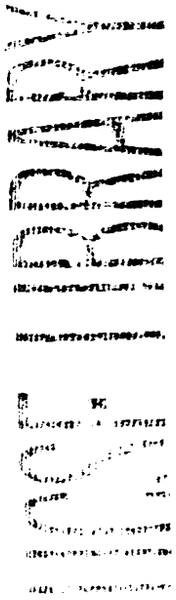
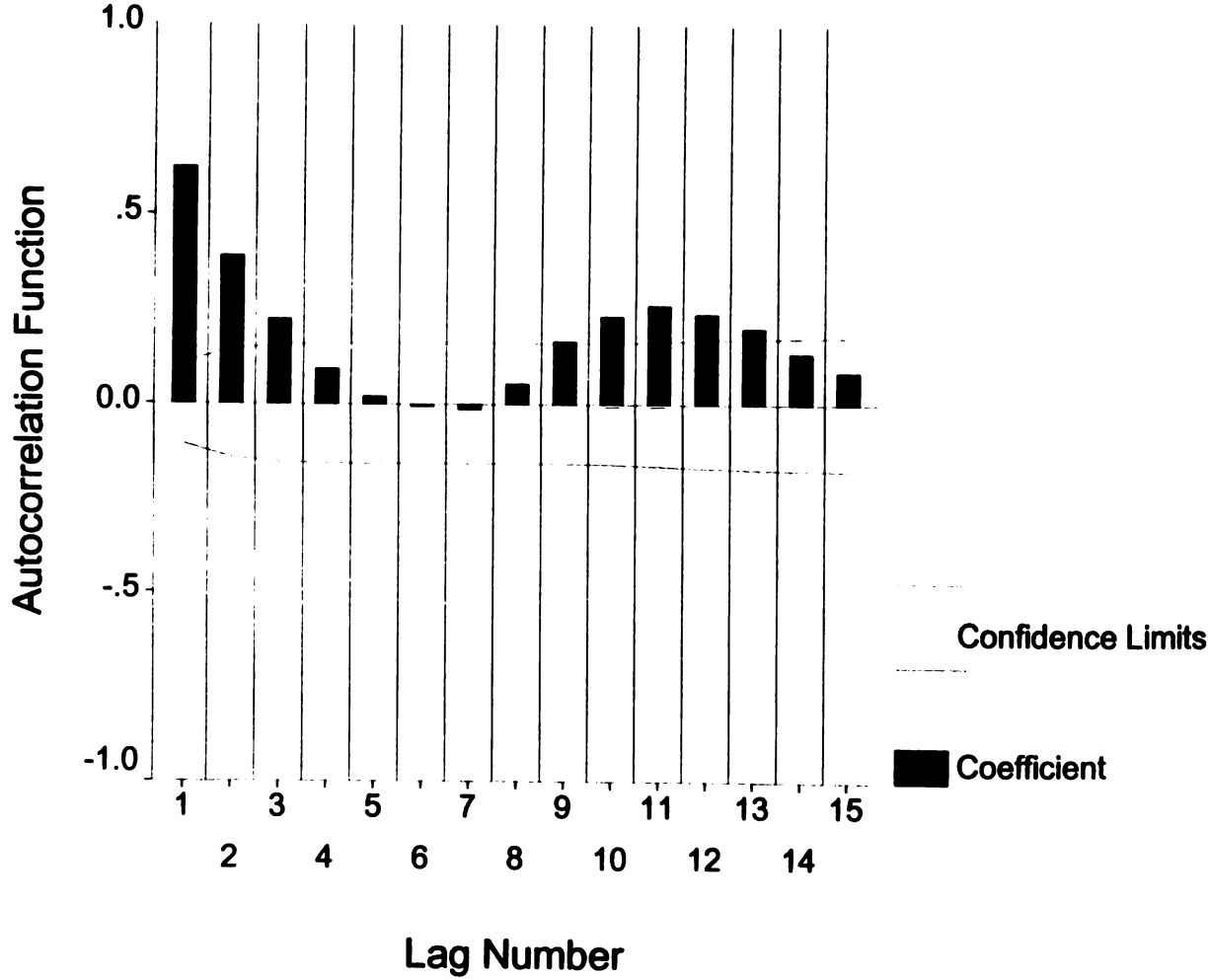


Figure 14: Correlogram with a Lag of 15 Minutes of an Animal Who Did Establish a Cyclic Pattern in the Percent of Absolute Power at 7 & 8 Hz



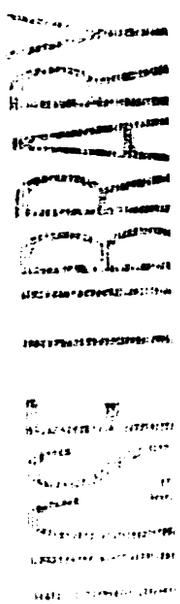
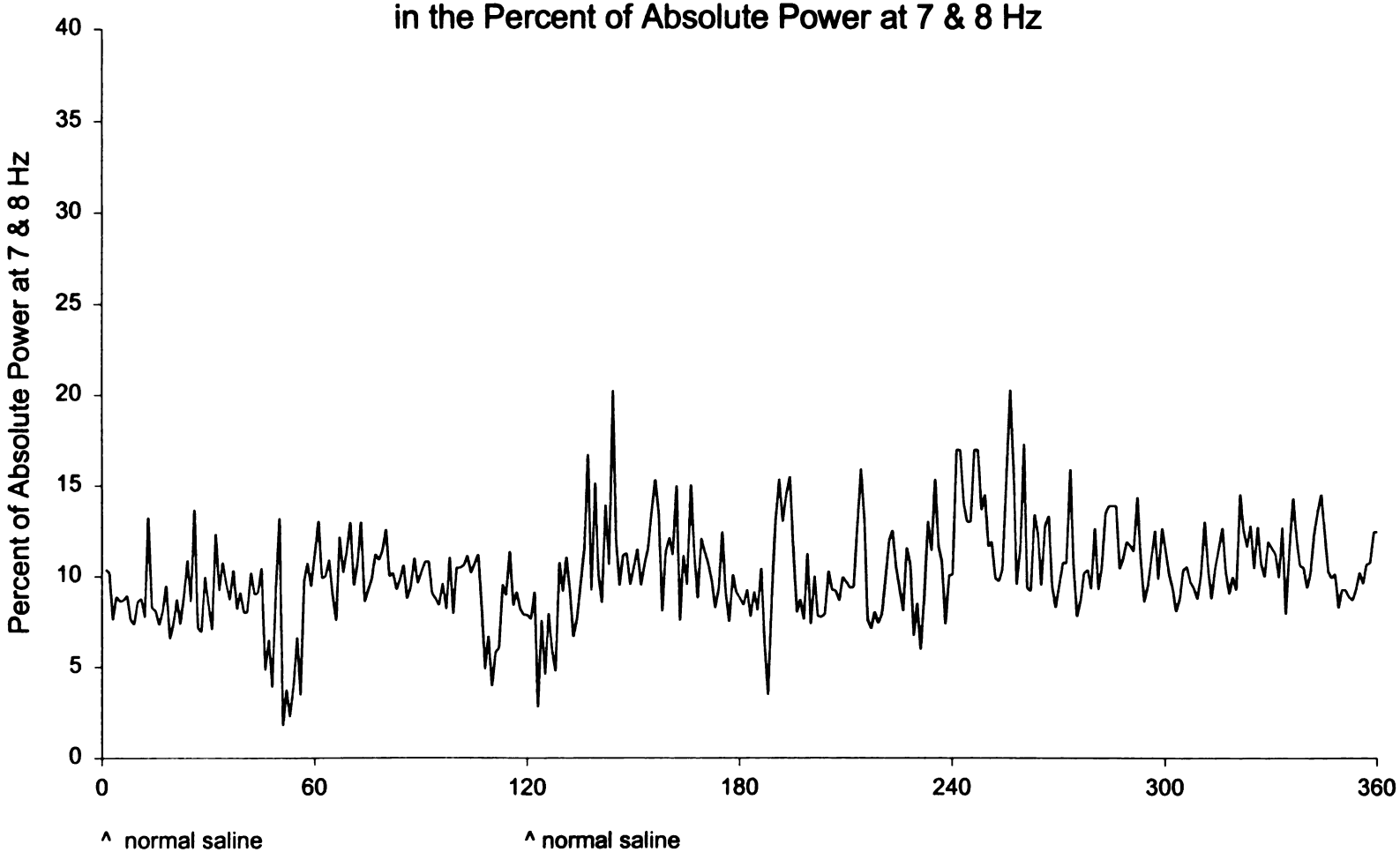


Figure 15: Example of an Animal Who Did Not Establish a Cyclic Pattern in the Percent of Absolute Power at 7 & 8 Hz



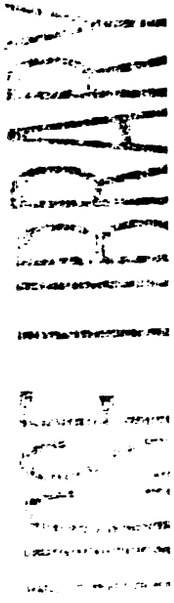


Figure 16: Correlogram with a Lag of 60 Minutes of an Animal Who Did Not Establish a Cyclic Pattern in the Percent of Absolute Power at 7 & 8 Hz

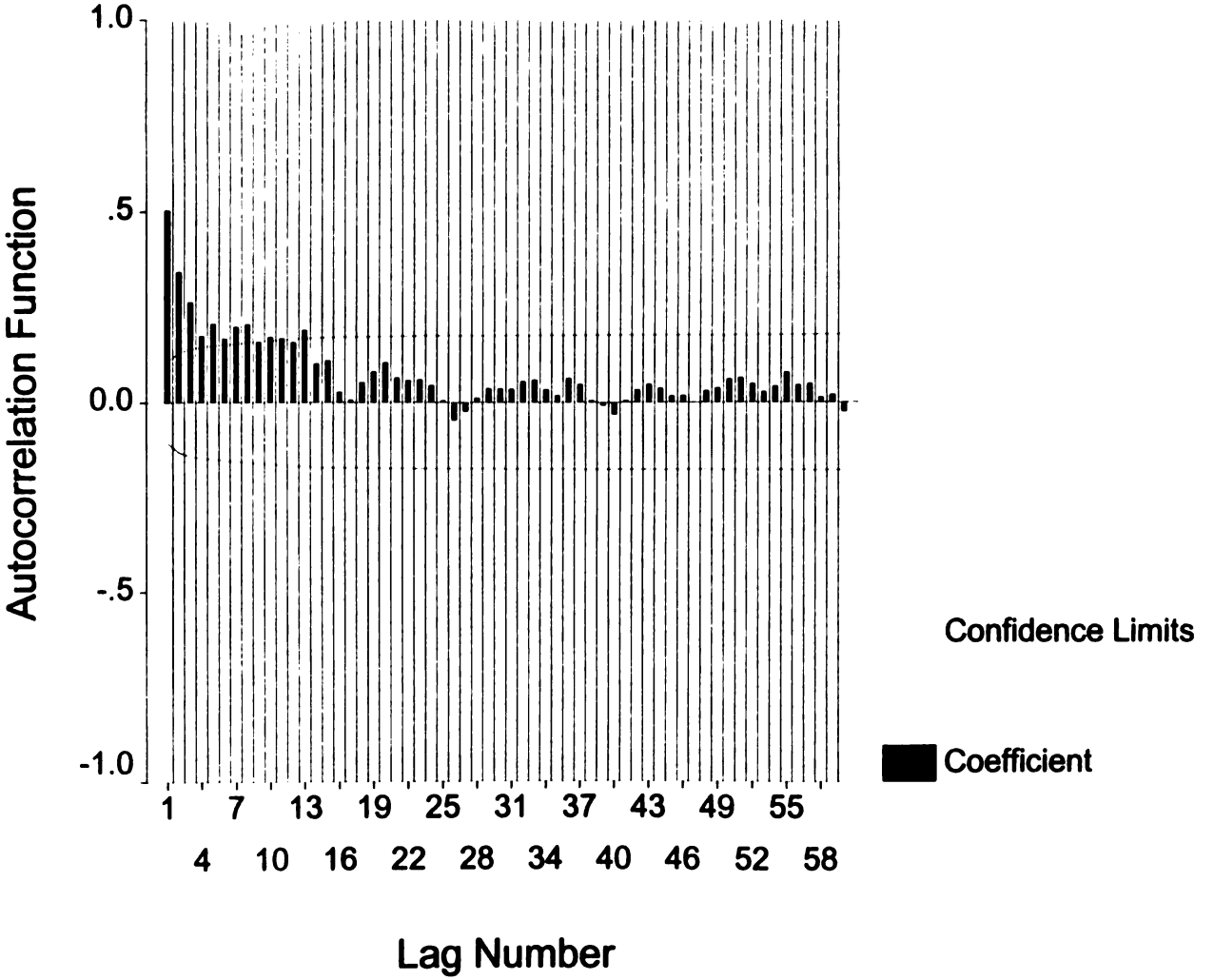


Figure 17: Effect of Morphine 5 ug/kg on Activity (n=16 for 8 animals)

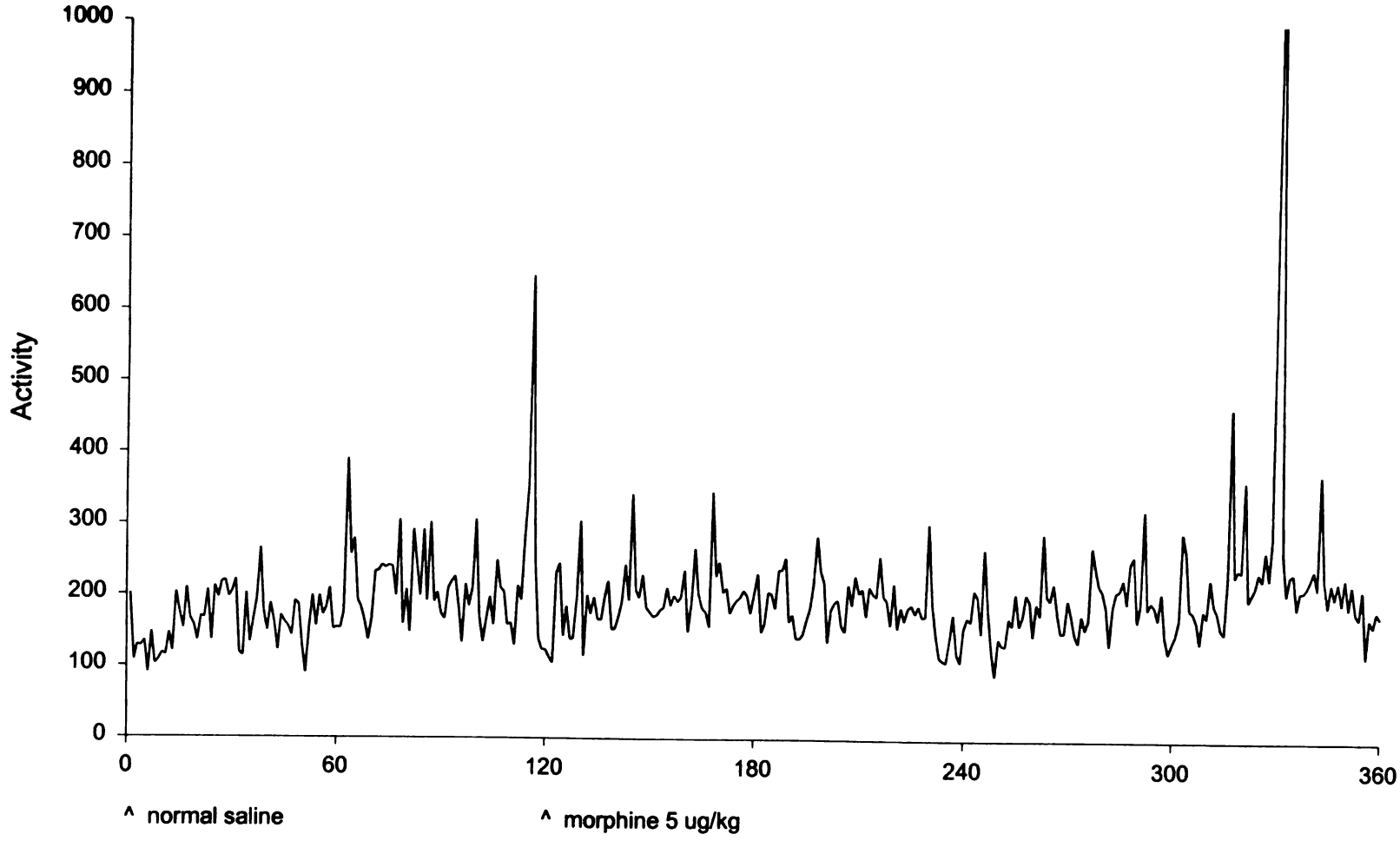


Figure 18: Effect of Morphine 5 ug/kg on Mobility (n=16 for 8 animals)

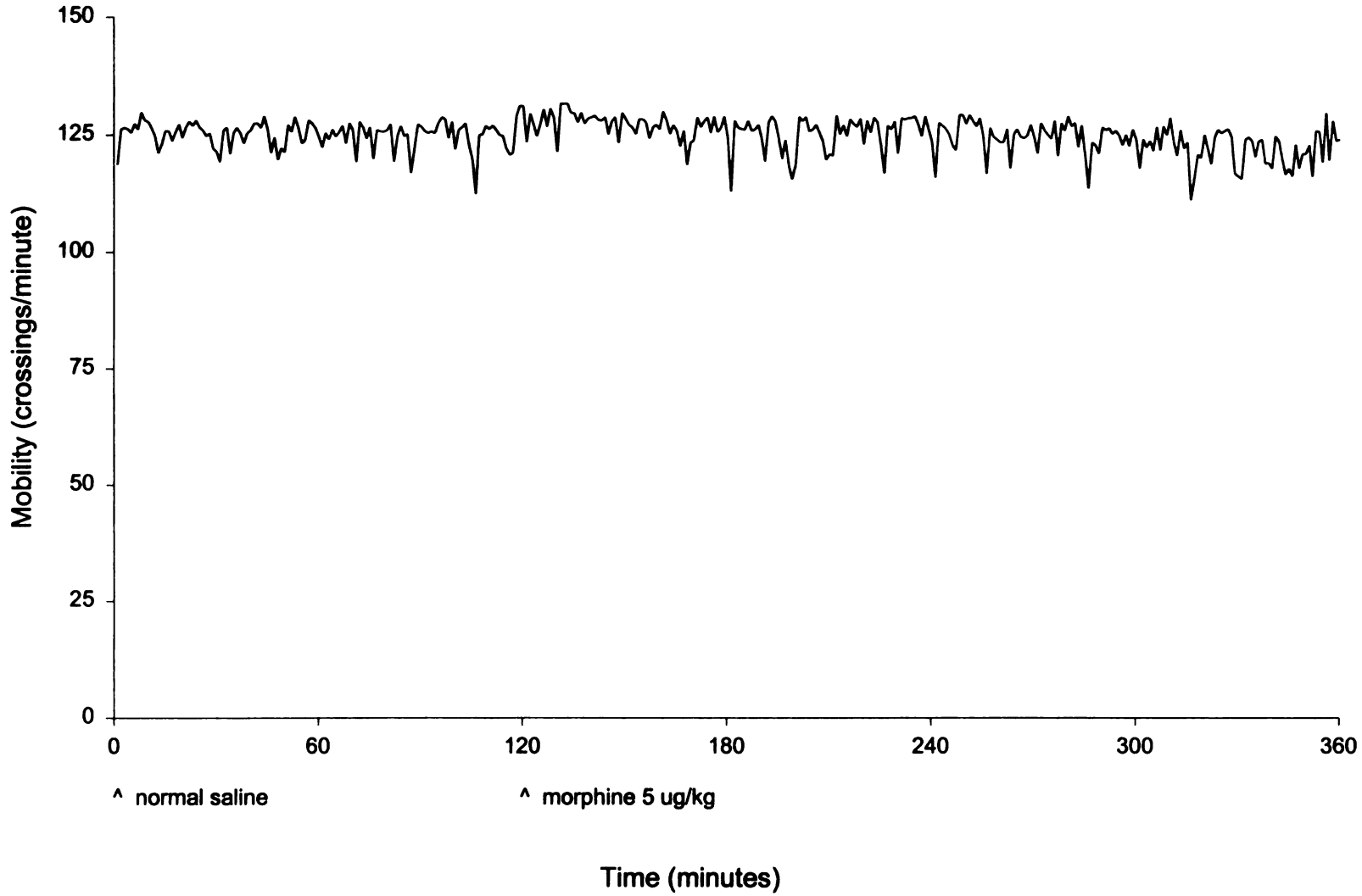
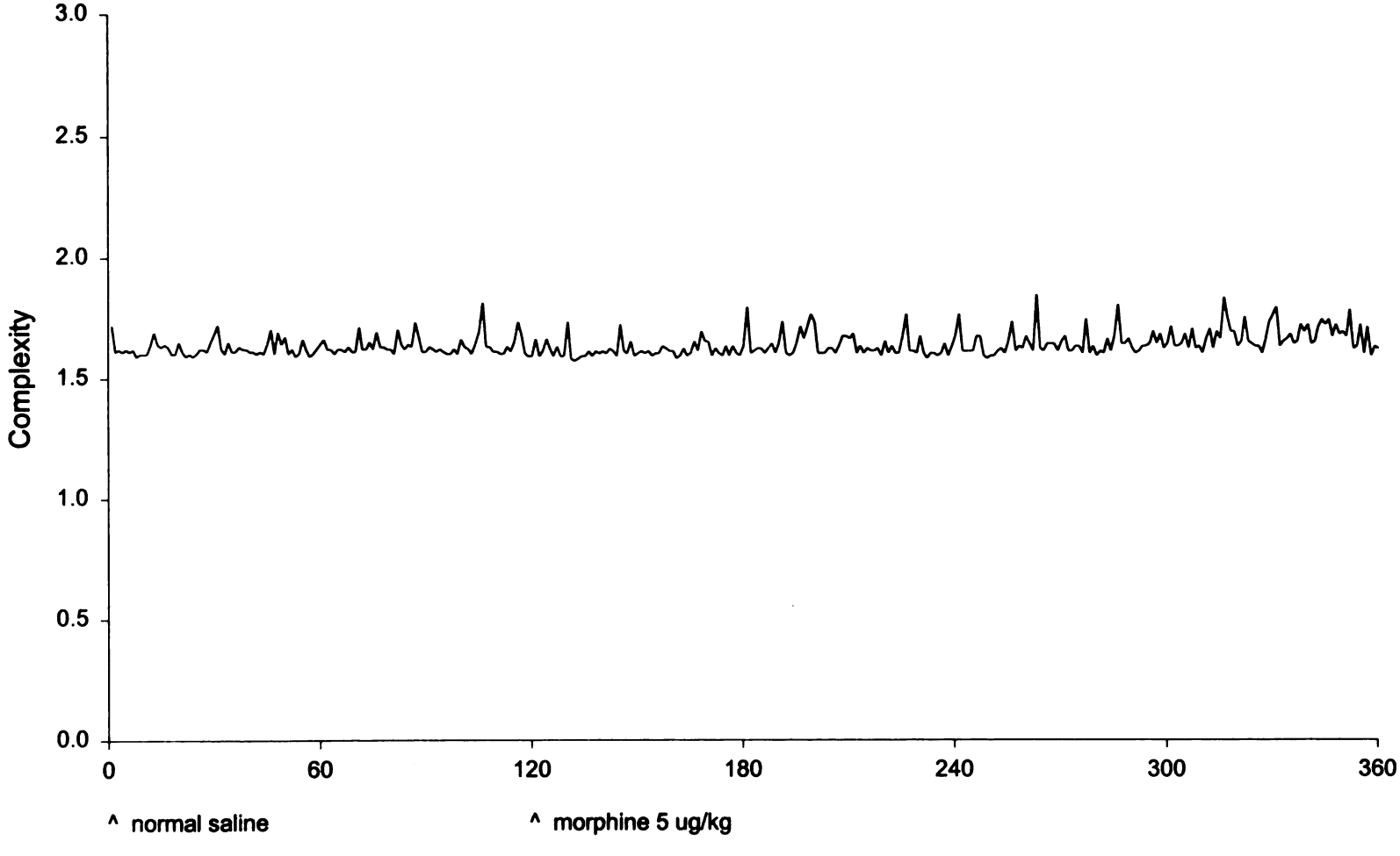


Figure 19: Effect of Morphine 5 ug/kg on Complexity (n=16 for 8 animals)



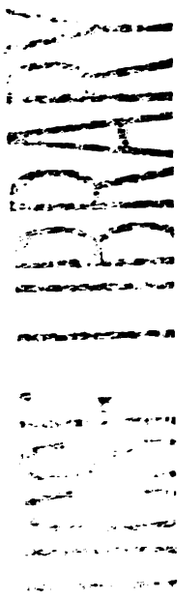
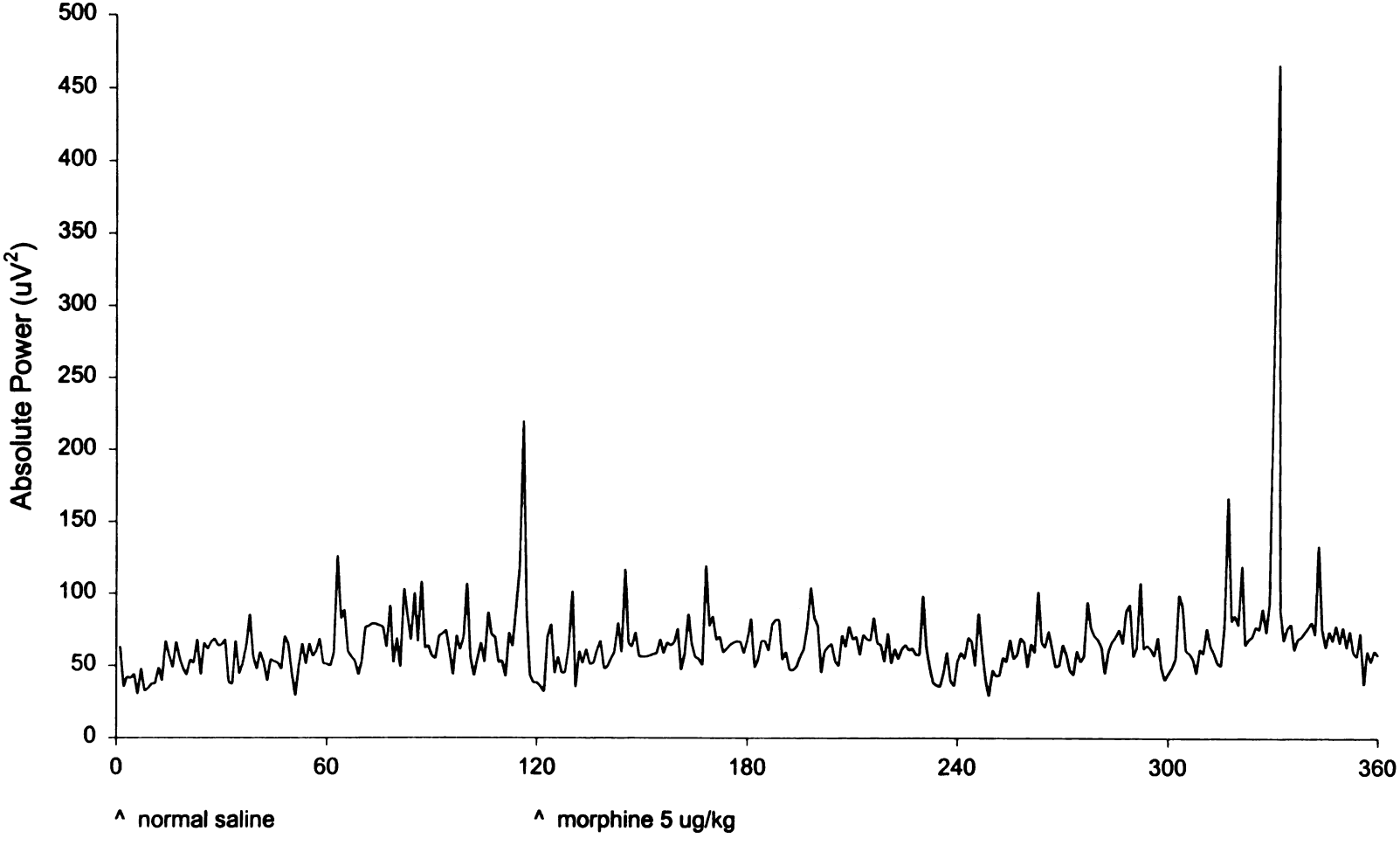


Figure 20: Effect of Morphine 5 ug/kg on Absolute Power (n=16 for 8 animals)



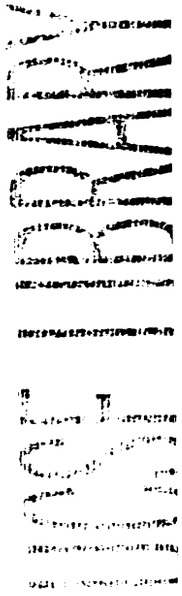


Figure 21: Effect of Morphine 5 ug/kg on Peak, Median & Edge Frequencies (n=16 for 8 animals)

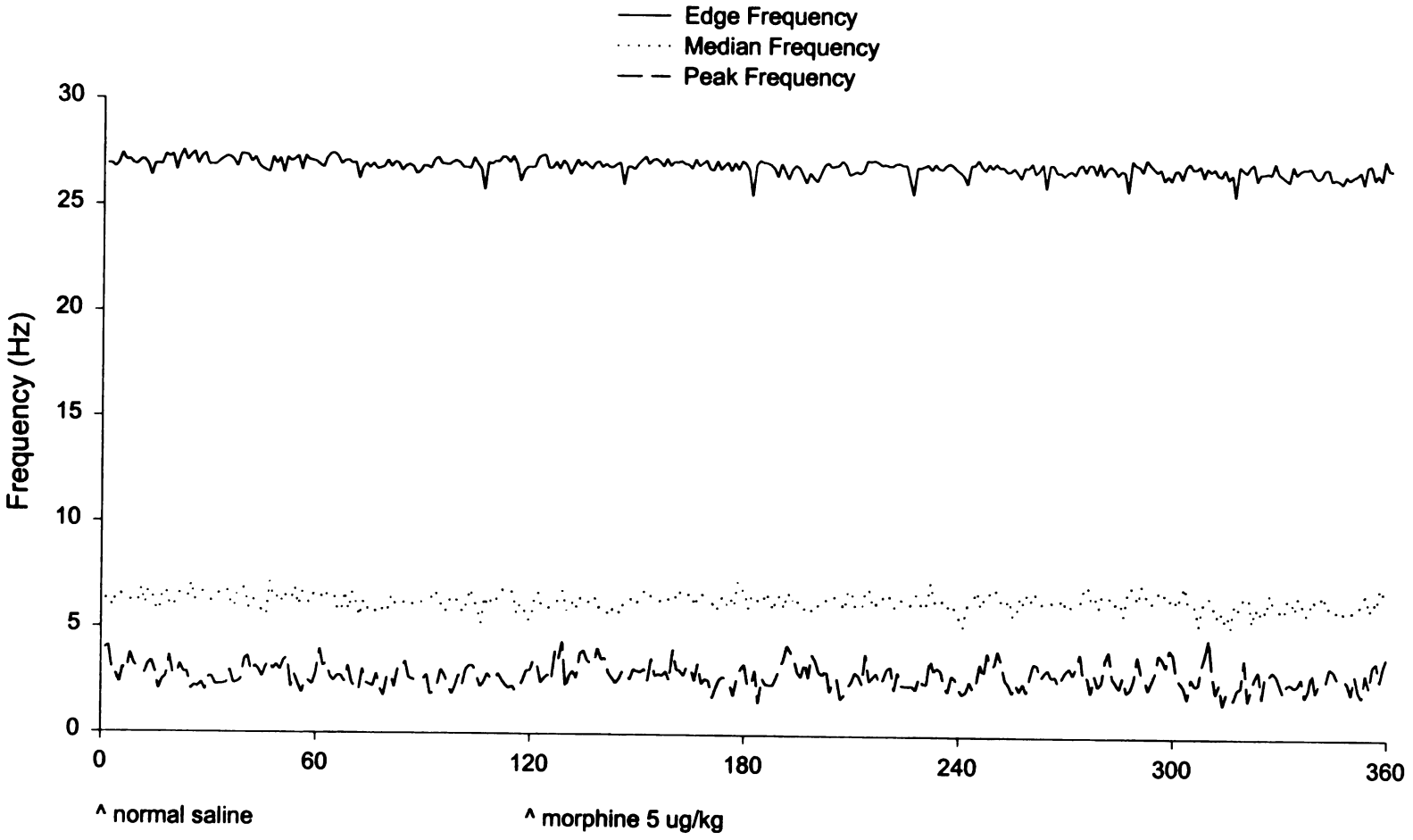


Figure 22: Effect of Morphine 5 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 0800-1000 (n=16 for 8 animals)

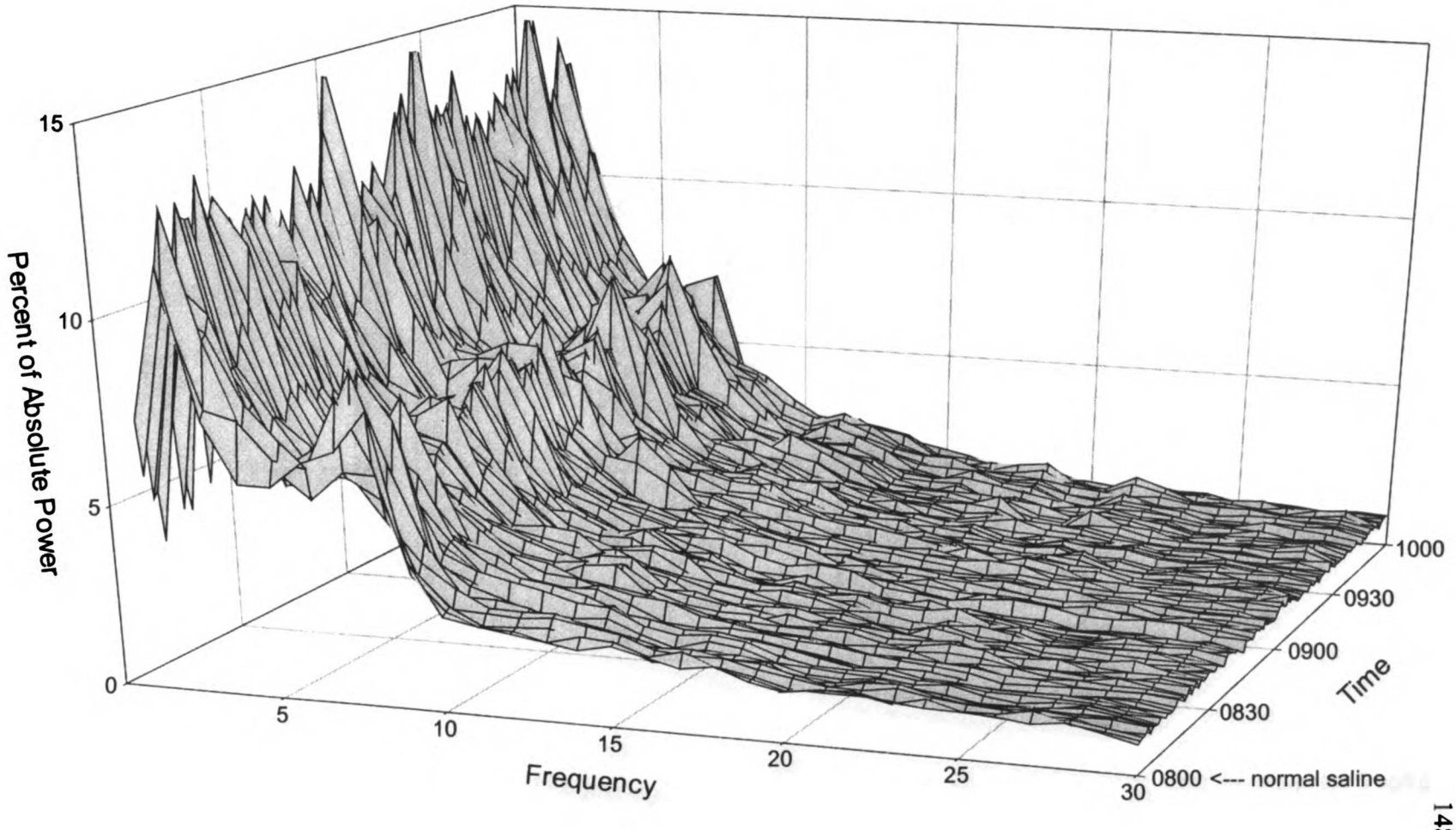
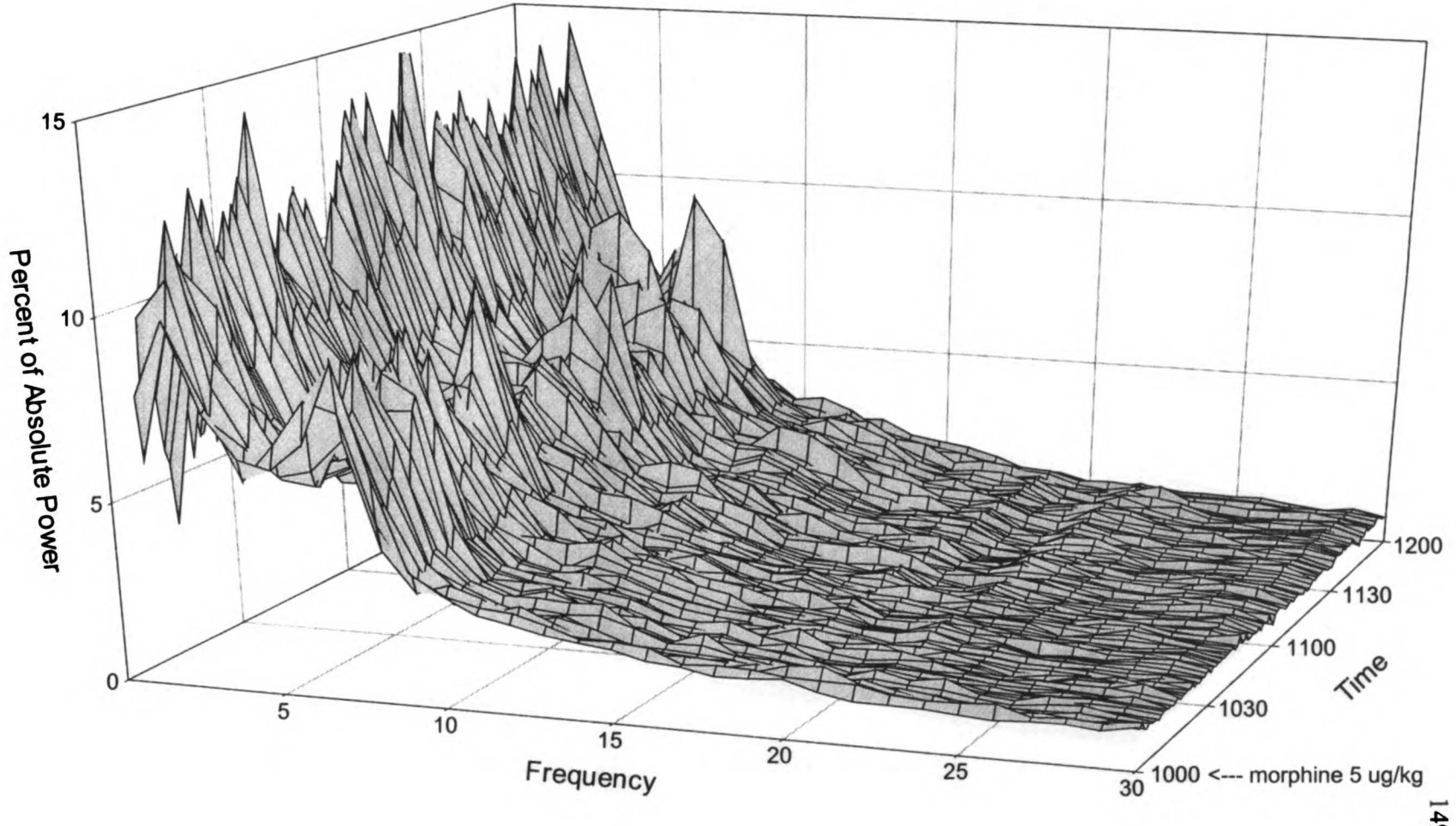
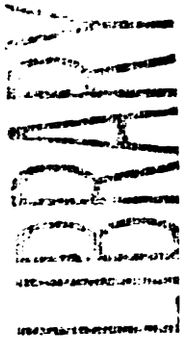


Figure 23: Effect of Morphine 5 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1000-1200 (n=16 for 8 animals)





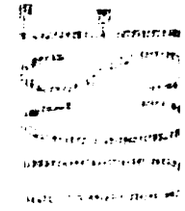
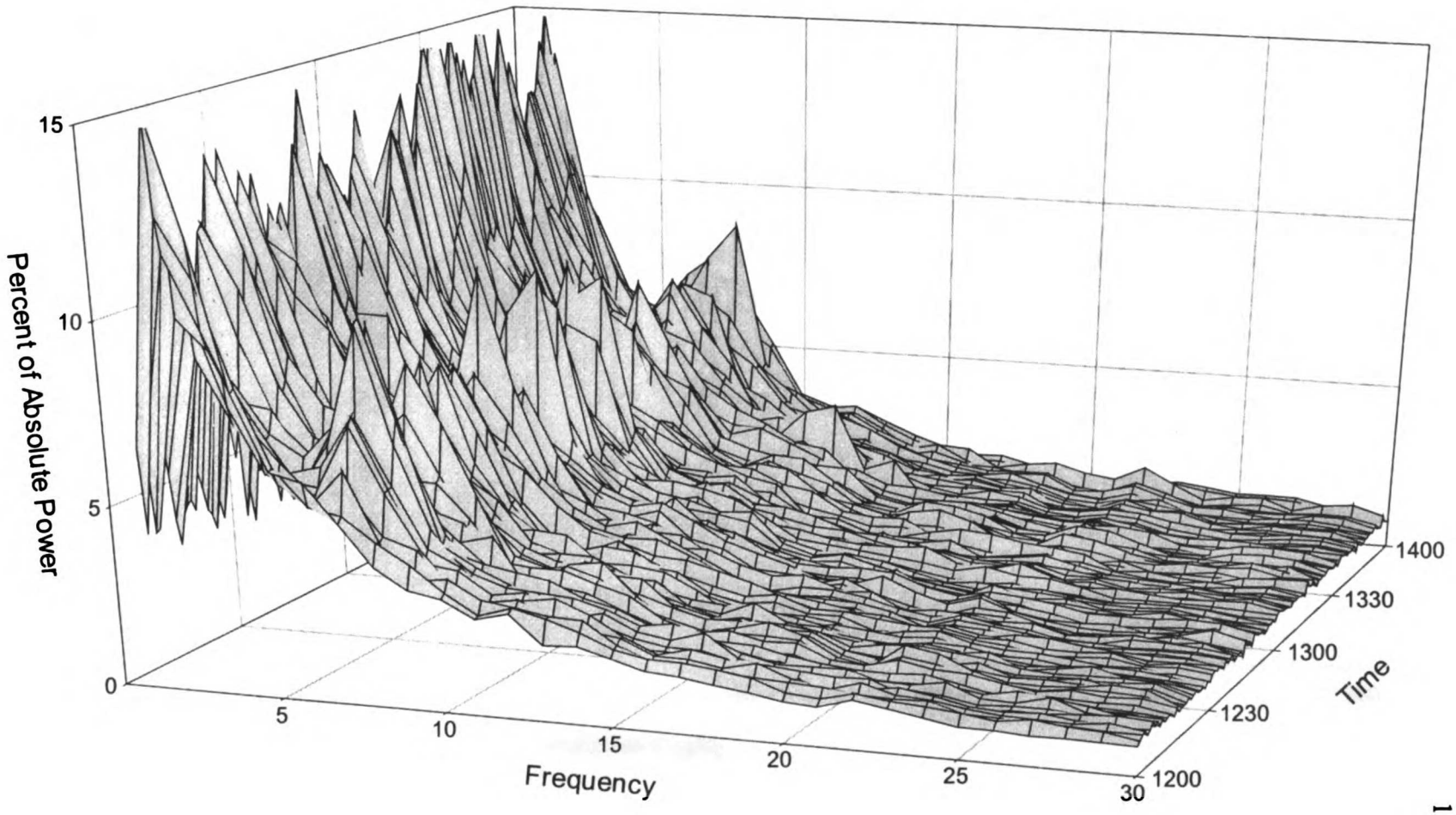


Figure 24: Effect of Morphine 5 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1200-1400 (n=16 for 8 animals)



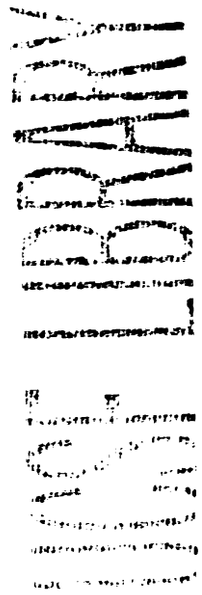
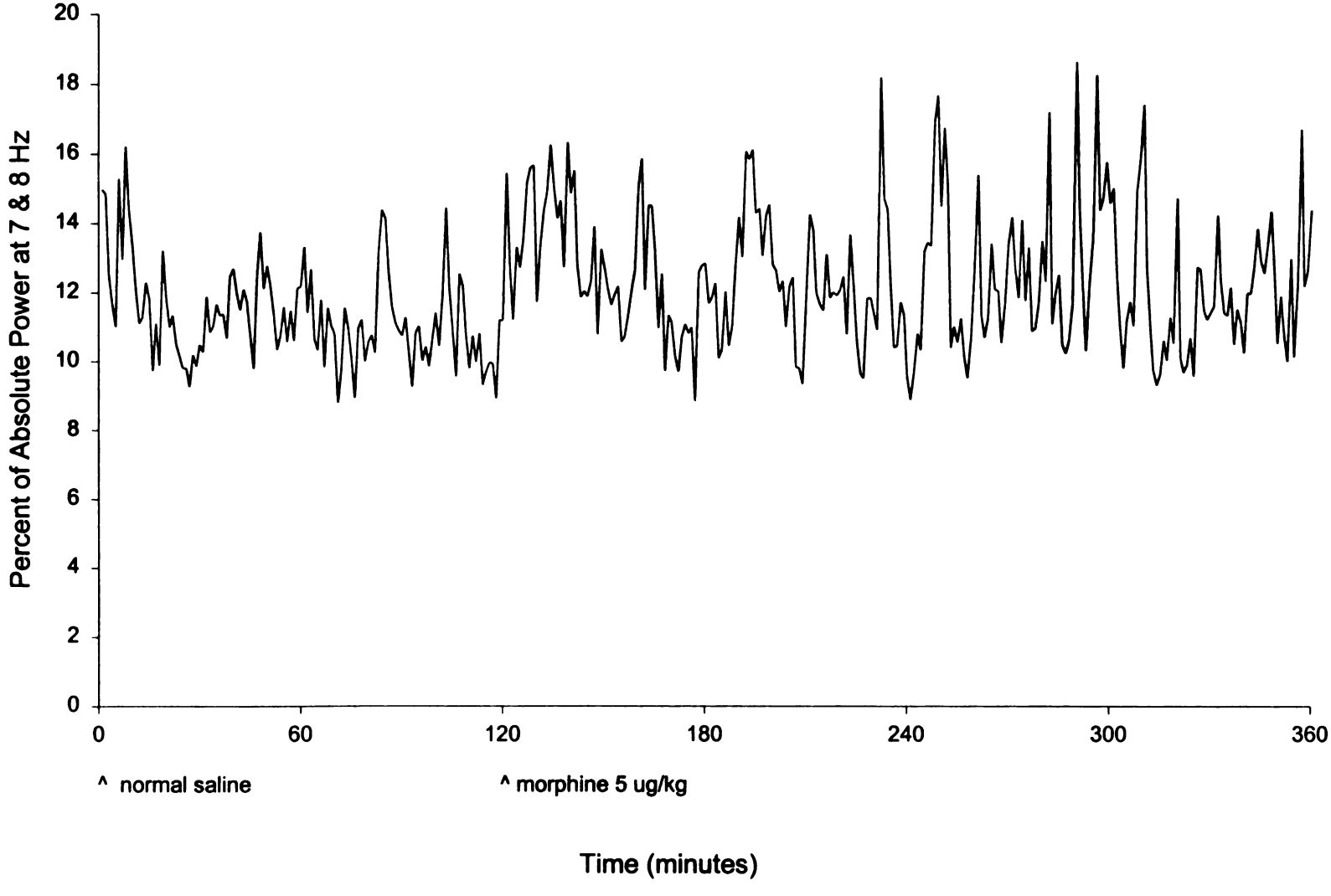
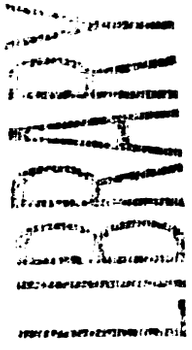


Figure 25: Effect of Morphine 5 ug/kg on Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)





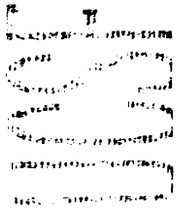
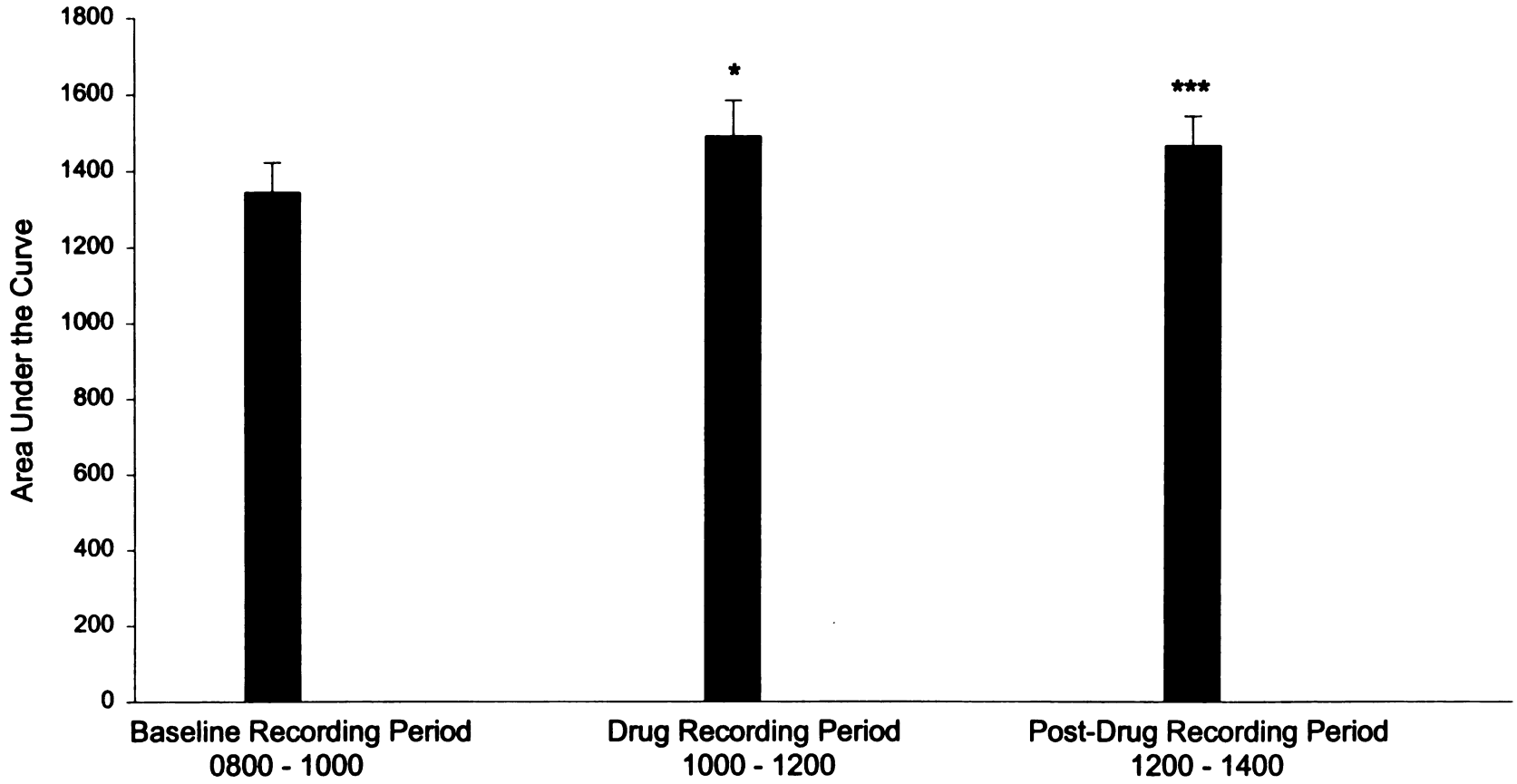


Figure 26: Effect of Morphine 5 ug/kg on Area-Under-the-Curve for Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)



* indicates that p = .05, *** indicates that p = .04

Figure 27: Effect of Morphine 500 ug/kg on Activity (n=16 for 8 animals)

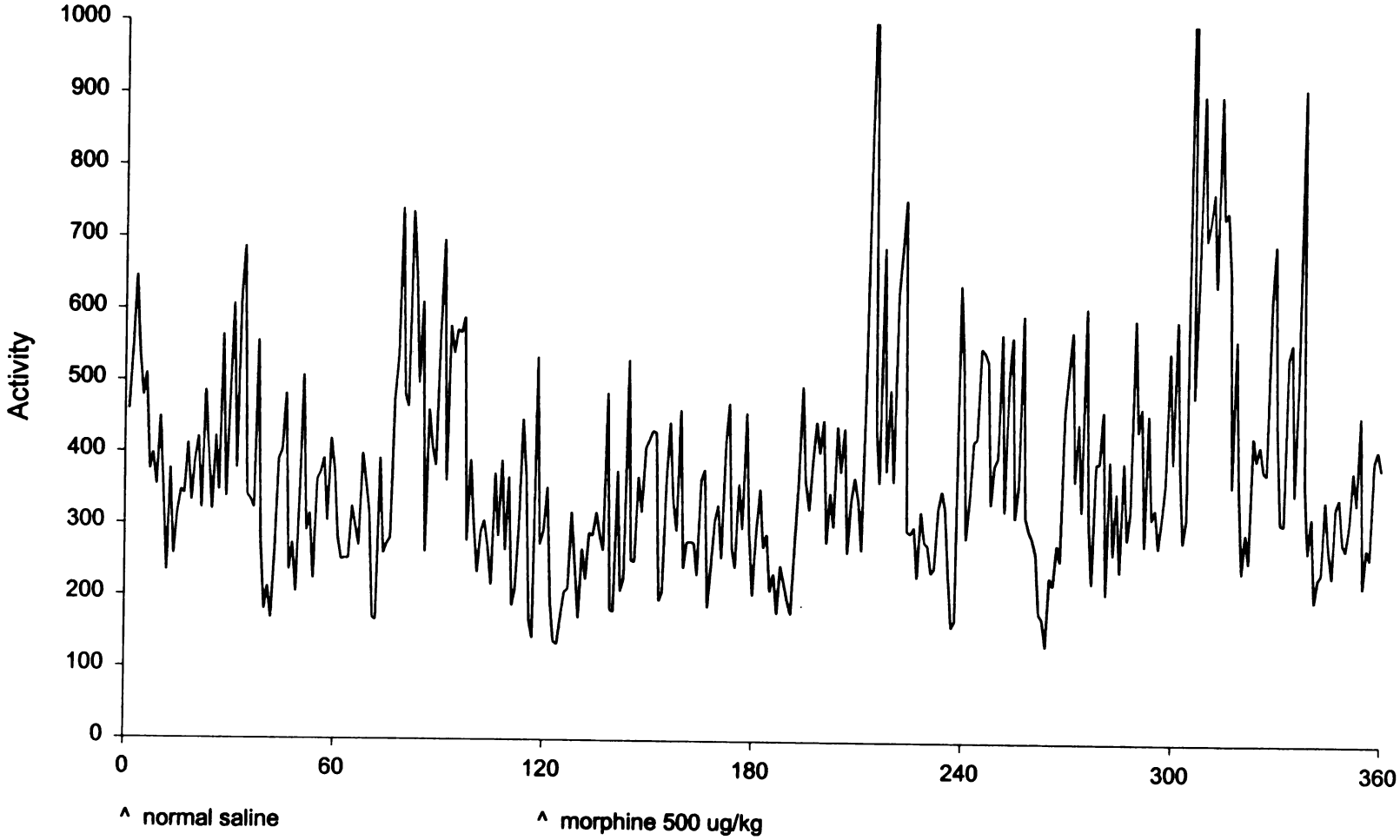
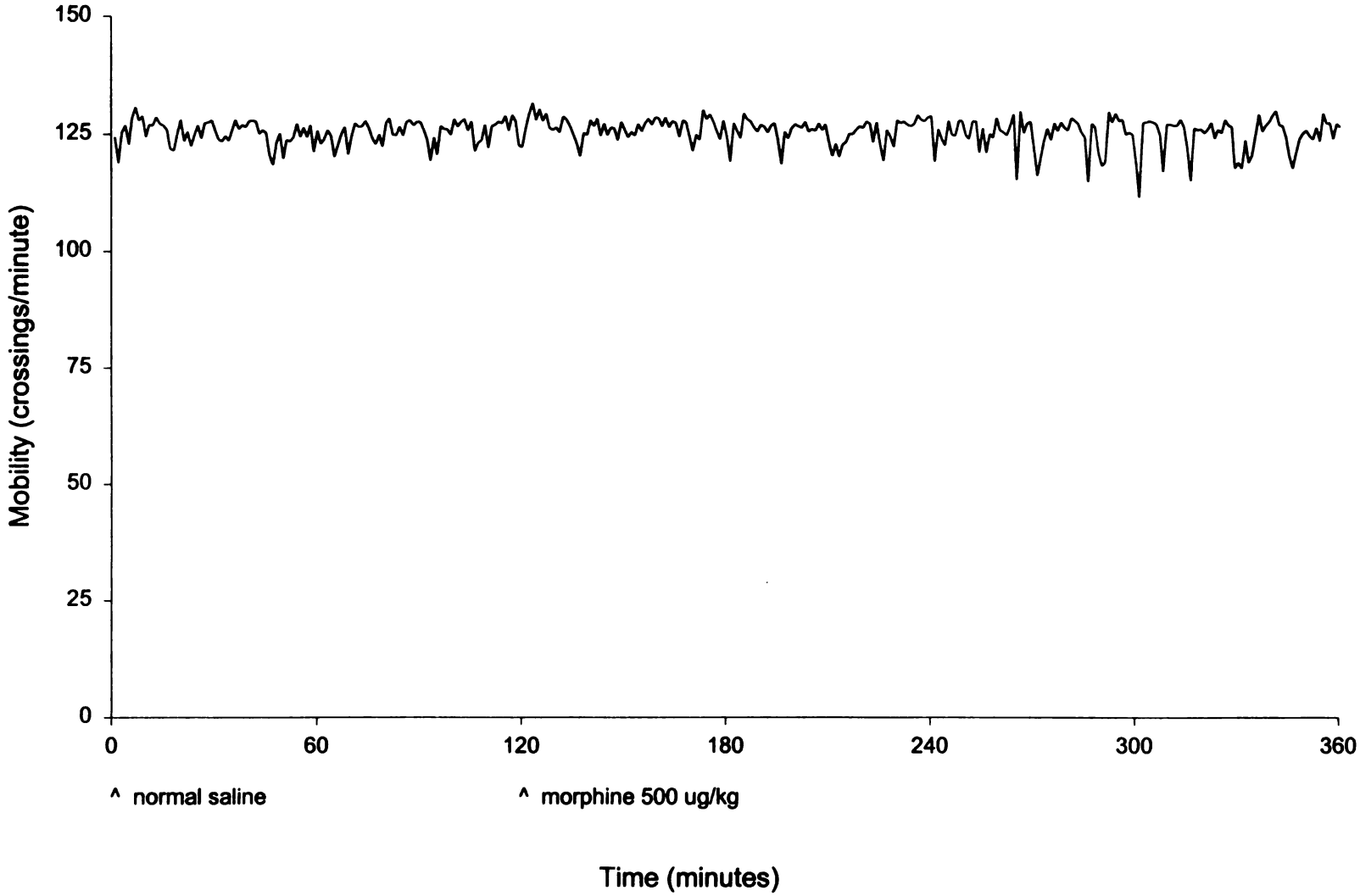
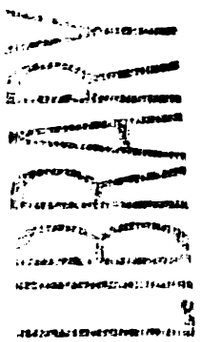


Figure 28: Effect of Morphine 500 ug/kg on Mobility (n=16 for 8 animals)





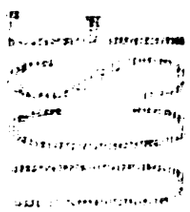
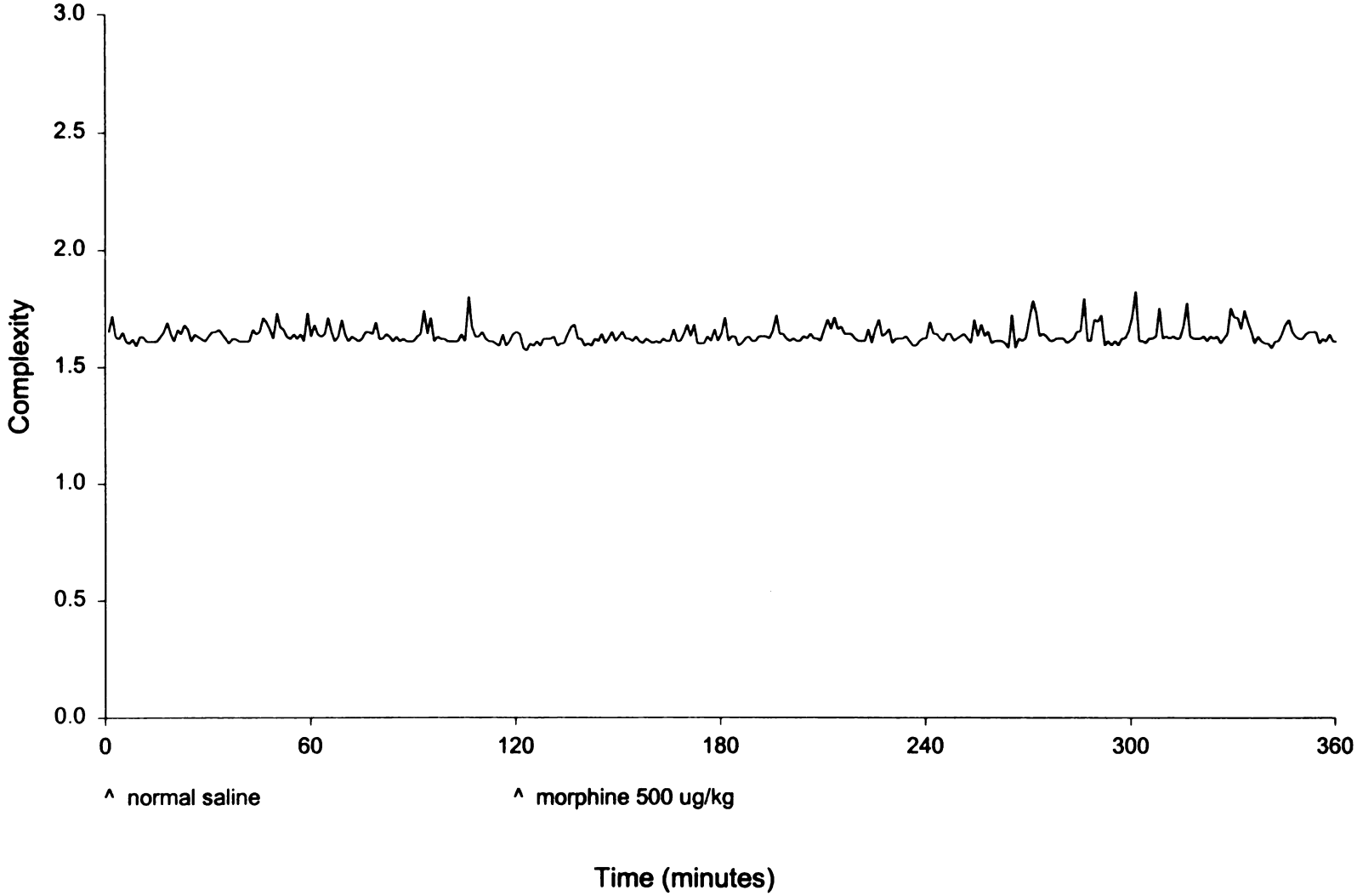


Figure 29: Effect of Morphine 500 ug/kg on Complexity (n=16 for 8 animals)



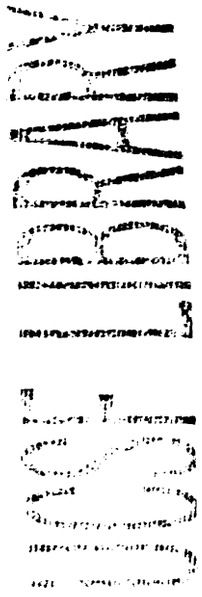


Figure 30: Effect of Morphine 500 ug/kg on Absolute Power (n=16 for 8 animals)

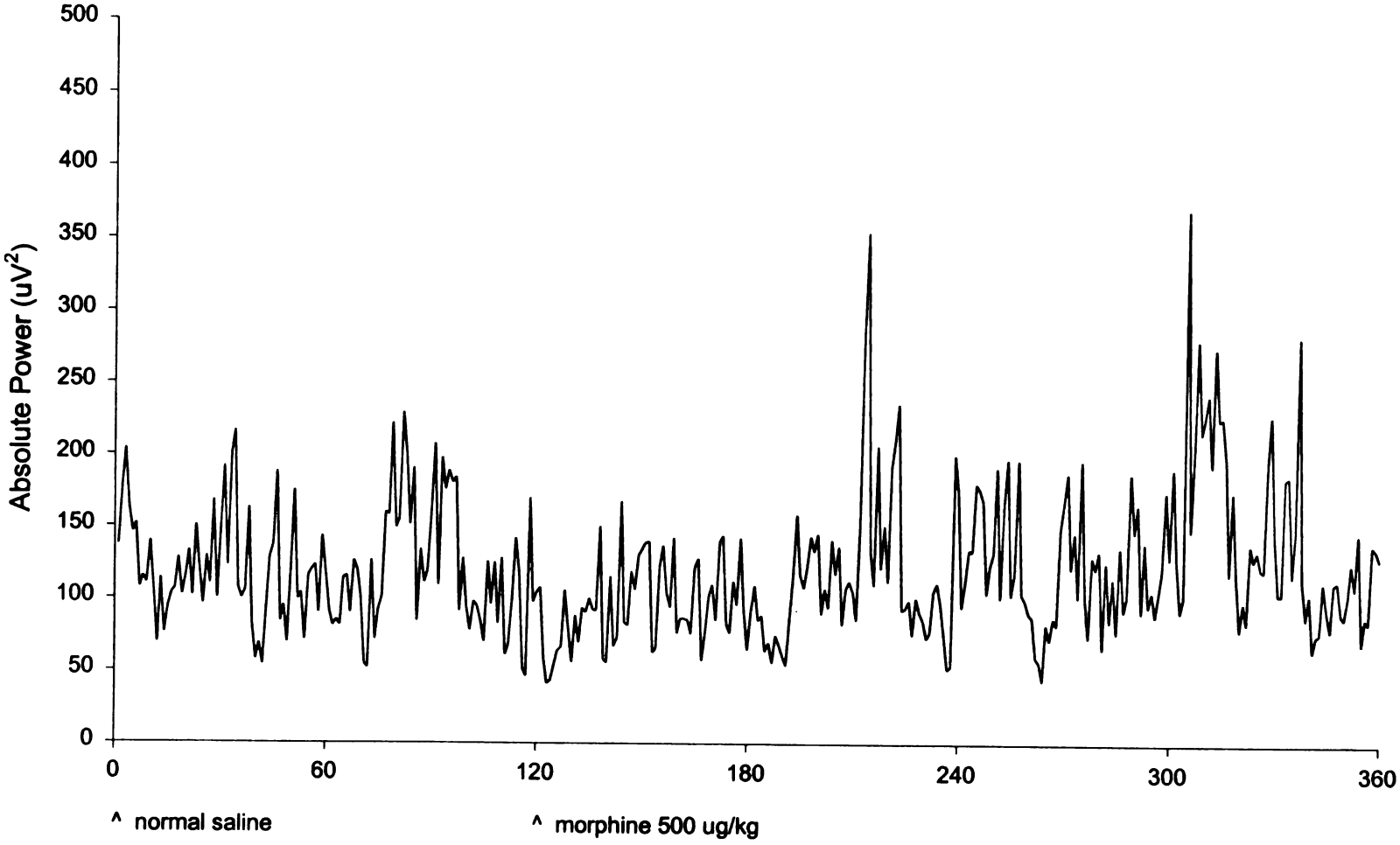


Figure 31: Effect of Morphine 500 ug/kg on Peak, Median & Edge Frequencies (n=16 for 8 animals)

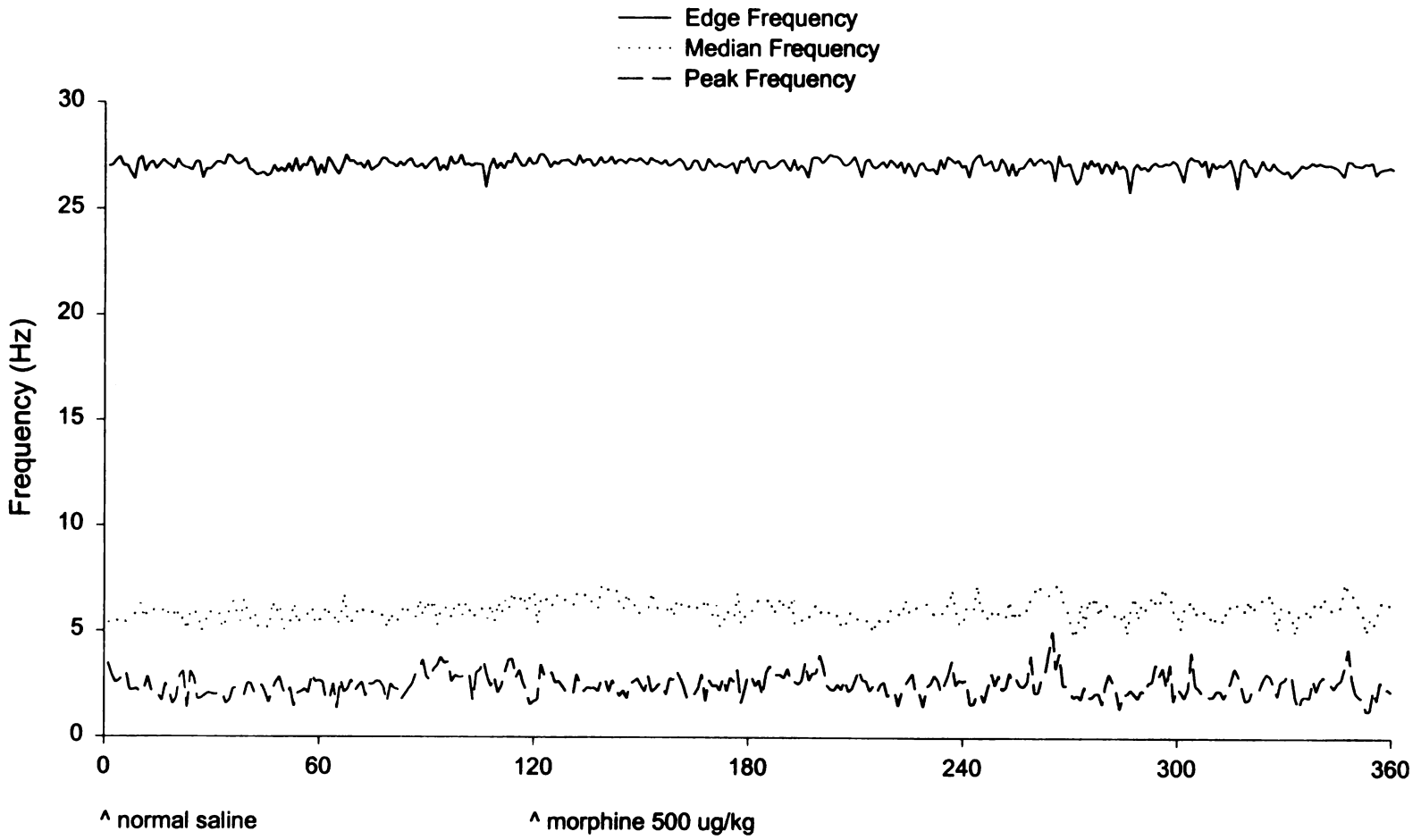


Figure 32: Effect of Morphine 500 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 0800-1000 (n=16 for 8 animals)

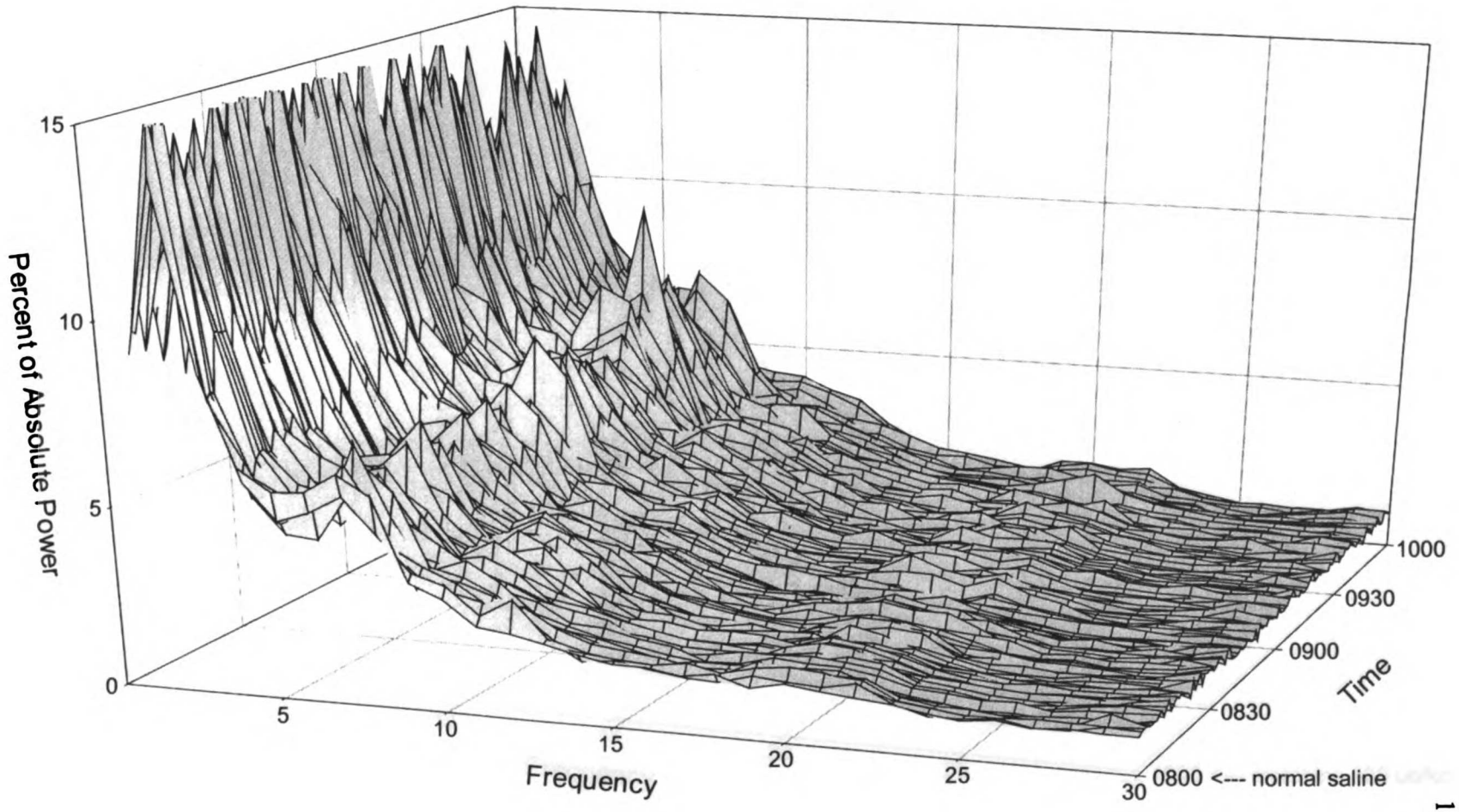
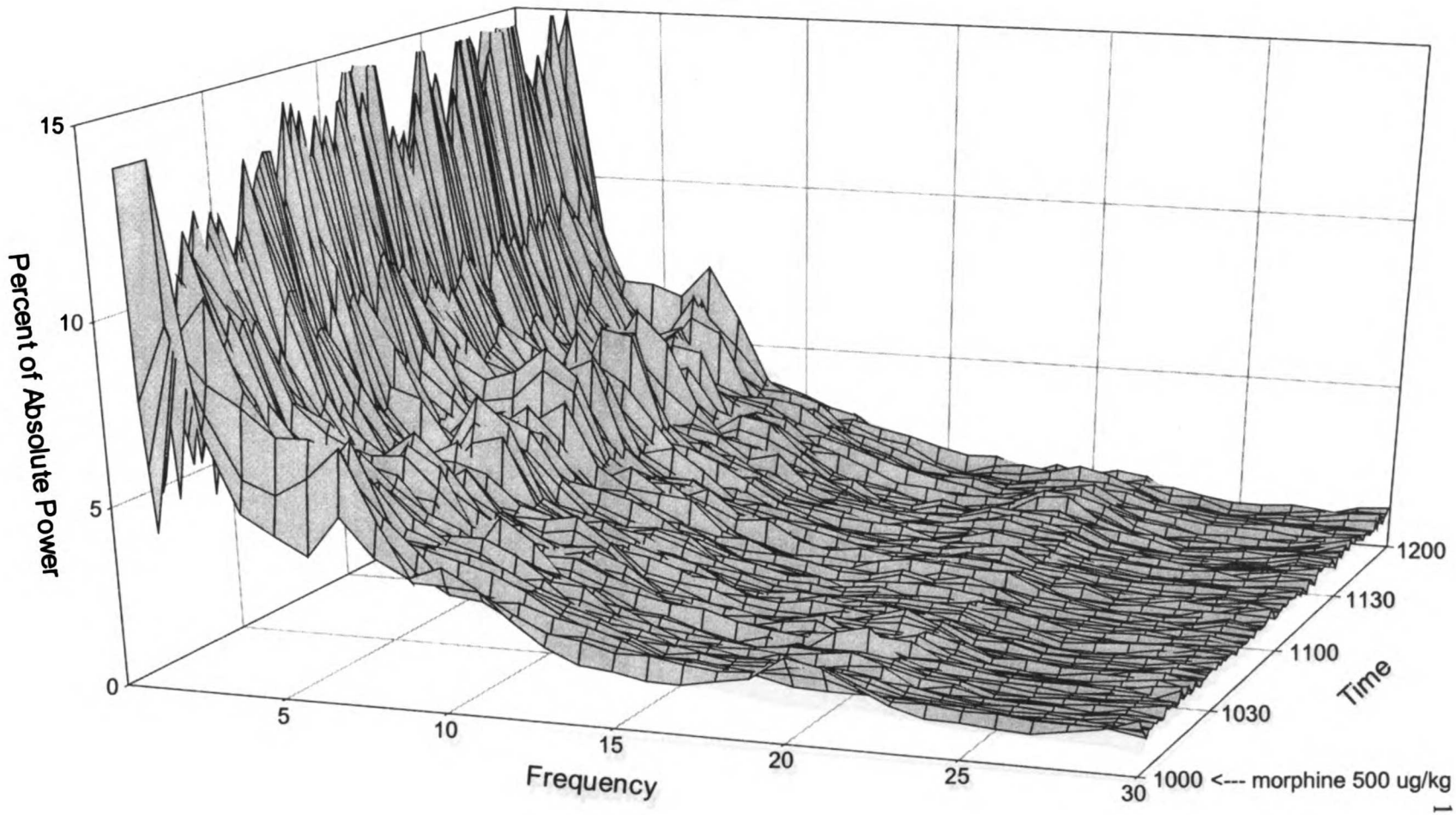


Figure 33: Effect of Morphine 500 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1000-1200 (n=16 for 8 animals)



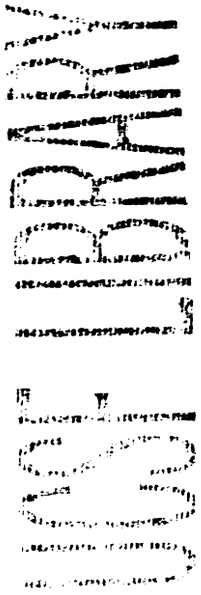
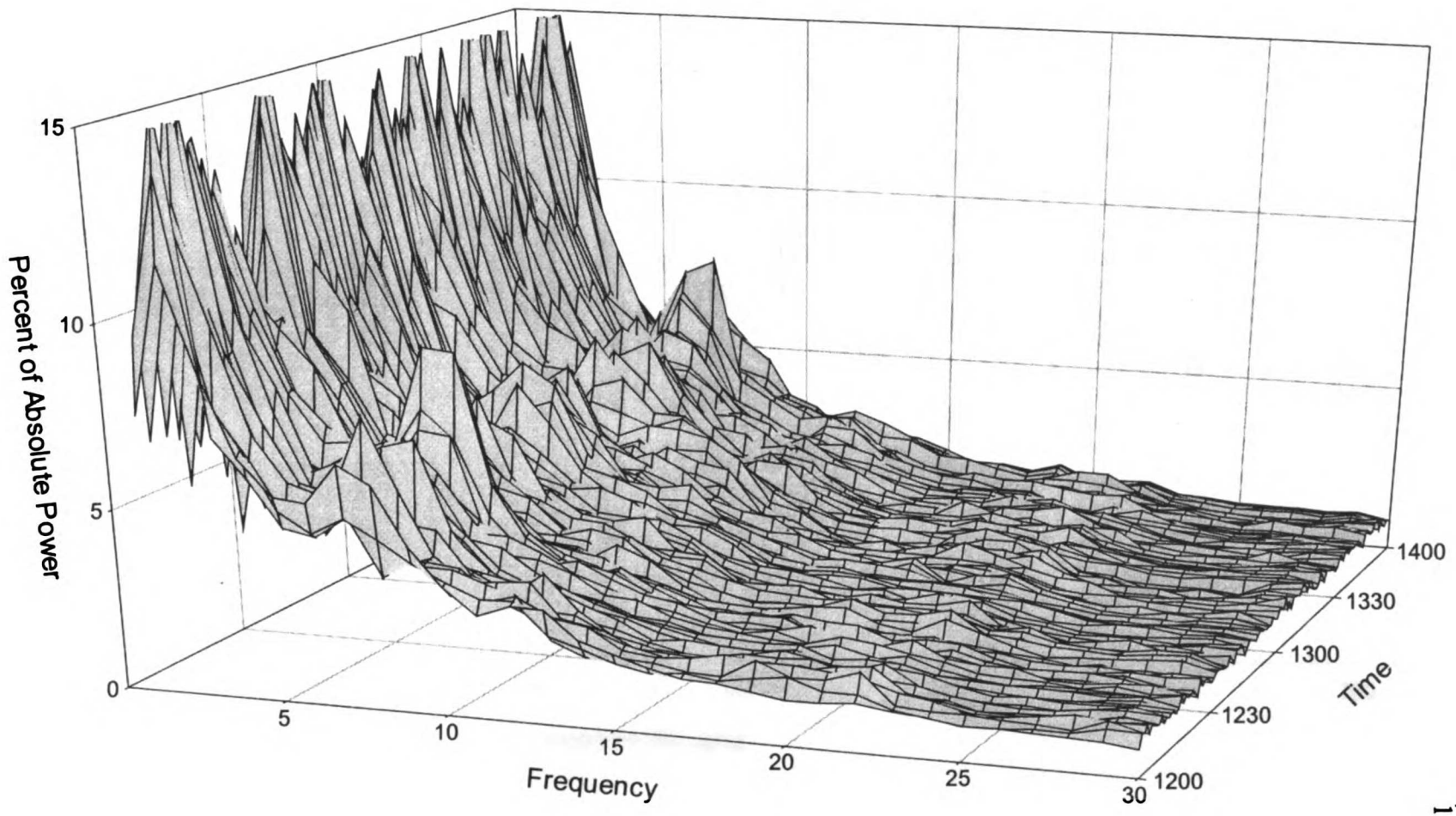
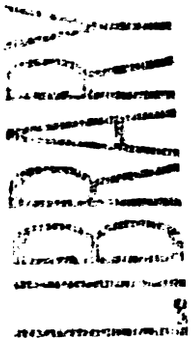


Figure 34: Effect of Morphine 500 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1200-1400 (n=16 for 8 animals)





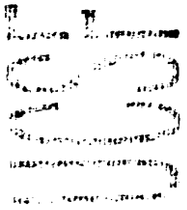


Figure 35: Effect of Morphine 500 ug/kg on Percent for Absolute Power at 7 & 8 Hz (n=16 for 8 animals)

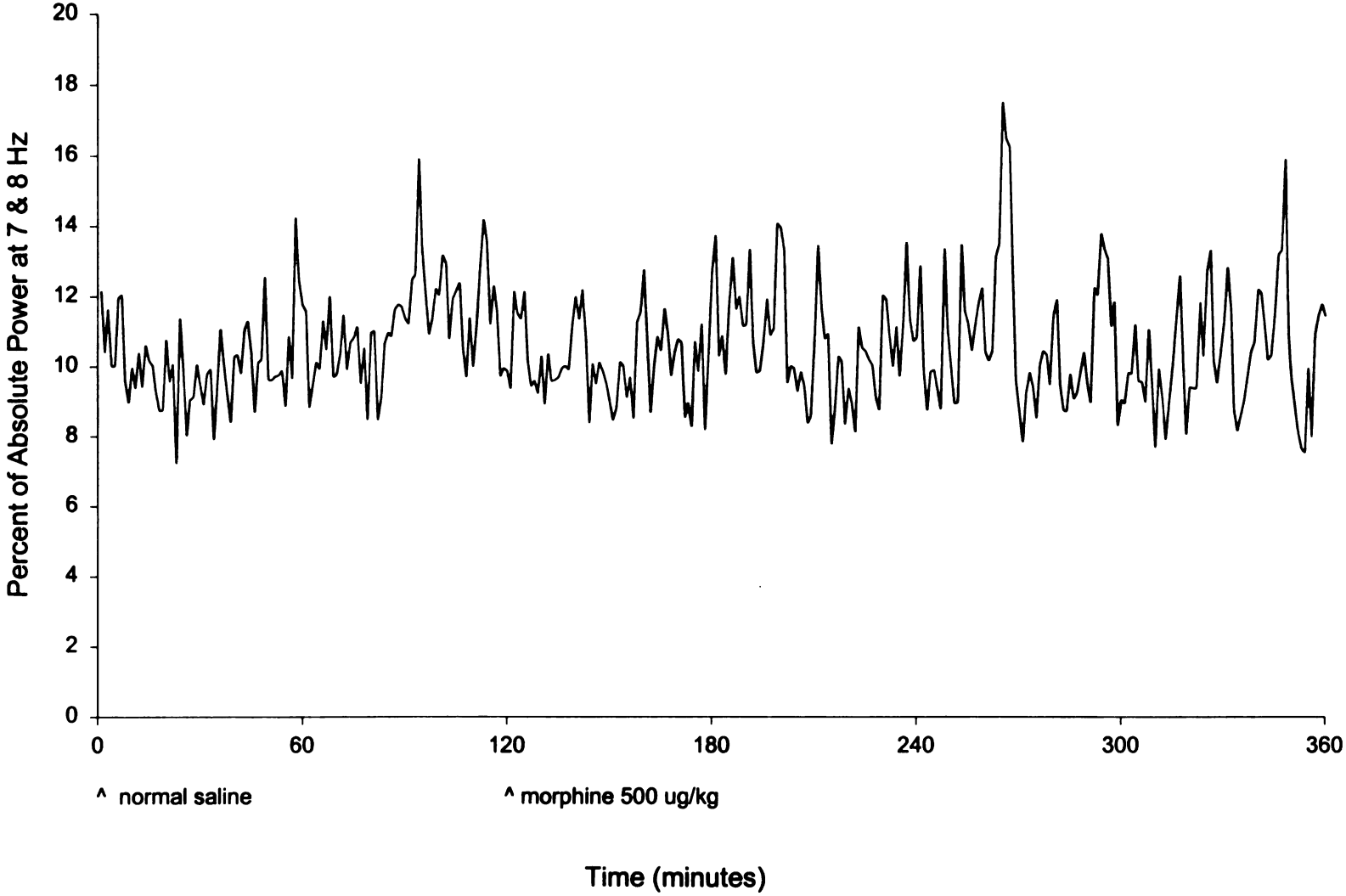


Figure 36: Effect of Morphine 500 ug/kg on Area-Under-the-Curve for Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)

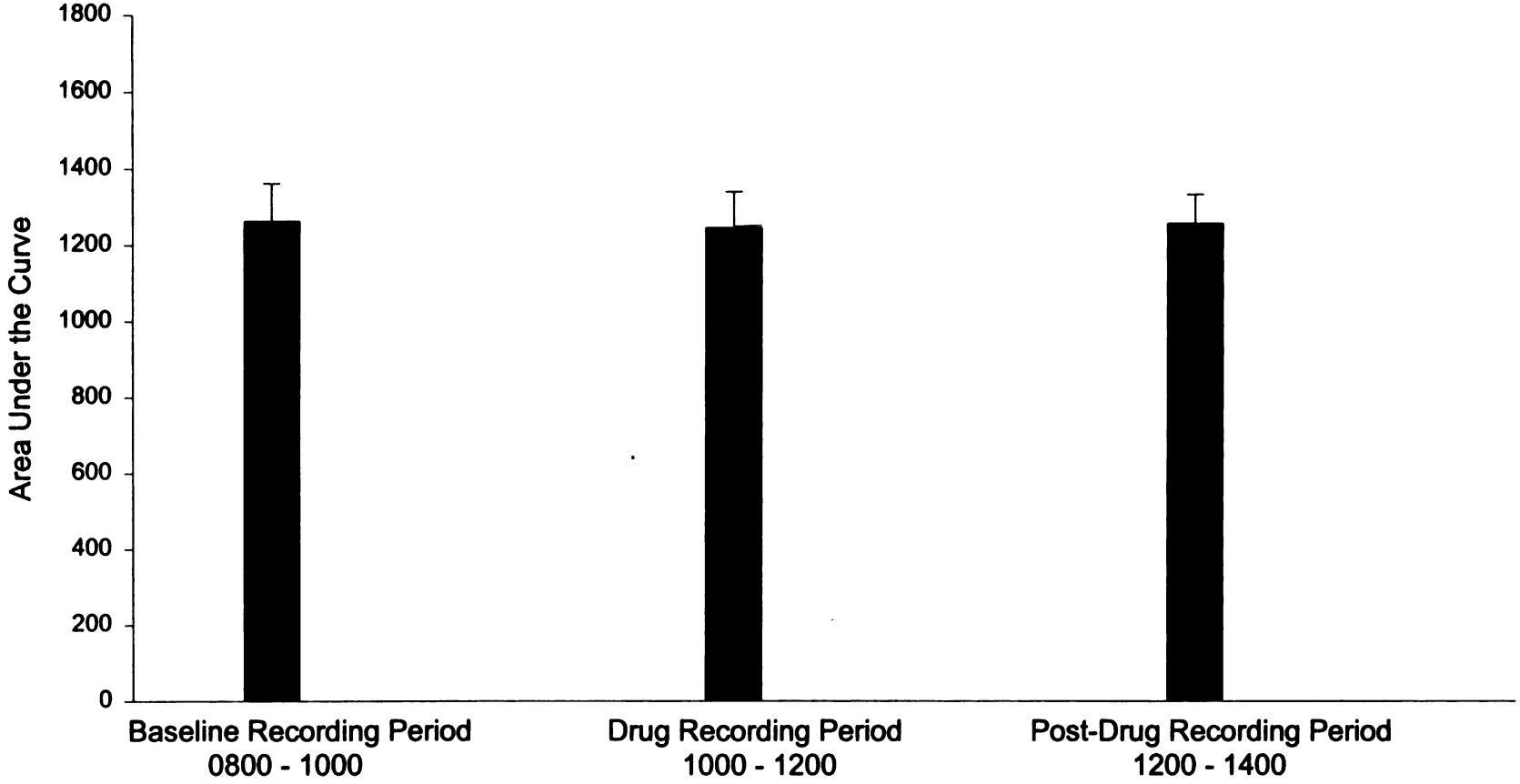


Figure 37: Effect of Pentazocine 50 ug/kg on Activity (n=16 for 8 animals)

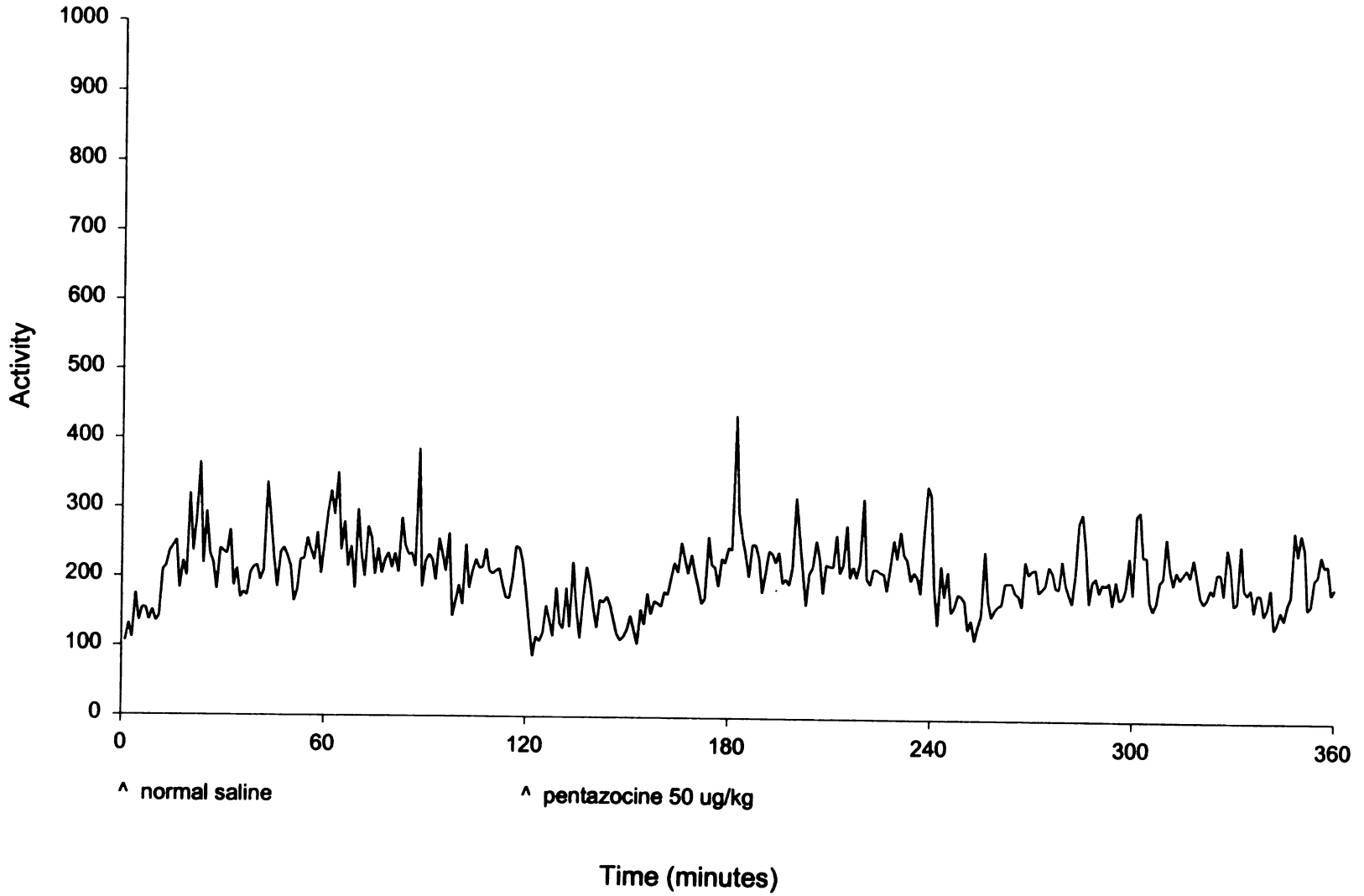
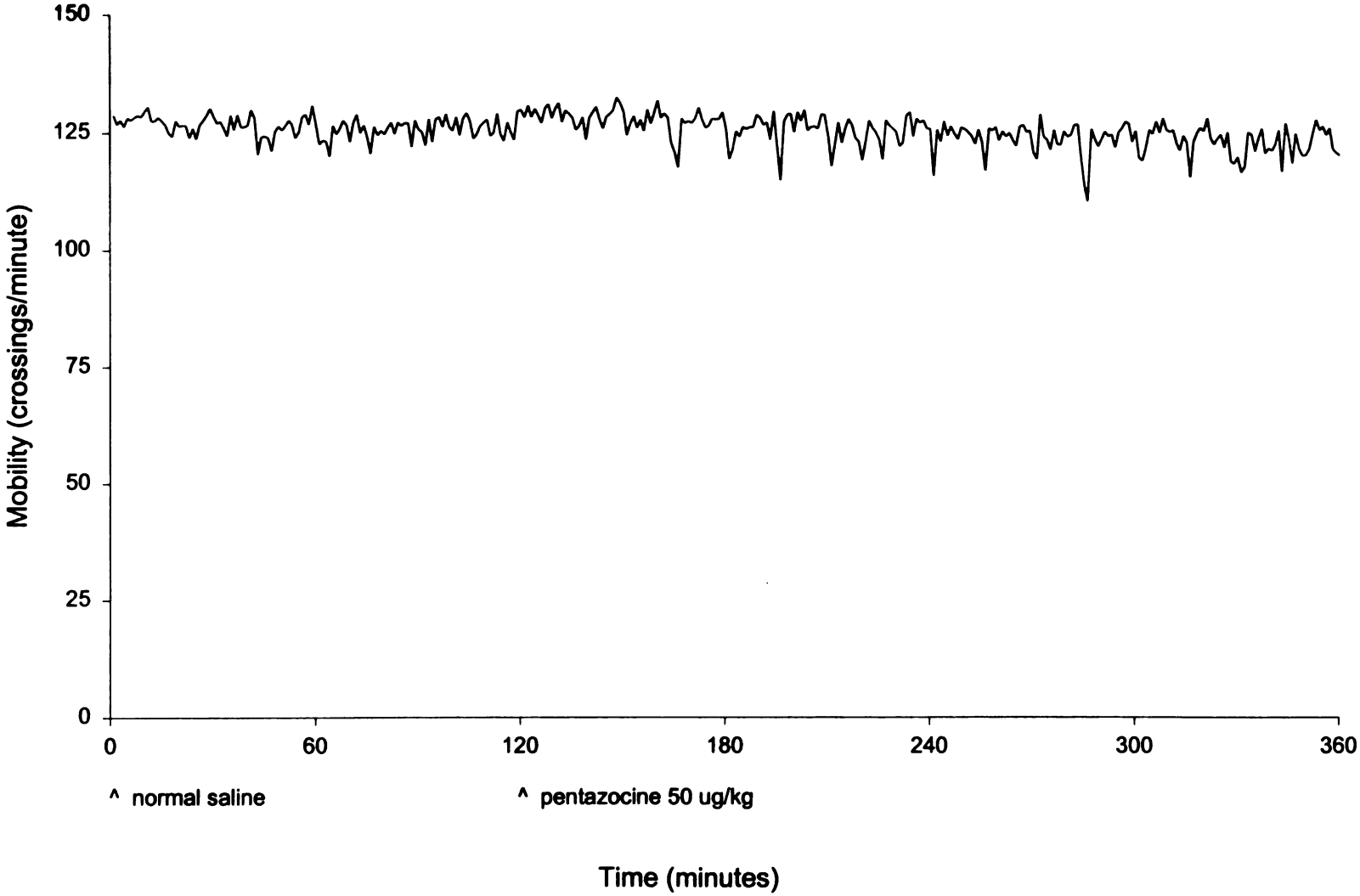
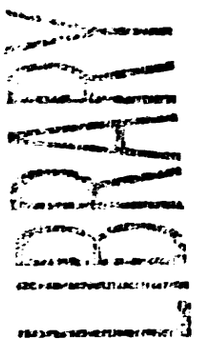


Figure 38: Effect of Pentazocine 50 ug/kg on Mobility (n=16 for 8 animals)





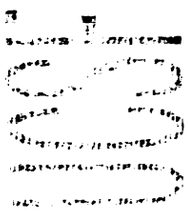
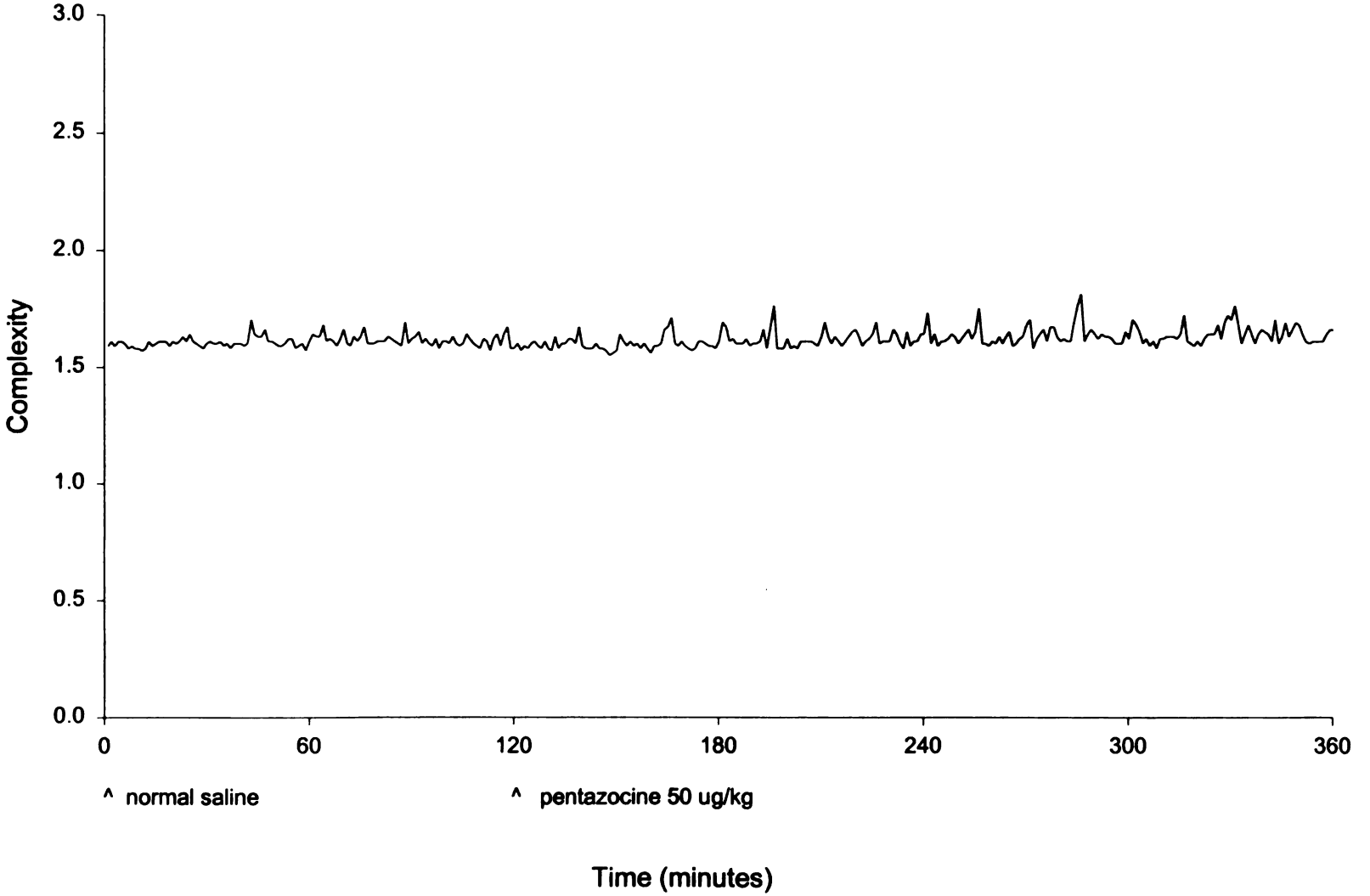
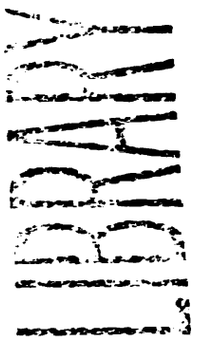


Figure 39: Effect of Pentazocine 50 ug/kg on Complexity (n=16 for 8 animals)





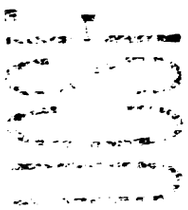
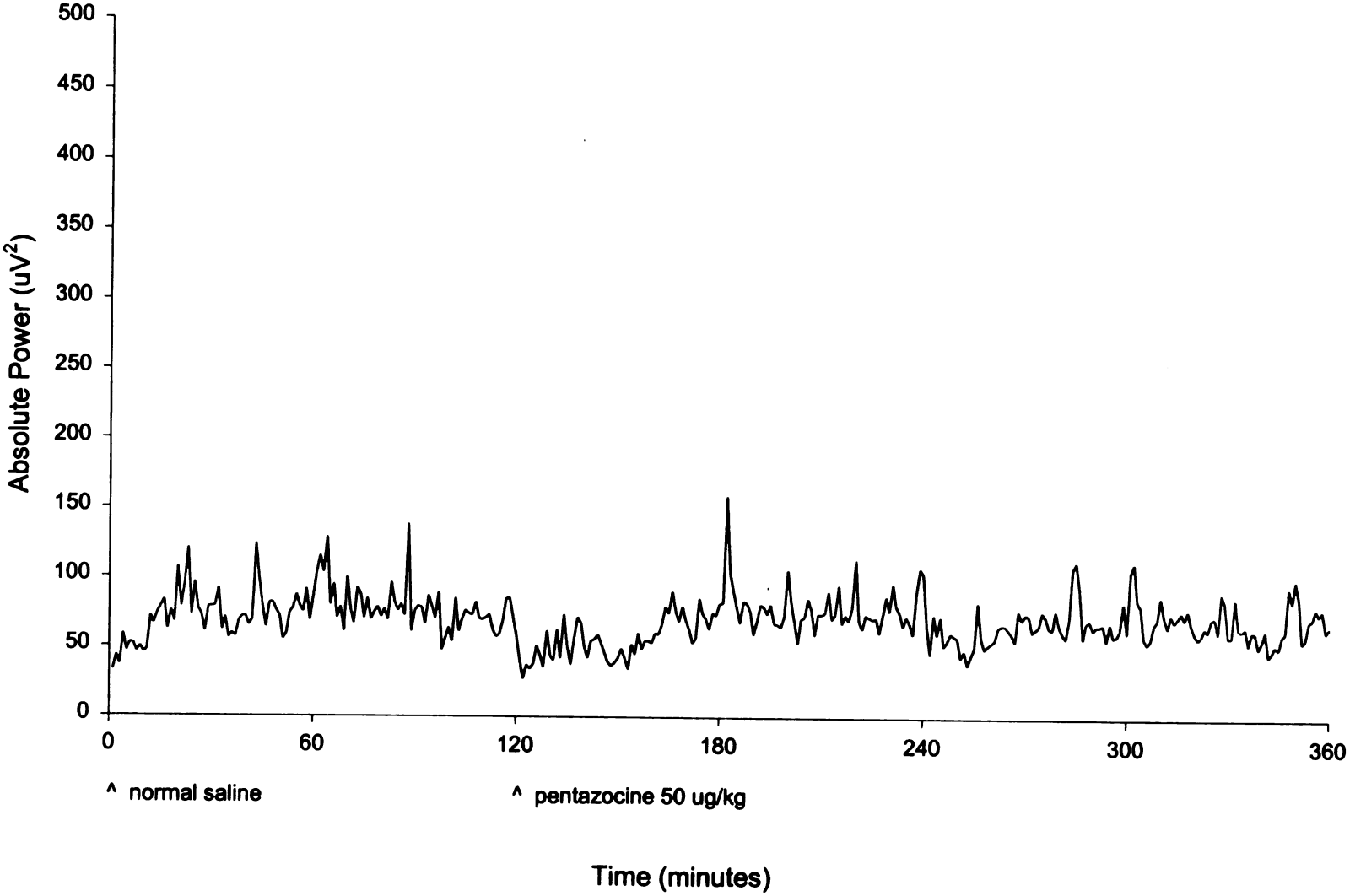
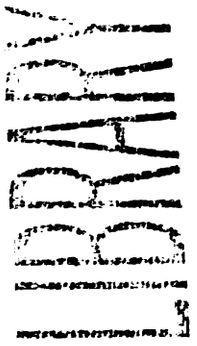


Figure 40: Effect of Pentazocine 50 ug/kg on Absolute Power (n=16 for 8 animals)





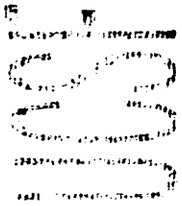
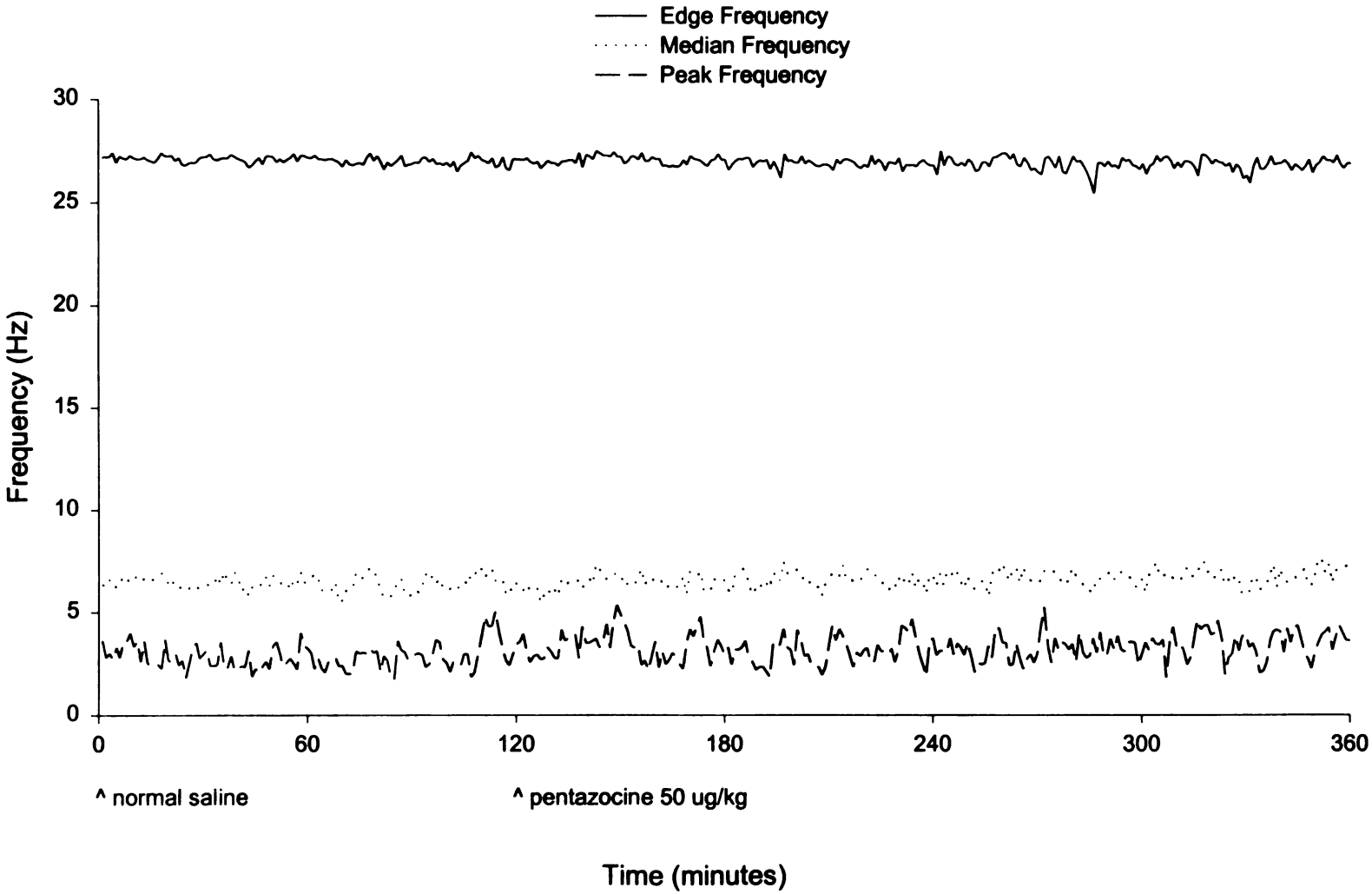
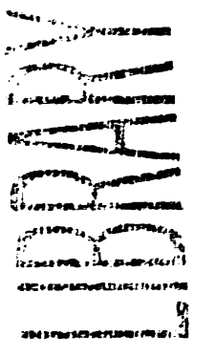


Figure 41: Effect of Pentazocine 50 ug/kg on Peak, Median & Edge Frequencies (n=16 for 8 animals)





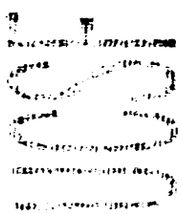
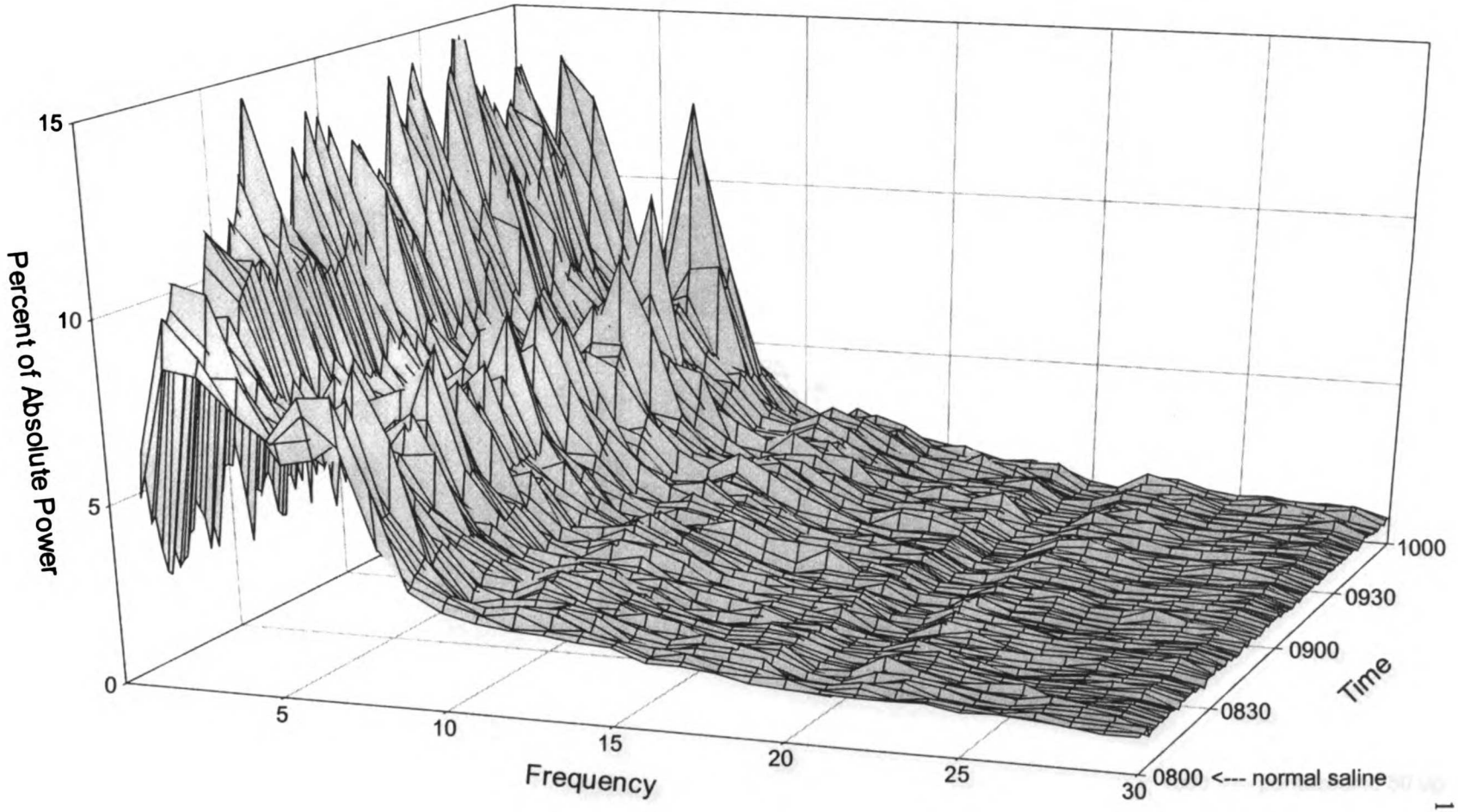
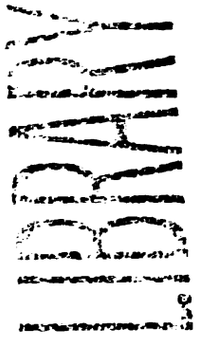


Figure 42: Effect of Pentazocine 50 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 0800-1000 (n=16 for 8 animals)





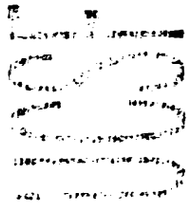


Figure 43: Effect of Pentazocine 50 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1000-1200 (n=16 for 8 animals)

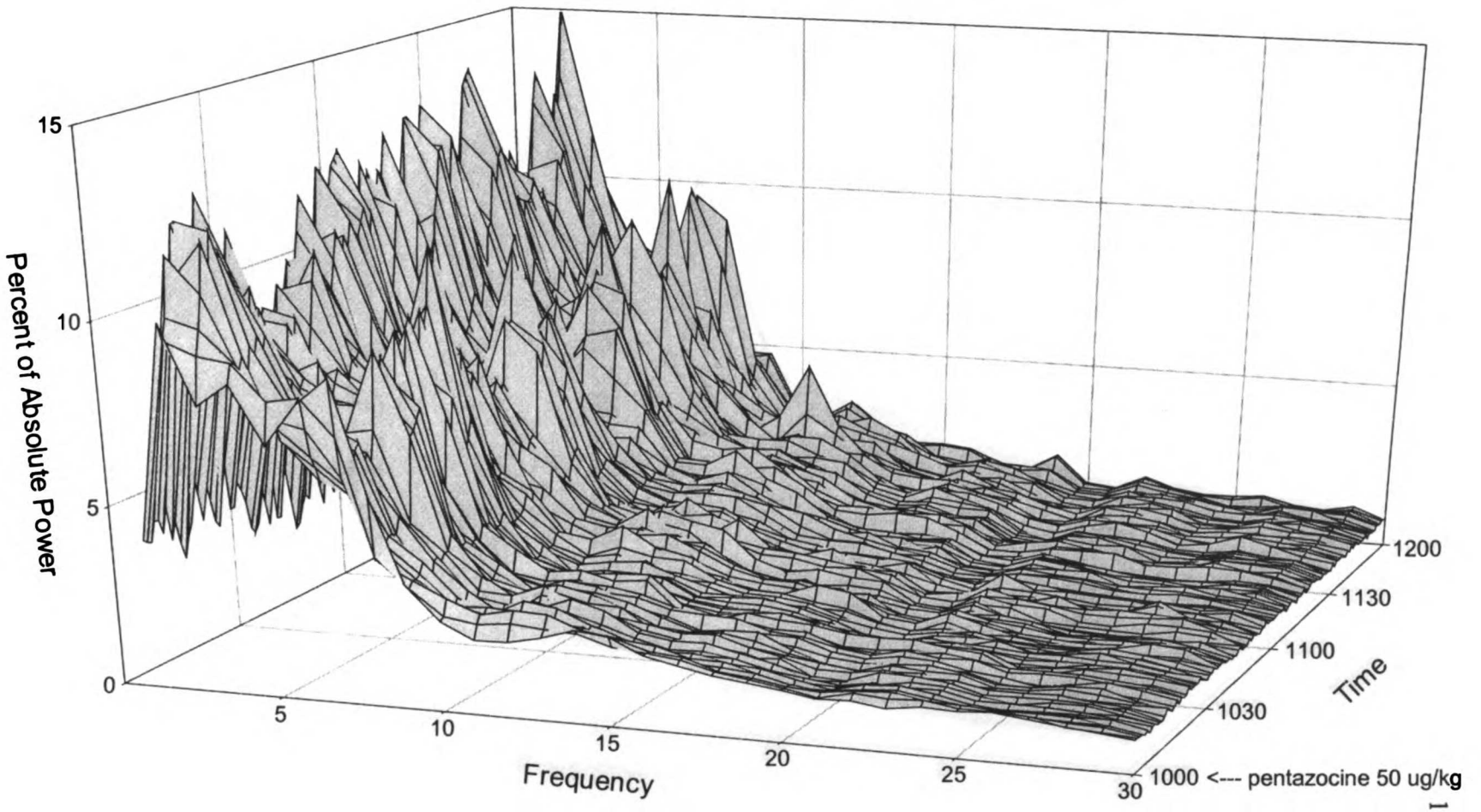
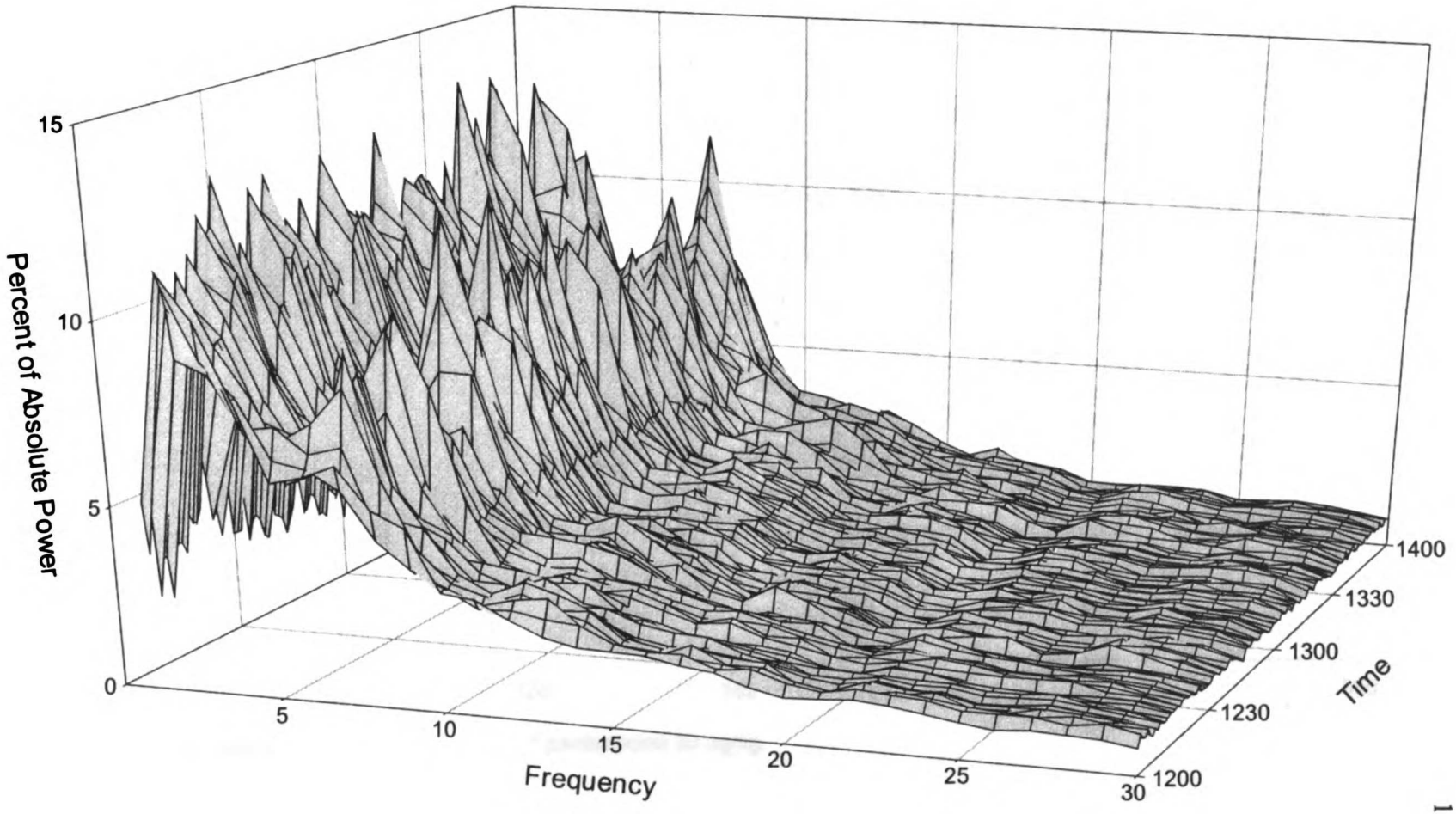


Figure 44: Effect of Pentazocine 50 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1200-1400 (n=16 for 8 animals)



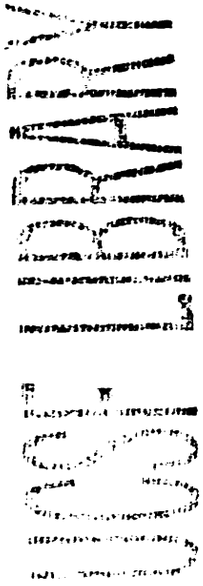


Figure 45: Effect of Pentazocine 50 ug/kg on Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)

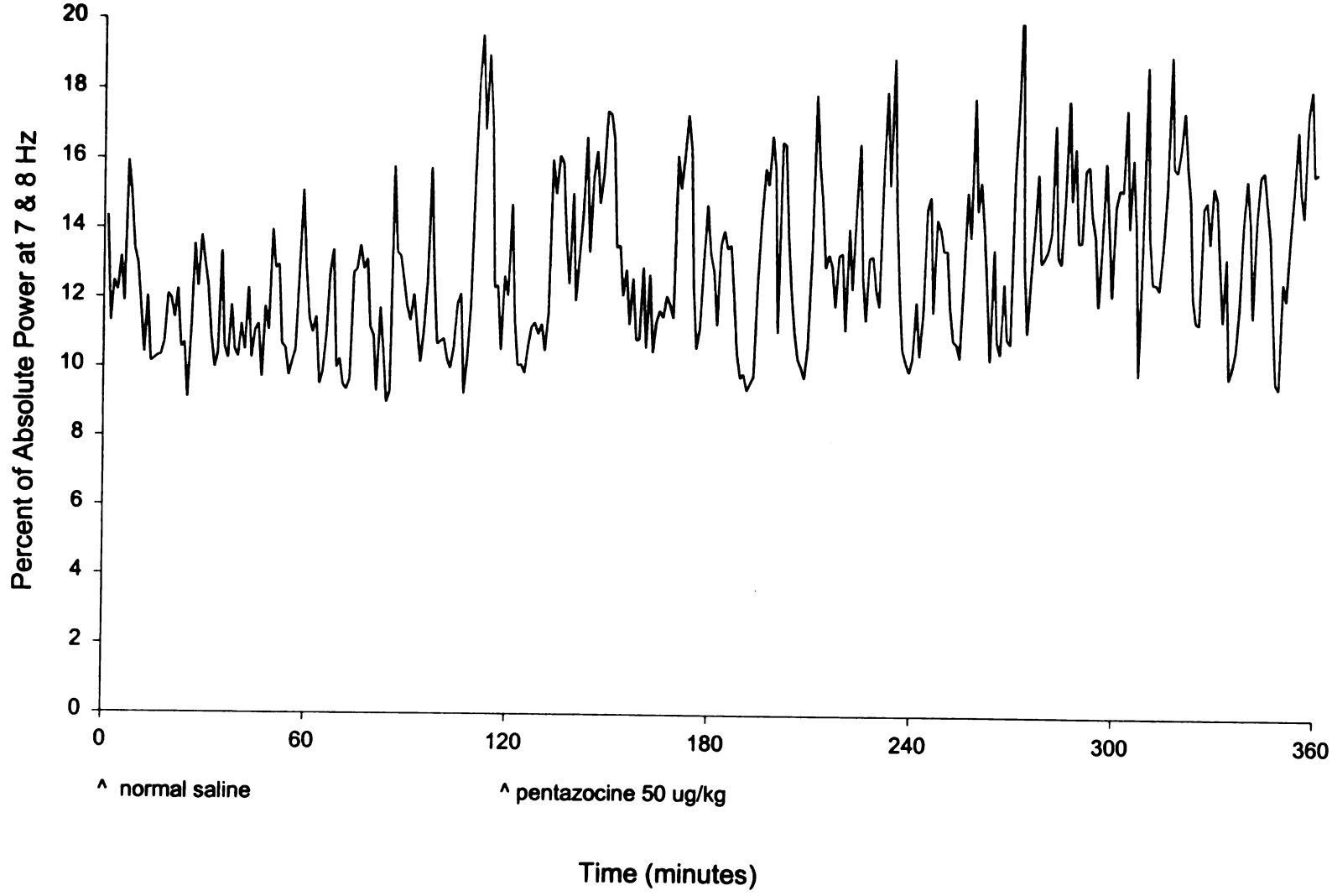
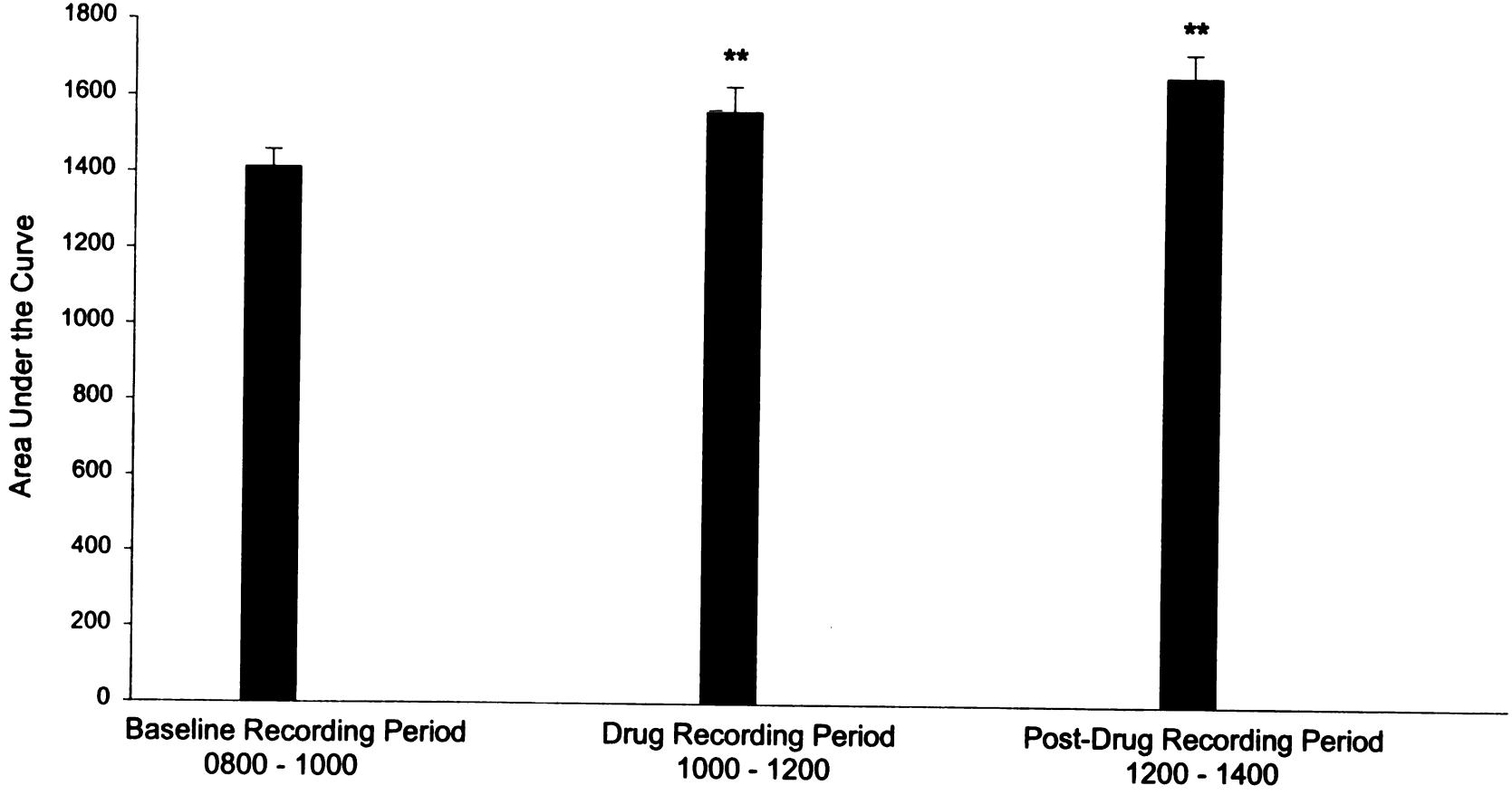
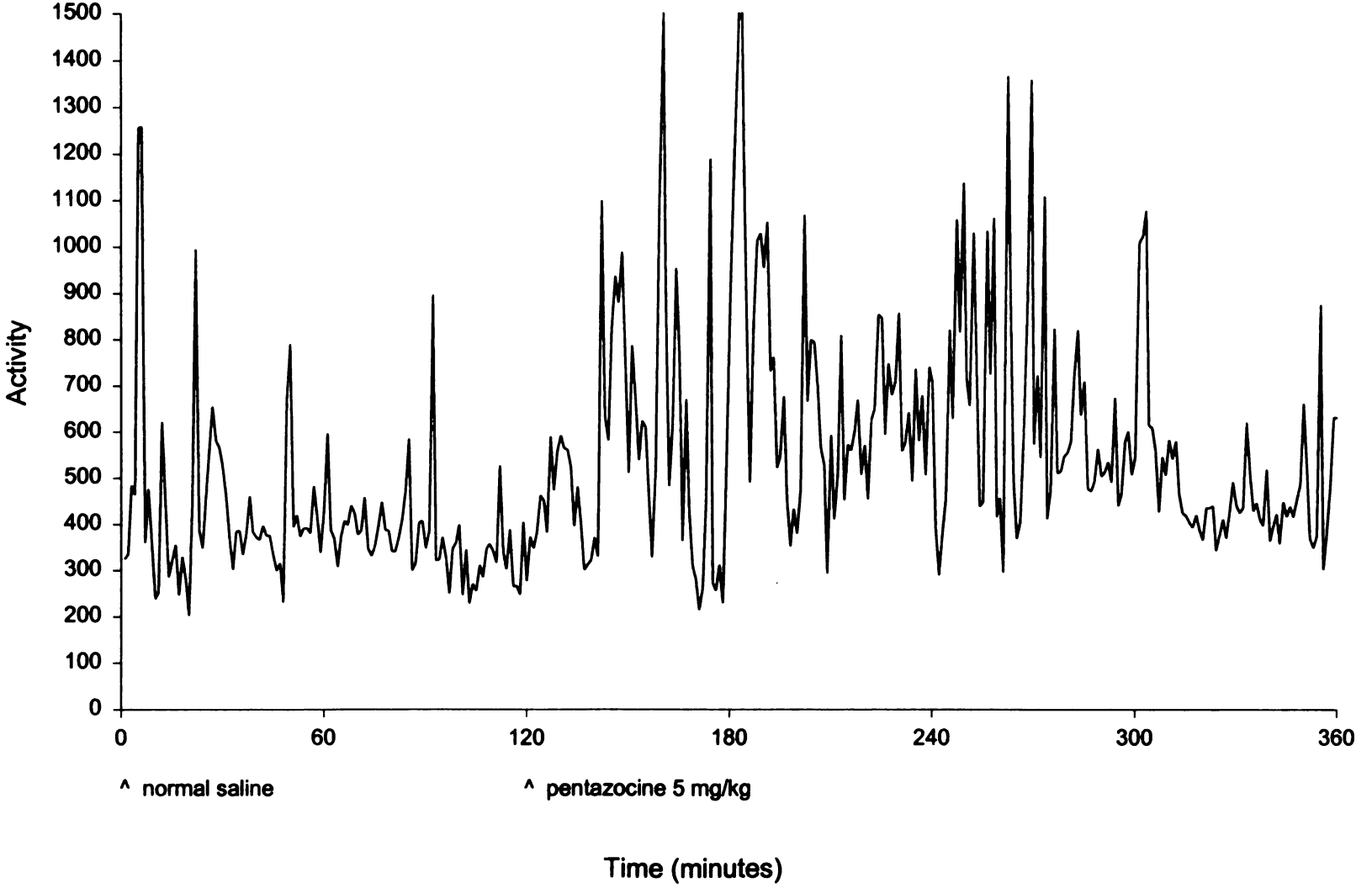


Figure 46: Effect of Pentazocine 50 ug/kg on Area-Under-the-Curve for Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)



** indicates that p = .01

Figure 47: Effect of Pentazocine 5 mg/kg on Activity (n=16 for 8 animals)



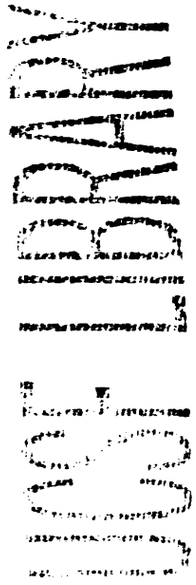


Figure 48: Effect of Pentazocine 5 mg/kg on Mobility (n=16 for 8 animals)

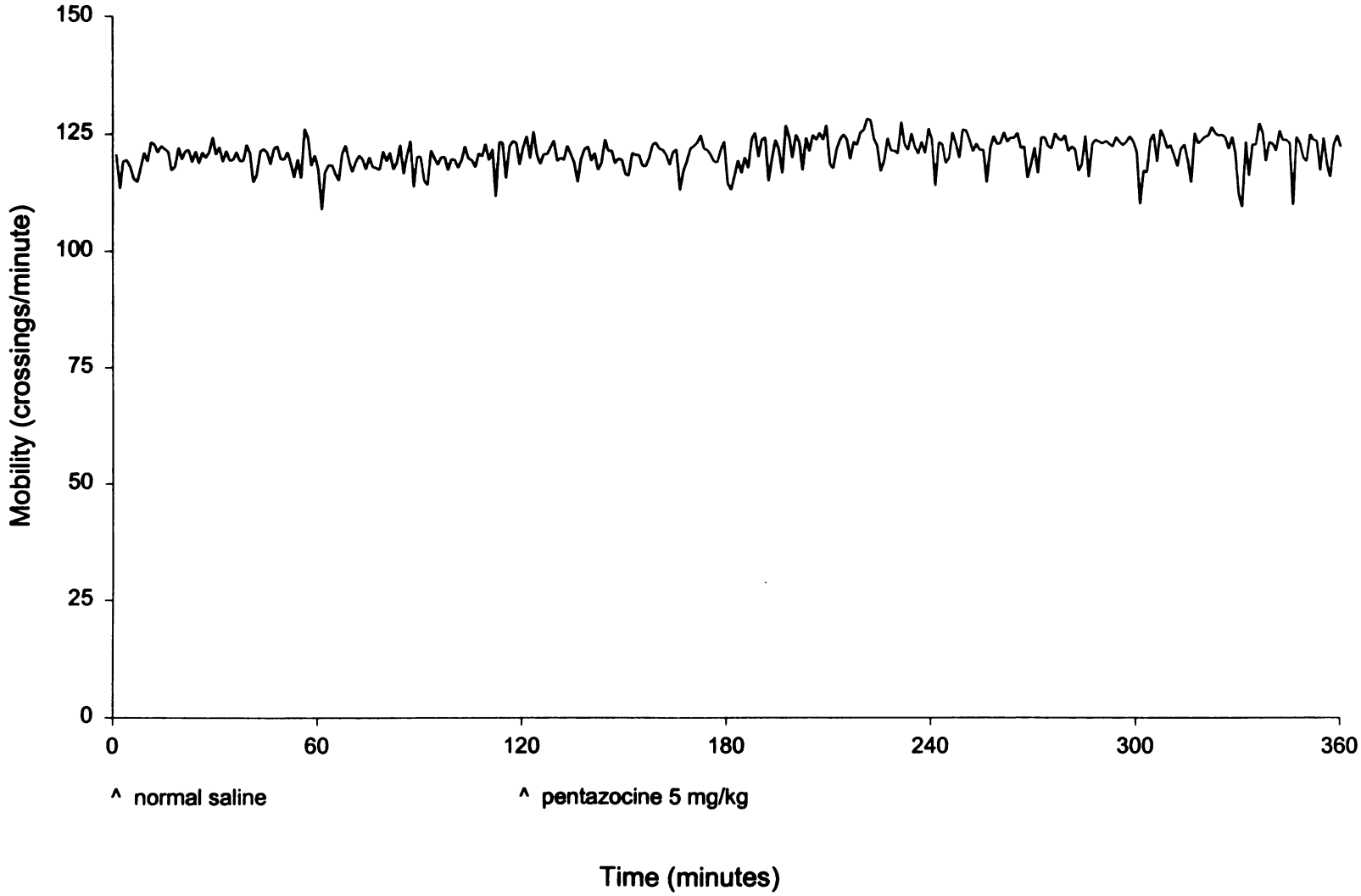


Figure 49: Effect of Pentazocine 5 mg/kg on Complexity (n=16 for 8 animals)

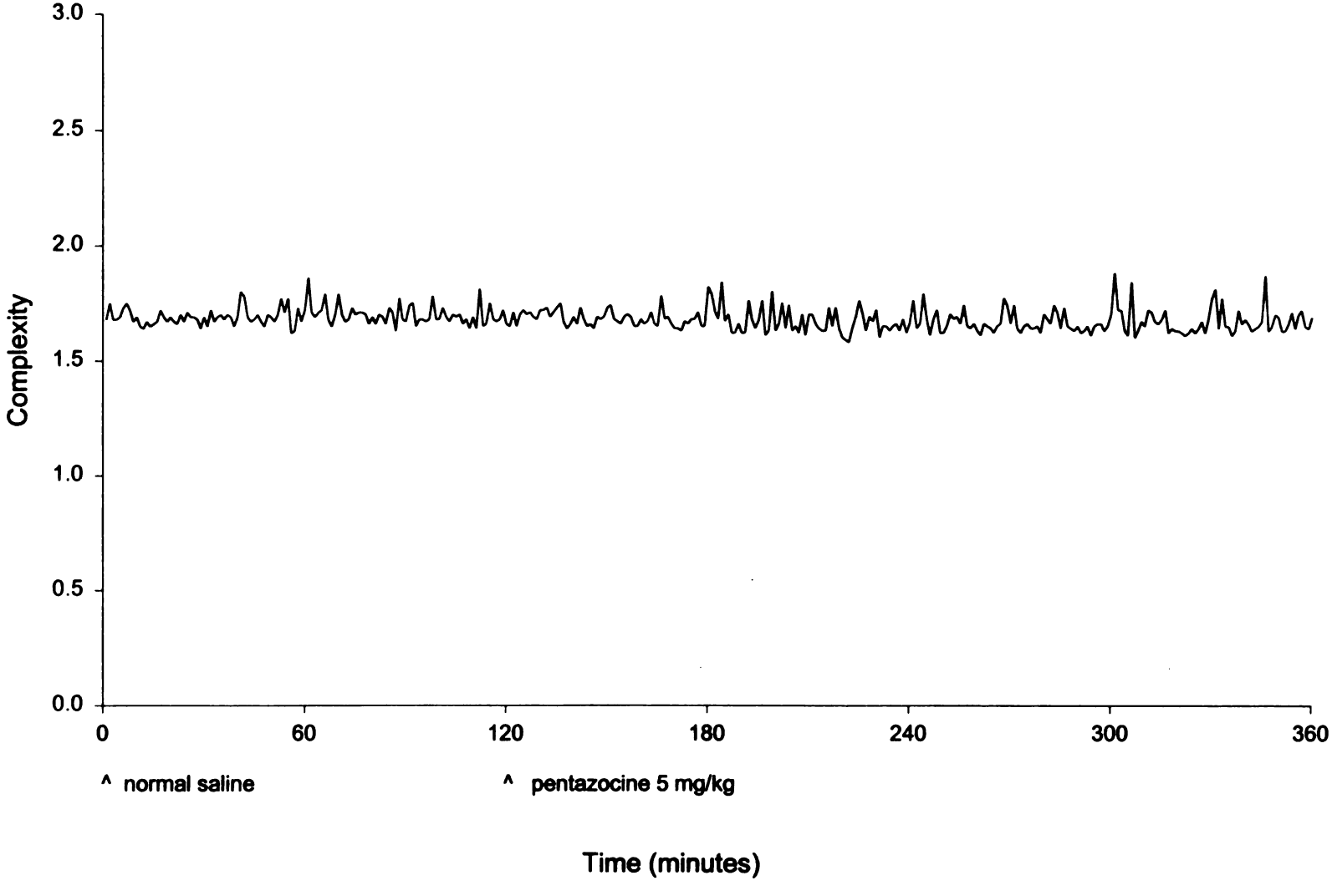
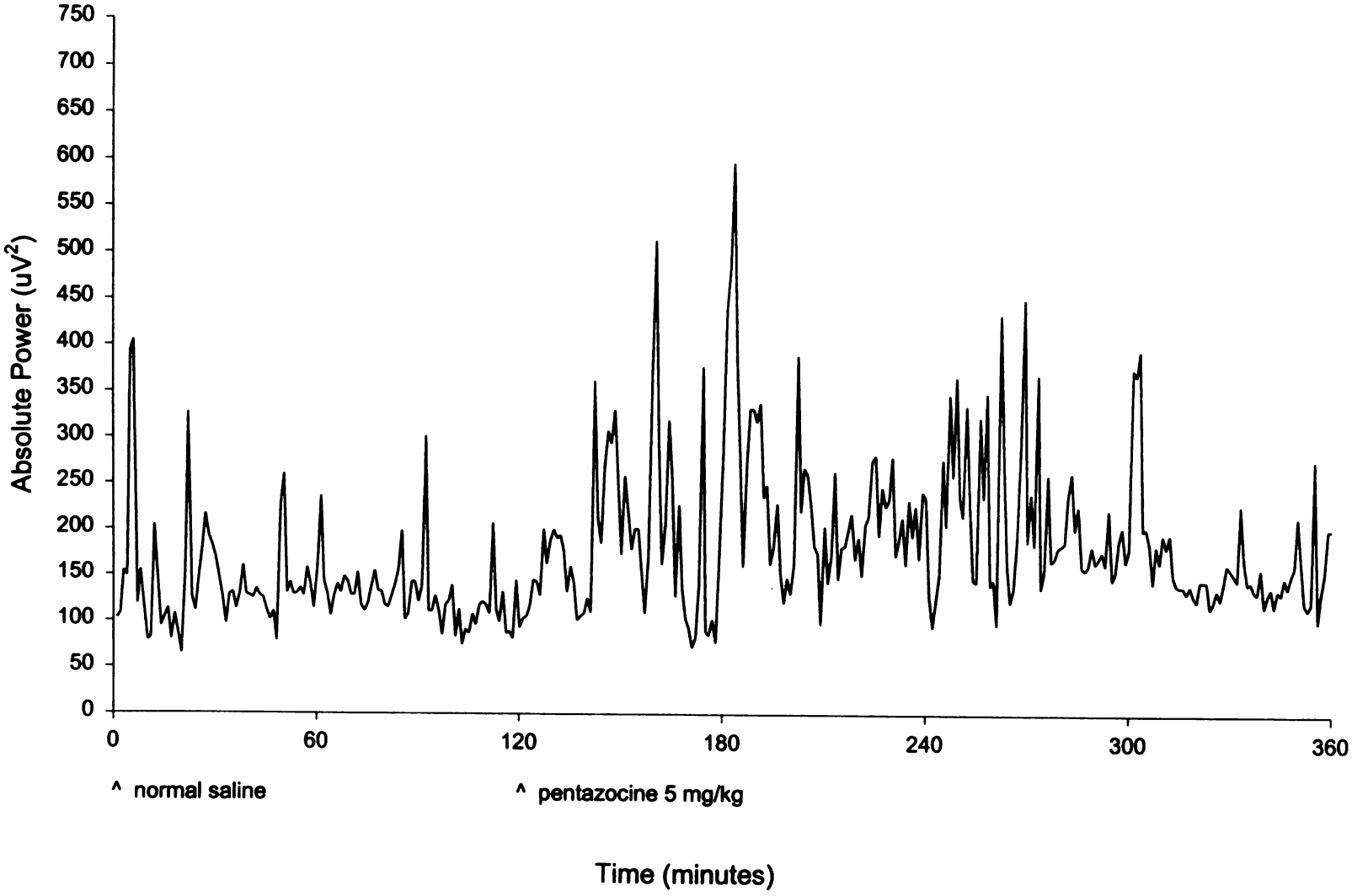
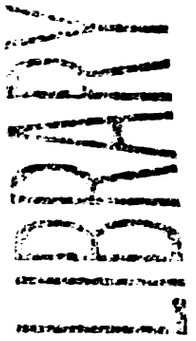


Figure 50: Effect of Pentazocine 5 mg/kg on Absolute Power (n=16 for 8 animals)





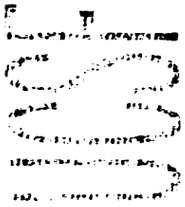


Figure 51: Effect of Pentazocine 5 mg/kg on Peak, Median & Edge Frequencies (n=16 for 8 animals)

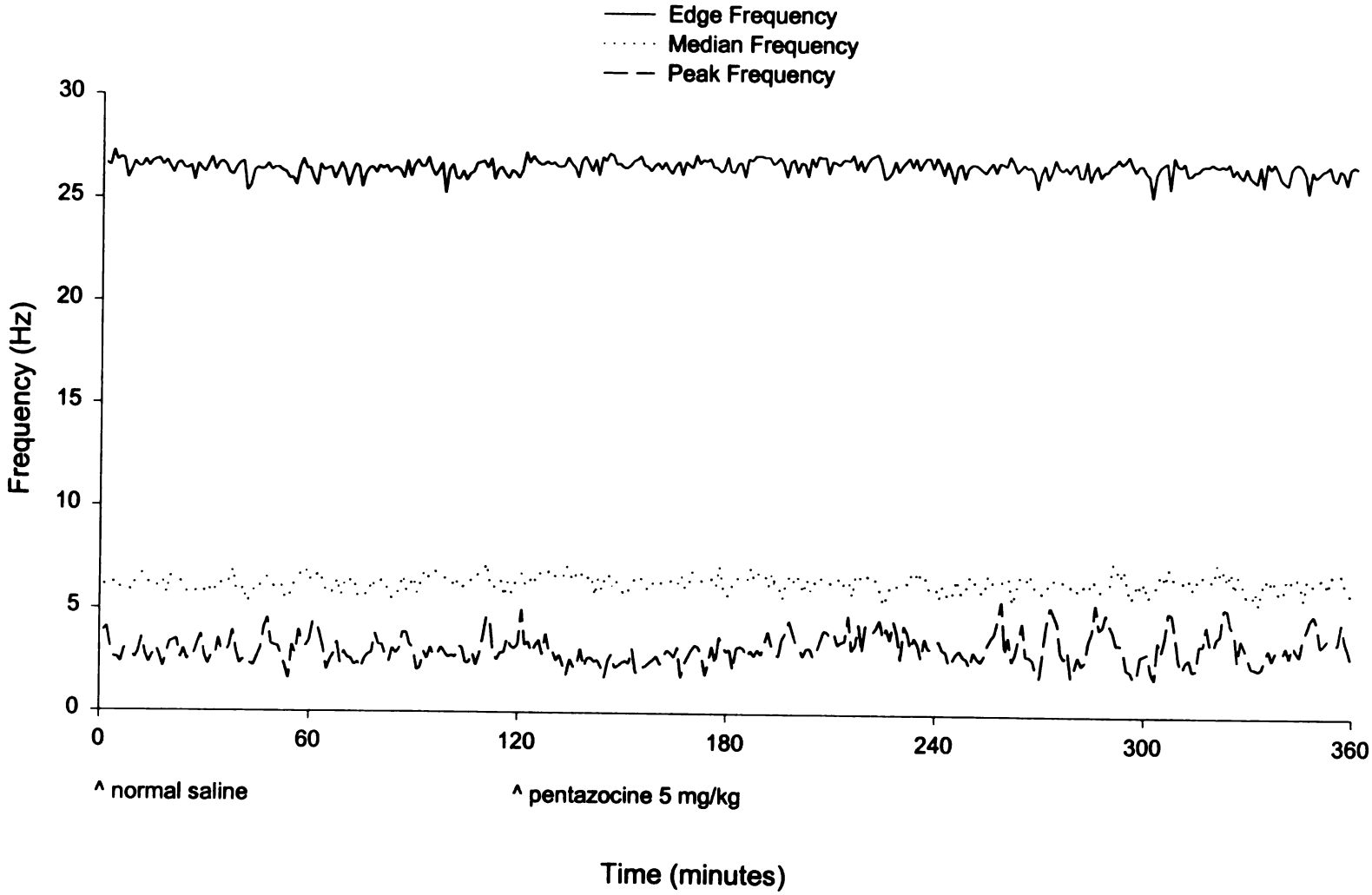


Figure 52: Effect of Pentazocine 5 mg/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 0800-1000 (n=16 for 8 animals)

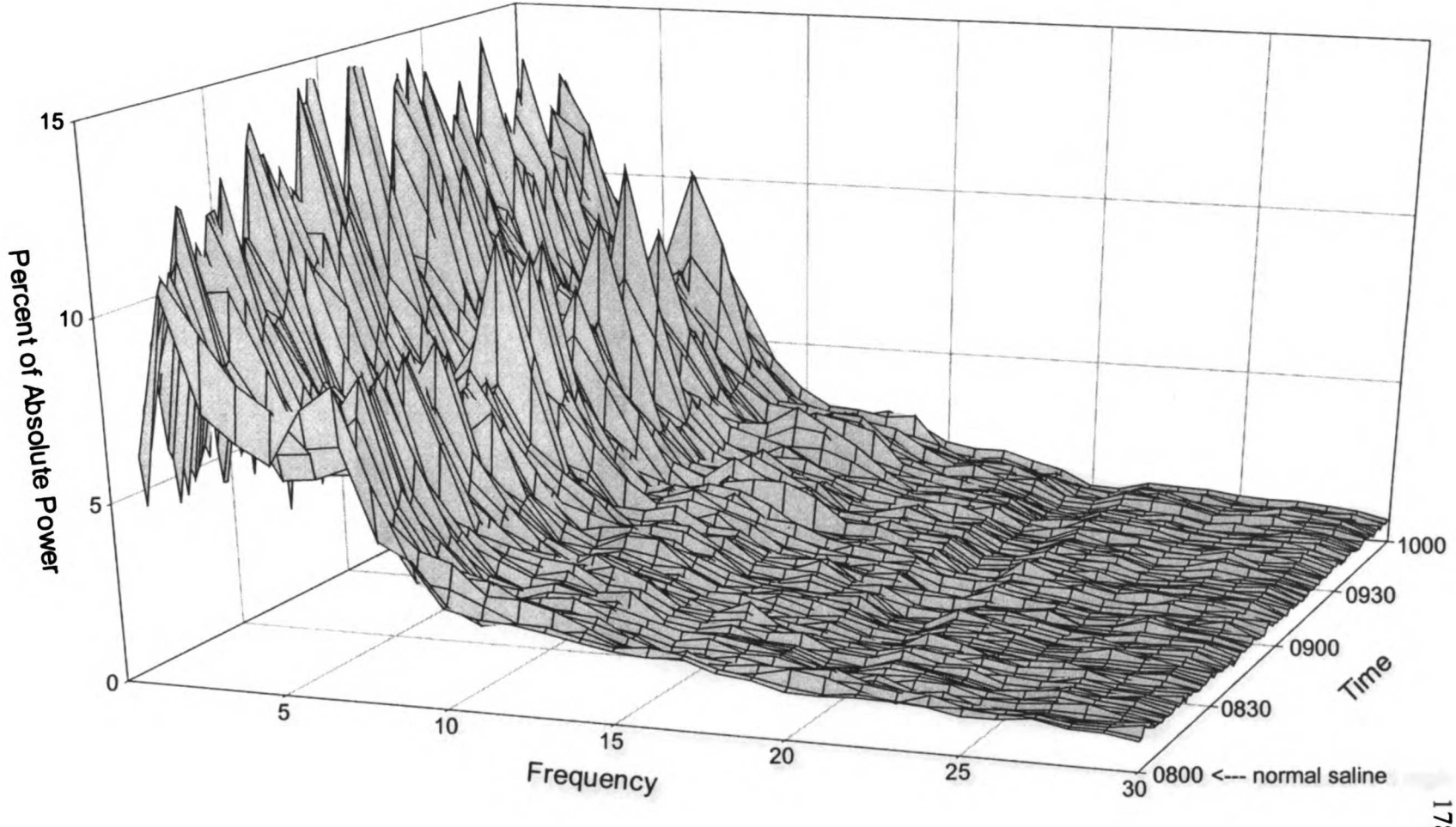


Figure 53: Effect of Pentazocine 5 mg/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1000-1200 (n=16 for 8 animals)

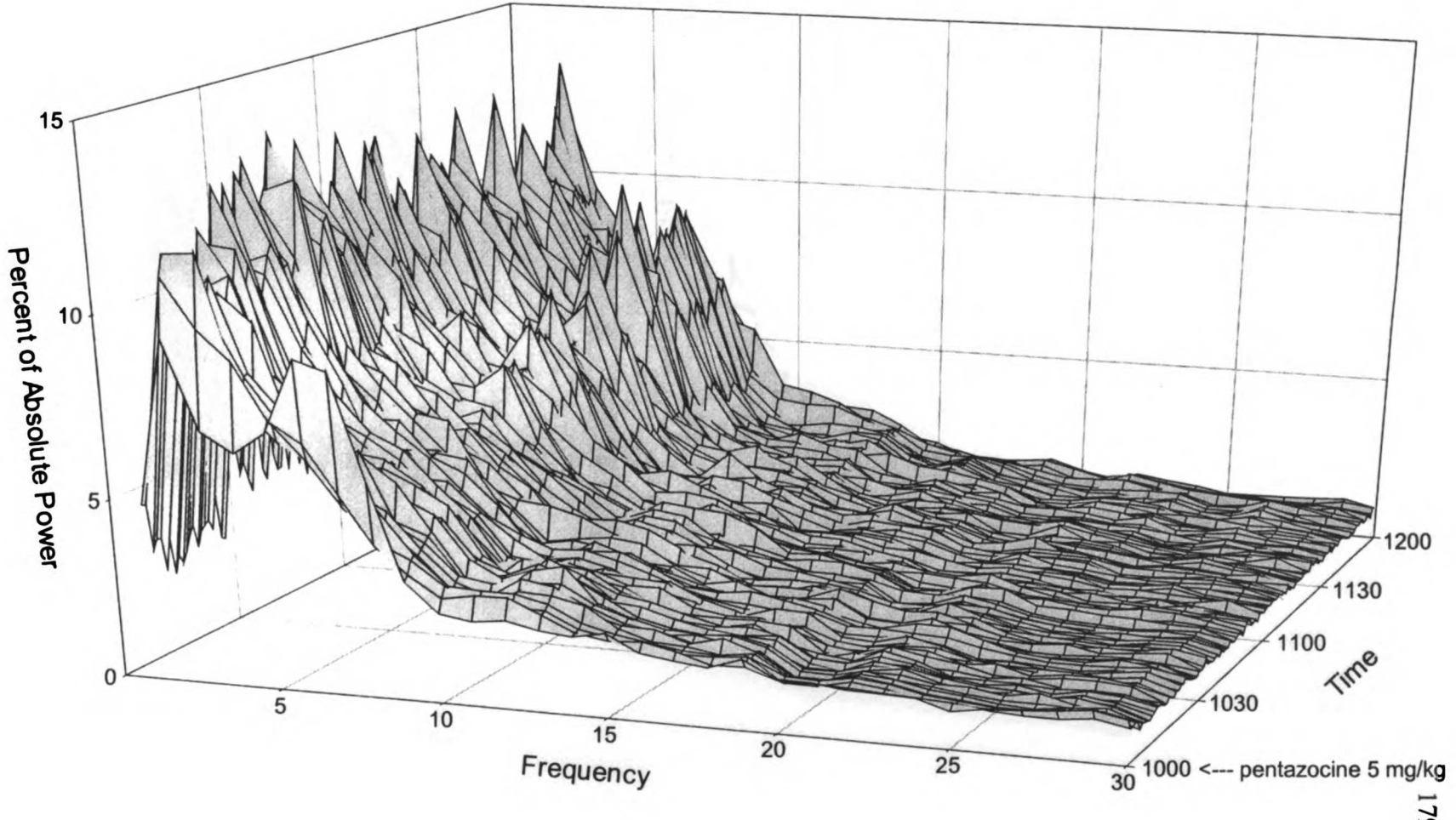


Figure 54: Effect of Pentazocine 5 mg/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1200-1400 (n=16 for 8 animals)

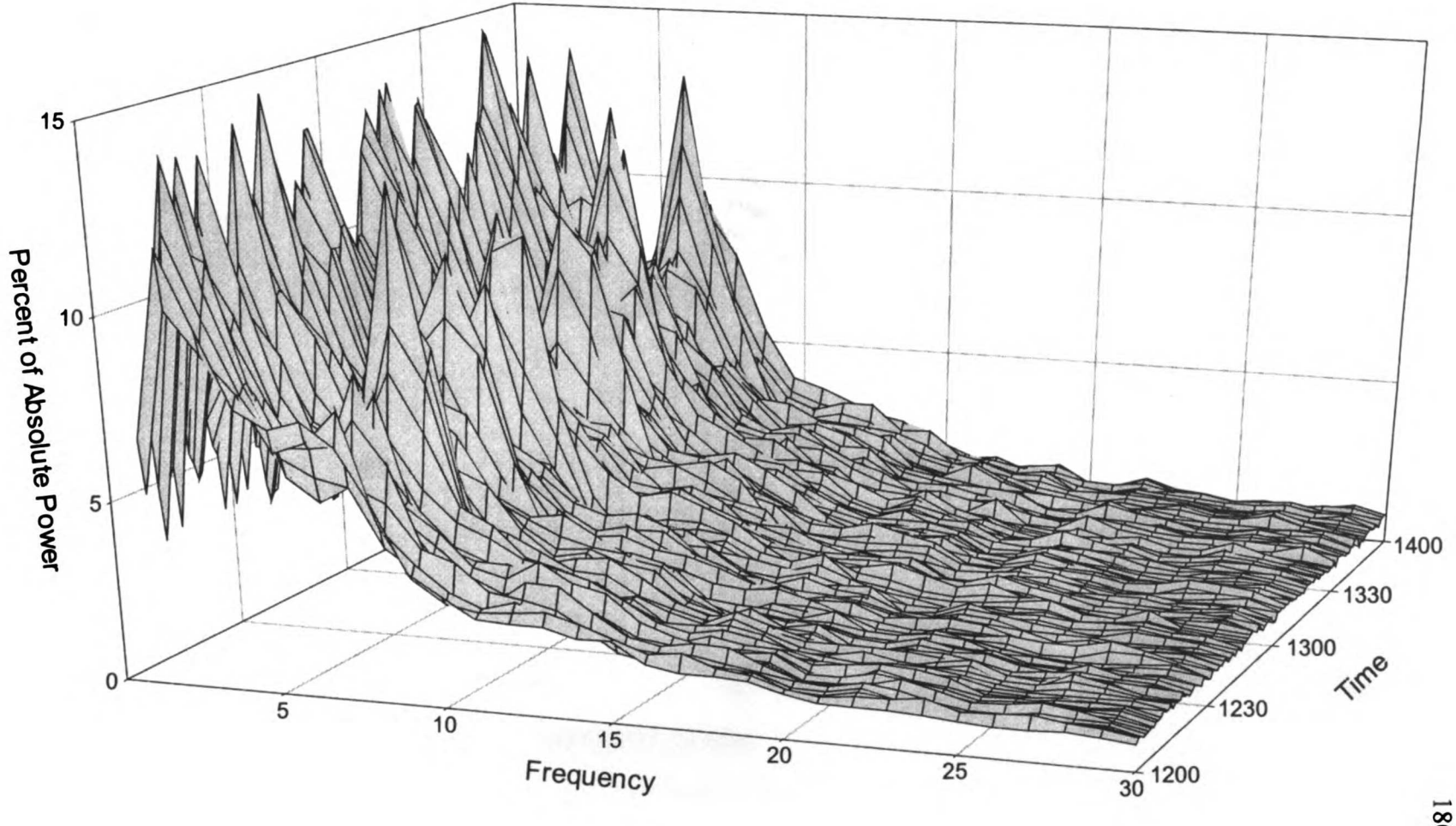


Figure 55: Effect of Pentazocine 5 mg/kg on Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)

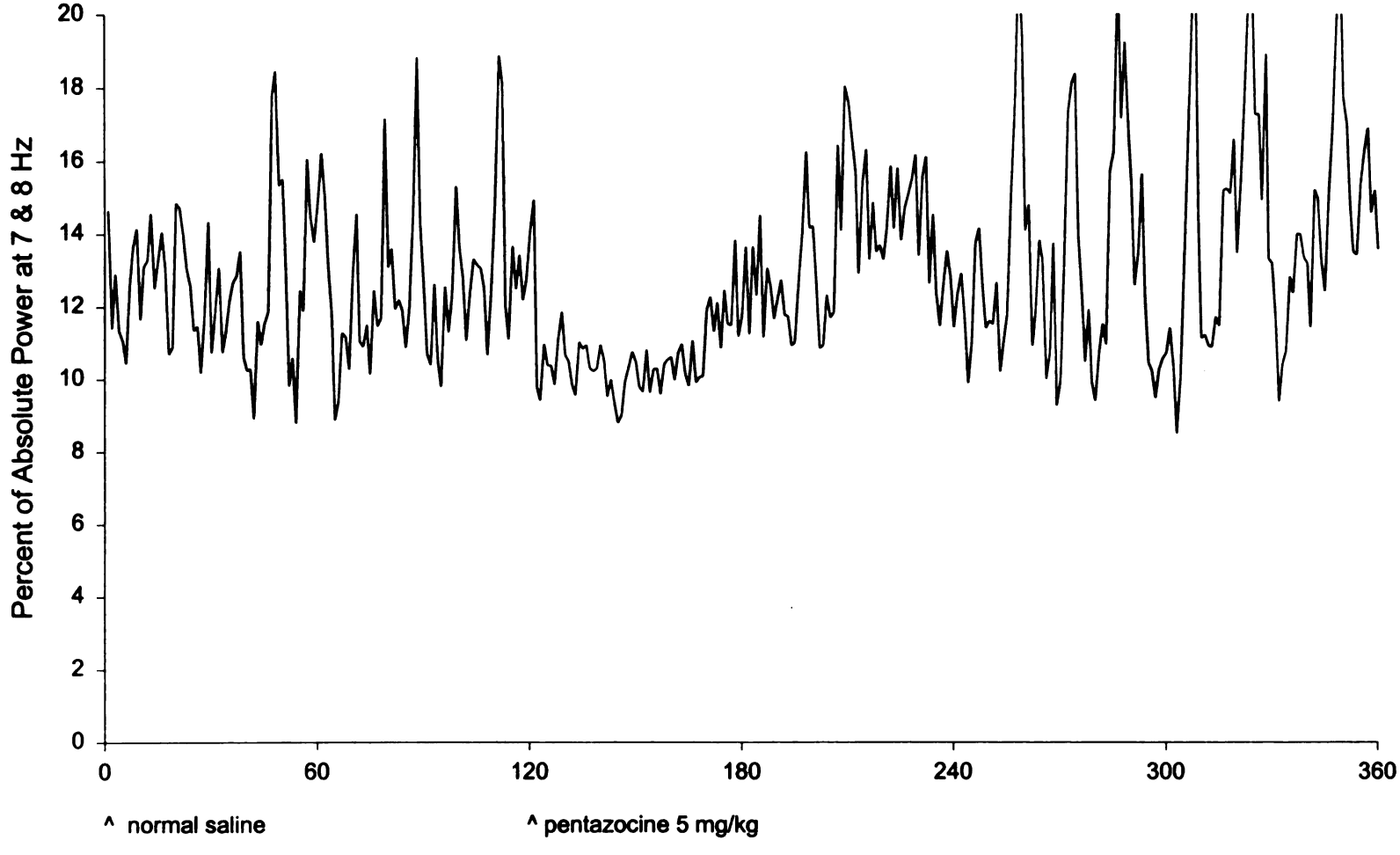


Figure 56: Effect of Pentazocine 5 mg/kg on Area-Under-the-Curve for Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)

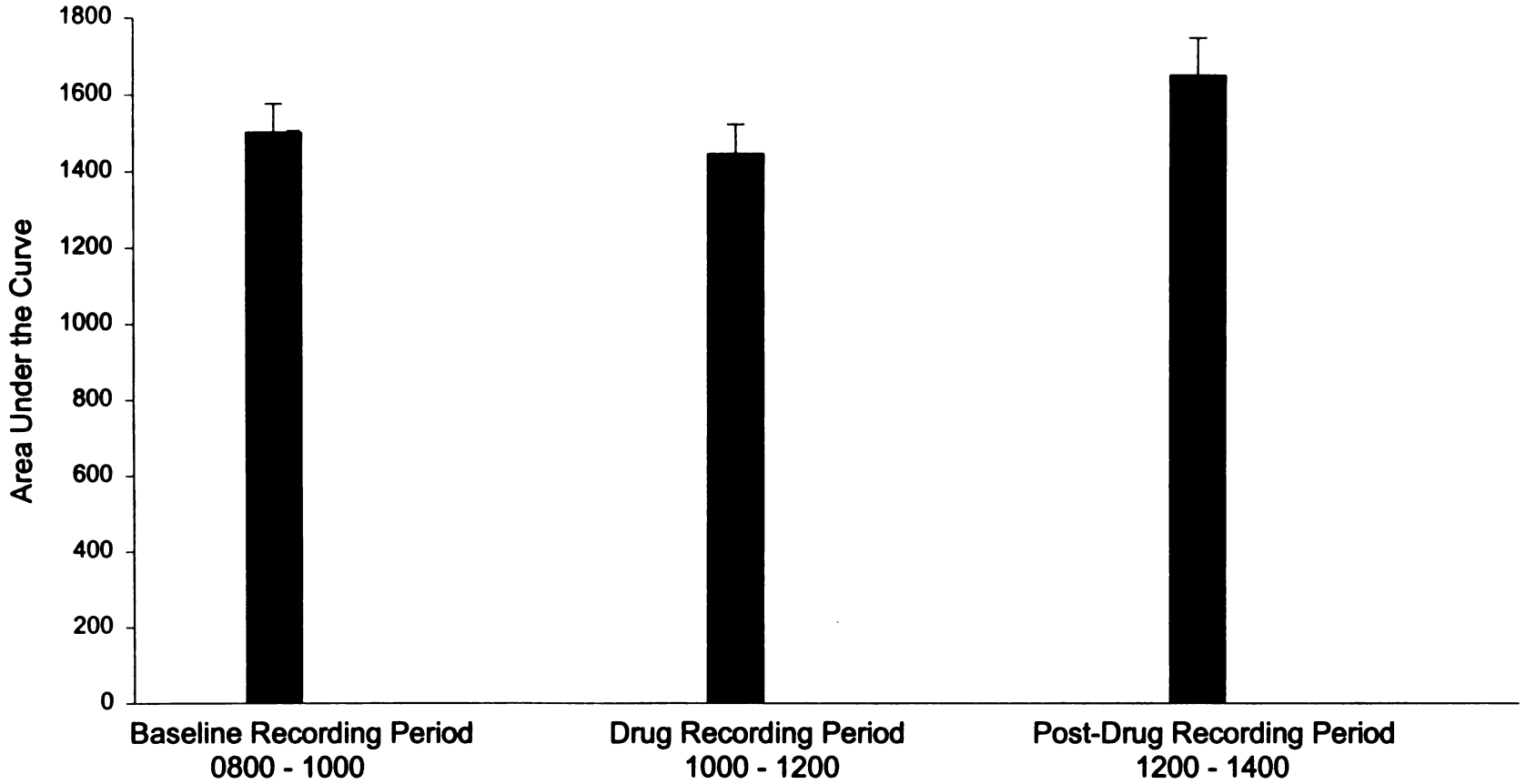


Figure 57: Effect of Naloxone 5 ug/kg on Activity (n=16 for 8 animals)

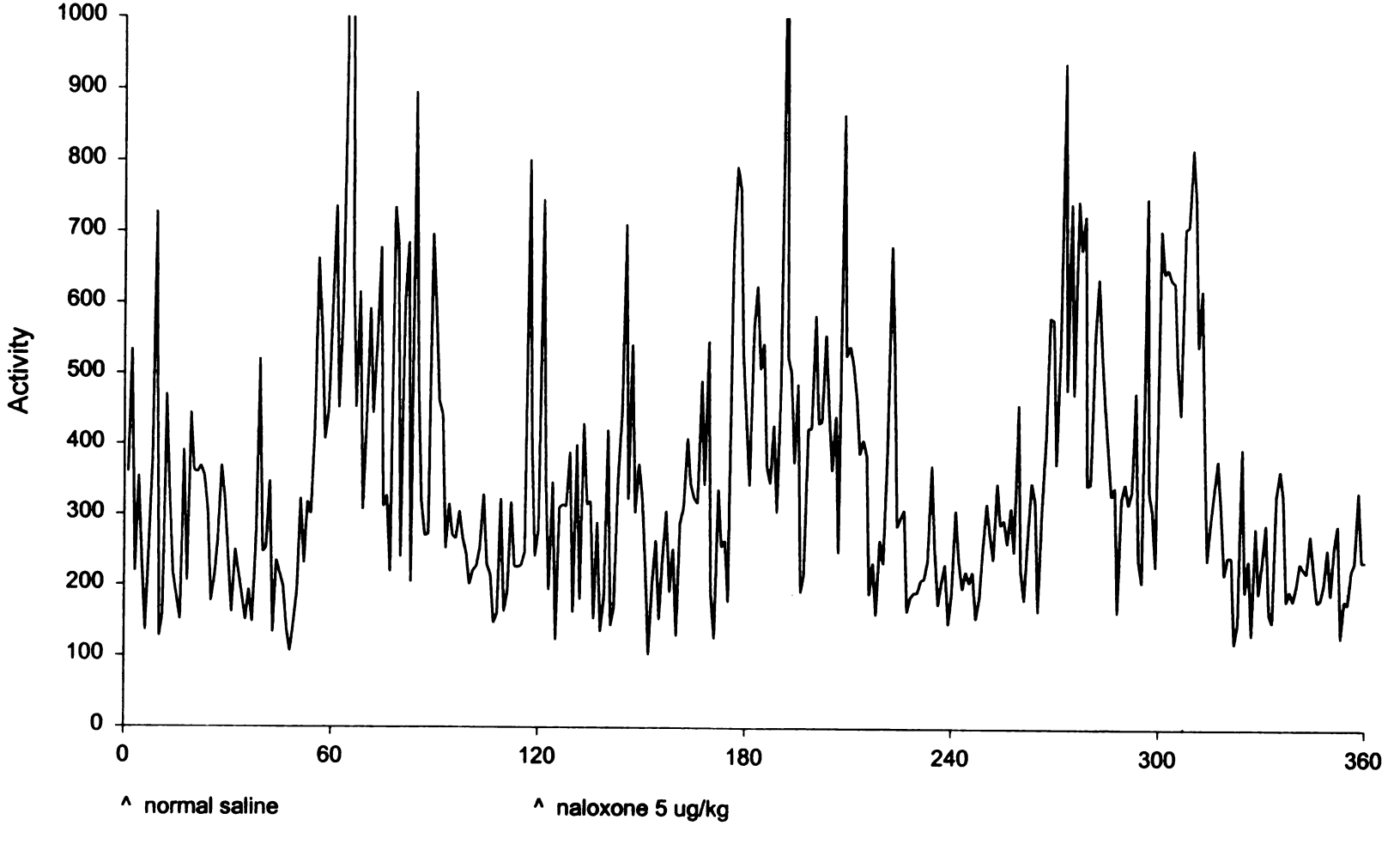


Figure 58: Effect of Naloxone 5 ug/kg on Mobility (n=16 for 8 animals)

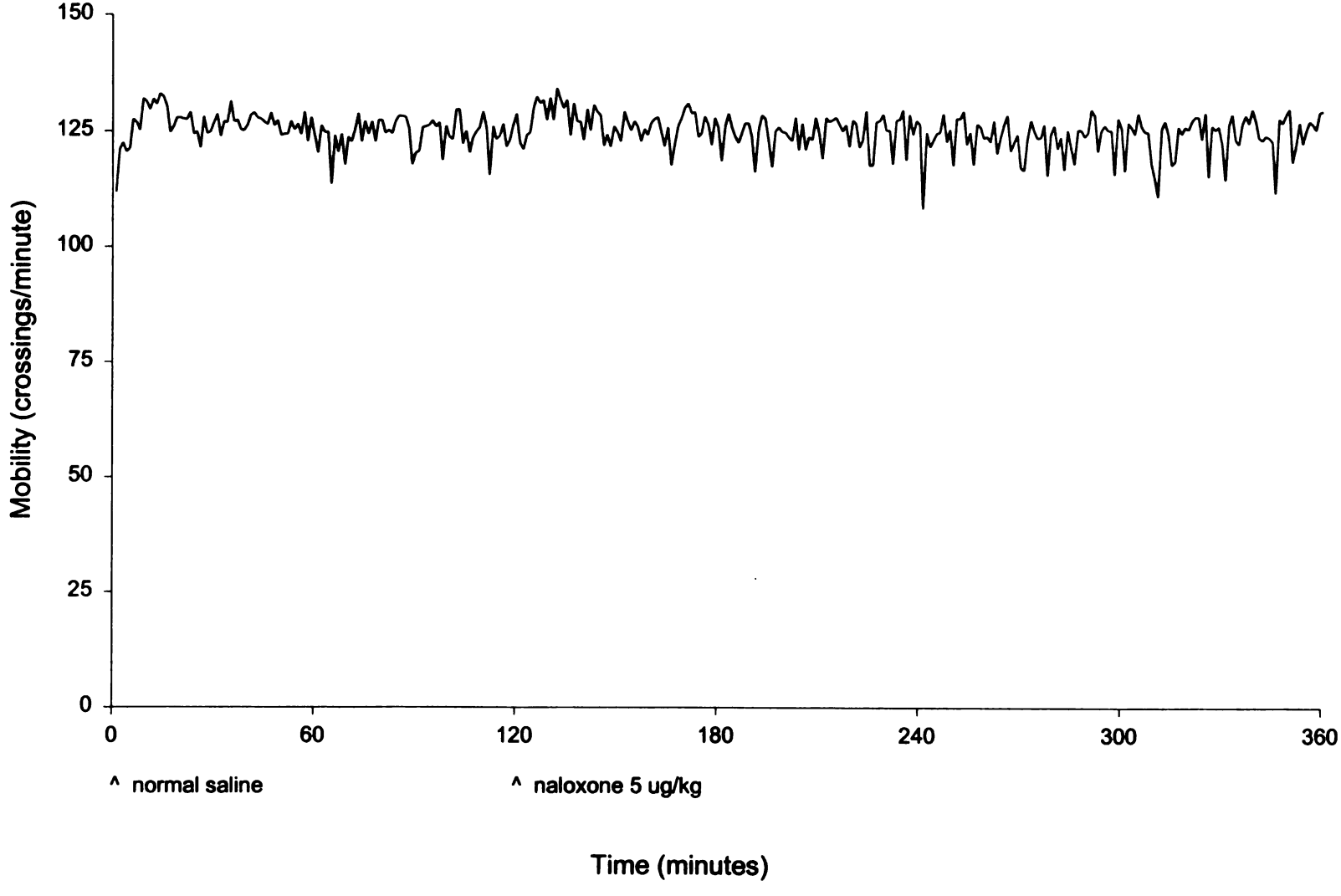


Figure 59: Effect of Naloxone 5 ug/kg on Complexity (n=16 for 8 animals)

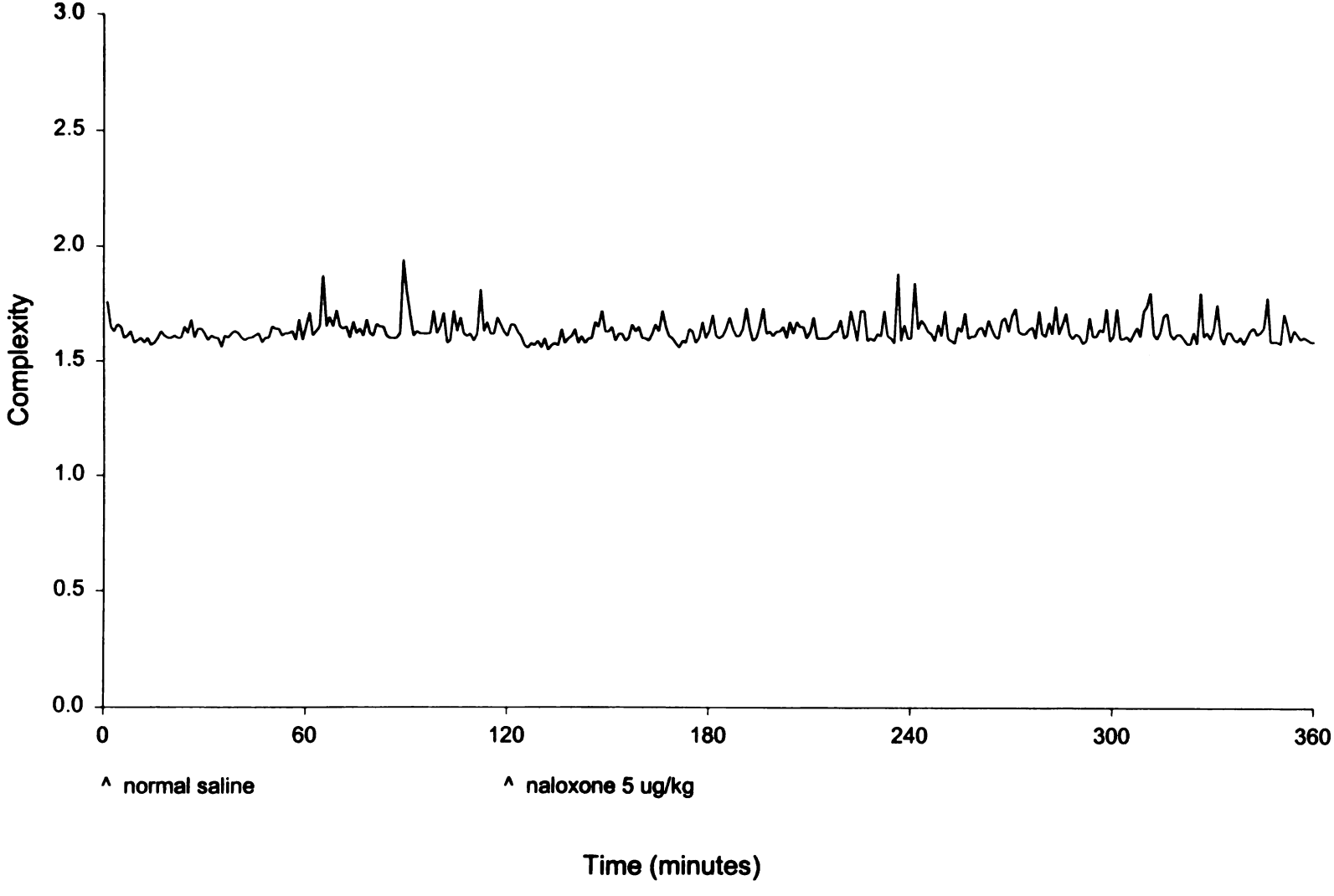


Figure 60: Effect of Naloxone 5 ug/kg on Absolute Power (n=16 for 8 animals)

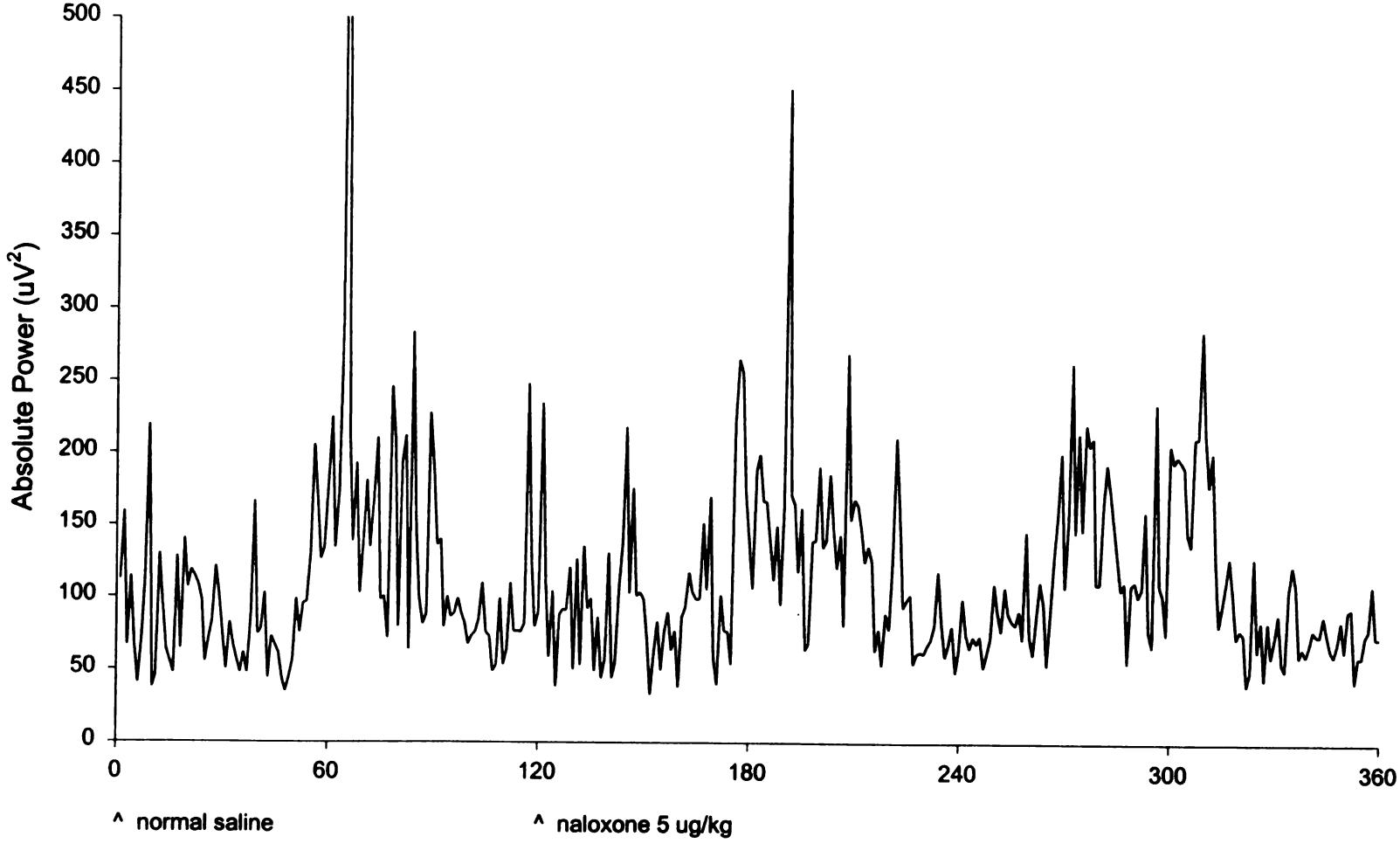


Figure 61: Effect of Naloxone 5 ug/kg on Peak, Median & Edge Frequencies (n=16 for 8 animals)

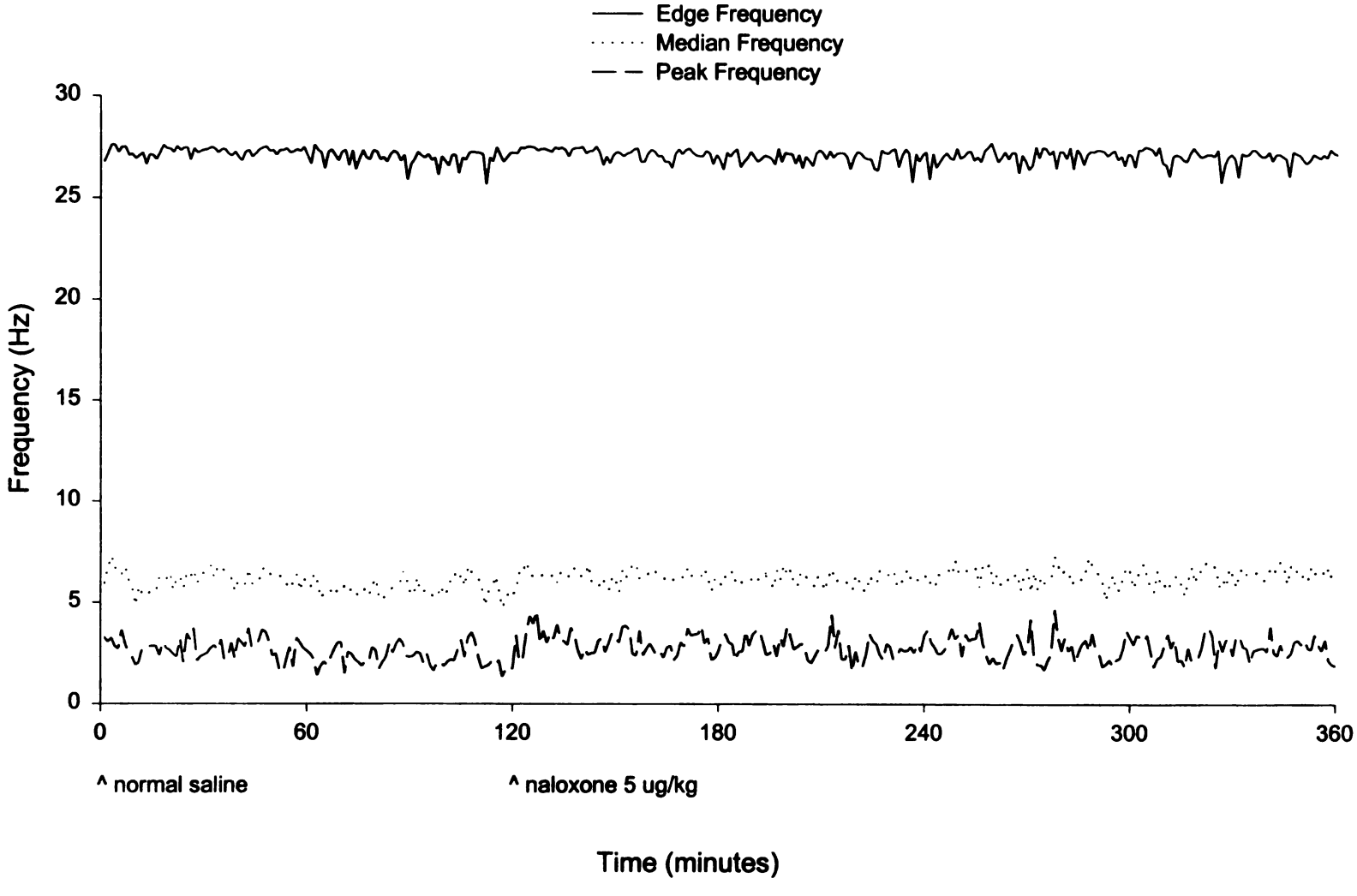


Figure 62: Effect of Naloxone 5 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 0800-1000 (n=16 for 8 animals)

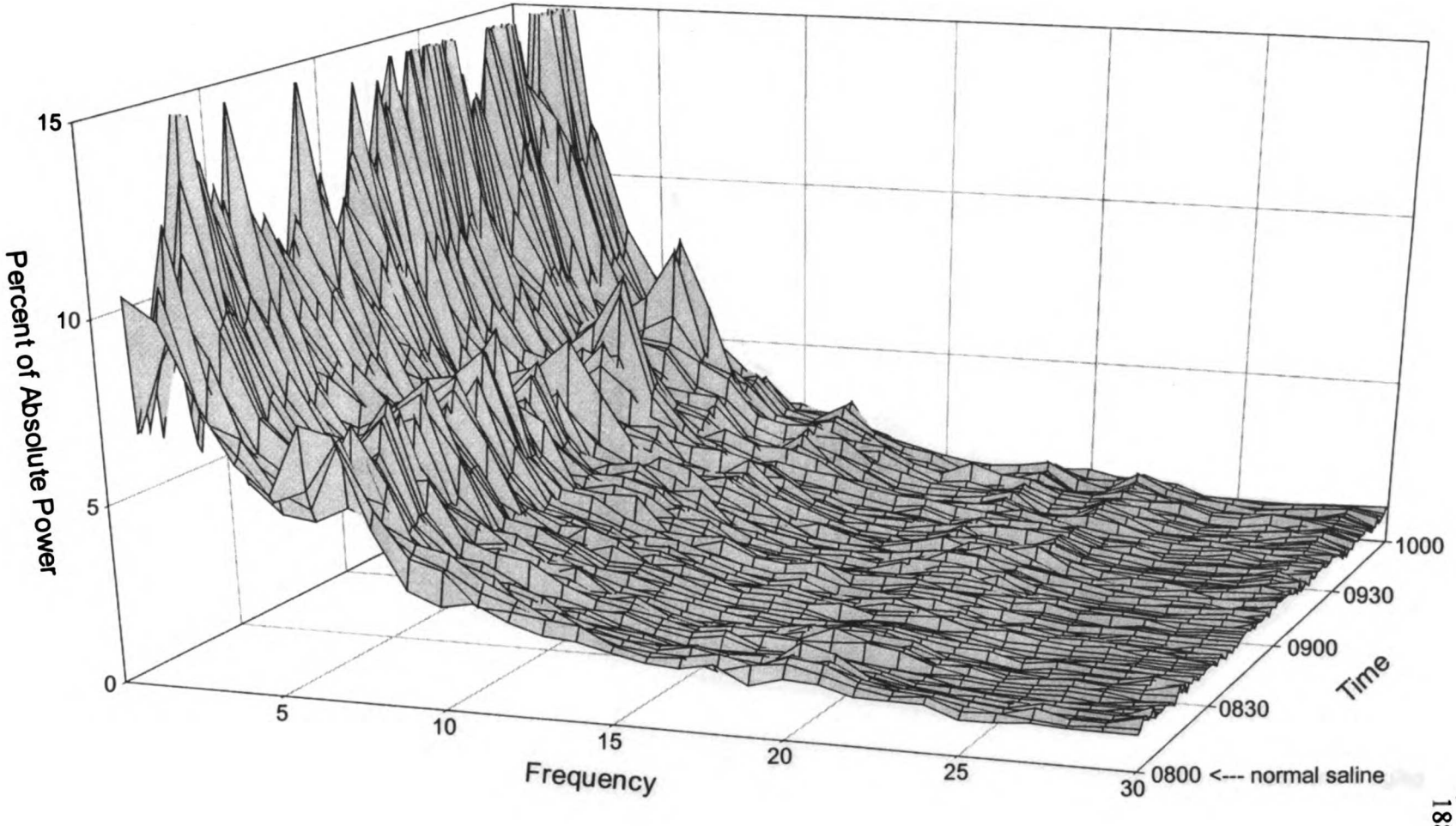


Figure 63: Effect of Naloxone 5 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1000-1200 (n=16 for 8 animals)

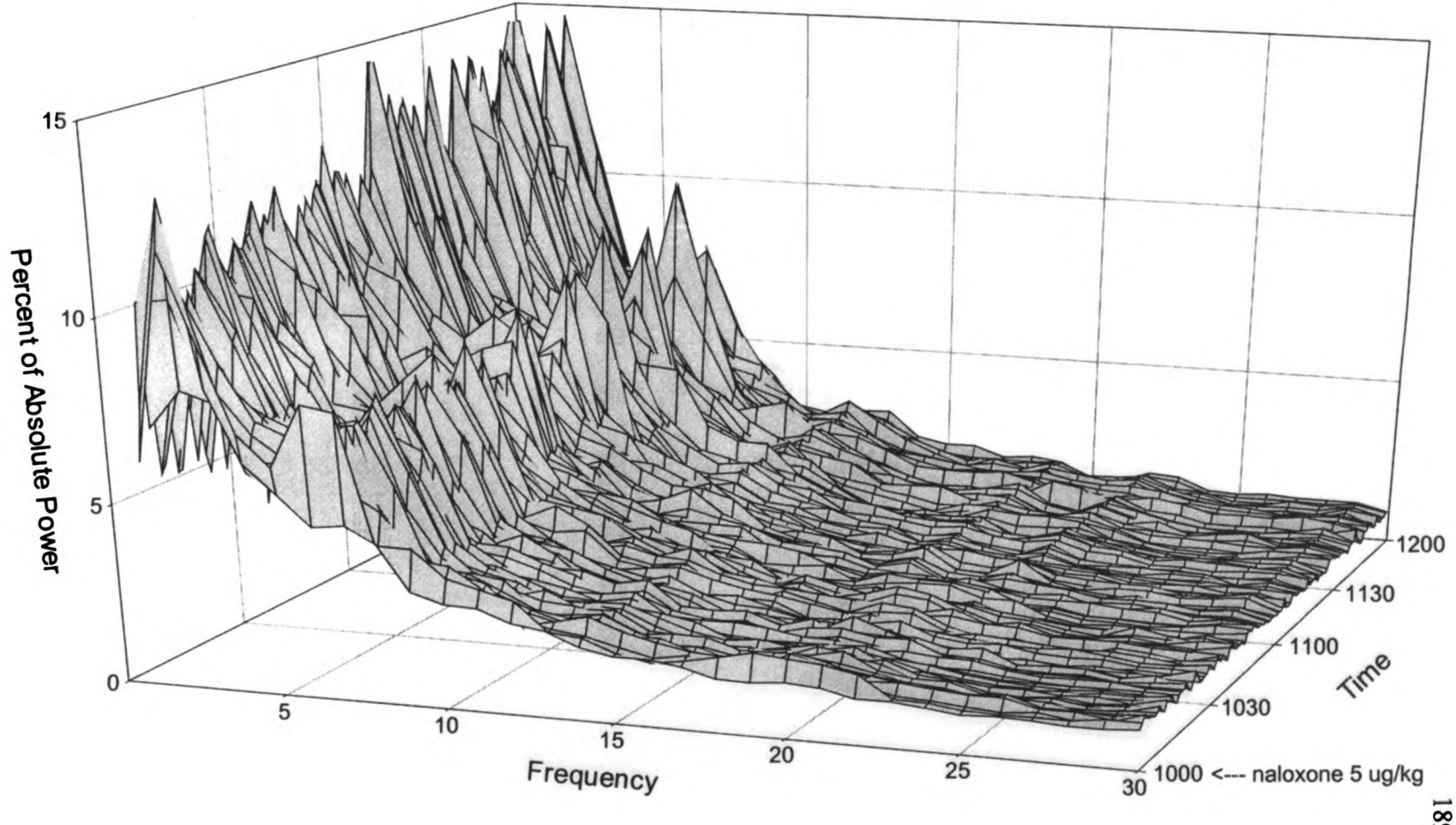


Figure 64: Effect of Naloxone 5 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1200-1400 (n=16 for 8 animals)

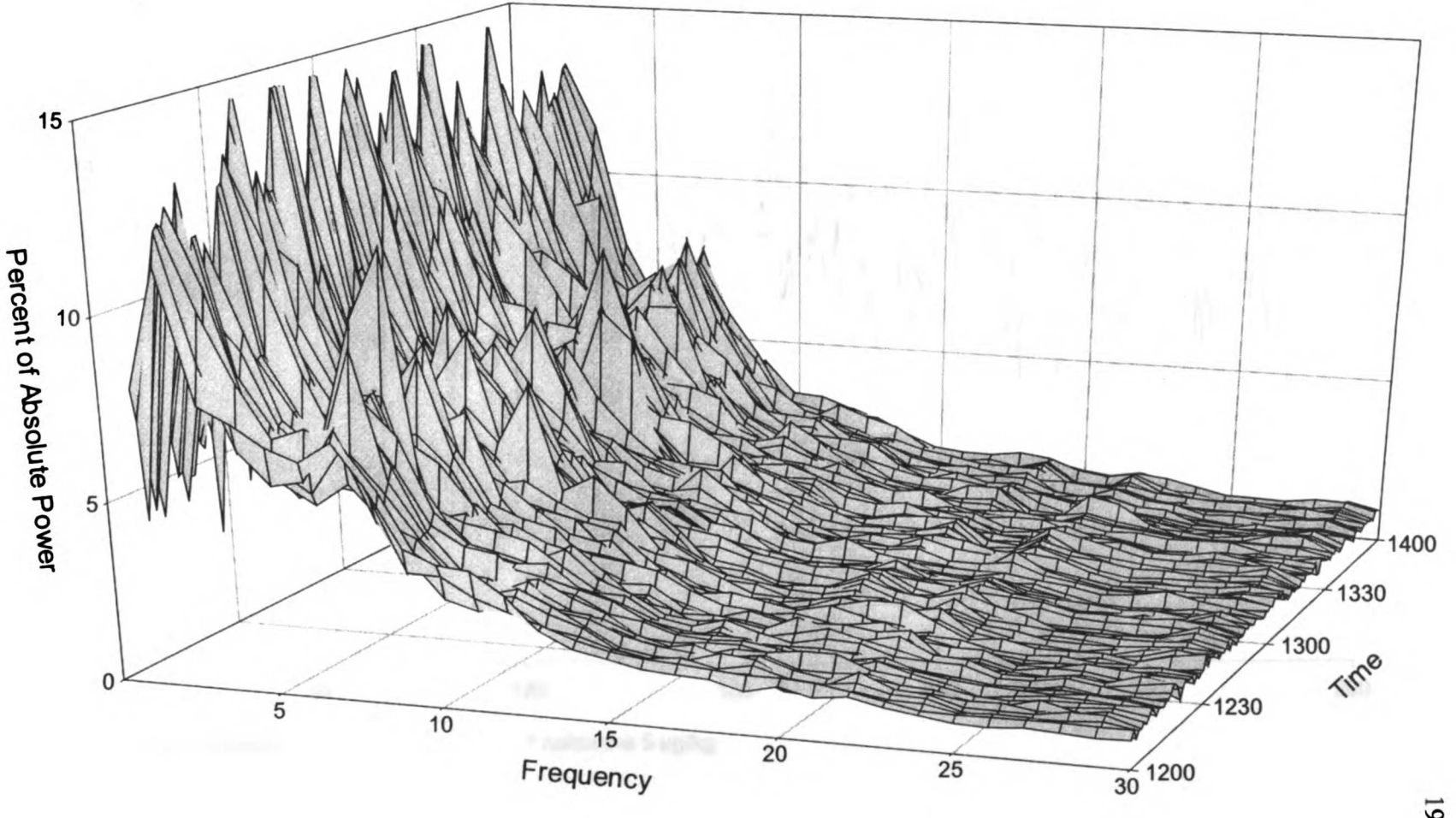


Figure 65: Effect of Naloxone 5 ug/kg on Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)

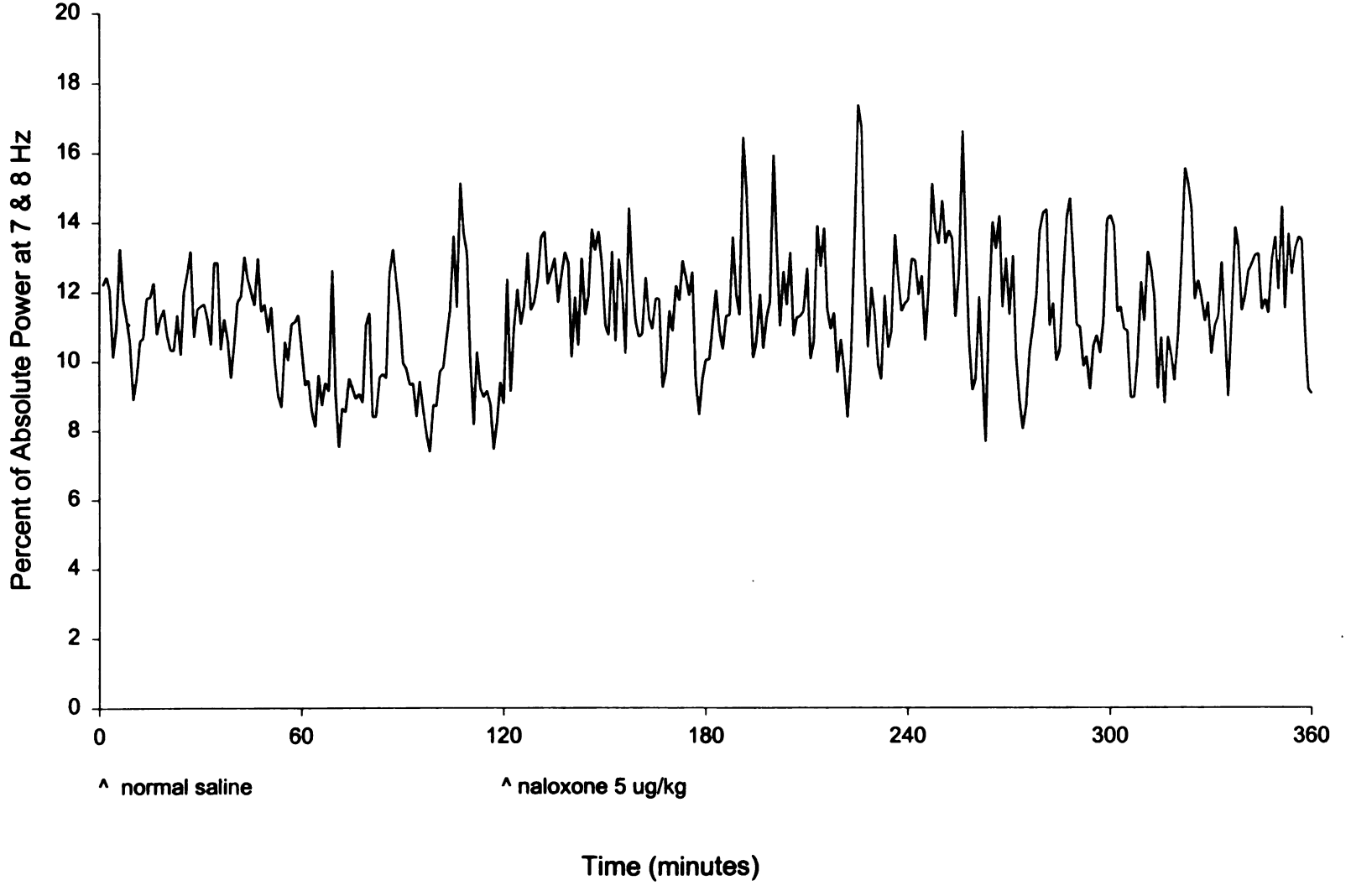
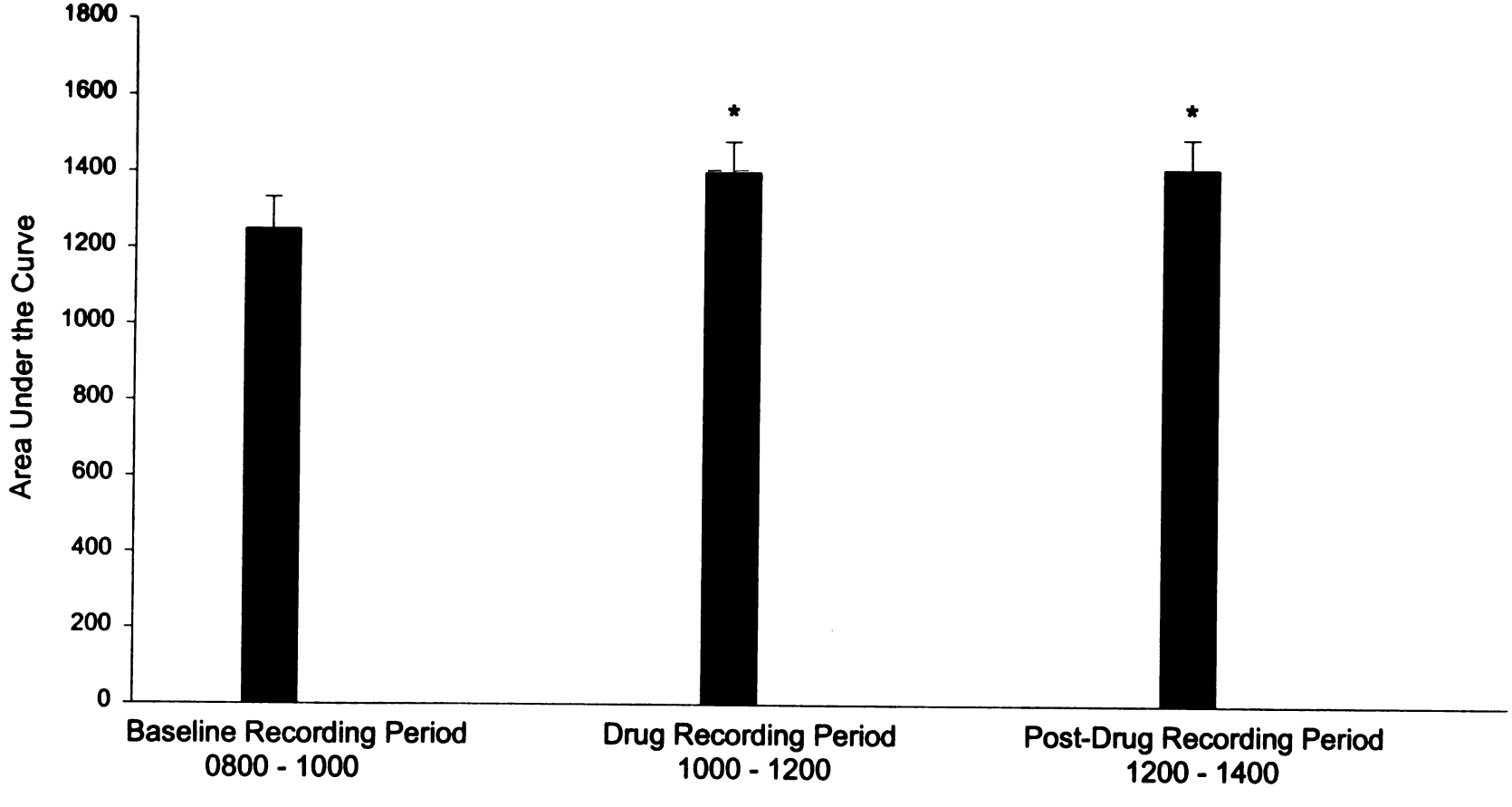


Figure 66: Effect of Naloxone 5 ug/kg on Area-Under-the-Curve for Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)



* indicates that p = .05

Figure 67: Effect of Naloxone 500 ug/kg on Activity (n=16 for 8 animals)

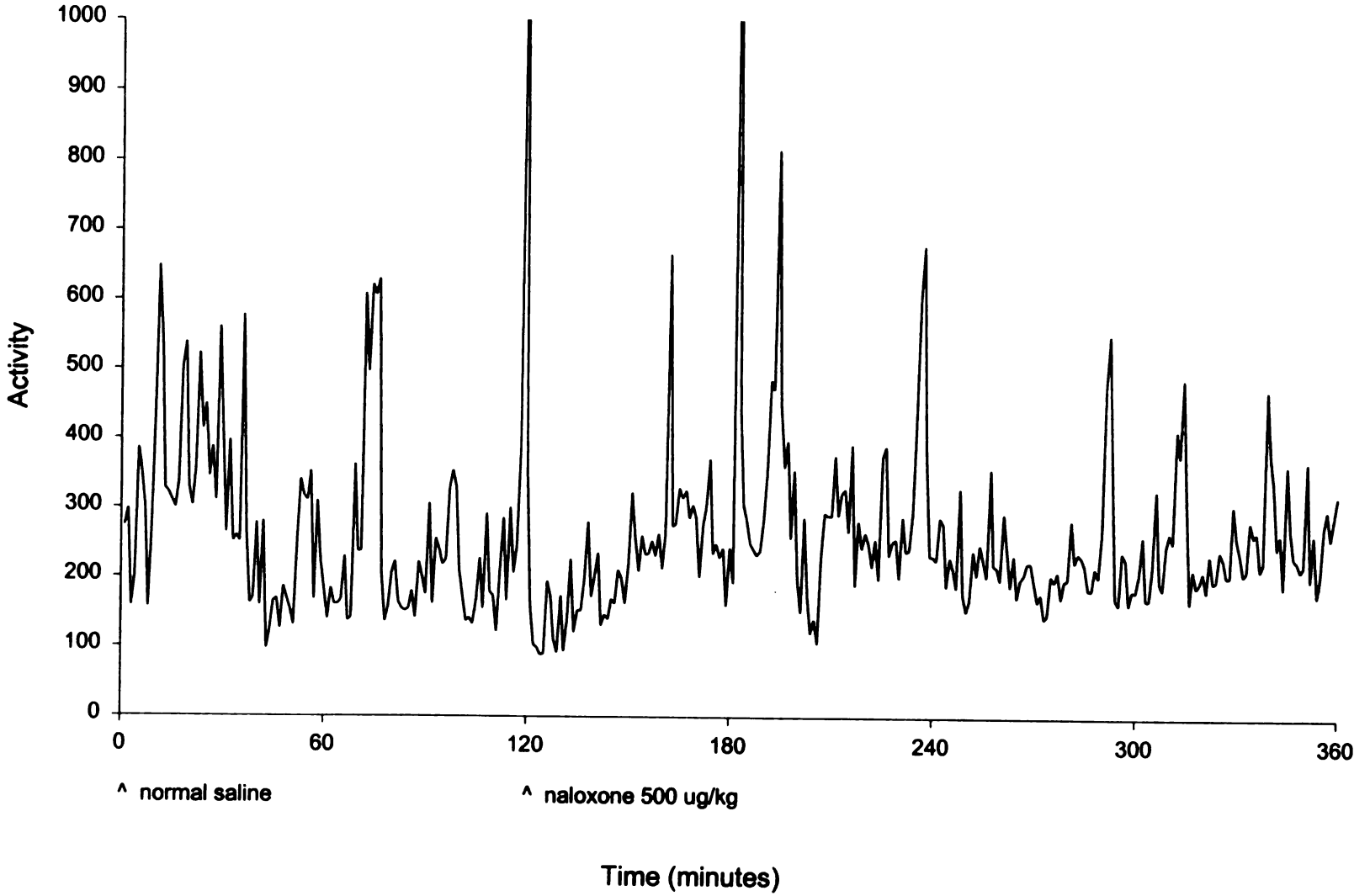


Figure 68: Effect of Naloxone 500 ug/kg on Mobility (n=16 for 8 animals)

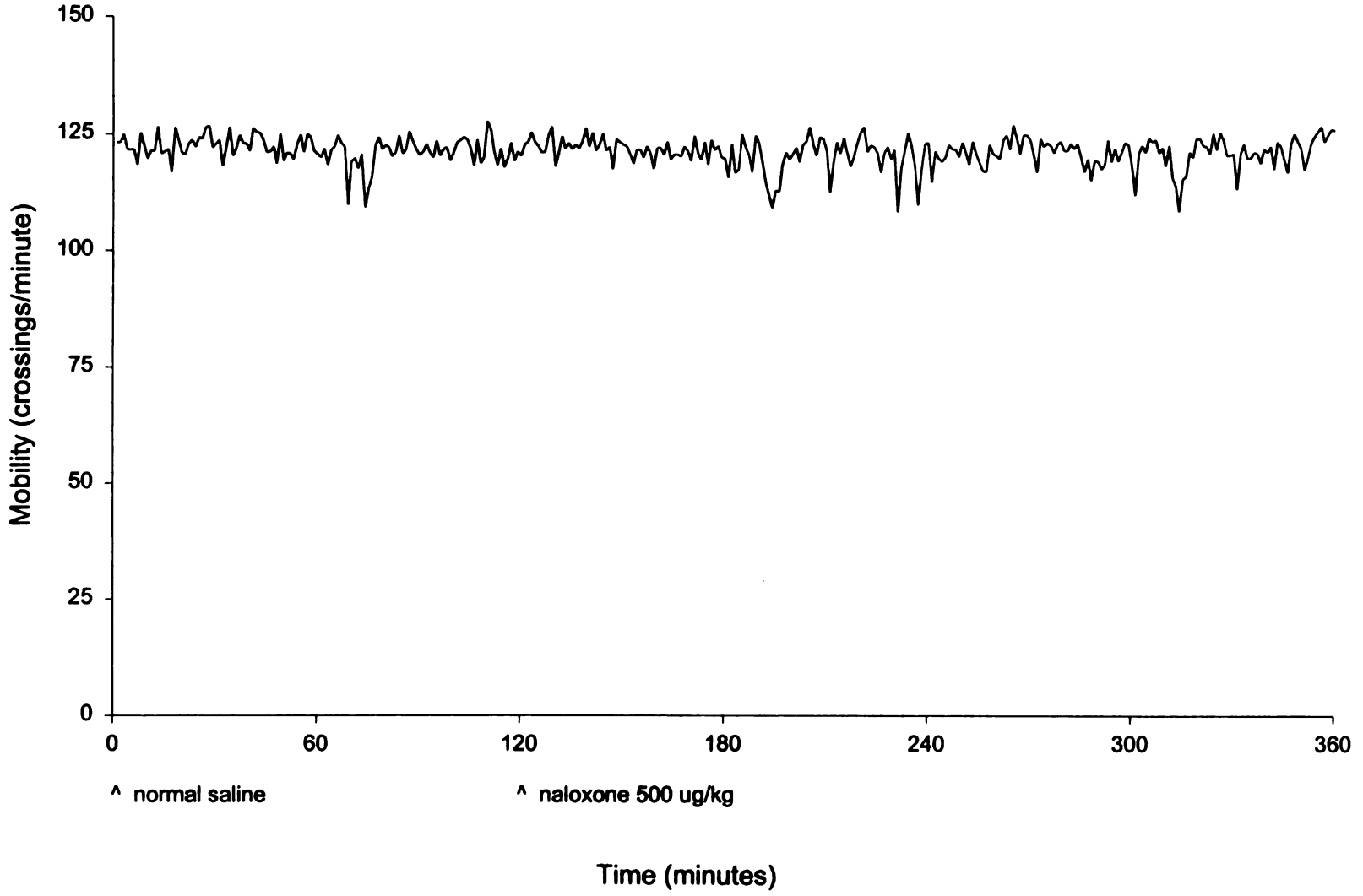


Figure 69: Effect of Naloxone 500 ug/kg on Complexity (n=16 for 8 animals)

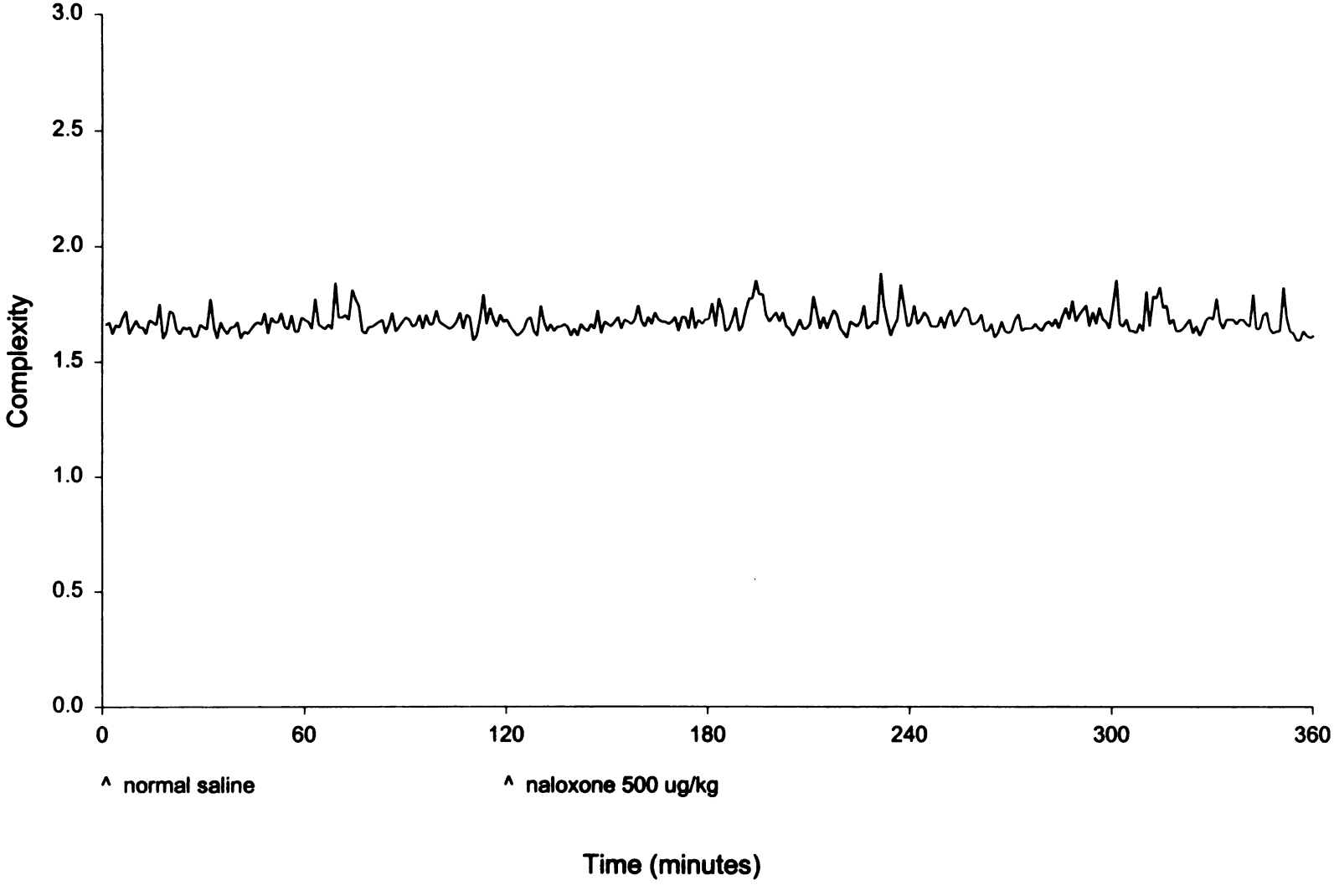


Figure 70: Effect of Naloxone 500 ug/kg on Absolute Power (n=16 for 8 animals)

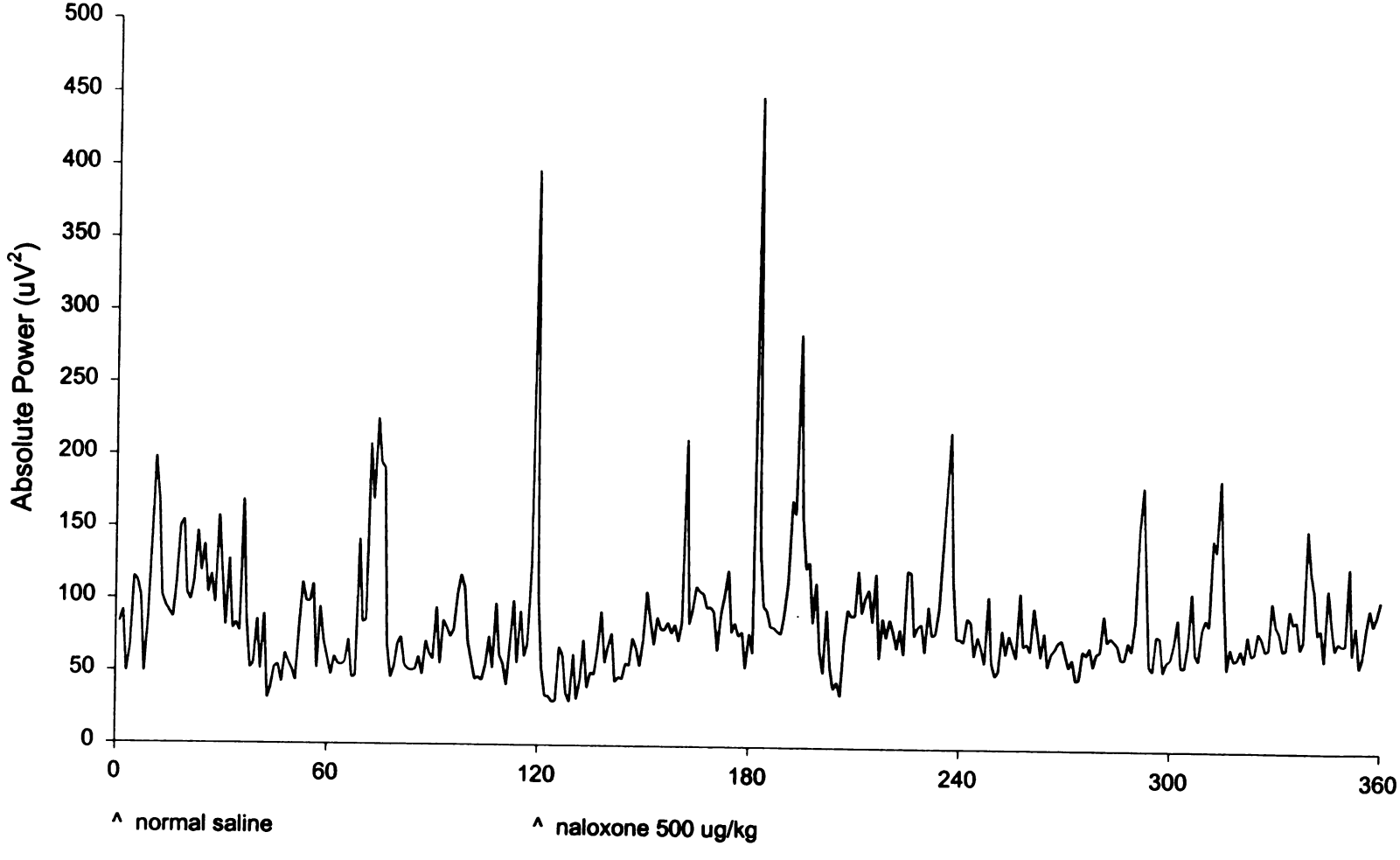


Figure 71: Effect of Naloxone 500 ug/kg on Peak, Median & Edge Frequencies (n=16 for 8 animals)

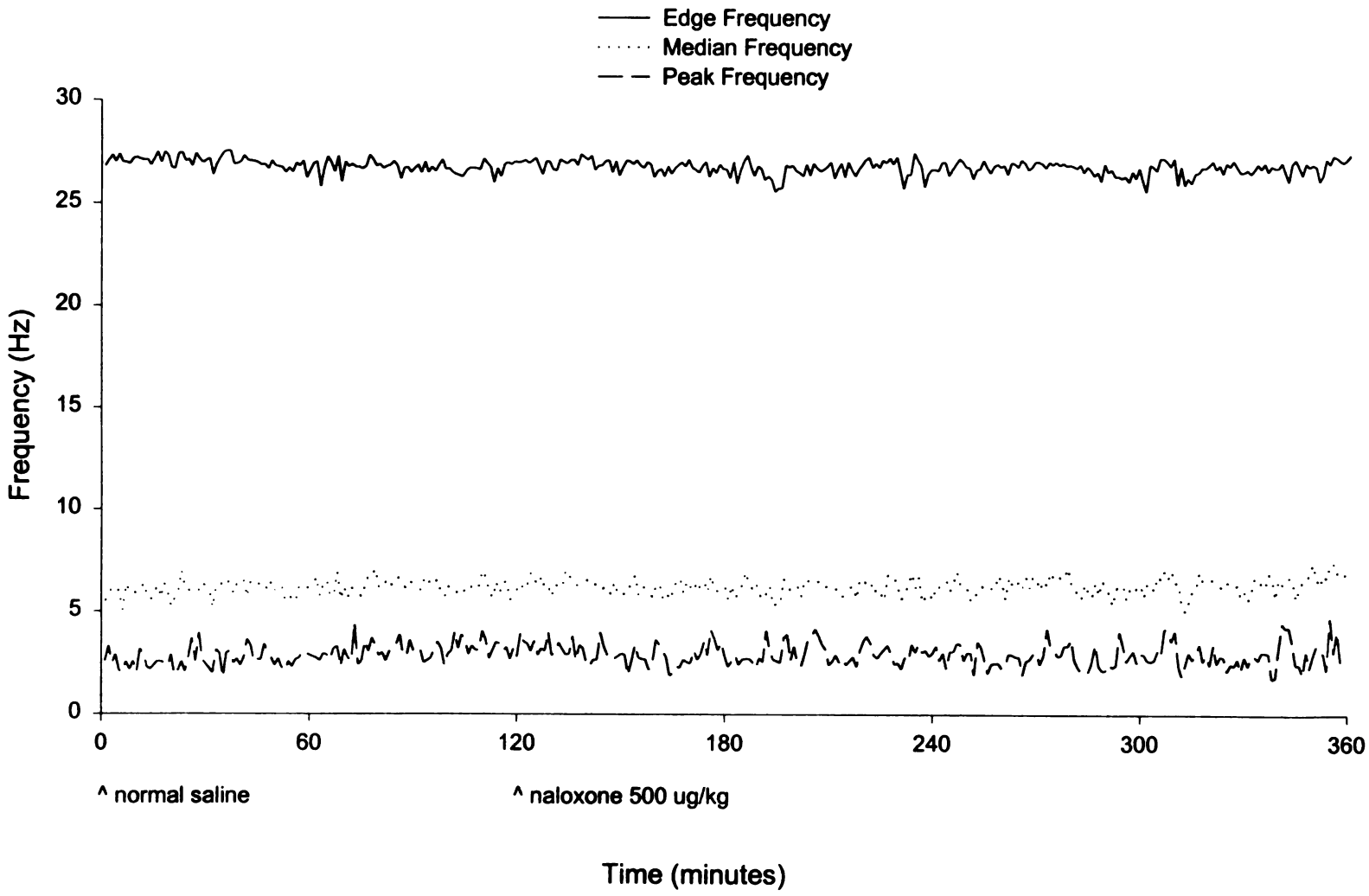


Figure 72: Effect of Naloxone 500 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 0800-1000 (n=16 for 8 animals)

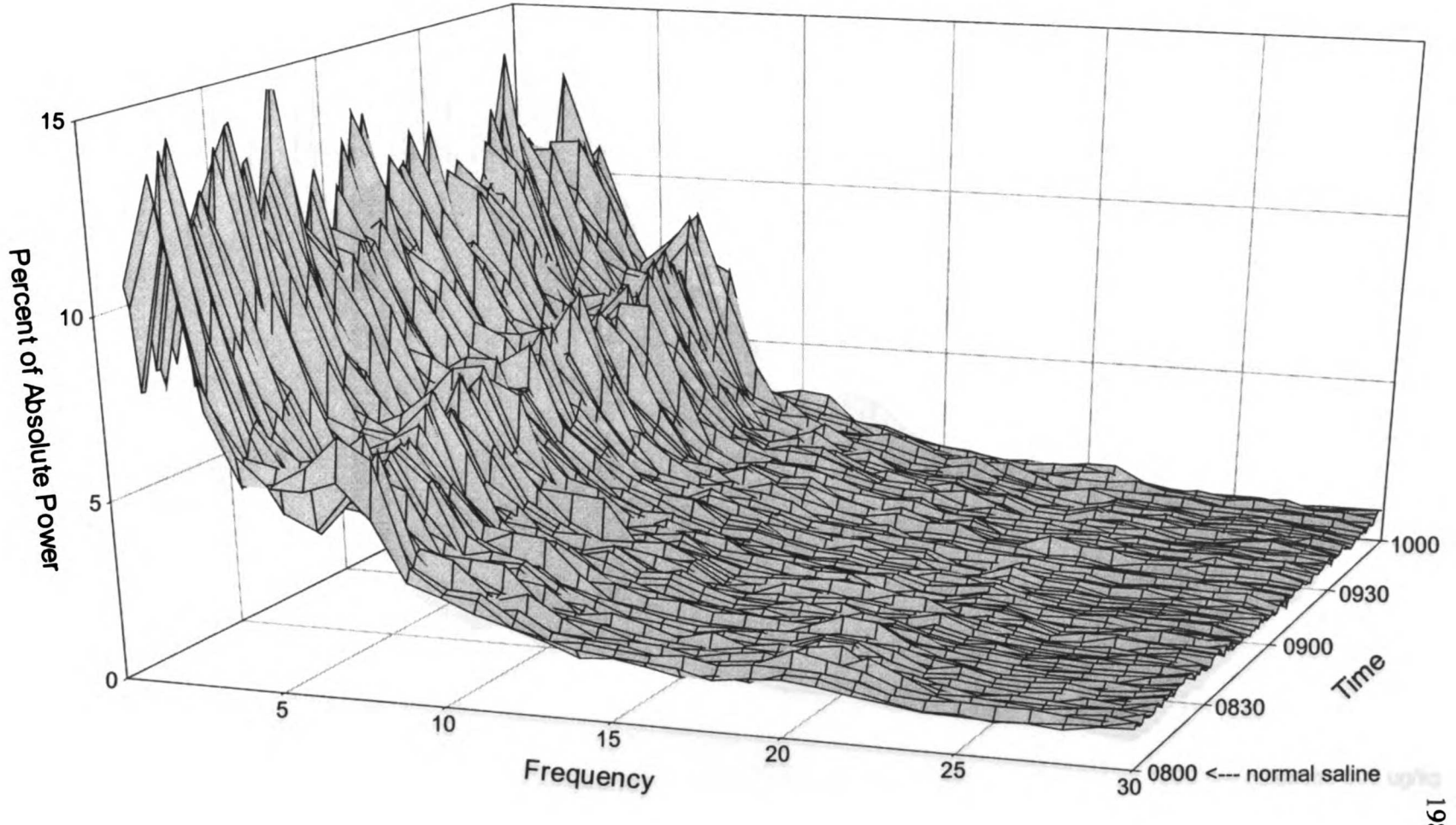


Figure 73: Effect of Naloxone 500 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1000-1200 (n=16 for 8 animals)

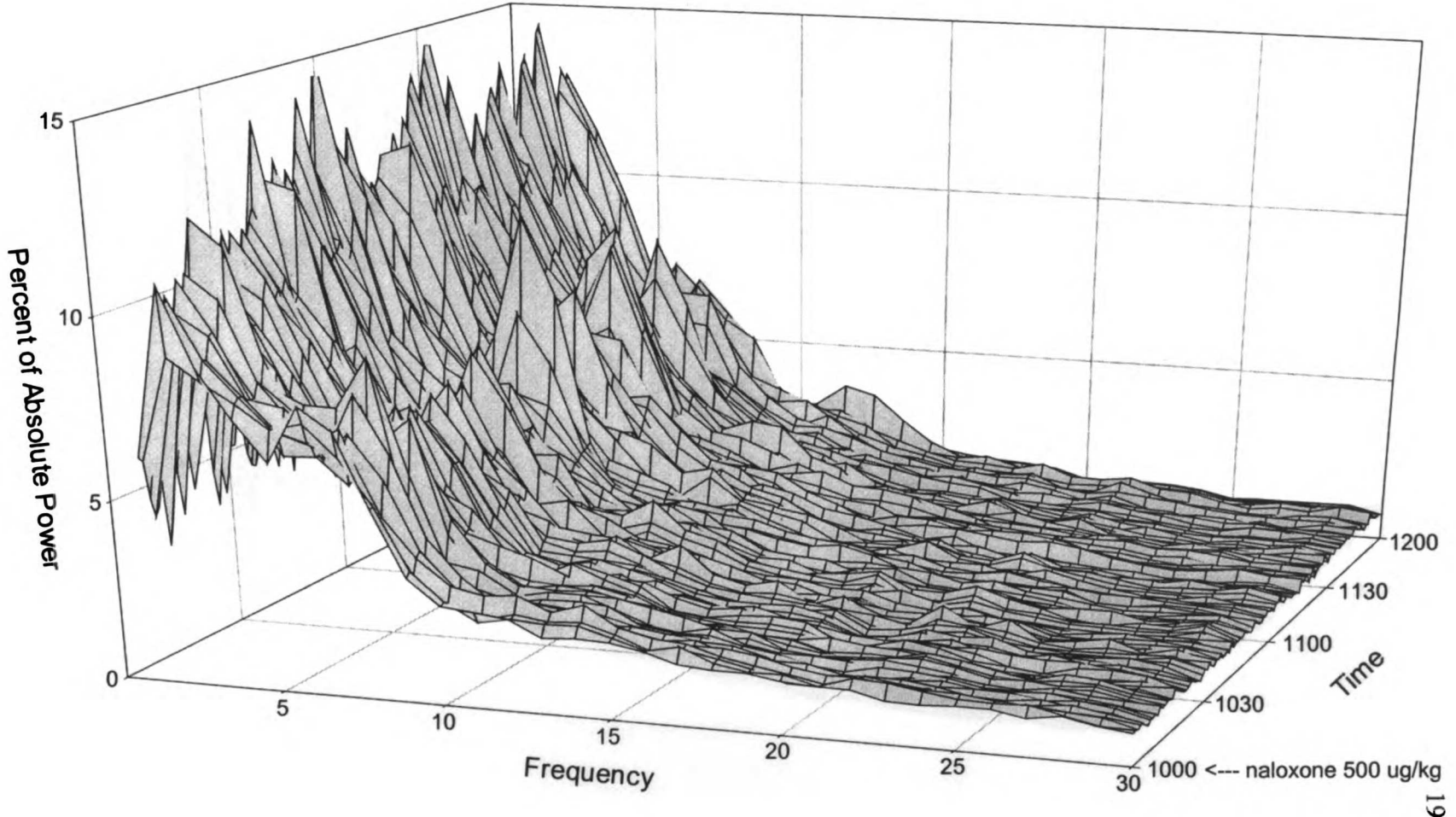


Figure 74: Effect of Naloxone 500 ug/kg on Percent of Absolute Power Attributable to Individual One Hz Frequencies Over Time, 1200-1400 (n=16 for 8 animals)

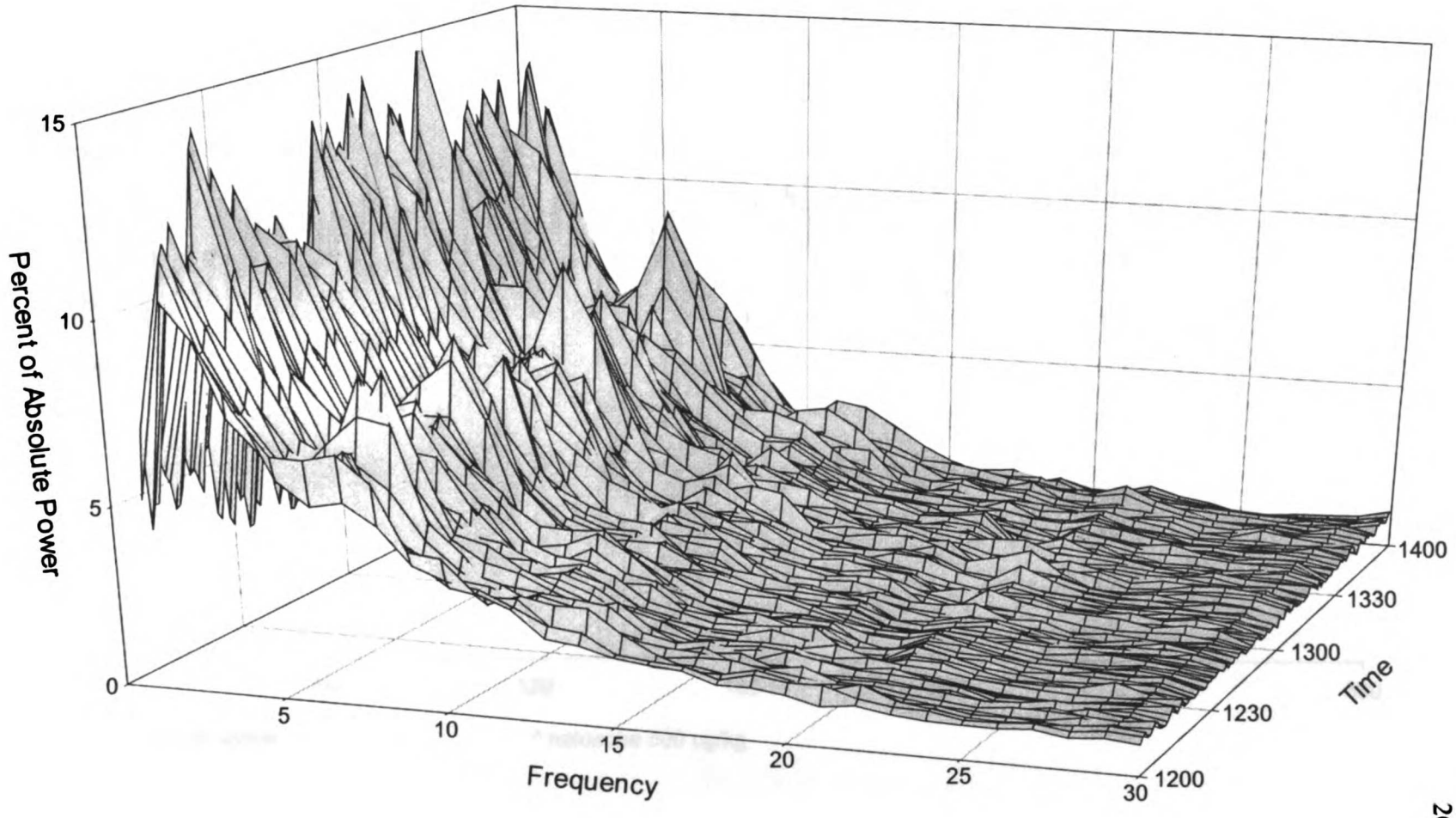


Figure 75: Effect of Naloxone 500 ug/kg on Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)

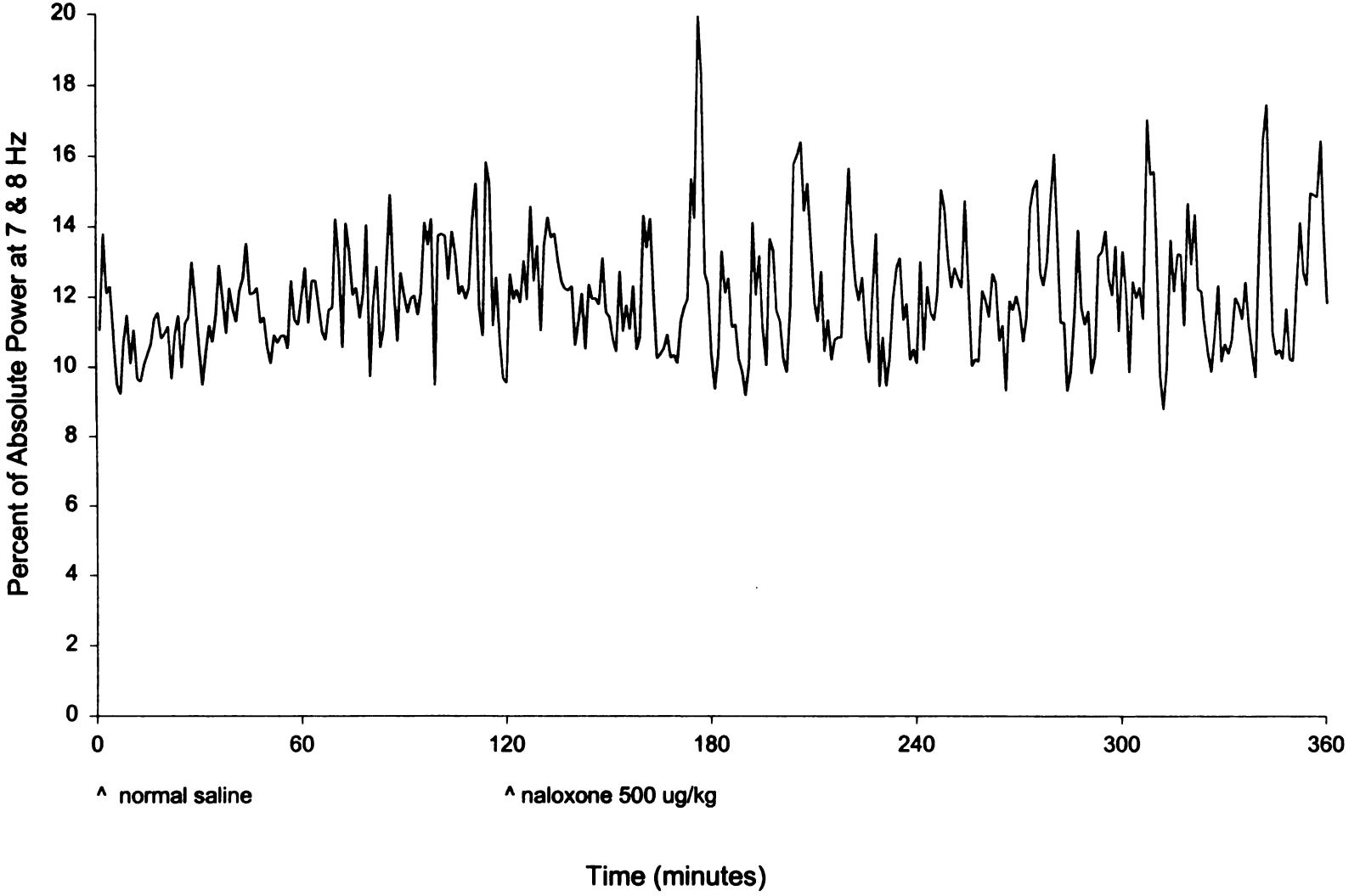
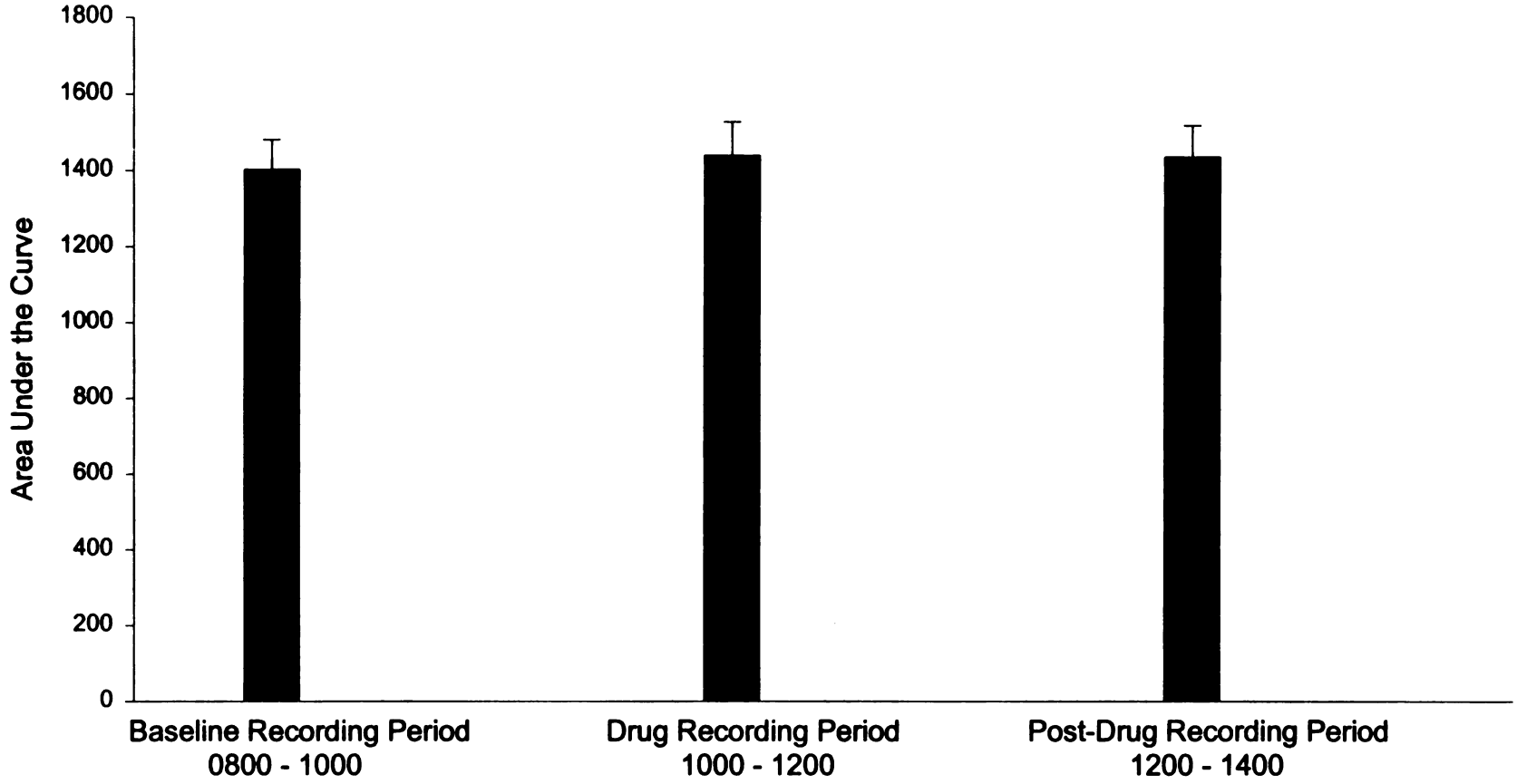


Figure 76: Effect of Naloxone 500 ug/kg on Area-Under-the-Curve for Percent of Absolute Power at 7 & 8 Hz (n=16 for 8 animals)



Appendix A: University of California San Francisco Animal Care Committee Approval

COMMITTEE ON ANIMAL RESEARCH
Office of Research Affairs, Box 0962
University of California, San Francisco

CAR APPROVAL LETTER
Project # 95011249

January 19, 1996

Christine Miaskowski, Ph.D.
Box 0610

Dept.: Physiological Nursing
Phone No.: x9407

Study Title: Opioid-Induced Changes in Consciousness in Rats

APPROVAL NUMBER: A7025-11249-02

Approval Date: 01/16/96
Expiration Date: 02/15/97

This number is a UCSF Committee on Animal Research (CAR) number which should be used for ordering animals for this study. This number may only be used by the principal investigator and those listed as participants included in the protocol and should be referenced in any correspondence regarding this study. The committee must be notified in writing of any changes to the approved protocol including changes in personnel.

Please distribute the final approved protocol to all individual participants so that they are familiar with the procedures that have been approved. Please remember that all personnel are to be fully trained before undertaking any procedures independently.

If you have any questions, please contact the Committee on Animal Research office at (415) 476-2197, Suite 11, Laurel Heights or Box 0962.

SPECIES NAME	TOTAL NUMBER APPROVED		
	Category A	Category B	Category C
Rats	0	66	66

Michael A. Heymann, M.D., Chairman
Committee on Animal Research