BEHAVIOURAL AND ELECTROENCEOPHALOGRAPHIC EFFECTS OF INTRACEREBROVENTRICULAR ADMINISTRATION OF β-ENDORPHIN IN NAIVE AND KINDLED RATS

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Abstract

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The epileptogenic effects of intracerebroventricular administration of β-endorphin was studied in non-kindled and electrically kindled rats. Three groups of rats were used, with stimulating/recording electrodes implanted in the ventral hippocampus, caudate-putamen or dorsomedial thalamus. Both behavioural and electroencephalographic (EEG) effects of β-endorphin were evaluated in these animals, before and after kindling. The three groups followed a similar time course for the development of kindled seizures. The hippocampus was found to require the lowest stimulus intensity for eliciting afterdischarges in the EEG, and this structure was also the most responsive to the ictal effects of β-endorphin administration. β Endorphin failed to elicit behavioural convulsions in kindled animals, even though EEG seizure activity was present. After kindling, β-endorphin-induced wet dog shakes were found to decrease in frequency while flinching (myoclonic jerks) and spiking in the EEG were increased. It is concluded that electrical kindling somewhat alters the behavioural and electrophysiological effects of β-endorphin and that dissociation can occur between limbic epileptiform EEG activity and behavioural convulsions in kindled rats.

Acknowledgements

With thanks to Roy Wise for his advice and patience; to my family, friends and colleagues for their constant encouragement; and to the Research Management of Merck Frosst Laboratories for that most precious of all commodities, time to pursue my studies.

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Introduction

The endogenous opiate peptidergic system:

The opioids dihydromorphine and etorphine and the opioid antagonist naloxone bind selectively to specific tissues with peripheral autonomic innervation, suggesting specific "recognition sites" where these agents may interact with neural substrates. Limited binding of these substances suggest a finite number of binding sites in a given tissue. Only the biologically active stereoisomers bind, indicating that the binding sites can discriminate between chemically similar but functionally dissimilar molecules (Kosterlitz and Hughes, 1978). Similar specific opiate binding sites have been discovered in many species of vertebrates, in both brain and spinal cord, with differing densities of receptor populations in specific areas. Since these binding sites or "receptors" evolved long before man's use of opiates, it was reasoned that substances with similar binding characteristics, and perhaps similar physiological activity to opiates might reside naturally in the brain and might act normally at these receptor sites. The fact that electrical brain stimulation in certain brain regions can cause morphine-like analgesia fits with the view that there are natural brain circuits which can function to suppress pain (Akil and Liebskind, 1975) and the fact that the opiate antagonist naloxone reverses the electrically-induced analgesia suggested that the target of opiate action is in such endogenous opioid neural circuits.

Using the known properties of *in vitro* isolated tissue preparations such as the guinea pig ileum and mouse vas deferens, which respond to morphine and other opiates, and the reversal of such opiate effects by naloxone, it was possible to use these test systems to study various brain extracts for potential opiate-like activity. Terenius and Wahlstrom (1974) obtained evidence for the presence of endogenous peptides with affinities for the opiate receptors and in 1975 the structures of the 5-amino acid peptide enkephalins were established. Within a short time it was also found that the amino acid sequence of one of the enkephalins corresponded with a portion of the 91 amino acid chain which comprises the hypothalamic prohormone β-lipotrophin, and that the 5-amino acid fragment, methionine-enkephalin had a high affinity for the opiate receptor (Bradbury, *et al.*, 1976). Subsequently, the amino acid sequences of several peptides with opiate-like activity were determined, permitting their extraction or synthesis thus making them available for physiological and pharmacological study.

Distribution of the short, 5-amino acid enkephalins, and the longer chain endorphins in the central nervous system is uneven and there are thought to be subtle differences between their physiological effects, even though they share many common features of pharmacological activity (e.g. analgesia, depression of respiration, release of prolactin, etc.).

It has been suggested that the rapid onset and termination of the activity of the enkephalins makes them good candidates as neurotransmitters, while the slower onset and degradation of β-endorphin, and its presence in high concentrations in the hypothalamus, suggests a possible endocrine control function (Kosterlitz and Hughes, 1978). However, as yet the specific functions of the opiate peptides are still only speculation.

Actions of β-endorphin

Profound analgesia, catalepsy, motor effects and epileptogenesis have been attributed to the opiate peptides upon intracerebral and systemic administration, leading to speculation that the physiological effects of these agents may be related to their possible roles in pain modification or the development of schizophrenia and epilepsy. In addition to morphine-like behavioural effects, iontophoretic application of β-endorphin directly to cells in various areas of the brain has been found to result mainly in reduced cell activity. This has been found to be the case for cells in the cerebral cortex, the lateral reticular nucleus of the brainstem, caudate nucleus and thalamus. The exception has been found to be hippocampal pyramidal cells which are excited rather than inhibited by β- endorphin. This inhibition occurs whether β-endorphin is administered iontophoretically, by microinjection into brain tissue or cerebral ventricles, or by systemic administration

(Bloom, et al., 1976) and it has been reported that this change in cell excitation results from β-endorphin induced inhibition of an inhibitory interneuron (Siggins, et al, 1978; Zieglgansberger, et al., 1978).

Systemic injection of high doses of β-endorphin, or microinjection into several brain sites have been found to cause epileptiform activity without behavioural convulsions (Bloom, 1978). Also, intracerebroventricular administration in rats produces epileptiform electroencephalographic (EEG) changes at extremely low concentrations (Henriksen, et al., 1978) and this effect can be reversed or prevented by administration of the opiate antagonist naloxone. It has thus been suggested that the endogenous opiate peptides, particularly β-endorphin, may function as epileptigens (Urca, et al., 1977; Wise et al., 1978) since the epileptic EEG effects are seen at doses below those required to induce analgesia or catalepsy (Bloom et al, 1978). The study of this epileptigenic effect of β-endorphin is particularly important if this and related substances are to be considered useful in terms of their potent analgesic effects. Also, an understanding of the epileptigenic activity of β-endorphin is a necessary preliminary to attempts to understand other more behavioural effects.

Electrical kindling

Low level electrical stimulation of various limbic and cortical structures can also cause epileptiform EEG activity without behavioural convulsions (Racine, 1972a). The epileptiform afterdischarge activity induced by electrical stimulation resembles that seen following intracerebroventricular or systemic administration of β-endorphin. Chronic low intensity electrical stimulation of susceptible limbic and cortical sites has been found to produce permanent synaptic alterations as evidenced by progressively decreasing thresholds for afterdischarges and the appearance, after several stimulations, of psychomotor behavioural convulsions (Racine, 1972a, Goddard, et al., 1969). This effect of chronic application of electrical stimulation has been termed "kindling".

With initial electrical stimulation, afterdischarges are induced at the stimulation site. These afterdischarges are relatively short and do not spread to adjacent or remote brain areas. As kindling progresses the afterdischarges become longer in duration, more complex in form, and propagate to contralateral limbic structures. At this stage the initial signs of behavioural convulsions are observed. Also, the stimulation threshold for eliciting afterdischarges is progressively decreased (Racine, 1972a). The behavioural convulsions at first appear as arrest and masticatory movements immediately following

stimulation. As daily stimulations continue the motor phenomena progress through specific phases of rearing, forepaw clonus and finally full psychomotor seizures with loss of righting reflex. After several months of stimulation animals can become susceptible to spontaneous seizures in the absence of electrical stimulation (Pinel, et al., 1975).

At least two separate and permanent synaptic changes appear to develop as a result of kindling. First, a significant and long term decrease in afterdischarge threshold, which can be 50 per cent or more, can occur in response to only one second of stimulation per day for several weeks (Racine, 1972b). This permanent lowering of the afterdischarge threshold is neither prevented nor reversed by treating the animals with anticonvulsant drugs such as diazepam or phenobarbital (Wise and Chinerman, 1974).

Second, propagation of the afterdischarges to contralateral structures and the triggering of behavioural convulsions also reflects a permanent synaptic change. These differ from the lowering of the afterdischarge threshold in that they can be prevented by pretreatment with anticonvulsant drugs (Racine, 1972b; Wise and Chinerman, 1974). The propagation of the afterdischarges and eliciting of convulsive behaviours is thus independent of the decrease in afterdischarge threshold.

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These phenomena are of general interest because of the progressive nature of the EEG changes and development of convulsions, and the apparent permanence of the synaptic alterations involved which may be related to clinical epilepsy and other progressive neural disorders. They are of special interest here because of the similarities between the effects of limbic stimulation and those of intracerebroventricular injections of endorphins and enkephalins. β-Endorphin, like low intensity electrical stimulation, causes bursts of electrical activity, or ictal episodes, in the limbic system, without concomitant behavioural convulsions and it is of interest to discover what relationship, if any, exists between the peptide effects and electrically-induced epileptiform EEG effects.

Although acute administration of β-endorphin causes epileptiform EEG effects, chronic administration has not been shown to induce progresssive seizure development such as occurs following chronic electrical stimulation (Wise, et al., 1978). Also, previous treatment with β-endorphin for 11 days prior to amygdaloid kindling did not facilitate the subsequent development of complex afterdischarges or convulsive behaviours during kindling. Conversely, administration of β-endorphin to animals already kindled by amygdala stimulation failed to initiate behavioural convulsions even though limbic ictal episodes were present. Thus, it would appear that simple initiation of ictal

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episodes is insufficient in itself to trigger convulsions even in fully kindled animals.

It has been reported that in amygdaloid kindled rats, administration of morphine enhances both ictal episodes and behavioural seizures, and these effects are reversed by naloxone (LeGal LaSalle, et al., 1977). So, while prior treatment with β-endorphin was not found to accelerate amygdaloid kindling, it would appear that kindling can potentiate some aspects of the seizure activity of morphine, Thus, there is some evidence that kindling can alter an animal's responsiveness to epileptigenic effects of opiates.

It is thought likely that the epileptigenic and analgesic effects of opiates are subserved by different anatomical sites and different receptors. Frenk, et al (1978[a]) have reported that endorphin microinjection into the periaqueductal gray area causes analgesia without limbic epileptiform activity, while injections into medial-thalamic nuclei cause epileptiform effects without analgesia.

Also, although transfer between kindling sites occurs it does not occur uniformly. Amygdaloid kindling can facilitate subsequent hippocampal kindling, but it has been reported that hippocampal kindling does not facilitate, and may even retard subsequent amygdaloid kindling. Therefore it is possible that kindling can alter the effects of β-endorphin if the right site is kindled.

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The present study was therefore designed to consider the possibility that kindling at sites other than the amygdala can affect subsequent ictal responses to intracerebroventricular administration of β-endorphin, and conversely to see if β-endorphin-induced afterdischarge activity would activate behavioural seizures in animals kindled at sites other than the amygdala. The sites chosen were ventral hippocampus, caudate-putamen and dorsomedial thalamus. The hippocampus has been found to be extremely susceptible to the kindling procedure, even though a longer time course of stimulation is required for the development of seizures (Goddard, et al., 1969). The hippocampus has been reported not to contain endorphins (Hughes, 1975); Terenius and Wahlstrom, 1974) or a significant density of opiate receptors (Pert, et al., 1975), but this area is unique in that endorphins have been reported to excite hippocampal pyramidal cells while other areas studied were, as a rule, uniformly inhibited (Bloom, et al., 1978).

The caudate has been reported to have a high threshold for afterdischarge, but if afterdischarges are elicited, kindling proceeds with moderate speed (Goddard, et al, 1969). The involvement of this area in the motor aspects of behavioural convulsions makes it an area of interest for the study of the susceptibility of animals kindled at this site to subsequent administration of β-endorphin.

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Methods:

Surgical preparation:

Under pentobarbital anesthesia (Nembutal 50 mg/kg i.p.) thirty adult female Sprague Dawley rats (Canadian Breeding Laboratories) weighing 225-260 grams were implanted with bipolar stimulating-recording electrodes (Plastic Products). Three groups of 10 rats each were implanted with electrodes in (1) ventral hippocampus (AP 1.8, left lateral 5.0, 6.0 mm from the dorsal skull surface), (2) caudate-putamen (AP 7.8, left lateral 3.5, 5.0 mm from the dorsal skull surface) or (3) dorsomedial thalamus (AP 5.2, left lateral 1.0, 6.0 mm from the dorsal skull surface). In all cases a ground wire was attached to a skull screw. In addition, each animal was implanted with an intracerebral guide cannula (Plastic Products) through which an inner cannula could be inserted for drug administration into the lateral ventricle. A dummy cannula was kept in place except during injections in order to keep the opening patent. Each rat was individually housed and allowed to recover for two weeks following surgery. During the recovery and early experimental periods 2 rats from each group developed infections or died so that the experimental groups were finally composed of 8 rats each.

Electrical stimulation (kindling)

Afterdishcarge thresholds: At least 24 hours after administration of the pre-kindling β-endorphin (see below) all rats were electrically stimulated with a 1 second train of biphasic square wave pulses, with 1 millisecond pulse width at 100 Hz. Monitoring of

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Seizure development (kindling)

Each animal was stimulated daily for 1 second between 1600 and 1900 hours at its pre-determined afterdischarge threshold, until kindled psychomotor seizures occurred on three consecutive days. A full seizure was determined by the presence of facial clonus, rearing and falling with forelimb and/or hindlimb pedalling. The number of afterdischarge days to the first full seizure was noted, with the two days of afterdischarge threshold determination included as Days 1 and 2. EEG was recorded before and after each stimulation and progressive behavioural changes which developed as a result of the repeated electrical stimulation were also noted.

Administration of β-endorphin

Prior to kindling, each rat was administered 10 microliters (µl) of sterile saline intracerebroventricularly (icv) and the electroencephalographic activity was recorded from the hippocampus, caudate or thalamus, for 15 minutes following injection. The following day each animal was given β-endorphin (10 micrograms/ 10 µl icv) dissolved in sterile saline and the EEG activity was recorded for 15 minutes after injection. A second icv injection of β-endorphin (10 µg) was administered at least 3 days after the first administration. In fully kindled animals β-endorphin (10 µg) was re-administered at least twice - first, the day after the determination of post-kindling afterdischarge thresholds and again at least three days later. In each case a 15 minute EEG recording was made following administration of the peptide.

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Changes in locomotor activity were assessed subjectively and note was taken of unusual behaviours such as scratching, biting, "wet dog shakes" or abnormal posturing.

Also, at the end of the 15 minute observation period, analgesia was roughly estimated by presence or absence of vocalization in response to manual pinching of one hind foot. Animals were tested for the presence of catalepsy by placing the forepaws on a horizontal bar 10 cm above the surface of the bench. An animal was considered to be cataleptic if it remained in position for 10 seconds.

After kindling, several animals in each group were given naloxone (10 mg/kg i.p.) either before or a few minutes after the administration of β-endorphin.

For all β-endorphin administrations and electrical stimulations, the animals were placed in a plexiglass cylinder (12 inches in diameter and 12 inches high) in which they could move freely and easily be observed.

Histology

Animals were sacrificed with an overdose of pentobarbital (Nembutal) injected intraperitoneally. Brains were injected (icv) with 10 µl of a 0.1% solution of toluidine blue to verify cannula placement. The brain was then removed, fixed in 5% formalin for 24 to 48 hours and sectioned to establish electrode placements. Sections were viewed under a stereomicroscope (10X) with a calibrated reticule in the eyepiece. Location of the

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electrode tip was determined and noted on a reproduction of the histological section (Pellegrino and Cushman, 1967).

Seven of the hippocampal electrodes were placed in the ventromedial (dentate) area of the hippocampal formation. One (H-5) was found to also impinge on the lateral geniculate body.

All of the thalamic electrodes invaded the area of dorsomedial nucleus, and the caudate-putamen electrodes were situated in the mid to lateral area of this structure (Figure 1).

RESULTS

Seizure development:

The behavioural responses to electrical stimulation varied between the three groups of rats. Initial stimulation of the hippocampus caused an arrest behaviour, with the animal assuming a hunched posture and staring. Stimulation of the caudate and thalamus however, caused violent ipsilateral muscle contraction which resulted in facial grimacing, flexion and occasionally clonus of the ipsilateral fore and hindlimbs and in many instances complete rotation about the body axis. These motor effects only occurred during the one second stimulation period. The post-stimulus posture was also very different from that seen in rats receiving hippocampal stimulation in that the body was flattened on the floor of the cage rather than hunched up. Shortly after the stimulation, these animals began calm exploratory behaviours, in most cases with mild to severe ataxia and some with continuous 'teeth chattering'.

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resemble the pattern of motor activity seen as the kindled seizures developed and did not appear to interfere in any way with the subsequent kindling procedure which progressed through the defined stages of myofacial and forelimb clonus, rearing and falling.

Animals stimulated in the hippocampus exhibited the expected development of kindled seizures without any severe motor effects of the electrical stimulation itself. Wet dog shakes were seen in all animals 1 to 2 minutes after hippocampal stimulation. The wet dog shakes varied in frequency from one animal to another but were consistent for each animal from day to day. However, as motor seizure activity progressed the incidence of wet dog shakes decreased and finally disappeared in all the rats.

None of the animals in the other two stimulation groups exhibited wet dog shakes in response to electrical stimulation.

Differences were observed in the time of onset and duration of behavioural seizure activity. In animals stimulated in the hippocampus, there was a latency of from 12 to 30 seconds from the time of stimulation until the animals displayed rearing, clonus and falling. The animals stimulated in the thalamus developed full seizures within 3 to 10 seconds after stimulation and those stimulated in the caudate responded most rapidly with full seizure behaviours evident within 2 to 5 seconds after stimulation. The duration of the seizures were similar for the hippocampal and thalamic groups of animals, lasting from 20 to 45 seconds while the seizures elicited by caudate stimulation only lasted from 5 to 20 seconds.

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The stimulation site also determined the behaviour of the animals following a fully kindled seizure. Those animals which had been kindled in the hippocampus appeared to be sedated following the seizure, but were very sensitive to sound and touch and could not be easily handled until 5 to 6 minutes later. This behaviour pattern corresponds with that seen after kindling of the amygdala (Rackham and Wise, in press). Those animals stimulated in either the caudate or the thalamus, however, were active once the seizure was over and could be readily picked up at that time.

No significant difference in the number of days required for kindling were observed between the hippocampus, thalamus or caudate stimulated groups of animals (Table 1). The median number of days to full seizure were 22 for the hippocampal group, 28 for the caudate group and 23 days for those animals stimulated in the thalamus.

Electroencephalographic (EEG) activity during kindling:

Afterdischarges:

Afterdischarges (AD's) elicited from the hippocampus began during the stimulation, with high frequency, high amplitude spiking which changed polarity after approximately 10 seconds and gradually decreased until the EEG became silent (20 to 60 seconds after stimulation). A second set of AD spikes occurred in seven of the eight rats at from 65 to 90 seconds after stimulation. The delayed AD's were unidirectional, low frequency (1.1 to 2.4 Hz) high amplitude spikes whose duration was more variable than that of the initial

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set of AD's (Figure 2, Table 2). The pattern and duration of AD's did not vary significantly from the start of stimulation to the development of full behavioural seizures. During stimulation of the hippocampus the animals 'arrested' in a hunched posture (back arched somewhat and back feet slightly splayed). During the AD period the animals were staring with some masticatory movements. As the AD's finished and the EEG became silent there was a period of up to one minute of agitated exploration, with a great deal of climbing and sniffing and grooming behaviours. In some animals a second arrest period preceded the onset of the delayed (rebound) AD's but usually the behaviour was still that of exploration, interspersed with grooming and wet dog shakes. The wet dog shakes ceased after the delayed AD's and the animal returned to exploratory activity.

Rat H-5 did not exhibit the delayed AD's and in this animal the AD threshold was found to be higher than for the other animals in the hippocampal group. It was noted at the end of the experiments that the electrode in this rat was partially in the lateral geniculate nucleus which could account for the slightly differing EEG pattern.

In the animals stimulated in the thalamus, initial AD's were very short but became more complex and of longer duration (25 to 45 seconds) as kindling progressed (Figures 2 & 7; Table 3).

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Caudate stimulation elicited only very short AD's, lasting from 2 to 20 seconds (Figures 4 & 8; Table 3). Primary AD's from the caudate, but not thalamic stimulation were followed by a short 'silent' period (approximately 15 seconds) and then some spiking and slow wave activity occurred before the EEG returned to pre-stimulation baseline (Figure 4). This pattern somewhat resembled that seen following hippocampal stimulation (i.e. AD's - silent period - spiking) although the time course and EEG frequencies were different.

In kindled animals, the thalamic and caudate groups had behavioural seizures only during the primary AD period (Figures 3 & 4). In the hippocampus group, however, behavioural seizures generally began in the latter half of the primary AD period and often continued during the hippocampal EEG silent period (Figure 2).

Afterdischarge Thresholds:

Initial (pre-kindling) afterdischarge thresholds for animals undergoing hippocampal stimulation were significantly lower than those for caudate or thalamus stimulation. Afterdischarge thresholds were reduced in all three groups after three kindled seizures had occurred (Table 4). The group stimulated in the thalamus had the smallest decrease in afterdischarge threshold (27%) while the hippocampal and caudate afterdischarge thresholds decreased an average of 42% and 47% respectively.

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Effects of intracerebroventricular injection of β-endorphin

Behavioural effects: With one exception, no signs of behavioural convulsions were seen after β-endorphin injections in any of the animals despite full ictal EEG responses as noted below. The one rat which did have a β-endorphin induced convulsion had been kindled in the hippocampus. Convulsions were only observed following the first post-kindling administration of β-endorphin; no convulsion occurred after a second peptide injection in the same animal even though ictal episodes were recorded in the EEG. No animals in the other two experimental groups had β-endorphin induced convulsions, either before or after kindling.

Increased exploratory activity including climbing the wall of the observation chamber, and exaggerated sniffing at the air and at the wire mesh floor, as well as suppression of corneal and pinna reflexes were observed in the majority of β-endorphin treated animals, both before and after kindling. These behaviours were present throughout the 15 minute post-injection observation period. Some signs of catalepsy, ataxia, teeth chattering, arrest in the 'kangaroo posture', biting at the paws, or chewing were also observed but these effects occurred in less than half the animals in response to the injection of β-endorphin. No measureable analgesia was observed with this dose of β-endorphin.

Wet dog shakes were observed in 12 of the 24 rats before kindling but in only 6 of the animals after they had been kindled (Table 5). As already mentioned, wet dog shakes were also observed following hippocampal stimulation but failed to be elicited as the animals developed the kindled seizures.

Scratching with one or both hind feet, unlike wet dog shakes, increased in occurrence in animals that had been kindled. Before kindling 9 of the 24 animals were observed to scratch after injection of β-endorphin, but after kindling 15 of the animals exhibited this behaviour (Table 5).

Prior to kindling, one animal in the hippocampus stimulated group exhibited a 'flinching' response, which corresponded with spiking in the EEG. After kindling, however, 21 of the 24 animals were observed to flinch after injection of β-endorphin, and in 20 of these animals the flinches corresponded with spiking in the EEG (Table 5). The flinching behaviour somewhat resembled a mild startle response, such as that elicited by blowing in a rat's face. This was a mild response but is of interest in that its occurrence does seem to be related to the fact that the animals had been kindled.

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Ictal episodes: In non-kindled animals, the hippocampus was found to be more susceptible to β-endorphin induced ictal episodes and spiking in the EEG than either the caudate or

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Ictal episodes: In non-kindled animals, the hippocampus was found to be more susceptible to β-endorphin induced ictal episodes and spiking in the EEG than either the caudate or

thalamus (Figures 5, 7, 8; Table 6). Six of the eight animals from which hippocampal recordings were made exhibited ictal episodes after injection of β-endorphin, while no animals in the caudate group, and only 2 in the thalamic group had this response. However, in 5 of the caudate and in 2 of the thalamic animals, slow wave EEG was observed after the administration of β-endorphin.

After kindling the number of animals having β-endorphin induced ictal episodes from the hippocampus was reduced to 3 out of 8 compared with 6 out of 8 before kindling. As already mentioned only one of these kindled animals had a psychomotor seizure in response to β-endorphin administration. As observed before kindling, no animals in the caudate group showed ictal responses to β-endorphin even after kindling. In the group of rats from which thalamic recording was made, only one had β-endorphin induced ictal episodes after kindling and these did not trigger a behavioural convulsion in this animal.

Spiking induced by β-endorphin

Before kindling, rhythmic spiking was observed in all eight rats in the hippocampal group and in three and four rats in the caudate and thalamus groups respectively (Table 6, Figures 5, 7, 8). The number of rats with spiking in the hippocampal EEG did not significantly change after kindling while in the other two groups the numbers of animals with spiking in the EEG increased after kindling (Table 6). As

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previously discussed, β-endorphin induced a flinching response in kindled animals which occurred simultaneously with the spiking EEG activity (Figures 6, 7, 8).

EEG effects of β-endorphin in the hippocampus

Since the hippocampus seemed particularly susceptible to the epileptogenic effects of β-endorphin, a more detailed analysis of these effects was made. Before kindling, the latency for the onset of ictal episodes ranged from 2 to 7 minutes after injection of β-endorphin. After kindling, of the three animals which responded to β-endorphin with ictal episodes, there was some indication of a delay in the onset of the response (Table 7), although the small number of animals responding makes it difficult to know if this delay is significant. One rat had a psychomotor seizure following the first post-kindling administration of β-endorphin but this was not repeated after a second administration and did not occur in the other two responding animals.

The pattern of ictal episodes in the hippocampus in response to β-endorphin was observed to correspond with that elicited by electrical stimulation; that is, an initial burst of ictal activity followed 1 to 2 minutes later by a second set of 'delayed' afterdischarges, usually of higher amplitude and lower frequency than the initial episode (Figure 5). The ictal episodes and spiking in the EEG could always be abolished or prevented by the intraperitoneal administration of naloxone (10 mg/kg)(Figure 9).

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Discussion

Kindling: Although the purpose of this study was not to describe the kindling *per se*, the obtained results do confirm others reported in the literature while expanding the findings of Goddard *et al.* (1969) with regard to stimulation of the caudate. Goddard reported that the caudate was variable in its ability to be kindled. Since the EEG was not recorded it is probable that the caudate was being stimulated at an intensity which did not initially elicit the afterdischarges which are essential for reliable kindling (Racine, 1972b). In this case the number of days to seizure would be significantly longer than for structures with much lower afterdischarge thresholds such as the hippocampus or the amygdala. In the present study the caudate, thalamus and hippocampus were stimulated at their individual afterdischarge threshold so that afterdischarges were elicited from the first day of stimulation. This could account for finding no significant difference between the number of days to kindling from either the hippocampus, caudate or thalamus. The afterdischarge thresholds and number of days to elicit seizures from the hippocampus in this study correspond with those reported by Racine *et al*, 1977). Also, the shorter afterdischarge duration and seizures observed from thalamic stimulation in this study have been reported by Cain and Kilbreath (1978). The response pattern to stimulation of the hippocampus, while illustrated in a recent

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paper by Racine et al, 1977), a possible mechanism was not discussed. of interest that the motor seizures seen in the present experiments occurred for the most part during the period when the EEG was silent.

A recurrent inhibitory pathway has been reported between the basket and pyramidal cells of the hippocampus, as well as a weaker recurrent excitatory feedback pathway, especially in the dentate area (Lopes da Silva and Arnolds, 1978). These recurrent pathways could be contributing to the silent period and the delayed afterdischarges seen after hippocampal stimulation. It would be of interest to record simultaneously from several limbic and hippocampal sites in order to compare EEG activity in response to electrical stimulation and especially ongoing activity during behavioural seizures.

β-Endorphin in naive and kindled rats: Dissociation between limbic excitation and convulsions:

It was previously thought that the synaptic changes which occur as a result of kindling would render an animal susceptible to motor seizures in response to any stimulus or chemical agent capable of eliciting epileptiform spiking within the limbic system. The present results, and those from other studies with β-endorphin in kindled rats (Wise et al, 1978), would suggest that this is not the case. In this study effective breakdown of the synaptic barriers to motor seizures was obtained during the kindling procedure, as

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evidenced by the progressive development of psychomotor convulsions and reductions in afterdischarge thresholds. However, β-endorphin failed in all but one instance to elicit convulsive behaviours in these animals even though ictal episodes and abnormal spiking were seen in the EEG. A variety of behavioural effects were observed, both before and after kindling in response to administration of β-endorphin. These included exploratory activity, excessive grooming, licking, biting and scratching which have been reported by others (Iwamoto and Way, 1978; Ervin, et al., 1978; Bloom, et al., 1978). Wet dog shakes were observed in most animals before kindling, but did not occur as frequently in fully kindled animals. Some evidence of catalepsy was also observed following administration of β-endorphin.

The observed effects of β-endorphin could be a reflection of the simultaneous excitatory and inhibitory effects reported to occur within the central nervous system following administration of opiates (Domino, et al., 1974; Bradley and Dray, 1973, Seeber, et al., 1978). These combined excitatory and inhibitory actions of opiates are thought to contribute to the biphasic effects on locomotor activity seen after opiate administration. For example, morphine and the more potent etonitazine have been reported to excite extensor motorneurons while inhibiting flexor motorneurons (Seeber, et al., 1978).

Also, morphine administered iontophoretically to brain stem neurons can cause excitation as well as a longer lasting inhibition of neuronal firing (Bradley and Dray, 1973). These mixed depressant and stimulant actions are thought to contribute to the cataleptic and muscular rigidity effects of the opiates. It is possible that similar mechanisms are involved in the β-endorphin induced excitation of the limbic system which occurs without the expected motor effects in kindled animals. The EEG excitation could be accompanied by inhibition of the essential motor pathways required for expression of behavioural seizures.

Although the absence of convulsions in response to β-endorphin in kindled animals was surprising, it has been reported that dissociation can occur between epileptiform discharge in the EEG and behavioural convulsions in man (Jasper, 1972). For instance, during the rapid eye movement (REM) phase of sleep, epileptiform EEG activity has been recorded in the absence of behavioural convulsions. During REM sleep the recorded EEG normally resembles that of an awake individual, indicative of increased arousal relative to the other sleep phases. Increased levels of arousal are thought to inhibit convulsions in epileptic patients as well as decrease the amount of abnormal spiking seen in the EEG. The majority of temporal lobe epileptic seizures are reported to occur during non-REM sleep, a time when arousal is at its minimum (Halasz, 1972).

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In man, administration of morphine has been found to cause increased arousal and sleeplessness as well as EEG bursts which have no known behavioural correlates (Kay, et al., 1969; Kay, 1972). Increased arousal and altertness have also been reported from sleep studies in cats and rats following administration of morphine (Echols and Jewett, 1972; Khazan, et al., 1967). Possibly these opiate effects are also induced by administration of β-endorphin so that the arousal or alerting effects of the peptide are capable of suppressing behavioural convulsions in spite of excitation of the limbic system in seizure-sensitive animals.

Some of the effects of intracerebroventricular administration of β-endorphin appeared to be less severe in kindled than in non-kindled animals, especially wet dog shakes. Wet dog shakes were elicited in 50 per cent of the animals before kindling, but in only 25 per cent of the animals after kindling. It is possible that some type of tolerance to this behaviour developed during the kindling process inasmuch as it was observed to gradually disappear in the hippocampal group with successive electrical stimulations. The kindling procedure may have caused changes in pathways which are responsible for carrying out this response or caused changes in responsiveness of receptors which mediate this behaviour. Since the kindling process reduced the

ability of β-endorphin to induce the wet dog shaking behaviour, it may also have affected the peptide's effects on those motor systems involved in convulsive behaviours.

Another indication that kindling itself affected subsequent motor effects of β-endorphin was the dramatic increase in the number of animals which displayed β-endorphin induced flinching after kindling. Before kindling only one rat was observed to exhibit flinching, while in kindled animals flinching was elicited in over 85 per cent of the animals. Possibly the flinch response reflects initial stages of behavioural seizure activity in view of its rhythmic and repetitive nature and association with spiking in the EEG.

EEG effects of β-endorphin

Both before and after kindling the hippocampus was the most responsive area to administration of β-endorphin. Ictal episodes were accompanied by behavioural arrest and the delayed post-ictal psiking was associated with wet dog shakes in unkindled rats. Behavioural arrest was also seen in the animals in the thalamus- and caudate-stimulated groups even though ictal episodes were not observed in the EEG after administration of β-endorphin.

EEG spiking and behavioural flinching were observed in all groups after administration of β-endorphin. It is probable that a single 'generator' site is responsible for the EEG spiking

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and the observed ictal episodes. It has been reported that opiate induced EEG activity is not altered by lesions in the thalamus so that it is unlikely that this structure is the generator site for the observed EEG activity (Yeung, et al., 1978) Yeung postulates that the responsible area is possibly the reticular formation, septum, hippocampus or amygdala. However, lesions of the amygdala, as well as the pre-optic area of the hypothalamus, have also been reported not to affect β-endorphin induced EEG activity (Henricksen, et al., 1978).

Also, it has been reported that stimulation of hippocampal neurons by β-endorphin is not a direct effect but is a result of disinhibition of the pyramidal cells caused by drug induced suppression of inhibitory interneurons (Zieglgansberger, et al., 1978). Siggins et al (1978) have reported that the opioid peptide enkephalin antagonizes the recurrent inhibition from basket cells, possibly by reduction of the inhibitory effects of gamma-aminobutyric acid (gaba), thus causing excitation of the pyramidal cells. This antagonism of basket cell inhibitory firing could be a primary site for generation of β-endorphin induced EEG changes. It is also possible that the septum or reticular formation are the generator sites for β-endorphin induced EEG changes since alteration in the firing of these areas is known to affect EEG activity in the hippocampus (Macadar, et al., 1974).

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Inhibition of firing in the septal-hippocampal pathway can be induced by stimulation of the septum, reticular formation or lateral hypothalamus and results in changes in normal hippocampal theta activity (Maynert, et al., 1975)

Future experiments should perhaps pursue the possibility that the septum, or ascending reticular formation are primary targets for the observed effects of β-endorphin. Also, since the opioid peptides are known to have differential effects on a variety of neurotransmitter systems such as catecholamines and monoamines in normal animals it would be of interest to know if the same effects are observed in supersensitive kindled animals. Such studies into β-endorphin effects at particular sites and on particular transmitters could help to explain the apparent dissociation between EEG effects and motor activity in kindled rats.

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Table 1

The number of afterdischarge days required to elicit psychomotor seizures in rats*.

Stimulation site Rat number		Afterdischarge days to seizure
Ventral hippocampus		
H-1		31
H-2		28
H-3		28
H-4		31
H-5		16
H-6		23
H-8		20
H-9		28
	Median	28
	range	16-31
Caudate-putamen		
CP-1		29
CP-2		40
CP-5		18
CP-6		29
CP-7		29
CP-8		38
CP-9		23
CP-10		37
	Median	29
	range	18-40
Dorsomedial thalamus		
T-1		30
T-2		16
T-3		27
T-4		18
T-5		31
T-6		20
T-7		30
T-8		28
	Median	28
	range	16-31

* Each animal was stimulated for 1 second per day with a biphasic square wave (100 Hz) at an intensity found to elicit afterdischarges.

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H-2		28
H-3		28
H-4		31
H-5		16
H-6		23
H-8		20
H-9		28
	Median	28
	range	16-31
Caudate-putamen		
CP-1		29
CP-2		40
CP-5		18
CP-6		29
CP-7		29
CP-8		38
CP-9		23
CP-10		37
	Median	29
	range	18-40
Dorsomedial thalamus		
T-1		30
T-2		16
T-3		27
T-4		18
T-5		31
T-6		20
T-7		30
T-8		28
	Median	28
	range	16-31

* Each animal was stimulated for 1 second per day with a biphasic square wave (100 Hz) at an intensity found to elicit afterdischarges.

Table 2

Afterdischarge duration* in rats stimulated in the ventral hippocampus

Rat Number	Afterdischarge duration (sec)	
	Immediate**	Delayed***
H-1	22	11
H-2	20	26
H-3	28	40
H-4	25	20
H-5	22	-
H-6	40	40
H-8	22	5
H-9	40	45
Mean	28	30
S.D.	8	13

*Afterdischarge duration measured on first seizure day.

** Afterdischarges occurring immediately after electrical stimulation.

*** Afterdischarges occurring 60 to 85 seconds after electrical stimulation.

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Afterdischarge duration* in rats stimulated in the ventral hippocampus

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H-4	25	20
H-5	22	-
H-6	40	40
H-8	22	5
H-9	40	45
Mean	28	30
S.D.	8	13

*Afterdischarge duration measured on first seizure day.

** Afterdischarges occurring immediately after electrical stimulation.

*** Afterdischarges occurring 60 to 85 seconds after electrical stimulation.

Table 3

Relationship of afterdischarge duration and seizure activity in kindled rats

Stimulation site	Time after stimulation (sec)-mean(range)		
	Afterdischarges	Psychomotor seizures	
	Duration	Onset	Duration
Hippocampus	28.7* (20-40)	17.9 (12-32)	33.5 (20-45)
Caudate-putamen	14.0 (2-20)	3.4 (2-5)	6.5 (5-20)
Thalamus	32.2 (26-45)	6.5 (3-10)	26.8 (15-45)

* Duration of initial (primary) afterdischarges only.

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Caudate-putamen	14.0 (2-20)	3.4 (2-5)	6.5 (5-20)
Thalamus	32.2 (26-45)	6.5 (3-10)	26.8 (15-45)

* Duration of initial (primary) afterdischarges only.

Table 4.

Afterdischarge thresholds before and after the development of kindled seizures

Stimulation site / Rat number	Afterdischarge threshold (uA) Before kindling	After kindling
Hippocampus		
H-1	30	20
H-2	55	50
H-3	55	50
H-4	50	12
H-5	150	75
H-6	75	40
H-8	40	30
H-9	110	70
Median	61	38
range	30-150	12-70
Caudate-putamen		
CP-1	350	250
CP-2	250	125
CP-5	300	90
CP-6	400	200
CP-7	150	50
CP-8	500	375
CP-9	500	150
CP-10	125	75
Median	301	134
range	125-500	75-250
Thalamus		
T-1	350	250
T-2	500	325
T-3	300	200
T-4	400	-
T-5	200	150
T-6	400	150
T-7	400	400
T-8	400	175
Median	361	206
range	200-400	150-400

Table 4.

Afterdischarge thresholds before and after the development of kindled seizures

Stimulation site / Rat number	Afterdischarge threshold (uA) Before kindling	After kindling
Hippocampus		
H-1	30	20
H-2	55	50
H-3	55	50
H-4	50	12
H-5	150	75
H-6	75	40
H-8	40	30
H-9	110	70
Median	61	38
range	30-150	12-70
Caudate-putamen		
CP-1	350	250
CP-2	250	125
CP-5	300	90
CP-6	400	200
CP-7	150	50
CP-8	500	375
CP-9	500	150
CP-10	125	75
Median	301	134
range	125-500	75-250
Thalamus		
T-1	350	250
T-2	500	325
T-3	300	200
T-4	400	-
T-5	200	150
T-6	400	150
T-7	400	400
T-8	400	175
Median	361	206
range	200-400	150-400

Table 5

Behavioural effects of intracerebroventricular injection of B-endorphin before and after kindling

Electrode site	Number of animals					
	Wet dog shakes		Scratching		Flinching	
	Before	After	Before	After	Before	After
Hippocampus	6/8	3/8	0/8	5/8	1/8	6/8
Caudate-putamen	4/8	1/8	5/8	4/8	0/8	8/8
Thalamus	2/8	2/8	4/8	5/8	0/8	7/8

Table 5

Behavioural effects of intracerebroventricular injection of B-endorphin before and after kindling

Electrode site	Number of animals					
	Wet dog shakes		Scratching		Flinching	
	Before	After	Before	After	Before	After
Hippocampus	6/8	3/8	0/8	5/8	1/8	6/8
Caudate-putamen	4/8	1/8	5/8	4/8	0/8	8/8
Thalamus	2/8	2/8	4/8	5/8	0/8	7/8

Table 6

Electroencephalographic changes following intracerebroventricular injection of B-endorphin

Recording site	Number of rats having ICTAL EPISODES	
	Before kindling	After kindling
Hippocampus	6/8	3/8
Caudate	0/8	0/8
Thalamus	2/8	1/8

Recording site	Number of rats having RHYTHMIC SPIKING	
	Before kindling	After kindling
Hippocampus	8/8	7/8
Caudate	3/8	6/8
Thalamus	4/8	7/8

Table 6

Electroencephalographic changes following intracerebroventricular injection of B-endorphin

Recording site	Number of rats having ICTAL EPISODES	
	Before kindling	After kindling
Hippocampus	6/8	3/8
Caudate	0/8	0/8
Thalamus	2/8	1/8

Recording site	Number of rats having RHYTHMIC SPIKING	
	Before kindling	After kindling
Hippocampus	8/8	7/8
Caudate	3/8	6/8
Thalamus	4/8	7/8

Table 7

Latency of first ictal episode in the hippocampal EEG following intracerebroventricular injection of B-endorphin

Rat No.	Time after injection of B-endorphin (min)			
	Before kindling		After kindling	
	Injection 1	Injection 2	Injection 1	Injection 2
H-1	3.5	2.5	-	-
H-2	4.0	6.0	10.0	10.0
H-3	5.0	3.0	-	-
H-4	4.0	4.6	-	-
H-5	7.0	6.5	-	-
H-6	-	-	-	-
H-8	-	-	3.0*	4.3
H-9	1.8	4.5	1.8	14.5

* rat had psychomotor seizure

Table 7

Latency of first ictal episode in the hippocampal EEG following intracerebroventricular injection of B-endorphin

Rat No.	Time after injection of B-endorphin (min)			
	Before kindling		After kindling	
	Injection 1	Injection 2	Injection 1	Injection 2
H-1	3.5	2.5	-	-
H-2	4.0	6.0	10.0	10.0
H-3	5.0	3.0	-	-
H-4	4.0	4.6	-	-
H-5	7.0	6.5	-	-
H-6	-	-	-	-
H-8	-	-	3.0*	4.3
H-9	1.8	4.5	1.8	14.5

* rat had psychomotor seizure

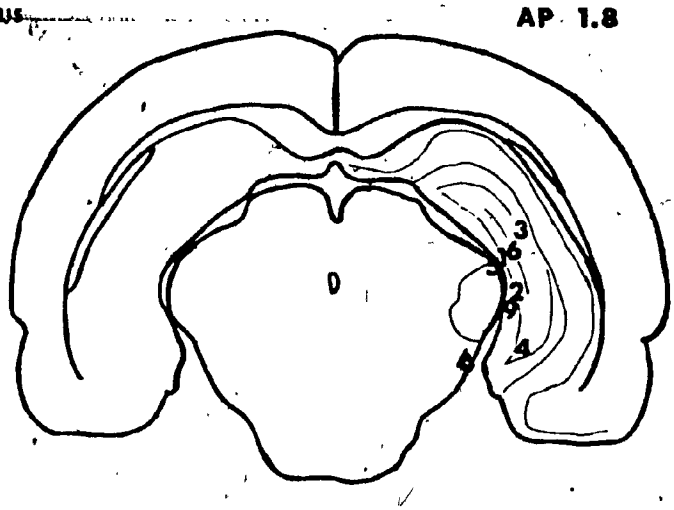
FIGURE 1

Histological reconstruction of electrode placements

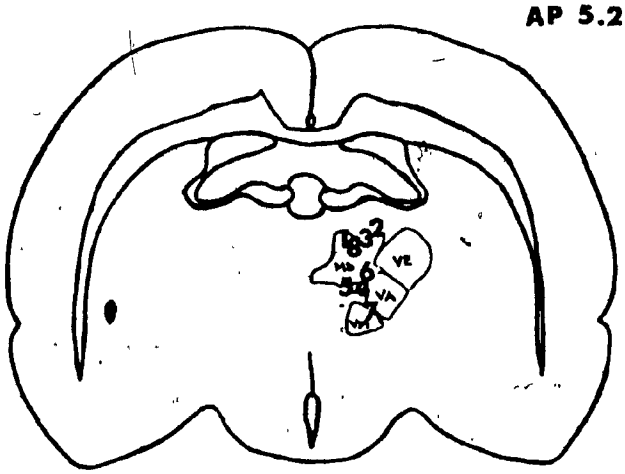
The numbers correspond with those assigned to each particular animal. For tables and figures each rat is designated by a number, prefixed with a letter which indicates experimental group i.e. H = hippocampus; CP = caudate-putamen; T = thalamus.

ELECTRODE PLACEMENT

HIPPOCAMPUS AP 1.8



THALAMUS AP 5.2



*CAUDATE-PUTAMEN AP 7.8

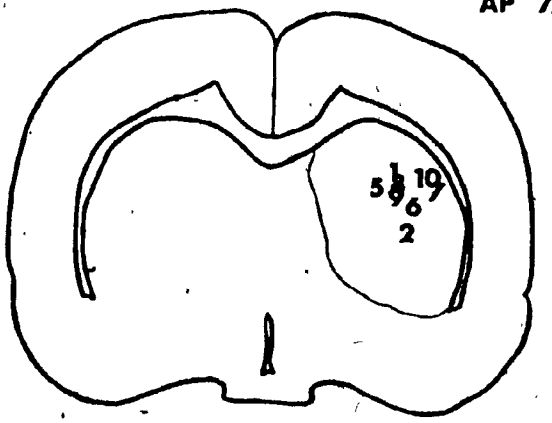


FIGURE 2

Electroencephalographic changes following electrical stimulation of the hippocampus

Rat H-1 on day 19 of kindling, before full seizure development, and on day 31 when fully kindled. Initial (primary) afterdischarges occur immediately after stimulation (30 uA), followed by a silent period, and then delayed (rebound) afterdischarges. Note that on day 31, the full seizure occurs mainly during the EEG silent period.

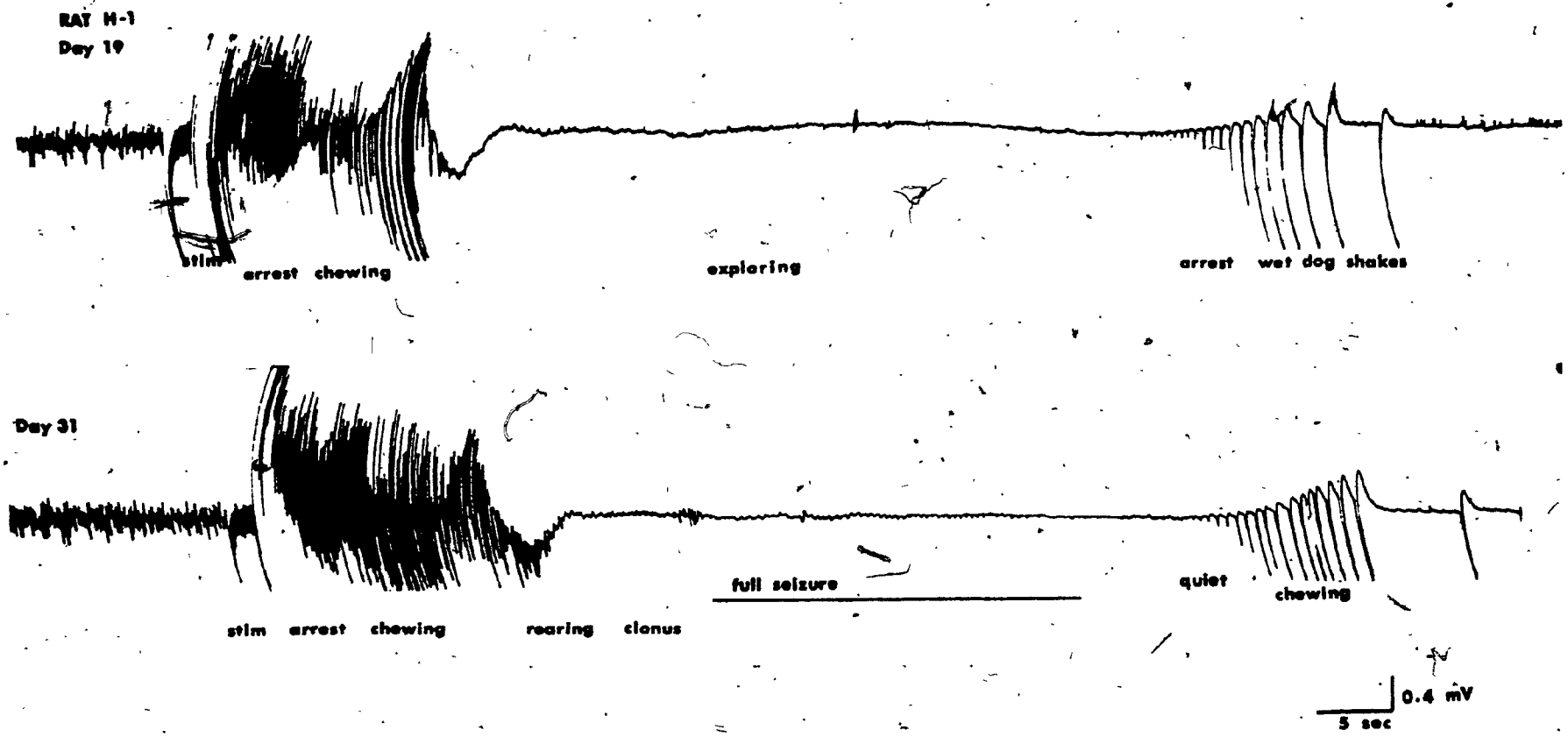

RAT H-1
Day 19
stim
arrest
chewing
exploring
arrest
wet dog shakes
Day 31
full seizure
quiet
chewing
stim
arrest
chewing
rearing
clonus
0.4 mV
5 sec

FIGURE 3

Electroencephalographic changes following electrical stimulation of the thalamus

Rat T-5 on day 23 of kindling, before full seizure development, and on day 35 when fully kindled. A short afterdischarge period follows the stimulation (200 uA) and on day 35 the seizure occurs during the afterdischarge period.

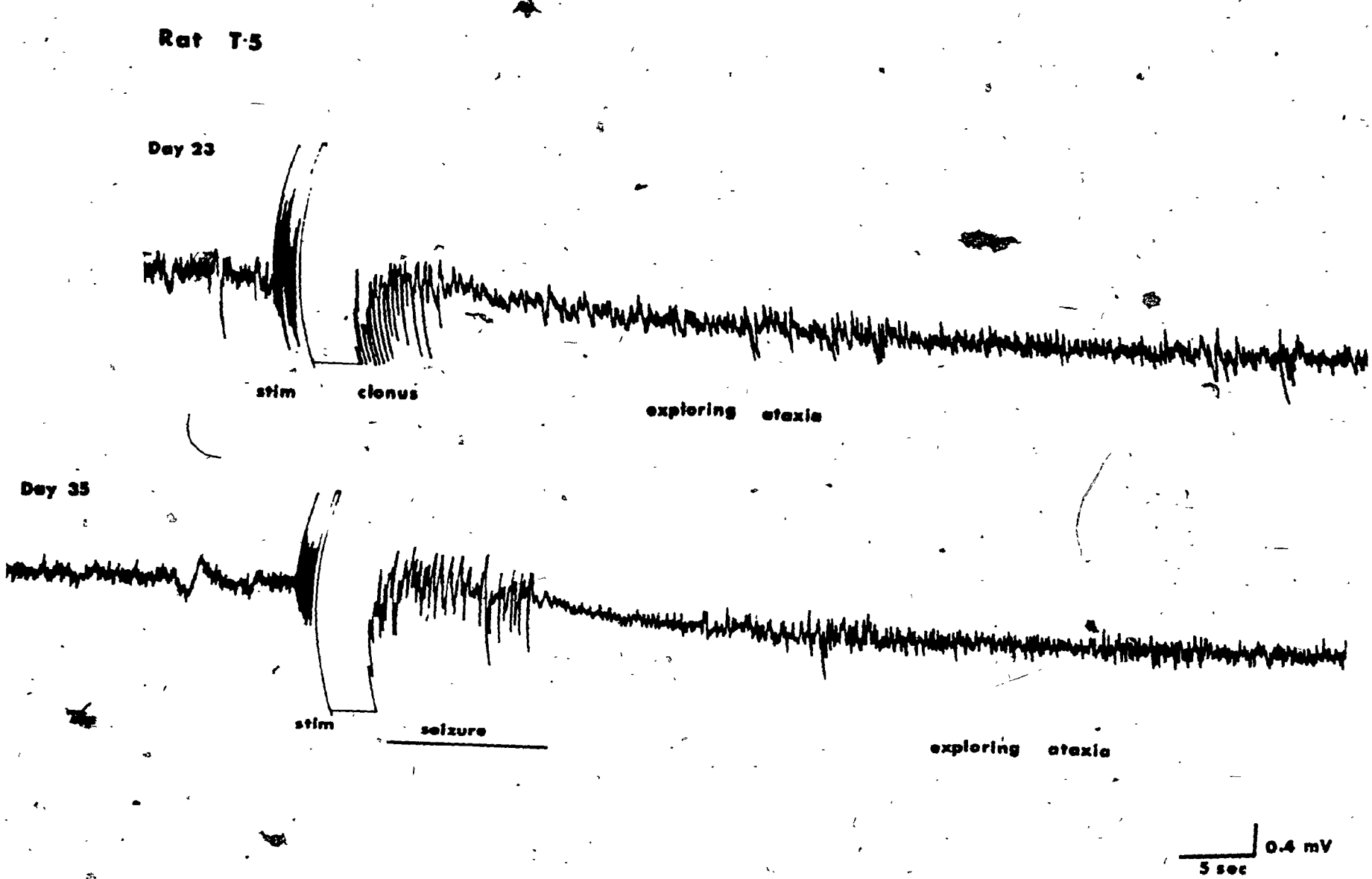

Rat T-5
Day 23
stim
clonus
exploring ataxia
Day 35
stim
seizure
exploring ataxia
0.4 mV
5 sec

FIGURE 4

Electroencephalographic changes following electrical stimulation of the caudate-putamen

Rat CP-10 on day 29 of kindling, before full seizure development, and on day 33 when fully kindled. A short afterdischarge period follows the stimulation (125 uA), then a short silent period. The silent period terminates with some spiking and slow wave activity in the EEG. The kindled seizure occurs during the afterdischarge period.

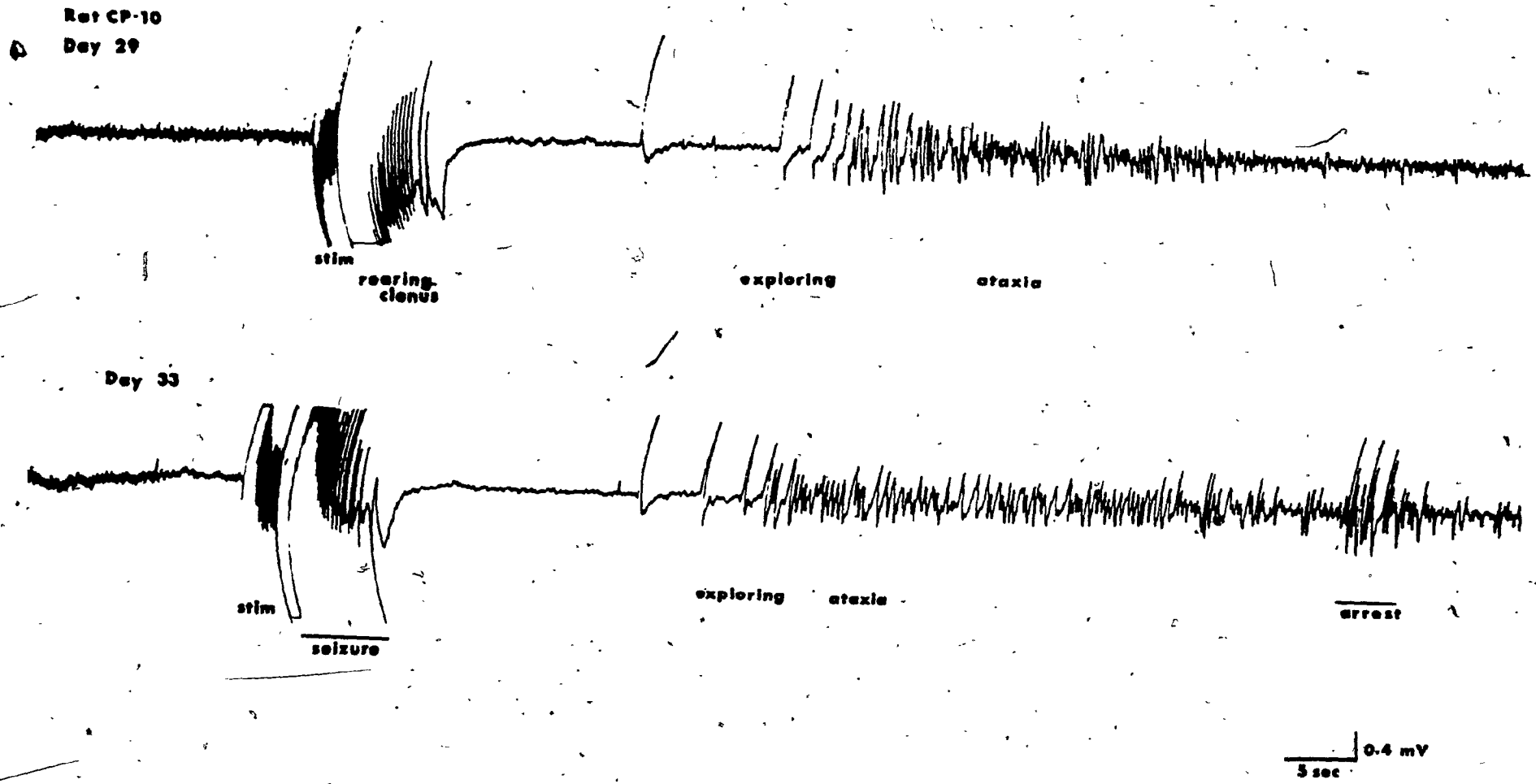

Rat CP-10
Day 29
stim
rearing-
clonus
exploring
ataxia
Day 33
stim
seizure
exploring
ataxia
arrest
0.4 mV
5 sec

FIGURE 5

The effects of intracerebroventricular injection of B-endorphin (10 ug/10 ul) on hippocampal electroencephalographic activity in a non-kindled rat

Rat H-2. Within 5 minutes after administration of B-endorphin spiking and ictal episodes can be seen in the EEG. The initial ictal episode is followed by a silent period and then spiking afterdischarge activity. Additional ictal episodes are seen at 10 and 14 minutes after injection, with continuous inter-ictal spiking.

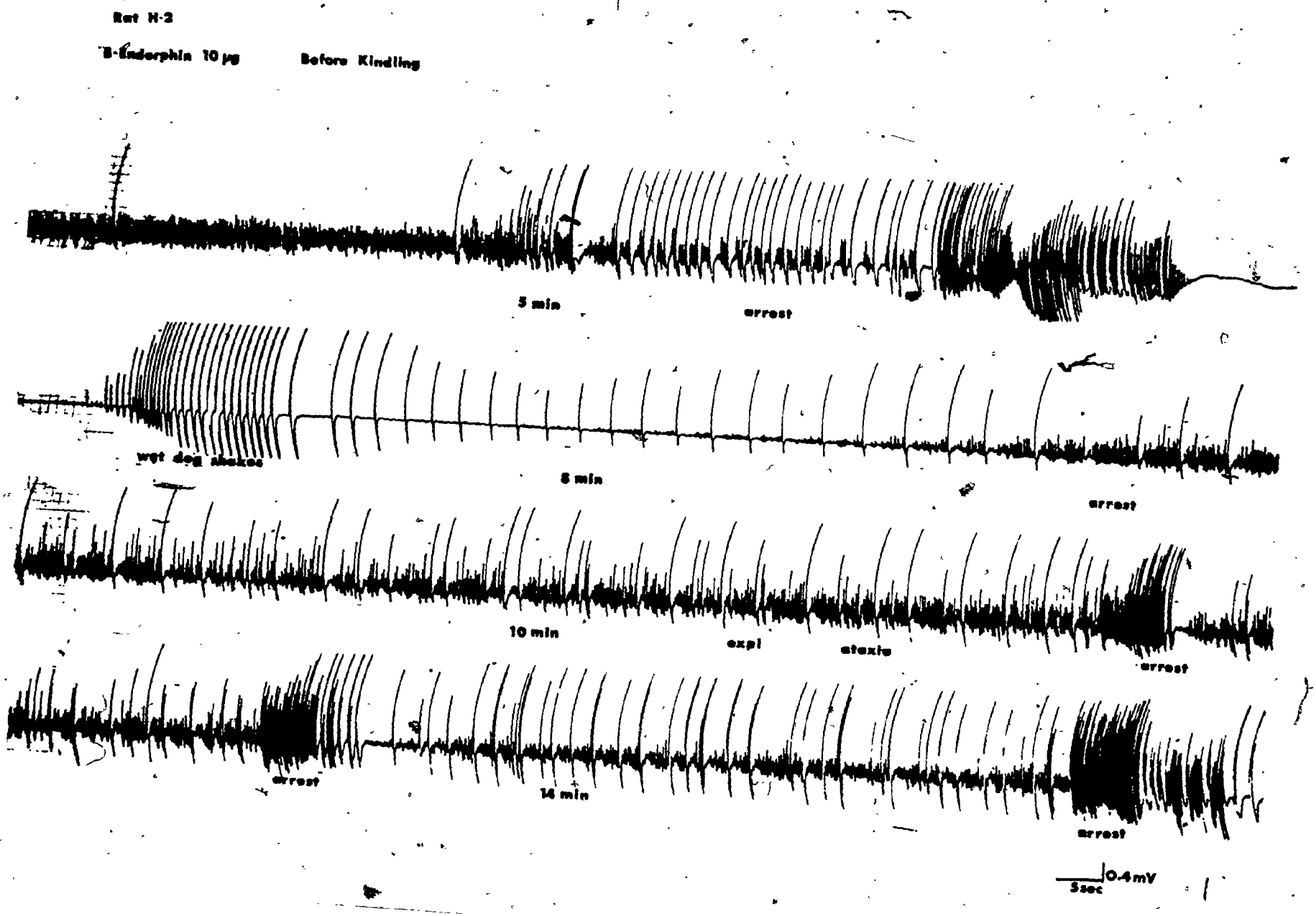

Rat H-2
β-Endorphin 10 µg
Before Kindling
5 min
arrest
wet dog shakes
8 min
arrest
10 min
expl
ataxia
arrest
arrest
14 min
arrest
0.4mV
5sec

FIGURE 6

The effects of intracerebroventricular injection of B-endorphin (10 ug/10 ul) on hippocampal electroencephalographic activity in a fully kindled rat

Rat H-2. Within 5 minutes after administration of B-endorphin spiking is seen in the EEG. No ictal episodes similar to those observed before kindling (figure 5) were elicited.

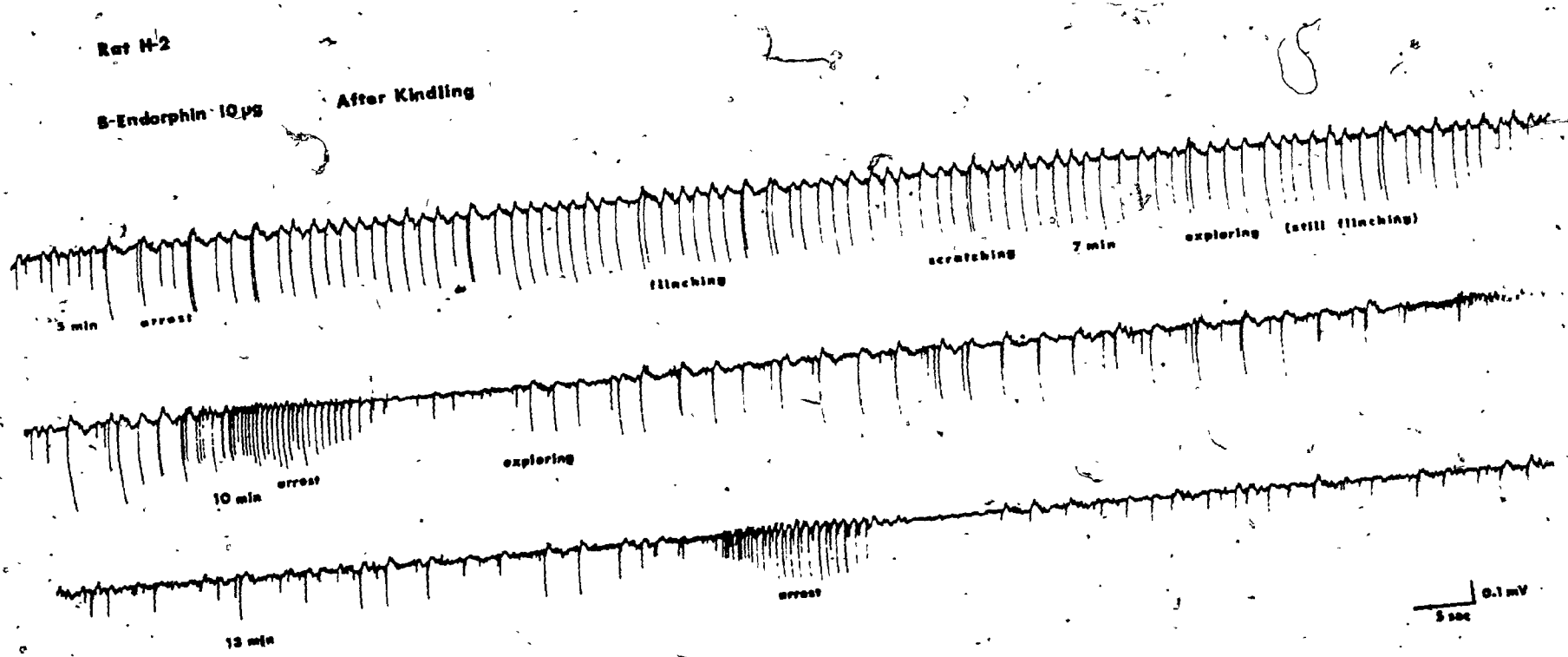

Rat H-2
β-Endorphin 10 µg
After Kindling
5 min
arrest
flinching
scratching
7 min
exploring
(still flinching)
10 min
arrest
exploring
arrest
13 min
0.1 mV
5 sec

FIGURE 7

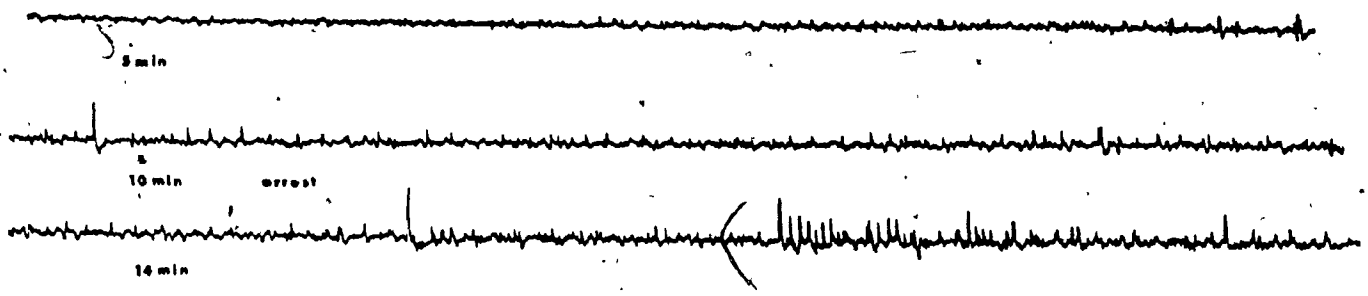
The effects of intracerebroventricular injection of B-endorphin (10 ug/10 ul) on thalamic electroencephalographic activity in a non-kindled and kindled rat

Rat T-2. A minimal amount of spiking in the EEG is seen in the unkindled animal (top three tracings). After kindling, spiking appears in the EEG and continues throughout the observation period.

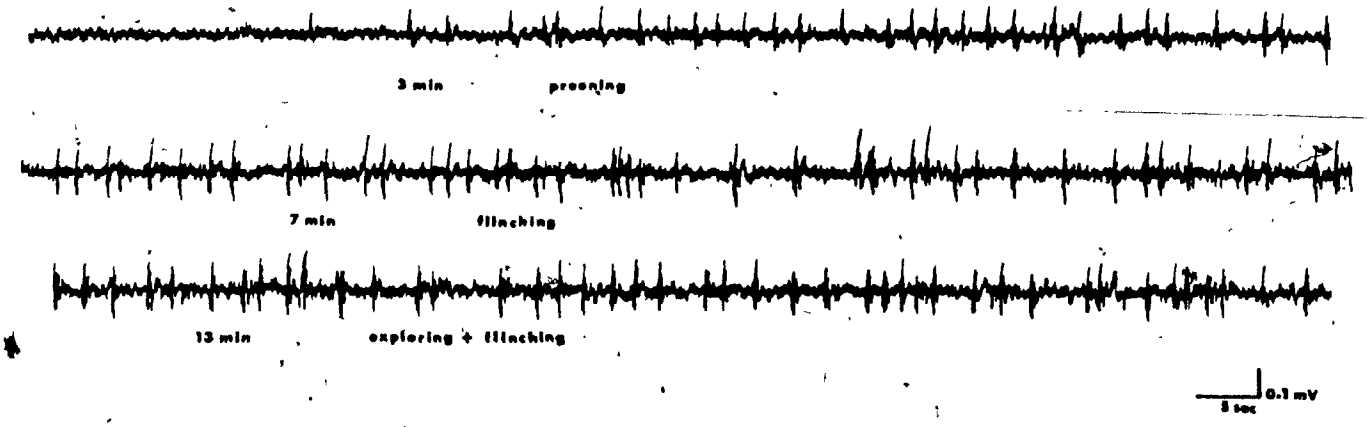
For comparative purposes, a sample record of the EEG of this animal during electrical stimulation is shown (bottom tracing).

Rat T-2 β-Endorphin 10 μg

Before Kindling



After Kindling



Kindling - Day 16

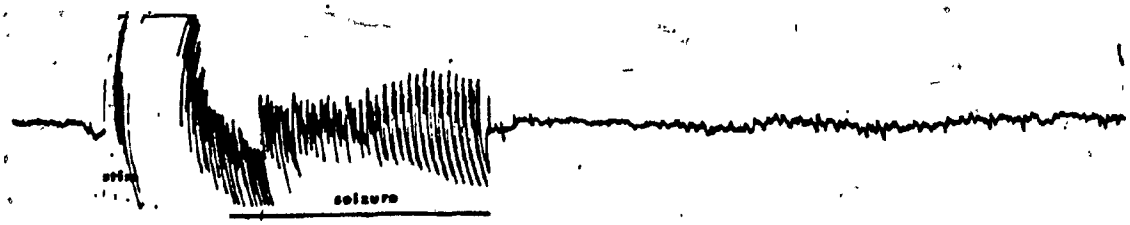


FIGURE 8

The effects of intracerebroventricular injection of B-endorphin (10 ug/10 ul) on caudate-putamen electroencephalographic activity in a non-kindled and kindled rat

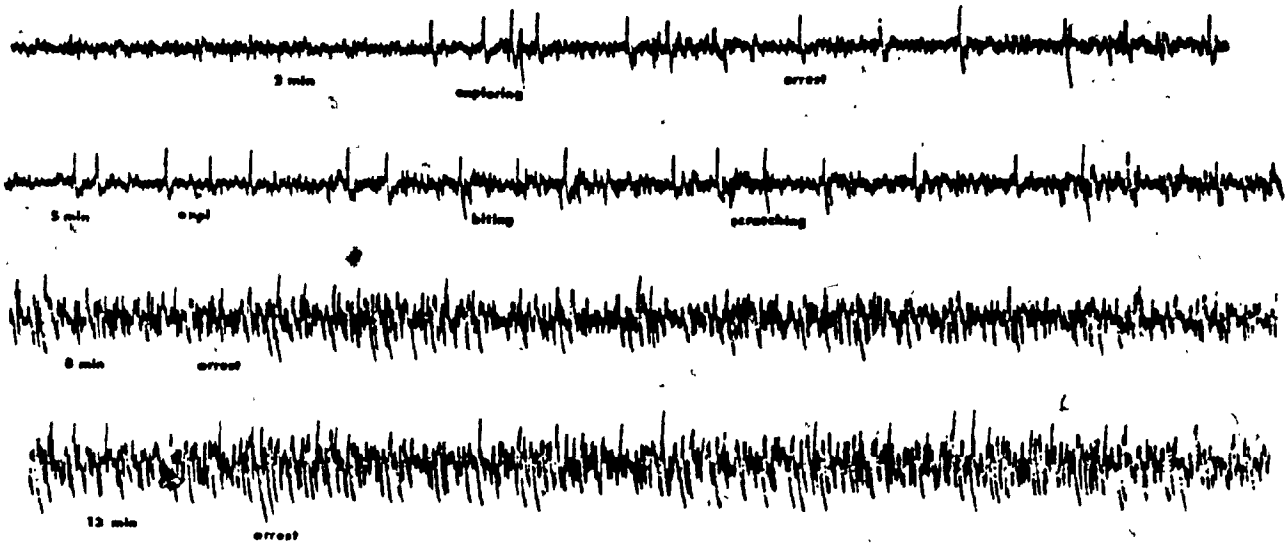
Rat CP-8. Before kindling, some spiking is seen in the EEG (top two tracings) as well as slow wave activity (next two tracings). After kindling, less spiking and slow wave activity is seen (bottom three tracings).

For comparative purposes, a sample EEG record from this animal during electrical stimulation is shown (last tracing).

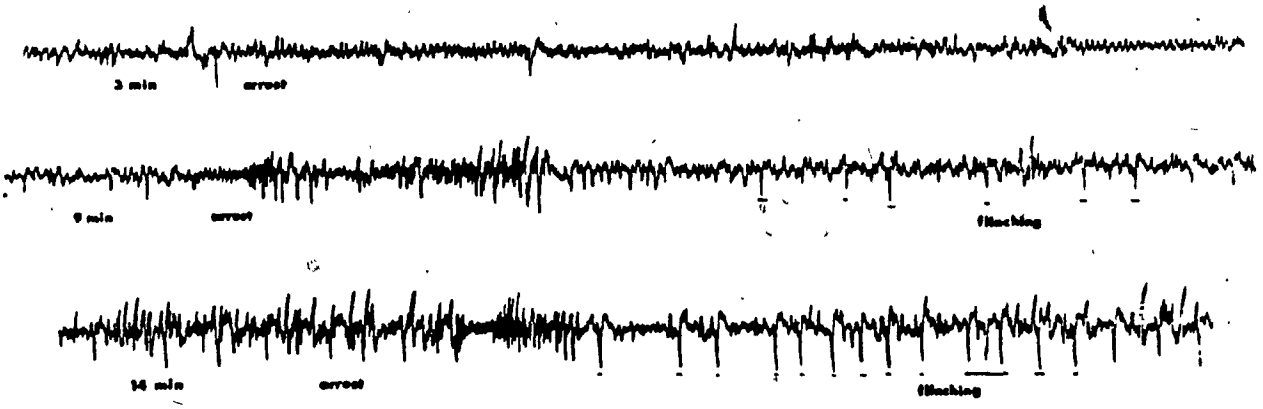
β-ENDORPHIN 10 pg

Rat CP-8

BEFORE KINDLING



AFTER KINDLING



0.1 mV
5 sec

KINDLING - DAY 21

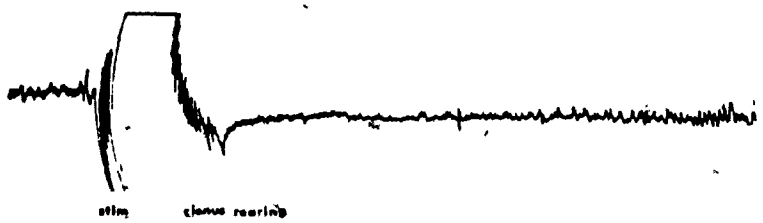


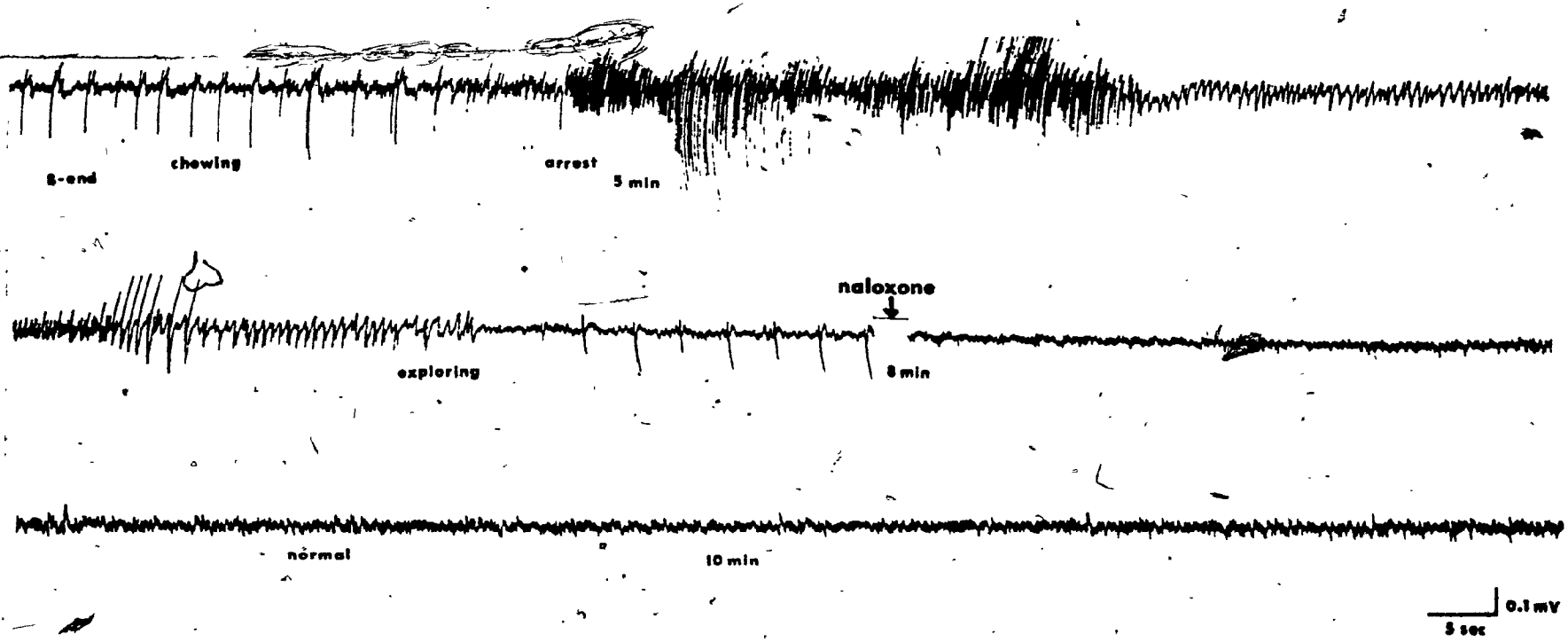
FIGURE 9

Reversal of electroencephalographic effects of B-endorphin by Naloxone (10 mg/kg i.p.)

Rat H-2, after kindling. Eight minutes after administration of B-endorphin, naloxone was injected. Following injection of naloxone, no B-endorphin induced electroencephalographic effects were recorded.

Rat H-2 β-Endorphin 10 µg + Naloxone 10 mg/kg

After Kindling



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