OBSERVATIONS OF THE PHENOMENON OF EXPERIMENTAL CEREBRAL CONCUSSION IN CONSCIOUS RATS

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INTRODUCTION

Trauma to the central nervous system has become the most common form of neurological disease (Caveness (1977). Loss of consciousness secondary to head injury is a frequent feature of such trauma. Many cases of permanent post-traumatic unconsciousness may be explained by the post-mortem observations of generalized neuronal damage in the cortex and/or brain stem. More frequently, however, the injury is less severe and produces unconsciousness followed by apparent complete recovery. This latter syndrome of transient loss of consciousness has been termed "concussion", and has been the subject of considerable investigation.

The word "concussion" is derived from the Latin "concutere" which means "to shake violently". Ambrose Paré (1510-1590) and his contemporaries used this term to describe the "ébranlement du cerveau" (shaking of the brain) which they believed was responsible for the sudden neuronal paralysis which followed a concussive head injury. These early investigators believed that no structural defect in the brain need occur to account for the clinical picture. Littré (1705) substantiated this opinion when he performed a post-mortem examination on a patient who ran head first into a wall, was instantly rendered unconscious, and died minutes later. There was no macroscopic evidence of damage to the brain. Similarly, Polis (1894) described patients who had suffered a concussion and died, in whom he could demonstrate no pathological lesions.

Before discussing the phenomenon of concussion, it is advisable to define the normal state of consciousness. This concept is best outlined in Ommaya's definition as:

> "that state of awareness in the organism which is characterized by maximum capacity to utilize its sensory input and motor output potential in order to achieve accurate storage and retrieval of events related to contemporary time and space."

The classic definition of concussion appears in the authoritative study of Denny-Brown and Russell (1941); "an essentially reversible syndrome (of neural dysfunction) without detectable pathology". The current generally accepted definition is that of the Committee to Study Head Injury Nomenclature (1966):

> "a clinical syndrome characterized as an immediate and transient impairment of neural function, such as alteration of consciousness, equilibrium, etc., due to mechanical forces".

It has been emphasized that, although usually reversible, a concussion may be fatal. For instance, if the respiratory and vasomotor centers of the medulla are transiently paralyzed, the affected individual may expire before these centers have had time to recover (Fulton, 1942; Symonds, 1974; Gurdjian, 1975).

For the reader who has not witnessed the instantaneous loss of neural function produced by a concussing blow, the eloquent description of Sir Charles Sherrington (1906) is instructive:

> "the knock-out blow...reduces in a moment a vigorous athlete to an unstrung bulk of flesh, whose weight alone determines its attitude, if indeed a reactionless mass can be described as having attitude at all".

Loss of consciousness and postural reflexes are fundamental signs of concussion in Sherrington's description of boxers. Maintenance of consciousness depends upon continuing interplay between the cerebral cortex and the brain stem reticular formation, while maintenance of posture depends upon a large number of complex reflexes encompassing almost the entire brain stem and spinal cord, with modulation by the cerebral cortex (Govons, 1968).

Walker (1973) also described the clinical picture:

> "the classic 'concussion' is featured by 'a fall like a log' with unresponsiveness for varying periods of time. However, other types may occur--a stiffening of the body in extension, a gradual slumping with glassy apparently unseeing eyes, and even retention of posture and some automatic movements with no response to the environment".

In man, concomitants of loss of consciousness are an impairment of higher neurological functions, especially memory (attested to by the frequent questions "where am I?" and "what happened?"); also, transient apnea, bradycardia, hypotension, and impairment of brain stem reflexes.

Many anecdotal reports have been derived from observations made in sports such as boxing, football, soccer and polo, where head injuries are not uncommon. The impairment of memory described by Walker is common to such reports. Amnesia may occur for minutes or hours prior to the injury, in which case, it is known as retrograde amnesia. Amnesia occurring for different time periods following the injury is known as post-traumatic, or antegrade amnesia. It is known that post-traumatic amnesia may follow trauma that does not produce unconsciousness (Fisher, 1966, Symonds, 1966). This period of post-traumatic amnesia has been likened to the "epileptic twilight state" (petit mal epilepsy) (Winterstein, 1937) in that the behavior of the individual appears to be "automatic". For example, after being concussed, the boxer or football player may continue, successfully, at his match and when questioned later, have no recollection of the concussing blow, nor of the events following it (Martland, 1928; Blonstein and Clarke, 1957; Yarnell and Lynch, 1970, 1973).

Following concussion, return of awareness to stimuli usually precedes sensory and motor recovery, which in turn, recover before complete restoration of memory and other cognitive functions (Ommaya and Gennarelli, 1975). This sequence reflects the dictum that the higher levels of the nervous system are more susceptible to injury and require longer periods for recovery from such an injury.

Although less frequently recognized than loss of consciousness, concussion may also produce transient focal neurological deficits, such as paresis in an arm or leg, without clouding of the sensorium.

Discussion to this point has emphasized the transient nature of the disturbance of neural function. Several reports have claimed to show neuronal chromatolysis and diminished neuronal cell counts in the brain stem reticular formation and related areas following concussion in anesthetized animals (Windle, et al. 1944; Groat, et al. 1945 A, B; Groat and Simmons, 1950; Chason, et al. 1957; Brown, et al, 1972). As a result of such studies, several scientists and clinicians have accepted the conclusion that concussion always produces irreversible brain damage. The quoted studies were poorly controlled and used relatively primitive techniques, but are still widely quoted. More recent, well-controlled studies, using both light and electron microscopy have demonstrated temporary mitochondrial swelling and abnormal microvascular permeability, but have not confirmed the presence of permanent structural damage (see below).

The controversy regarding the presence of permanent structural alterations following concussion is far from resolved. However, when head injury is viewed as a spectrum, ranging from mild concussion to severe cerebral laceration, it is obvious that there will be an overlap between the different gradations of severity. Hence, concussion occurs as a clinical syndrome that may be associated with, but unrelated to a variety of pathological states (Kaplan and Browder, 1954). In these complicated cases, one would be mistaken to attempt to relate the dramatic, generalized, transient loss of neural function to the permanent loss of a small circumscribed population of neurons. In an attempt to avoid the controversy that surrounds the term concussion, Walker (1973) has introduced the term "traumatic unconsciousness". Traumatic unconsciousness is common to a wide variety of head injuries--it is the basic response of the nervous system to trauma. It may be seen alone--in concussion--or may be complicated by irreversible brain damage in more serious injuries. Less common than cerebral concussion and also of unknown etiology are concussion of the spinal cord or of a peripheral nerve, which may

produce a transient loss of neural function. The latter entities are not within the scope of this paper and will not be discussed further.

Since the syndrome of concussion is one of the minor forms of injury to the nervous system, appears to be reversible, and requires no special treatment, why then has it attracted so much continuing attention? One reason is that the pathophysiology of the syndrome remains unknown in spite of numerous investigations. Another is that the cellular substrate of concussion may contribute in some way to the prolonged traumatic unconsciousness frequently observed in humans, which may last several weeks, to be followed eventually by a good recovery (Becker, 1978). Becker speculated that neurons may have been rendered "dysfunctional" during this period, although morphologically intact and therefore, capable of recovery. His views are in agreement with those of Govons (1968), who stated that "within the limits of our present knowledge, no definite time limit can be set for maximum duration of coma following a concussive blow".

Understanding the pathophysiology of concussion may conceivably lead to improved treatment of central nervous system trauma.

MECHANISMS OF HEAD INJURY

The brain of all species is well-protected from low velocity blunt injuries by a series of protective layers. The scalp although thin and vascular, is important in that it is free to slide, which helps to absorb applied tangential forces (Gurdjian, 1975); it also absorbs significant energy when it is disrupted. In both examples, the scalp aids in absorbing forces that otherwise would be transmitted to the brain. Similarly the skull, consisting of two layers of cortical bone, separated by a delicate lattice of marrow, is analogous to a corrugated cardboard box. Significant forces must be applied to this structure before it is penetrated, and hence, it is another shock-absorber. From this point onwards, the protective devices are rather flimsy. The dura, arachnoid and their processes aid in the support of the brain, as does the cerebrospinal fluid in which the brain "floats".

No combination of the above-mentioned protective layers will protect the brain from blunt injuries which apply acceleration or deceleration forces to the head. The importance of acceleration-deceleration forces in the production of concussion was elucidated by Denny-Brown and Russell (1941) and Denny-Brown (1945) who were the first investigators to appreciate the fact that damage to the brain is not proportional to the energy of the striking blow alone. They found that it was difficult, if not impossible, to produce experimental concussion in cats when the head was fixed, but relatively easy when the head was free to move. Holbourn (1943, 1945) further underscored the importance of acceleration, in particular rotational acceleration. Employing gelatin models of the brain, he surmised that shear strains would be more severe with rotational (angular) than with translational (linear) acceleration. This fact has become generally accepted because of the large body of clinical and experimental evidence suporting it. (Pudenz and Shelden, 1947; Rowbotham, 1964; Sellier and Unterharnscheidt, 1966; Ommaya, et al.

1968; Yarnell and Ommaya, 1969; Joseph and Crisp, 1971; Adams and Graham, 1972; Ripperger, 1975). Translational (linear) acceleration is such that all parts of the impacted body are accelerated uniformly. Rotational (angular) acceleration is more complex in that there occurs asymmetrical acceleration of the impacted body, in relation to its center of gravity (Fig. 1). During rotational acceleration, the brain tends to remain essentially stationary as the skull rotates. This can be compared to water in a bucket which remains at rest while the bucket is twirled around its geometric axis. Such rotation produces shear stresses, which are maximal at the brain surface, and decrease at increased distance from the surface (Joseph and Crisp, 1971; Ripperger, 1975; Ommaya and Gennarelli, 1975). Nature also supports this theory. May, et al. (1979) studied a natural model in which the effects of acceleration may be observed. The woodpecker may pound its head incessantly without the development of concussion. The reason is probably that, as high-speed cinematography has shown, the acceleration of the woodpecker's head in linear, rather than rotational. This movement requires more complex neuromuscular innervation than if the acceleration were rotational. The authors suggested that linear acceleration had been "selected" because it is safer.

Ommaya and Gennarelli (1975) have shown that while translational acceleration is most likely to produce focal lesions (such as temporal lobe contusions and hemorrhages), such focal lesions do not often result in concussion.

Rotational acceleration, on the other hand, is more effective in producing diffuse brain injury and hence, resulting in cerebral concussion. More recently, Joseph and Crisp (1971) have documented elegant mathematical analyses of the mechanics of brain movement within the skull at the time of injury and confirmed Holbourn's theories. The most convincing clinical correlate is the efficient use of an "upper-cut" blow in boxing. This type of a blow--striking the chin sideways and upwards--is very effective in producing a concussion, secondary to rotational

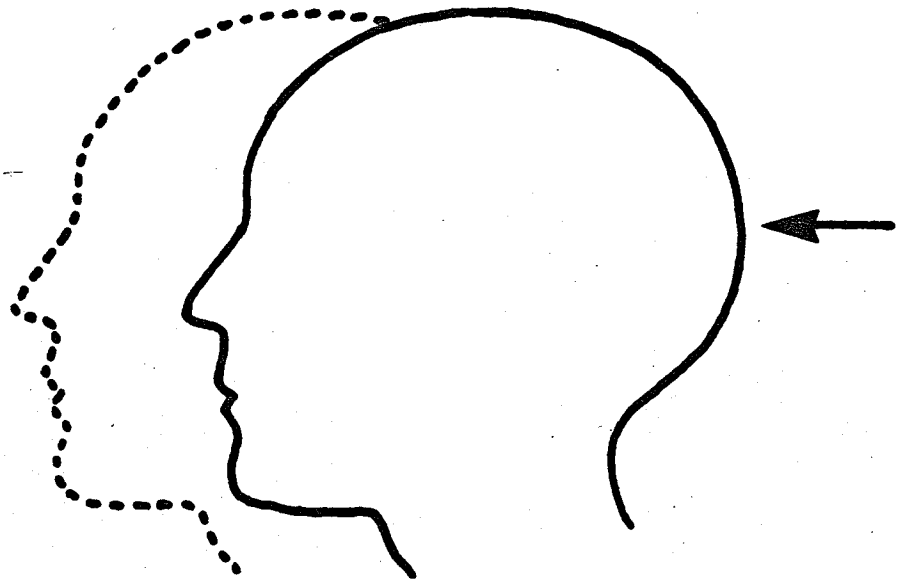
Figure 1

Ommaya (1976) has confirmed the more dramatic effects of rotational acceleration (the head is accelerated asymmetrically in relation to its center of gravity) compared to translational acceleration (all parts of the head are accelerated uniformly).

The solid lines indicate head position pre-acceleration. The interrupted lines indicate head position post-impact.

The accelerating force is from right to left (arrow).

TRANSLATION (LINEAR)



ROTATION (ANGULAR)

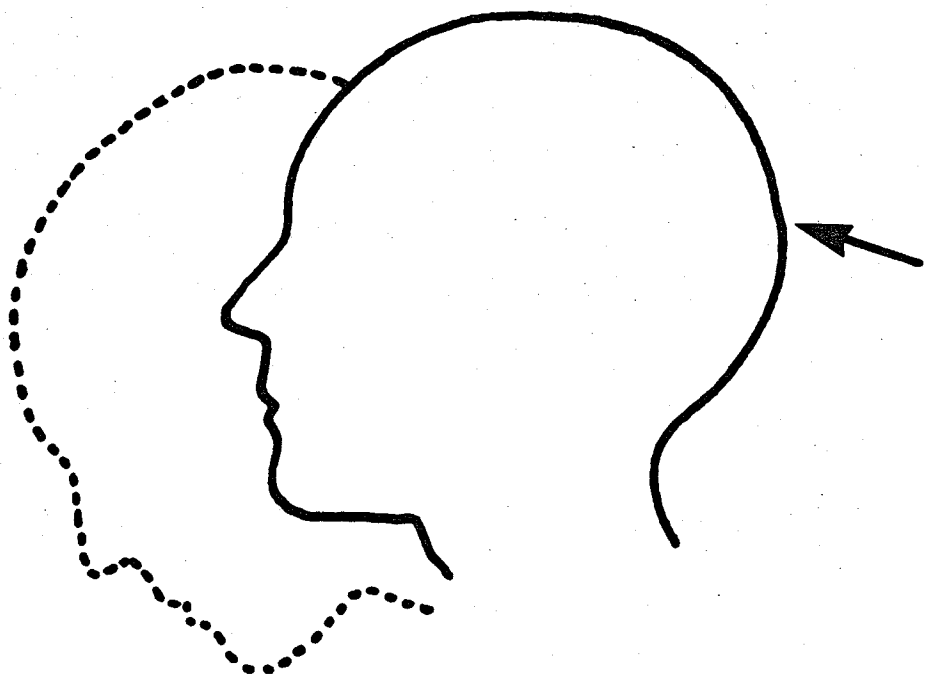


FIGURE 1
(modified from Ommaya, 1976)

acceleration to the head.

Several authors have recorded movement of the brain within the skull at the time of experimental concussion (Shelden and Pudenz, 1947; Gurdjian, et al. 1968; Gosch, et al. 1970; Ommaya, et al. 1974; and Shatsky, et al. 1974). The original experiments of Shelden and Pudenz were ingenious. These authors replaced the calvaria of monkeys with form-fitting lucite "calvaria", through which the brain could be observed. When an impact producing rotational acceleration was applied to the skull, the brain could be observed, by high-speed cinemaphotography, to "swirl" around within the calvarium.

Having witnessed this swirling movement of the brain within the skull at the time of concussion, succeeding experiments focussed upon the question as to whether or not this sudden, violent movement of the brain was accompanied by any variation in intracranial pressure (ICP).

Scott (1940) measured intracranial pressure in anesthetized dogs subjected to a head injury by a weight dropped on a fixed, immobile head. This study reported results from a model which would now be described as a compression-type injury. However, Scott did record an instantaneous, but transient elevation of intracranial pressure, which averaged 300 mm Hg. and lasted .2-.6 seconds.

Walker, et al. (1944) also demonstrated elevation of intracranial pressure in an experimental model of concussion. These authors believed that it was not the absolute height of the ICP, but rather oscillations in the ICP that were important. They believed that, through the production of pressure-waves, damaging shearing forces would be produced within the brain substance.

Gurdjian, et al. (1954) demonstrated that, although acceleration was important in the production of concussion, unless the impact acceleration produced a

rise in intracranial pressure, the "concussive reponse" was not observed.* These authors suggested that concussion following acceleration or deceleration trauma results from this increased ICP at the time of impact. This observation was substantiated by the later studies of Lindgren, et al. 1967; Rinder, 1969; and Stahlhammar, 1975, who also illustrated the correlation between increased ICP and the "concussive response" in anesthetized rabbits. When a decompression (eg. a large craniectomy) was provided prior to striking the head, the ICP was not changed significantly by the blow and the "concussive response" was either absent or less severe.

Following these preliminary observations, the role of alterations in ICP became the focal point of research on concussion. Thomas, et al. (1966, 1967), using human cadaver skulls, demonstrated that acceleration impact to the skull was associated with the abrupt development of pressure gradients within the skull; high pressures at the impact site and reduced pressures at the site opposite the impact (the counter-pole). These pressure gradients were observed to become steeper with increasing degrees of acceleration imparted to the skull (Fig. 2). Similar studies using cat cadaver heads (Clarke, et al. 1948), fluid-filled spheres (Sellier and Unterharnscheidt, 1965), anesthetized rabbits (Stahlhammar, 1975) and computer models (Ward, et al. 1978) have confirmed the existence of gradients in the instants following traumatic acceleration of the head. Unterharnscheidt (1969) and Ripperger (1975) have elucidated the mechanism of development of such pressure gradients (Fig. 3). Gurdjian (1975) has also studied this problem. He relates the development of ICP gradients to the phenomenon of "inertial loading" of the brain at the time of impact acceleration. When a striking object contacts the

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*since the animals in these experiments were anesthetized, "concussion" could not be correlated with changes in levels of consciousness. Hence, the degree of concussion was correlated with the "concussive response" consisting of transient apnea, associated with absent brain stem reflexes, bradycardia, and hypotension).

Figure 2

Thomas, et al. (1966, 1967) and Gurdjian (1972) have demonstrated the development of intracranial pressure gradients at the time of acceleration impact. The site of impact is marked at (A). The counterpole is marked at (B). Positive pressures were recorded at (A), negative pressures at (B). Lines a, b, and c demonstrate the increase in the gradient which accompanies increased acceleration (acceleration a > b > c).

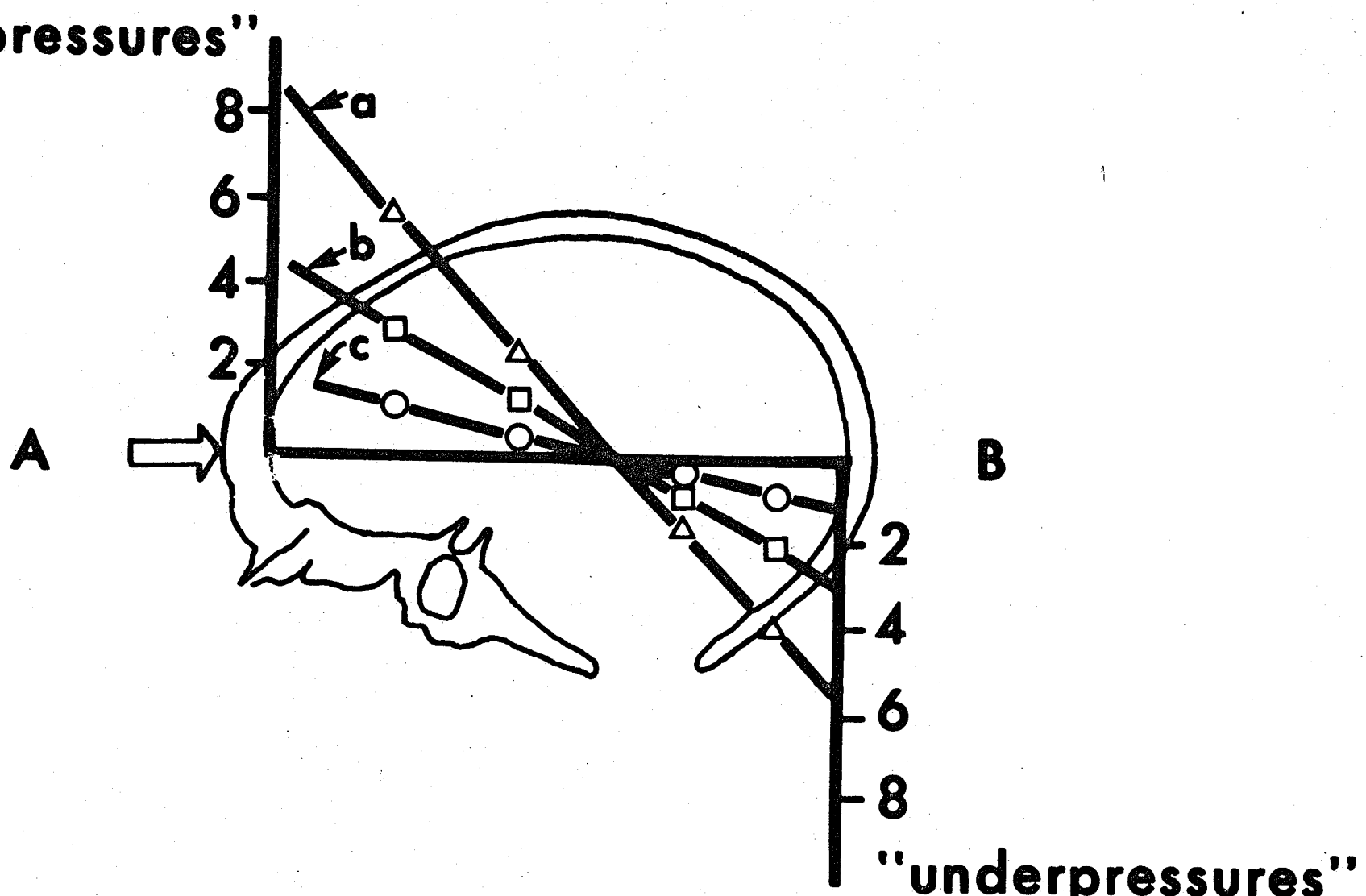


FIGURE 2

(from Gurdjian, 1972)

Figure 3

A = a "cube" of cerebral tissue

F = the applied force

P = pressure

a = direction of acceleration

Ripperger (1975) analyzed the forces which are involved in the acceleration of a "cube" of brain tissue (A) at the time of impact. If the pressures P1 and P2 are equal, no acceleration can occur. Therefore, P1 must be greater than P2 and a pressure gradient is produced across (A) in the direction of (a). The maximum (P1) and minimum (P2) pressures will have the same magnitudes. Compressions and rarefactions then alternate as pressure waves (Unterharnscheidt, 1969).

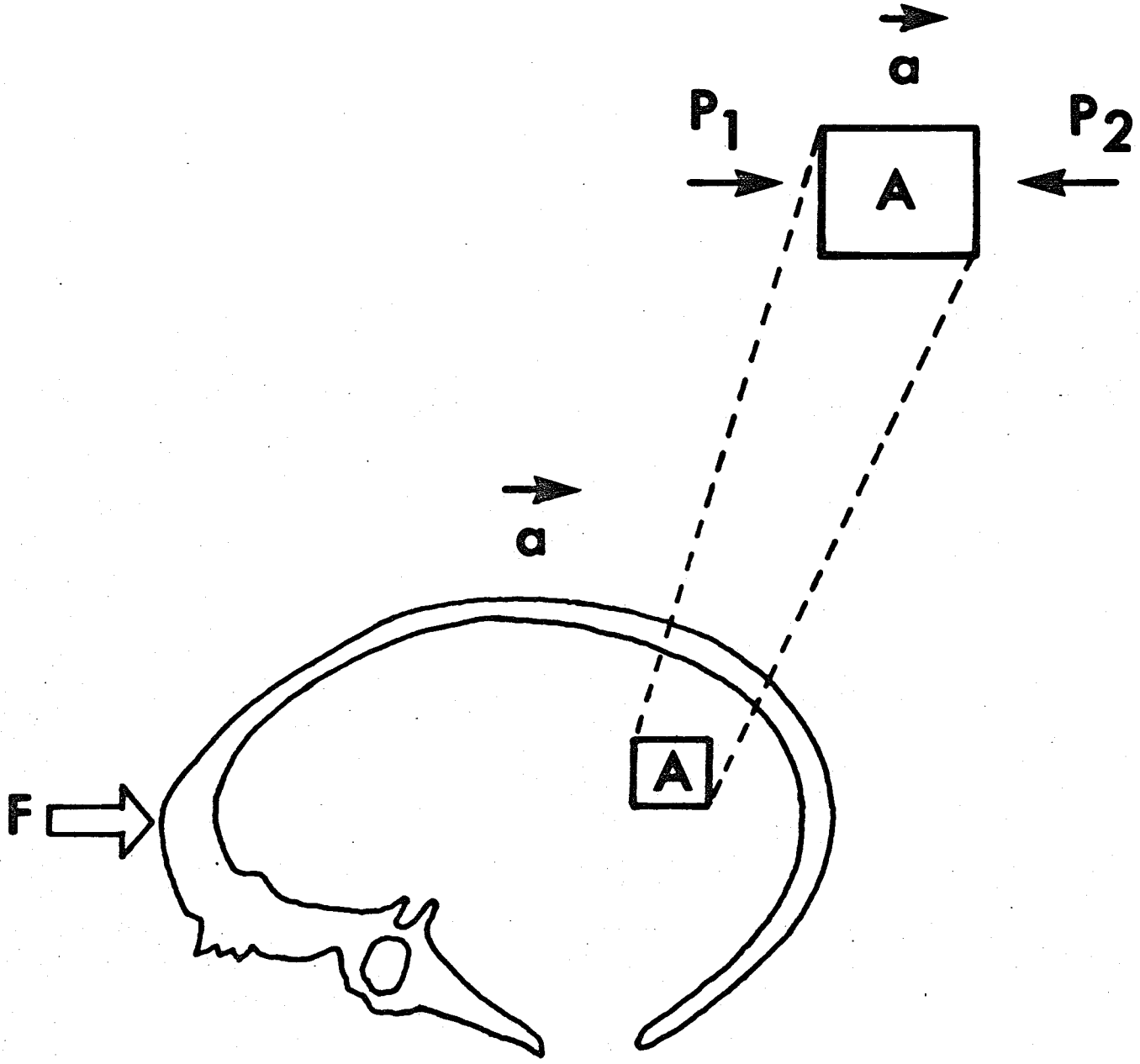


FIGURE 3
(modified from Ripperger, 1975)

skull in the occipital region, for example, the skull is instantly accelerated in the opposite direction. Because of the relative inertia of the brain, it is "slapped" by the occipital bone, producing a sudden increase in pressure in the occipital region. In the frontal region, however, the skull is moving away from the brain, creating an empty space, where reduced pressures may be recorded. Such inertial loading of the brain has been recorded photographically by Pudenz and Shelden (1947) and Gosch, et al. (1969, 1970). Shatsky, et al. (1974), in an elegant study, observed brain movements indirectly at the time of impact acceleration in anesthetized monkeys. These investigators performed angiography during the injury, and employing flash x-ray techniques, recorded 1000 films/sec. The distortion of the outlined cerebral blood vessels at the time of trauma allows an indirect interpretation of the accompanying distortion in the surrounding brain tissue, occurring in milliseconds. The results correlated with previous direct observations of the brain through "transparent calvaria". At the instant of impact, the vessels underlying the impacted area of the skull were 'emptied'of contrast material. Midline vessels were displaced towards the side of a lateral impact, as predicted by Gurdjian's theory of 'inertial loading'. The importance of such pressure gradients, particularly negative pressures, in producing shearing strains and tissue damage have been emphasized in several studies (Clarke, et al. 1948; Gross, 1958; Sellierand Unterharnscheidt, 1966; Unterharnscheidt, 1969; Joseph and Crisp, 1971). The same authors have speculated that such forces may be responsible for functional changes in the absence of structural changes (i.e. concussion). The inertial loading theory predicts that concussion may be produced without cranial impact. For instance, if the head is suddenly accelerated or decelerated by indirect means, a concussion may follow. This fact was confirmed by Ommaya, et al. (1968) and Yarnell and Ommaya (1969), who strapped anesthetized monkeys into speed sleds with their heads free to rotate on the cervical spine.

Sudden acceleration of the sled produced a concussive response. (the amount of force required to produce a concussive response was increased by 50 percent when a cervical collar was applied in order to restrict rotational acceleration of the head). This phenomenon explains the occasionally observed concussion following a fall on the buttocks, or a sudden hyperextension of the cervical spine in a motor vehicle accident.

At present, it is generally agreed that experimental concussive injuries produced by impact acceleration are accompanied by the production of pressure waves within the brain substance. However, the pathophysiology of concussion--how the combination of impact, acceleration, and changes in intracranial pressure produce reversible alterations in neurological function--remains unknown.

EARLY THEORIES REGARDING PATHOPHYSIOLOGY

1) Vascular alterations: a) Ischemia
b) Hemorrhage
c) Cerebral Edema

a) Among the earliest theories attempting to explain the pathophysiology of concussion, changes secondary to vascular abnormalities were popular. Stromeyer (1864) considered, for instance, that concussion resulted from flattening of the skull at the point of impact. This distortion was thought to produce cerebral anemia by expulsion of blood from brain capillaries. Kramer (1896) believed that concussion occurred consequent to cerebral vascular compression produced by the observed momentary rise in intracranial pressure. This cerebral "anemia" was proposed to be responsible for the transient neurological disturbances witnessed. The famous physiologist W.B. Cannon (1901) supported this theory, relating the loss of consciousness in concussion to "a checked blood flow" resulting from a sudden increase in intracranial pressure. In company with Cannon, several eminent physiologists and clinicians, including Sir Wilfred Trotter (1924), Wolff (1936), and Fulton (1942) subscribed to the theory of impaired cerebral circulation. More recently, Dott (1960) has resurrected this theory, proposing that ischemia-- particularly of the diencephalon--may be the etiology of concussion. He suggested that, as the brain moves in relation to the skull, the blood vessels of the brain stem are stretched, develop spasm, and produce subsequent brain stem ischemia.

It has been known for some time (Cushing, 1901) that raised intracranial pressure will result in diminished cerebral blood flow. However, even complete interruption of cerebral blood flow will not result in instantaneous loss of consiousness (as is observed in concussion). For instance, Beauvieux (1905), during observations of the bizarre method of execution by guillotine, noted that following decapitation the subject's eyes would turn and look at the observer if the name was called in the first 25 seconds. The corneal reflex was obtainable in some cases for as along as two minutes, indicating continuing neural function in the

absence of circulation. Rossen, et al. (1943) have also demonstrated that sudden interruption of the cerebral circulation is not accompanied by instantaneous unconsciousness; this occurs at about six to seven seconds. This evidence argues against the theory of a sudden diminution of cerebral blood flow being responsible for the clinical signs of concussion.

b) From ischemia, the focal point turned to hemorrhage. Schaller (1941) proposed that concussion was due to multiple petechial hemorrhages which were dependent upon the combination of vasodilatation, vascular stasis, and anoxemia. However, in the experiments which he reported, the majority of experimental head injuries were complicated by torn nerve fibers, gliosis, and torn blood vessels at post-mortem examination. Schaller postulated that the reversible effects of concussion were accounted for by resorption of perivascular petechiae. Denny-Brown and Russell (1941) disagreed that the histological changes attributed to vascular injury were essential to the production of concussion. They argued that a transient neurological disturbance could not be explained by a permanent pathological change.

It is well recognized that continued intracranial hemorrhage will eventually produce loss of consciousness, but the hemorrhage must be massive in order to produce an instantaneous loss of consciousness; such as that observed in spontaneous subarachnoid hemorrhage.

Several authors have demonstrated increased vascular permeability following concussion (Ommaya and Rockoff, 1964; Rinder and Olsen, 1968; Povlishock, et al. 1978, 1979). These changes were usually localized to the brain stem and cervical spinal cord, but have been inconstant. The authors postulated that the increased vascular permeability may have been due to either a) direct mechanical vascular damage; b) a result of the increase

in blood pressure observed after such experiments, or c) a reflection of uptake of tracer by damaged neurons or glia.

c) Cerebral edema will certainly result in neurological dysfunction, which may be reversible. This dysfunction is a result of either pressure effects, or the dilution of the extracellular ions which are involved in neuronal transmission. However, cerebral edema does not develop within seconds, and therefore, cannot explain the instantaneous neurological dysfunction. The original studies of Eichelberger, et al. (1949) in anesthetized dogs concluded that cerebral edema did not result from "pure" concussion--i.e. uncomplicated by vascular damage. The water and electrolyte contents of brain tissue remained normal when tested up to 24 hours post-trauma. The one exception was a decrease in brain tissue calcium content observed in the brains of animals sacrificed 24 hours post-trauma. This interesting observation has not been followed up, but may have a role to play in altered neuronal membrane permeability.

Recent studies by Faas and Ommaya (1968) using more modern techniques, and measuring water, sodium, potassium and chloride in separated gray and white matter of concussed monkeys reached the same conclusions. The monkeys showed no evidence of cerebral edema following experimental concussion, and showed no demonstrable neurological deficits following the trauma. The authors postulated that the increased brain volume reported by White, et al. (1943) may have been due to increased cerebral blood volume secondary to transient vasoparalysis. Grubb, et al. (1970) could not incriminate cerebral edema as part of the pathology of concussion, either.

In recent studies, Povlishock, et al. (1978, 1979), using light and electron microscopy, investigated low-grade compressive-type head injuries. A 2-2.5 atmospheric pressure blow to the opened cranium of anesthetized cats

produced a "concussive response", without neuropathological changes. The authors noted that horseradish peroxidase, injected into the animals prior to trauma, could be traced to leak across the walls of brain stem arterioles, venules, and capillaries within three minutes. The authors stressed that the endothelial cell membranes and tight junctions had remained intact. They postulated vesicular transport of the horseradish peroxidase across the endothelium of the brain stem vasculature. The tracer could eventually be localized within neural, glial, and pericytic elements. The relationship of these findings to the pathophysiology of concussion is unclear, but it was questioned whether or not the observed changes may be a precursor of chromatolysis. They are more likely to represent epiphenomena of concussive injury than the etiology of the instantaneous neural dysfunction.

STRUCTURAL CHANGES

Greenfield (1939) first suggested that concussion may be due to reversible alterations in the anatomy of neurons, or their axons. He reasoned that transient stretching and distortion of nerve fibers might produce a transient neurological disturbance. In subsequent studies, Windle, et al. (1944, 1945) opposed the view that concussion was purely functional and reversible, without permanent anatomical changes. They studied anesthetized guinea pigs that were "concussed" and later sacrificed. The authors observed fragmentation and disarrangement of Nissl bodies, accompanied by chromatolysis. These changes were delayed; maximal at six to eight days following concussion. They affected mainly the lateral vestibular nuclei, pontine tegmentum, and the reticular formation. Groat, et al. (1945 A, B; 1950) described similar changes in anesthetized primates and guinea pigs and suggested that the loss of neurons observed was directly proportional to the severity of the concussion. Chason, et al. (1958, 1966) described chromatolytic changes in the medial and lateral reticular substances of the medulla. Friede (1961) found chromatolysis and loss of nerve cells in the reticular formation, lateral vestibular nucleus, and red nucleus in experimental "concussion" of anesthetized cats, produced by stretching of the cervical cord at the cranio-vertebral junction. Friede interpreted these cellular changes as secondary to injury to the axons of these cells in the high cervical spinal cord. He concluded, however, that a head injury may produce functional changes without concomitant morphological changes. Gurdjian, et al. (1966) observed neuronal damage in the brain stem and reticular formation, medial temporal lobes, and posterior thalamus in the experimental concussion of anesthetized dogs.

Bakay, et al. (1977) observed swollen neuronal mitochondria in the occipital cortex and cerebellar cortex, along with increased permeability of the

blood-brain barrier to injected ferritin. The changes were observed within a half-hour of the injury, were maximal at one hour, and had disappeared by 24 hours. Chromatolysis was not observed. The authors wisely concluded that the morphological changes did not, by themselves, explain the loss of consciousness in concussion. Similar electron microscopic studies by Povlishock, et al. (1978, 1979) discussed earlier also did not indicate permanent pathological changes.

Clinical pathological studies of human concussion are few, since it is rare that a concussion proves fatal. Strich (1961) studied the pathology of the brains of young patients who had been injured in motor vehicle accidents, and who remained comatose until time of death. Although the white matter appeared grossly normal, there was severe neuronal degeneration microscopically. The nerve fibers in the white matter were torn, with the formation of "retraction balls". From these findings, Strich postulated that a lesser injury, such as "stretching" of the nerve fibers, may at some stage be associated with the transient neural dysfunction characteristic of concussion. Oppenheimer (1968) supported Strich's theory. He studied five patients who had suffered a concussion, and died later of unrelated causes. He inferred that previous authors had failed to discover anatomical injuries because the latter took about 72 hours to develop. He recorded axonal injuries in all of his patients. This finding has yet to be confirmed by others.

As a result of their investigations into the pathology of human head injuries, Peerless and Rewcastle (1967) concluded that the definition of concussion should not exclude the fact that a small number of neurons may have perished as a result of the head injury. The number may be "so small as to be negligible at the time, but which may become clinically significant after further damage".

Several criticisms of the above-quoted studies can be levied. The experimental studies were all performed with the animals under general anesthesia, which excluded the possibility of neurological examination. Hence, the diagnosis of concussion was based upon the "concussive response"; alteration of brain stem reflexes and associated autonomic disturbances--both of which may occur with more serious head injuries. In spite of poor controls, lack of statistical analysis and the use of relatively primitive anatomical techniques in the earlier studies, they continue to be widely quoted. As stated in the introduction, this search for irreversible cell damage is not germane to the investigation of the pathophysiology of uncomplicated concussion per se. It cannot be reasonably argued that the generalized transient neural dysfunction is explainable by the loss of a small, circumscribed population of neurons. As Kaplan and Browder (1954) have succinctly stated; "cerebral concussion is a clinical syndrome that may be associated with, but unrelated to a variety of cerebral pathological states."

ELECTROPHYSIOLOGICAL STUDIES

Ranson's work (1939) first focussed on the importance of the integrity of the basal brain region in the maintenance of normal consciousness. He demonstrated that bilateral lesions in the hypothalamic area of monkeys were effective in producing somnolence and catalepsy. Jefferson (1944) provided a record of the clinical correlate of such lesions. He observed that, in addition to the loss of consciousness, alterations in blood pressure, respiratory rate, pulse rate, and brainstem reflexes were common in concussed subjects. His neurosurgical practice had demonstrated that tumors or vascular lesions affecting the brain stem invariably produced stupor and coma. Consequently, he reasoned that trauma most probably exerts its effects on consciousness by disturbances in the same area. His acute insight also led him to comment that "...it is no more 'required' to show histological changes (in concussion) than it is in post-epileptic stupor and in Todd's paralysis". In effect, he was comparing the transient neural dysfunction--of unknown etiology--occurring post-ictally, with that occurring post-concussion.

The classical work of Moruzzi and Magoun (1949) confirmed that the brain stem was indeed important in the modulation of the conscious state. They demonstrated that electrical stimulation of the brain stem reticular activating system was extremely effective in producing a state of arousal in experimental animals. Lindsley, et al. (1949) continued these studies in unanesthetized "encephale isolée" preparations. These authors demonstrated that those lesions in the reticular activating system that interfered with wakefulness led to abolition of the cortical activation pattern, with the production of high voltage slow waves, spindles, and increased synchrony. The EEG patterns following lesions in the reticular activating system represent "opposite extremes of these contrasting states". (that is, wakefulness and sleep). French and Magoun

(1952) reported that the volume of destruction of the area governed the degree of behavioral and EEG effect on the animal. In some cases of incomplete injury, the animals would recover consciousness, at which time the EEG also improved. They postulated that their lesion had not destroyed all the cells in the reticular activating system, but had "functionally inactivated" some of them.

Foltz, et al. (1953, 1956) examined the role of the reticular activating system in experimental concussion of anesthetized monkeys. These authors implanted recording electrodes in the medial reticular formation, the medial lemniscus, and cerebral cortex in order to record evoked potentials resulting from stimulation of the sciatic nerve. Following a blow to the head, the evoked potentials were abolished from the reticular formation, but remained unchanged in the medial lemniscus and cortex. Spontaneous return of the evoked potentials occurred at varying intervals following concussion. However, this return to normal could not be correlated with return of consciousness, since the animals were anesthetized. (Since this experiment was performed, Magoun (1963) has shown that anesthesia--either barbiturate or ether--produces a marked depression, or abolition of reticular potentials, while the primary surface potentials of the cortex remain unattenuated. This is an excellent example of how the use of an anesthetic may complicate the interpretation of such an experiment. For instance, fluctuations in the depth of anesthesia undoubtedly affected the recording of evoked potentials from the reticular formation). Ward (1966) postulated that the observed diminished medial reticular formation activity may be due to a restriction of spread of activity within the multi-synaptic system of the reticular formation. Early support for this theory is found in the writings of Gurdjian (1966) and Ommaya (1974). Currently, most investigators subscribe to the theory of transient dysfunction of the reticular

activating system as the cause of unconsciousness following concussion. It must be remembered, however, that pathology affecting the cerebral cortex diffusely may also result in coma (French, 1952; Plum and Posner, 1972). The role of the cerebral cortex in concussion has been neglected until relatively recently. Dysfunction of the reticular activating system is unlikely to explain the amnesia, or impaired learning that accompany a concussion.

Several studies of electroencephalographic recordings from concussed experimental animals have been published. Interpretation of these studies has been complicated, however, by the use of general anesthetics at the time of, or just prior to concussion. These drugs alone have the ability to produce profound EEG changes, with the appearance of widespread delta waves and discontinuous recordings characterized by burst-suppression patterns (Scott, 1976). More importantly, the presence of an anesthetic clouds the subject of study, that is, the level of consciousness. It is impossible to define the relative contributions of concussion and anesthesia to the production of coma and EEG dysrhythmia. Nor have EEG studies in humans documented the immediate response to concussion. It is difficult to transport a patient from the site of injury to a medical facility where an EEG may be recorded. Also, of course, there is no "baseline record" for comparison. Minor abnormalities present on a post-concussion EEG may have been present prior to injury. Previously reported studies also had in common the fact that they were restricted to a qualitative analysis of the EEG, because of the limitation of the methods available at the time. Presently, the EEG power spectrum can be analyzed by computer; statistical studies of each frequency band are generated and hence a quantitative study can be performed (Havlicek, et al. 1977).

The following studies have recorded depression of the EEG in anesthetized animals following experimental concussion: Denny-Brown and Russell, 1941; Williams and Denny-Brown, 1941; Gurdjian, et al. 1944, 1975; Dow, et al. 1944, 1945B; Ward and Clark, 1948; Foltz, et al. 1953; Meyer and Denny-Brown, 1955; Ward, 1966; Meyer, et al. 1970; Grubb, et al. 1970; Brown, et al. 1972; Letcher, et al. 1973; Ommaya, et al. 1973, 1974, 1976; and Sullivan, et al. 1976.

In addition to the EEG depression, most investigators also witnessed the appearance of high-voltage slow waves sometime after concussion. Some interpreted these as evidence of cortical injury, others as an early stage in recovery of the EEG. Several stated that the EEG began to recover at various stages after concussion (Williams and Denny-Brown witnessed complete recovery), but most did not accurately document the time period of the EEG abnormality, or continue the EEG record in order to verify complete recovery. Again, it must be stressed that in these studies the added variable of an anesthetic was present. The only exception of which I am aware is the study by Letcher, et al. (1973), in which monkeys were anesthetized in order to be restrained, but were alert at the time of impact. In the four monkeys studied, EEG depression was observed at the site of impact, with the later appearance of slow waves. No statistical, detailed analysis of the EEG records was performed.

Walker (1944) suggested that concussion was accompanied by increased EEG activity, due to a sudden intense neuronal discharge within the brain. However, his recordings probably represented the "cortical injury potential" (following local trauma to the cortex, the cortex becomes negative in respect to the white matter by three to nine millivolts--Meyer and Denny-Brown, 1955). Few investigators subscribe to Walker's interpretations.

Ward attempted to clarifv the question by comparing the changes due to concussion with those observed following "spreading depression". Leão (1944) in his original experiments produced depression of the EEG in response to local trauma to the exposed cortex. The depression of the EEG was first apparent locally, and then spread to involve the entire cerebral cortex. Complete recovery followed. Whether or not the phenomenon of "spreading depression" plays a role in the transient neural dysfunction of concussion has not been further investigated.

The literature is replete with studies of EEG's in human head injuries, some of which have concentrated upon the response to concussion (Williams, 1941; Dow, 1945A; Laufer, et al. 1947; Larsson, et al. 1954; Rodin, et al. 1965; O'Leary, 1966; Reid, et al. 1971). The shortest interval between trauma and recording was 50 minutes in Williams' series. No baseline records were available for comparison. The prominent finding in his study was the appearance of abnormal slow waves, which persisted as long as the level of consciousness was impaired. As the slow waves decreased in voltage, the frequencies increased. Very few patients could be followed until their records returned to normal. Dow (1945A) set up a recording lab in close proximity to a dock-yard, and therefore was able to obtain some records within ten minutes of head injury. Still, most cases were clinically recovered from concussion by the time their EEG was recorded, and any initially abnormal EEG had recovered within minutes. Follow-up records showed no difference between the 197 cases of head injury and 211 controls. (10 percent abnormal records in the first group, 8 percent in the second). To the authors, this suggested some mechanism in concussion other than permanent anatomical damage.

Kaplan and Browder (1954) obtained 1400 EEG's in 1043 boxers who had been under observation of the authors during their fights. Some records were

obtained within ten minutes of the end of a bout. They observed no changes suggestive of permanent structural damage.

Larsson, et al. (1954) studied boxers within 15-30 minutes of a concussing blow. They also observed generalized depression of the EEG and/or the appearance of slow-waves in 30 percent of the subjects. They interpreted this generalized depression to result from either general cortical damage, or injury to a system which projects to the entire cortex (such as the reticular activating system).

The study of Reid, et al. (1971) is worthy of comment, since these authors attempted to study the EEG response to concussion in a subject that had a baseline EEG, was unanesthetized, and had a high probability of being concussed. They prepared a football player so that his continuously recorded EEG would be observed by telemetry. A device was attached to his helmet in order to measure acceleration, then the plays in which he took part were recorded on videotape. After several plays, the subject was eventually concussed. The EEG record demonstrated depression of EEG activity over the cerebral hemisphere contralateral to the blow initally. A repeat record three days later demonstrated a "mild slow wave disorder". The record was then followed no further.

Ommaya, et al. (1973, 1974, 1976) have underlined the importance of employing the cortical sensory evoked potentials as an electro-physiological marker of concussion. These authors observed that the onset of "paralytic coma" following a head injury was accompanied by abolition of the P2 wave of the sensory-evoked potential at the cortex (the P2 wave represents the cortically-recorded signal of sensory afferents travelling via the extralemniscal pathway). Return of the P2 wave coincided with the return of the animal's responsiveness and motor performance. Long-lasting effects were observed at

the level of the cerebral cortex. The time taken for the P2 wave to travel from one hemisphere to the opposite hemisphere was increased following concussion. This impairment of cortical function persisted longer than the abnormality in the P2 wave. Whether or not it returned to normal was not stated.

The above discussions have concentrated on the role of the reticular activating system and the cortex in the production of the unconscious state. Other areas that deserve mention, but which have not been subject to specific study in concussion include:

a) The diencephalon, where Hess (1932) has shown that certain areas induce sleep when stimulated.

b) The amygdala and hippocampus. Bilateral hippocampal damage markedly impairs memory. Psycho-motor epilepsy--whose prime characteristic is some disturbance of consciousness--often originates in the medial temporal lobes. Also, stimulation of the medial temporal structures may produce a state of unawareness, often associated with retrograde and antegrade amnesia.

c) The frontal lobe. Acute bilateral sectioning of the prefrontal fibers passing to the thalamus may result in a state of somnolence. (Walker, 1973)

Although there have been many studies of the electrophysiological response to cerebral concussion, there has yet to be a study which incorporates a baseline EEG recording in a non-anesthetized animal, which is followed post-concussion in order to characterize the changes in EEG frequency and amplitude and follow their recovery. A statistical (quantitative) interpretation of the EEG would be mandatory in order to reach firm conclusions.

CHANGES IN NEUROTRANSMITTERS

One might expect that studies of putative neurotransmitters would provide important information regarding the pathophysiology of concussion. Except for a few early studies, however, there has been very little investigation regarding the role of these important substances in head injury. Bornstein (1946) postulated that concussion may be due to an alteration in synaptic transmission. He analyzed the cerebrospinal fluid (CSF) content of acetylcholine in animals that had been concussed, under anesthesia. With the bioassay he used, there was no acetylcholine detectable in the CSF under normal conditions. Following reversible head injuries, this investigator observed significant amounts of acetylcholine in the CSF. He also noted a crude correlation between behavior, EEG abnormality, and CSF acetylcholine concentration. Atropine sulfate, injected subcutaneously within 30 minutes of concussion would reverse the EEG changes. He claimed to be able to mimic the clinical and EEG effects of concussion by the application of acetylcholine directly to the cortex. Earlier, Jasper (1941) had demonstrated that although small, physiological concentrations of acetyl choline synchronize the EEG, large amounts depress the electrical activity of the cortex. Bornstein concluded that acetylcholine may be one of the factors involved in the "traumatic paralysis" of concussion. Tower and McEachern (1948, 1949) studied the CSF of patients with head injuries and supported Bornstein's laboratory findings. Fourteen cases of head injury were observed to have increased CSF acetylcholine concentrations, associated with low CSF cholinesterase concentration. Recovery of the patient with reversal of the EEG abnormalities was said to be accompanied by a parallel decrease in acetylcholine concentration. Sachs (1957) recorded similar findings in clinical and laboratory studies. He also demonstrated reduction of CSF acetylcholine values in head-injured patients following the administration of

atropine. (In 6 of 28 cases, he also described the presence of serotonin in the CSF) Metz (1971) substantiated the earlier work by his studies on anesthetized dogs. A blow directly to the cortex resulted in significantly increased release of acetylcholine from the cortical cells (measured by means of a push-pull cannula), which was noted within 20 minutes of trauma. The increase reached a plateau at 40 minutes and remained so for the duration of the experiment (170 minutes). The author wisely questioned whether the observed release of acetylcholine was a contributing factor to the effects of craniocerebral trauma, or a coincidental byproduct of no importance. Tower (1966) postulated that the increased release of ACh could be due either to neuronal excitation or direct traumatic release. On the basis of these findings, Ward (1950, 1966) administered high doses of atropine sulfate to head-injured patients. He claimed good results in "selected cases", but these evidently have not been substantiated.

Osterholm, et al. (1969) studied the brain levels of serotonin following trauma. Intraventricular administration of serotonin has been shown to produce decreased motor activity, lethargy, tremor, and convulsions. Large doses limit or diminish neural activity. Osterholm's studies were concentrated on injuries which were more serious than concussion. Most animals had demonstrated cerebral contusions. Six of the twenty-five experimental animals (cats) died from the injury; four had subdural hematomas, two had "pulped brain tissue". However, his results were interesting. He found that the concentration of serotonin in cortex underlying the site of impact was twice the control value at one hour post-trauma. At six hours post-trauma, the serotonin content of the hypothalamus, pons, and limbic system were four times as high as control values. This study postulated that the trauma induced release of serotonin from intracellular sites might act upon other neurons to depress their function, thus

resulting in post-traumatic neurological deficits.

Vecht, et al. (1975 A, B) and Bareggi, et al. (1975) have studied the turnover of the monoamines serotonin and dopamine in coma secondary to head injuries of varying severity. Comatose patients were given probenicid in order to inhibit the active transport of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) from the CSF and brain into the blood. These authors found decreased CSF levels of HVA following head injury. 5-HIAA levels were normal or increased during the state of unconsciousness, but decreased remarkably after regaining consciousness. In their series of 98 patients, Vecht, et al. (1975 A) claimed a good correlation ($r = -9.46$, $p < .05$) between the duration of coma and the decreased CSF HVA levels. Also, twenty-six days post-trauma, the 5HIAA levels of those in coma were greater than in those who eventually recovered ($p < .005$). The authors suggested that the observed CSF changes may indicate altered cerebral dopamine and serotonin metabolism after head injury (although the presence of blood-brain barrier damage, hemorrhagic CSF, and serotonin release from platelets may have affected the results).

Huger and Patrick (1978,1979) studied the effect of concussion on brain catecholamine levels and synthesis. They postulated that perturbations in the central catecholamine systems may be responsible for some of the signs of concussion (eg. loss of consciousness, hypertension, and motor dysfunction). Concussion was produced in non-anesthetized rats by means of an acceleration-deceleration device. Loss of consciousness lasted about two minutes; convulsions 30 seconds; and hyperventilation two to five minutes. Subarachnoid hemorrhage was commonly observed, but contusions were not consistently found. Controls consisted of a sham group who were accelerated, but had no head impact. Dopamine levels were found to be increased in the medulla-pons at five minutes post-concussion, and the midbrain-hypothalamus at 15 minutes and one

hour after trauma. Norepinephrine synthesis rates were significantly increased in the cerebella of traumatized animals at two hours post-concussion. In general, dopamine synthesis was slightly higher and norepinephrine synthesis slightly lower in the traumatized group than in the sham group (indicating possible accumulation of the norepinephrine precursor dopamine). Some of these alterations were probably due to stress, since they occurred also in the sham group (the animals were confined in a small animal holder for about two minutes). Their results are more interesting when compared to the previously quoted studies of Bareggi, et al. (1975) and Vecht, et al. (1975 A, B). If the increase in dopamine is due to interference with its degradative pathway, one might expect to find decreased CSF values of HVA, as Bareggi and Vecht have reported.

Cyclic AMP and cGMP, substances which mediate the central effects of bioactive amines and acetylcholines have recently been the subject of study in head injury. Rudman, et al. (1976) and Fleischer, et al. (1977) measured the levels of ventricular fluid cAMP by radioimmunoassay in 133 samples from 26 patients who were comatose following head injury. The levels of c AMP in these patients were decreased in comparison to controls (15-30 nM). The grade of coma correlated well with the cAMP level ($r = -.080$, $p < 0.01$), i.e. deeper coma--lower cAMP level. Remarkably, no patient recovered to a normal sensorium until the cAMP reached the lower limits of normal (15 nM). Plasma cAMP levels were normal, leading the authors to postulate that the low CSF levels resulted from a disorder of cAMP metabolism within the nervous system (eg. decreased production of adenylate cyclase, inadequate delivery of cyclase-activating hormones, decreased ATP, or increased transport of cAMP out of the CSF). It is interesting to speculate that the observed changes in cAMP levels may occur--much more rapidly--during the phenomenon of concussion. However, no such study has been reported.

DISTURBANCES IN CEREBRAL METABOLISM

Meyer, et al. (1970), in an excellent study, recorded the effects of concussion (administered with an airgun at a burr-hole site) in anesthetized baboons. The authors measured cerebral blood flow (CBF), cerebral lactate, and pyruvate and cerebral metabolic rate of oxygen consumption. The concussion apparatus produced a transient rise in intracranial pressure, associated initially with an increase in cerebral blood flow, and usually a delayed (about two to four minutes) decrease in CBF. "Mild concussion" was shown to produce an increase in cerebral metabolism, while "severe concussion" was associated with a decreased cerebral metabolism. The increase in cerebral metabolism in the mildly traumatized group was accompanied by a burst of low voltage past activity (14-20 Hz) on the EEG in 10 of 19 cases. One wonders whether these experiments in lightly anesthetized animals actually produced a "mild concussion", or perhaps, only a stressful pain stimulus, which in effect activated the EEG. The "severe concussion" group demonstrated, in addition to a later decrease in cerebral blood flow and decreased cerebral metabolism, transient slowing of the EEG. Despite decreased metabolism, the cerebral lactate production was not increased. The increased CBF in the face of decreased metabolism in some animals may reflect loss of cerebrovascular autoregulation.

The transient increase in CBF immediately following concussion was also observed by Walker (1944), Gurdjian, (1954), Rinder (1969), Stahlhammar (1975), and Nilsson (1977). In most studies, this transient increase was followed by a decrease below control values, which persisted for 20-40 minutes. Tindall, et al. (1972) studied 11 baboons subjected to "severe concussion" while in the alert state. Cerebral blood flow decreased to 30 percent of the original flow. Autoregulation later recovered, as tested with norepinephrine-induced hypertension. Rockoff and Ommaya (1964) also demonstrated slowing of the cerebral

circulation 15-30 minutes following concussion in monkeys. The transient increase has been explained, as above, as due to acute loss of regulation so that cerebral vascular resistance is suddenly decreased. The later decrease in cerebral blood flow may be a response to the decreased metabolic demand of transiently paralyzed neural tissue. An alternative explanation is the presence of "vasospasm". In an angiographic study of rats subject to acceleration concussion (7-9 meters/sec. trauma) under general anesthesia, Ekelund, et al. (1974) demonstrated a high incidence of vasospasm. However, their trauma model produced skull fractures in four of seven rats subjected to blows at eight to nine meters per second and subarachnoid hemorrhage in all rats in this group. One might argue that the vasospasm was obviously secondary to the subarachnoid hemorrhage, however, vasospasm was also observed in animals in whom no subarachnoid hemorrhage was observed.

The above studies support the evidence (discussed earlier) that the loss of consciousness observed in concussion is not due to decreased CBF; the CBF is often increased immediately. Ponten and Siesjo (1973) demonstrated that concussion could be experimentally produced without the depletion of brain energy reserves that were associated with cerebral contusion or laceration. These observations were in agreement with earlier reports by Gurdjian, et al. (1944, 1975).

Nilsson, et al. (1977 A, B, C, D) studied the immediate effects of an acceleration concussion model on cerebral metabolism. The striking velocity ranged from seven to nine meters/second. Most animals concussed (under anesthesia) at seven meters/second showed no detectable change of the energy states of the brain regions studied. After a nine meter/second injury, the changes were pronounced. The authors noted decreased phosphocreatine levels increased lactate, ADP , and AMP. The changes developed rapidly and about 50

percent of the animals studied showed a derangement of energy state in brain stem regions, to levels comparable to those obtained after 10-20 seconds of total cerebral ischemia. In this model, however, it is difficult to be sure that only a concussion was produced. Indeed, many animals demonstrated linear skull fractures, subarachnoid hemorrhage, cerebral and brain stem hemorrhages. The rats were not alert at the time of "concussion" and were not followed until they recovered. It is difficult in a study such as this to decide whether the energy changes were due to the concussion, or the associated pathology observed.

The initially increased metabolic rates described by Meyer, et al. (1970) and Nilsson, et al. (1977 A, B, C, D) led them to conclude that concussion represents a trauma-induced general neuronal excitation, followed by neuronal paralysis (similar to post-ictal depression). That is, the initial neuronal excitation is accompanied by increased cerebral metabolism. Energy depletion by this abnormal neurophysiological activity may then lead to a prolonged state of cerebral dysfunction, manifested by unconsciousness.

Chason, et al. (1966) proposed that, since nerve cell function depends upon the presence of glucose as a source of energy, perhaps concussion results from a sudden arrest of carbohydrate metabolism (possibly following destruction of the enzymes that metabolize glucose). This theory is yet to be substantiated by experimental evidence.

Another novel, but unsubstantiated theory was volunteered by Dixon (1962). Based upon previous studies that suggested that "memory" depends upon continuing neuronal protein synthesis, Dixon suggested that the amnesia of concussion may be produced by an alteration of the specific RNA responsible for the fabrication of such a protein.

> "Repetitive patterns of neuronal activity depend on the intra-molecular composition of neuronal proteins. The structure of the protein molecules may be modified by impulses reaching the neurons".

These changes would affect only recently acquired memories, thus producing a retrograde amnesia.

CLINICAL STUDIES OF CONCUSSION AND THE POST CONCUSSION SYNDROME

Some experienced neurologists have suggested that recovery is not always complete following concussion. Such claims are evident in discussions of the "post-concussion syndrome". The term describes a complex of symptoms consisting of headache, irritability, impaired concentration, memory, and learning which characteristically follow a mild head injury (Courville, 1953). The symptoms have in common the facts that they are entirely subjective, and are very rare following severe head injuries (Denny-Brown, 1945 A). In fact, Miller (1961,1966), who has studied this problem in detail observed that the severity and duration of post-concussional symptoms were inversely related to the severity of the injury, and that recovery often occurred coincidentally with the settlement of compensation claims. Furthermore, Miller reported that the most consistent feature of the syndrome was the patient's claim of unfitness for work, which was unrelated to any true disability, "even if its symptomatology is accepted at face value". Friedman (1969) drew similar conclusions and claimed that the syndrome "has no pathological counterpart". Symonds (1962) admits that the symptoms are of a psychologic nature but claimed that this did not necessarily mean that they did not have a physical basis. They are related, be said, to all those qualities which constitute the patient's personality. He found no difficulty in explaining the existence of the post-concussion syndrome in some patients and not in others. He interpreted the phenomenon of concussion as a graded spectrum, with the inclusion of structural damage at the severe end of the scale. This is a highly personalized definition and does not agree with most observations. Rutherford (1977) could not correlate existence of post-concussion symptoms with the severity of injury, in particular, the time period of post-traumatic amnesia, as might be expected if the symptoms were wholly due to organic causes. He concluded that each

post-concussion symptom might be explained on either an organic or functional basis. The fact that there was a strong correlation between symptoms and motivation suggested a neurotic element. Russell and Smith (1961) in their classical study drew attention to the strong correlation between the severity of an individual head injury and the duration of post-traumatic amnesia (P.T.A.) in that case. Their conclusions, in agreement with those of Rutherford (1977) were that post-injury signs and symptoms showing a positive correlation with an increased duration of P.T.A. were "primarily organic". Those unaffected by the duration of P.T.A. were said to be "nonorganic" or psychogenic. Taylor (1966), along similar lines, stated that post-traumatic symptoms (excepting amnesia and vertigo) "defy any attempt at anatomic localization". It is interesting that in injuries where compensation is not a question, eg. sports injuries, recovery is typically rapid and post-concussion symptoms rare. Cook (1969) documented this fact in a series of sport-related injuries. He concluded that:

> "the post-concussion syndrome with its stereotyped and persistent symptoms relate neither to concussion, nor to brain injury, nor possibly, even to any frightening aspect of the accident; its existence depends upon by whose hand the injury was caused".

Gotten (1956) recorded that 88 percent of his cases with post-concussion symptoms improved after settlement of litigation. Psychosomatic symptoms were expressed in some way in 85 percent of cases.

The main criticisms of clinical studies are that they are retrospective and poorly controlled. Although it is difficult to find two head injuries which are strictly comparable in all aspects, attempts at controlling the studies by taking such factors as the nature of the accident, the speed of the vehicle, associated injuries, and the period of amnesia, into account have been ignored. For example, Taylor's exacting study of cerebral blood flow in patients with

post-concussion symptoms concluded that such patients have an increased cerebral circulation time when compared to "controls". However, there was no attempt to match the two groups regarding severity of injury (eg. length of time of unconsciousness, amnesia, presence or absence of skull fracture, etc.). (Taylor, 1966). A similar example is the study by Gronwall and Wrightson (1974 A) This experiment compared controls with post-concussion patients in a "paced auditory serial addition test". The post-concussion patients were reported to perform poorly in this relatively simple arithmetical quiz, with return to normal weeks after the accident. The original paper does not note the fact, but personal communication has verified that all the post-concussion patients were being financially compensated (Gronwall and Wrightson, 1974 B).

A prospective study was begun at the Health Sciences Centre in 1978, in order to document factors influencing recovery from head injury. All gradations of severity have been included. Mitigating circumstances, as well as the influence of litigation are being documented. Tabulation of the multiple factors involved has been computerized. This is a unique study and should help to clarify the post-concussion syndrome. At this time, more than 1500 patients have been included. (Parkinson, et al. 1980).

Study of the behavioral effects of concussion in man is obviously complex. It is difficult or impossible to envisage a situation where a test of memory, performance, or learning ability could be compared before and after concussion. Although man is willing to expose himself to situations where concussion is predictable (eg. boxing, football), it is understandably difficult to recruit volunteers for a controlled study of the effects of head injury on higher functions. As a result, the scientist has turned to the laboratory animal in order to study the phenomenon. In experiments of this nature previously performed, the subjects have been anesthetized. This immediately eliminates the key

subject of attention, that is, the level of consciousness. "Concussion" in such preparations has been diagnosed on the basis of such alterations as decreased corneal reflexes, decreased pinna reflexes, abnormal righting reflexes, and particularly, changes in blood pressure, pulse rate, and respiratory pattern. These signs all have in common the fact that they may be produced by injuries which inflict pathology far more serious than uncomplicated concussion (Gurdjian, 1944, 1975). Also, the presence of an anesthetic adds an important variable to experimental concussion. Ommaya (1966), Gurdjian (1975), Parkinson (1977), and Shetter and Demakas (1979) have all emphasized the drawbacks of using anesthetics in such studies. Ommaya (1966) has stated:

> "Perhaps the greatest remaining difficulty with such a model (of experimental concussion) is evaluation of the state of consciousness in animals subjected to trauma under anesthesia. Reproducible control of degrees of anesthesia is not possible; then, determination of the relative significances of anesthesia and concussion in a reduced state of consciousness is very difficult".

An example is a paper by Ommaya, et al. (1971). These authors reported partial retrograde amnesia when concussion was produced in the rat less than seven seconds after training in a passive avoidance learning situation. Animals trained during the post-concussional period compared well with controls in performance, but when re-tested 24 hours later, their performance was impaired. In these experiments, the rats had received a dose of Brevital for preparation, but were allowed to recover from the anesthetic just prior to concussion (Ommaya, 1975 P). The results then obviously reflect the combined effects of an anesthetic and trauma. Which is predominant?

In a scholarly paper, Govons, et al. (1972) studied concussion in non-anesthetized rats. He pointed out the fact that a blow to the head is an accepted method of "humanely" sacrificing laboratory animals, and that:

> "a concussive blow to the head is a rapidly acting anesthetic, stunning an animal instantly and rendering it helpless and insensitive to pain...administration of drugs in the study of head blows is unnecessary".

He studied 35 undrugged animals and noted no evidence of fear, rage, or conditioning following experimental concussion. The animals could be handled with bare hands following concussion, suggesting the possibility of post-traumatic amnesia. In this study, concussion was frequently manifested by a seizure--usually most notable in the hind limbs. The seizure lasted 15 to 60 seconds. Postictally, the rats remained limp and motionless except for respiratory activity. One to eight minutes following concussion, the animal was able to right itself. For 24 hours following concussion, spontaneous movement was observed to be reduced as much as 50 percent. Within three days, most animals had regained full activity. In this experiment, concussion was produced in non-anesthetized rats. Pathological examination revealed no skull fractures, cerebral contusion, or hematomas and clinical examination revealed no detectable permanent neurological deficit.

In a review of over 200 papers studying the phenomenon of concussion, Govons, et al. (1968, 1971, 1972) and Letcher, et al (1973) have been the only investigators who studied the phenomenon of concussion in non-anesthetized animals. This, of course, is the most accurate experimental model of the clinical problem.

The remainder of this dissertation documents our studies of concussion in non-anesthetized animals; attempting to clarify the pathophysiology of this syndrome.

EFFECTS OF EXPERIMENTAL CONCUSSION UPON MEMORY AND LEARNING IN THE CONSCIOUS RAT

Studies of slow-motion film strips of boxing ring knock-outs have demonstrated that concussion appears to be a graded phenomenon clinically (Parkinson, et al. 1978). Concussion could be divided into four stages. In Stage 1, there is impairment of memory; in Stage 2, there is added impairment of somatic motor activity; in Stage 3, motor activity ceases and respirations are impaired; and in Stage 4, respirations cease transiently. Recovery from Stages 2, 3, and 4 is rapid in boxers, but recovery from Stage 1 takes minutes and sometimes hours. During this period, as noted in the introduction, the boxer may continue to fight and defend himself, without any recollection of such events later. Most knock-outs in the ring occur at Stage 2, since the boxer is incoordinated, with impaired locomotion and reflexes, and therefore, an easy target. The Staging system outlined above applied equally well to experimental concussion in rats. Occasionally, seizures were observed to follow a concussing blow. Invariably, the seizures were associated with transient apnea so that these animals were included in Stage 4. Preliminary studies determined that rats could be concussed to Stage 4 and exhibit recovery to normal gait, posture, and behavior. We then progressed to study the effects of such a concussion on the learning, memory, and performance traits of such rats; comparing these with non-concussed controls.

MATERIALS AND METHODS

Initial studies to determine a force that could concuss a rat to Stage 4 required trial and error. The initial concussion device consisted of a rubber-headed reflex hammer, fixed to a pivot. The hammer end was attached to the base of the device by elastic bands, which acted as the propelling device. A protractor, fixed to the pivot point, was used to determine the angle of release of the hammer, which was repeated for different rats. In an attempt to devise a more reproducible form of injury, a spring-loaded pistol was then used. The pistol propelled a padded, lead-tipped dart (mass 21.66 grams) at a velocity of 9.14 meters/second. The non-medicated rat was held manually in a position so that the occiput of the skull would be struck. Using such a model, the momentum and kinetic energy of the system could be calculated, and the absolute values would be reproducible between individual experiments (Table 1). As Shetter and Demakas (1979) have noted, a complete mathematical analysis of a head injury is virtually impossible. In these experiments, the mass, shape and velocity of the striking object were constant, as were the mass, shape, and size of the head. An important variable was the degree of tone of the cervical musculature of the rat at the time the blow was struck. Decreased tone would obviously result in a greater acceleration of the head in relation to the body, with more severe concussive effects. Similarly, the concussive effect would be greater if the rat's head were moving towards the striking object at the time of the blow. The degree of rotational acceleration of the head in relation to the body produced by the blow is demonstrated in Figure 4, a time lapse stroboscopic photograph made at the moment of concussion.

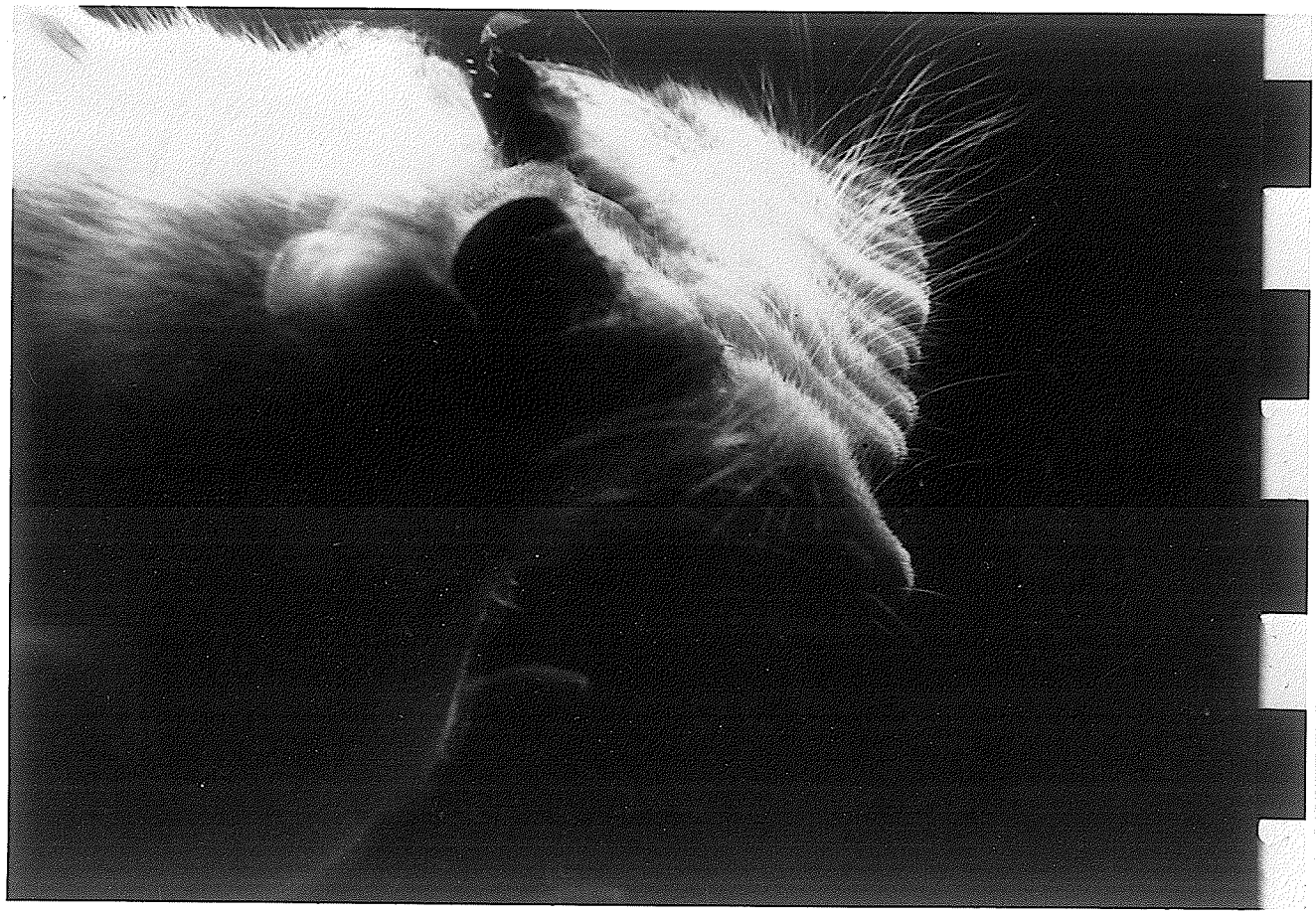
The resulting concussion was staged as previously outlined.

TABLE 1

Mass of dart (m) =	21.66 grams
Velocity of dart (v) =	913.5 centimeters/sec.
Kinetic energy ($\frac{1}{2}$ mv^2) =	9.037×10^6 ergs
Momentum (mv) =	1.979×10^4 gm. cm. sec.

Figure 4

Photograph taken at the moment of concussion. Illumination was provided by a strobe light, flashing at 50 times per second. The strobe light was triggered simultaneously with release of the dart from the spring-loaded pistol. The strobe flashed for 300 milliseconds. Scale at right = 1cm/bar. The degree of rotational acceleration following this blow to the head (which produced a Stage 4 concussion) can be appreciated.

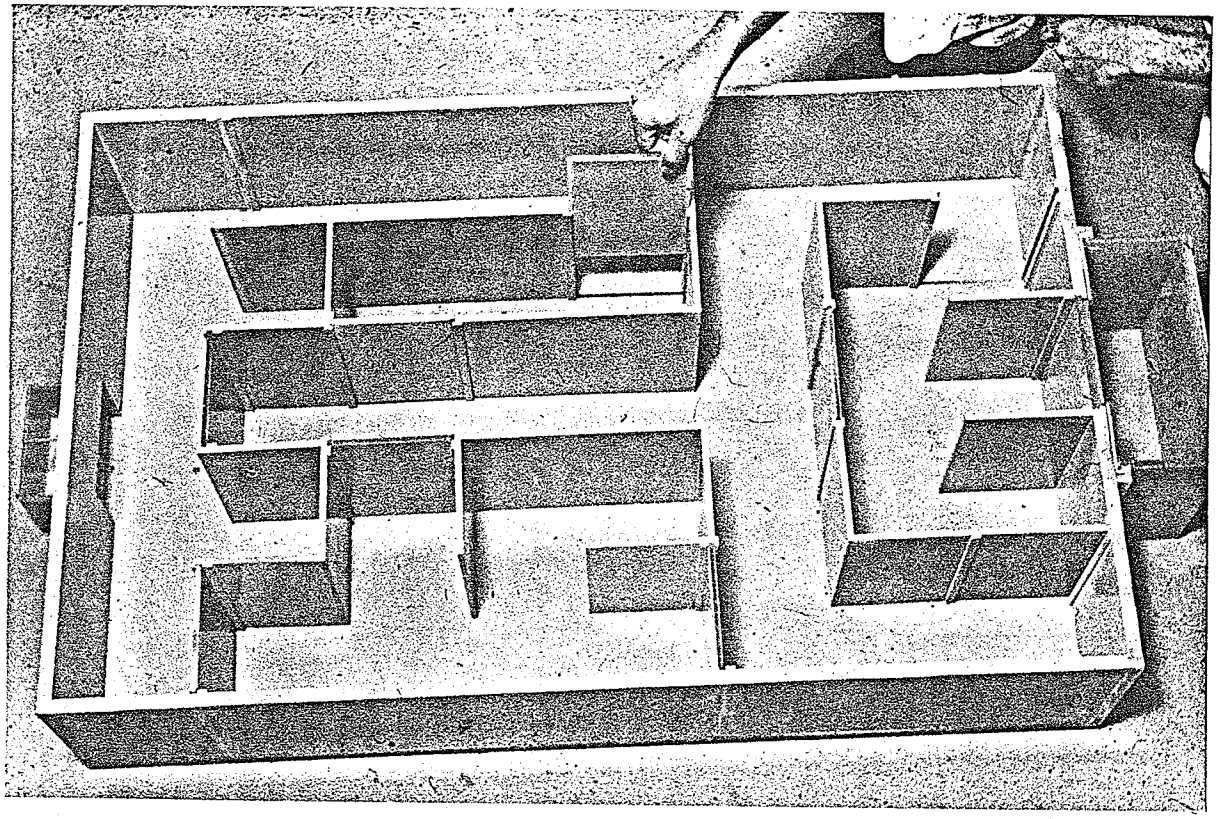


Analysis of the initial concussion experiments was performed with cinephotographic film at 64 frames per second (in later experiments, these records were replaced by video-tape recordings made at 32 frames per second). In either case, the visual records could be re-played in slow-motion in order to study the factors involved in each experiment; (eg. degree of head rotation).

In the initial studies regarding memory, learning, and performance, each rat was concussed to Stage 4. Those concussed to a lesser Stage were not used in the experiment. Three groups of 12 Sprague-Dawley rats (males, weighing between 170-200 grams, were used in the experiments). Each group consisted of two "feeding controls" and ten animals reduced to 70 percent of the control weight by diet restriction. The weight reduction by "fasting" was necessary because animals fed "ad libitum" were very difficult to train at food-rewarded tasks. Twenty rats from the first two groups were then trained in seven patterns of a fourteen pattern maze. The pattern of the maze could be changed readily by changing the position of one or more of a series of sliding doors (Fig. 5). The ten rats from the third group were trained in the same maze, but for these rats, the correct course was marked with 2 x 0.8 cm. stripes of different shades. A correct alley was marked with a light stripe, while an incorrect alley was marked by a dark stripe (see experiments of Anderson, et al. 1972). A protocol was kept for each rat, with the number of runs taken to learn each maze, and the time and the number of errors made for each run. A turn of the head or the body 90 degrees in the wrong direction was counted as an error. Vertical searching and "exploratory" behavior were common in the initial runs for each rat, and were manifested as extended time periods for each run. As the rat learned the principle of the maze, however, such exploratory behavior was sharply curtailed. During training, the greatest source of distraction appeared to be noise, which was carefully avoided.

Figure 5

There are at least 14 courses made possible by changing the position of a series of sliding doors in the same maze. The starting box is on the right; the food reward is in the small box on the left. The animal has to discover the correct course in order to reach a food reward.



A rat, on average, required seven to ten days of training, with six to ten runs daily to perform well in the maze. Initially, the tendency was to remain in the starting box, until urged out manually. With any minor interference at this stage (eg. a sudden movement, or sudden noise), the rat would abort its explorations and return to the starting box immediately. After about two to three days (12-20 trials), the rat would realize the principle of the maze and attempt to reach the food reward. The rats became so adept at the performance of their task that they reached the reward within five to ten seconds; an occasional "star" performer would take as little as three seconds. At optimal performance, the rat made no mistakes on a particular route (Figure 6,7). When the route was suddenly changed, two or three errors would occur (depending upon how many alleys had been changed) until the new route was learned. The learning of a new route occurred much more rapidly than with the original route. Interestingly, the rats trained in mazes in which the routes were marked with light or dark colored strips made fewer errors when the route was changed; they were able to adapt to a new route rapidly with this additional clue. In order to exclude the possibility that the rats were following olfactory clues, the maze was washed out between trials. Once a rat had learned a particular maze route, he would continue at his optimal performance indefinitely--as long as he was tested two or three times weekly. If the repeated testing was not carried out, the rat approached that maze as though it were a new maze.

Once the maze training was completed, five rats in each group were concussed (leaving five rats as trained controls in each group). Concussions were documented by cinephotography (at 64 frames per second).

RESULTS

Twelve rats were concussed to Stage 4 (apnea, associated with a period of somatic immobility lasting less than five seconds). Subsequent to this was

Figure 6

The time required to complete the maze and obtain the food reward (plotted on the ordinate) decreased with repeated training sessions. Each training session consisted of five to ten runs in the maze. After five or six training sessions, rats reached their optimal performance.

Points plotted indicate the means for rats tested in a particular session. Vertical bars in this and following figures indicate standard error.
(n = 30)

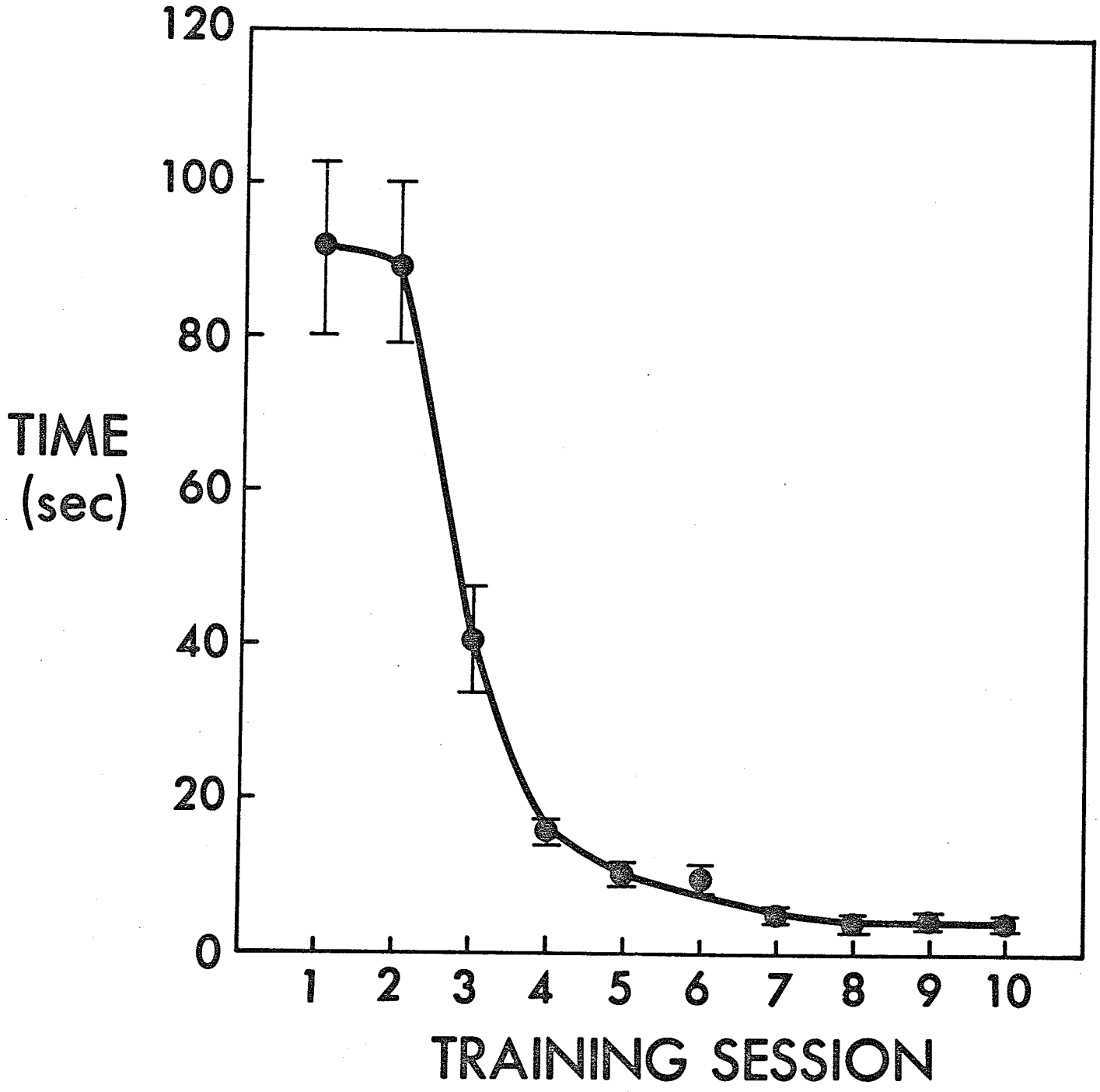
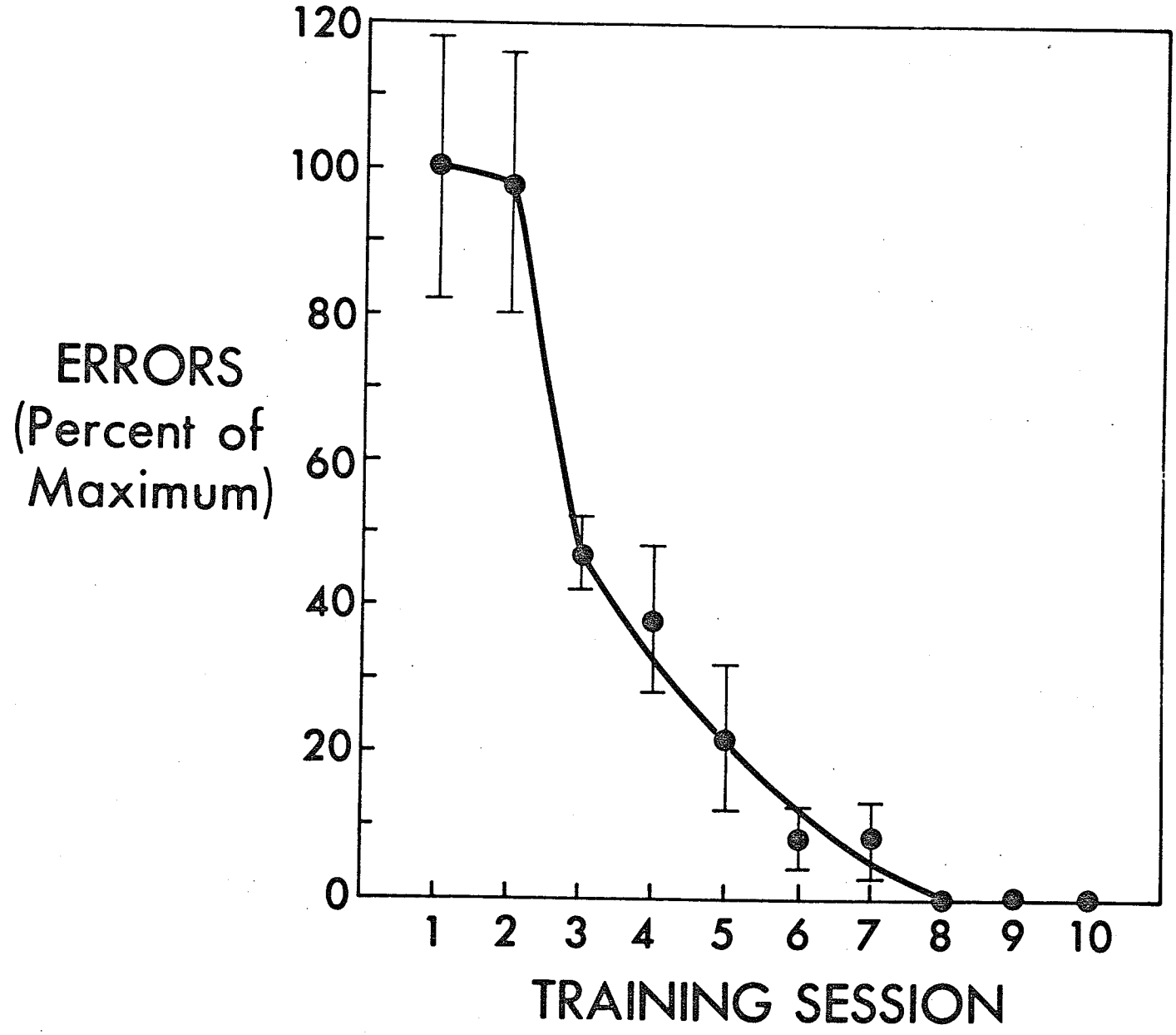

120
100
80
60
40
20
0
TIME
(sec)
1
2
3
4
5
6
7
8
9
10
TRAINING SESSION

Figure 7

The mean number of errors for rats tested in each training session are plotted on the ordinate, expressed as a percentage of the mean number of errors made in the initial training session. There is a sharp drop in the number of errors following the second session. When optimal performance is reached, rats make no errors on repeated testing in the same maze.

(n = 30)


ERRORS
(Percent of
Maximum)
120
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
TRAINING SESSION

a period characterized by irregular respirations, which lasted up to three seconds (Stage 3). Inappropriate, incoordinated movement occurred for a matter of minutes (Stage 2) and progressed to normal gait, posture, and speed.

When re-tested in the maze, the behavior of the rats was remarkably similar; they appeared as though they were encountering the maze for the first time. After 10-15 minutes, the rats ventured from the starting gate again. Completion of the maze post-concussion was characterized by three or four errors on a route which had previously been performed flawlessly (Figure 8). More dramatic was the increase in the time required to complete the maze; often a multiple of 20 to 30 times the pre-concussion value (Figure 9). After 50 to 60 minutes in the maze, and four to five runs, the performance of the rats returned to "optimal". It is most interesting to note that the gait, posture, and attitude of the rat returned to what would be considered normal within at most five minutes. However, impaired performance, suggesting amnesia, persisted for as long as 60 minutes. Another suggestion of post-traumatic amnesia was the fact that, if the rat were concussed a second time, he showed no evidence of fear or rage when he encountered the concussion apparatus.

Controls, which were held manually, while the hammer was allowed to drop beside their heads, showed no change in their maze performance, nor did immobilization stress or tail pinch impair their performance. When compared with controls, days and weeks later, in their ability to either perform a previously learned maze, or to learn a new maze, the concussed rats demonstrated no impairment (Figure 10). Post-mortem examination of the brains of concussed rats, demonstrated no pathological changes when compared to controls.

Using this simple model, an immediate transient impairment of memory and performance has been demonstrated following concussion. No permanent

Figure 8

After reaching optimal performance (i.e. no errors made upon repeated testing in the same maze), rats concussed to Stage 4 were re-tested in that maze. The number of errors made post-concussion increased from 0 to about 45% of the number made during the initial training session. After three or four trials over the next hour, the number of errors decreased to the pre-concussion level (0).

(n = 12)

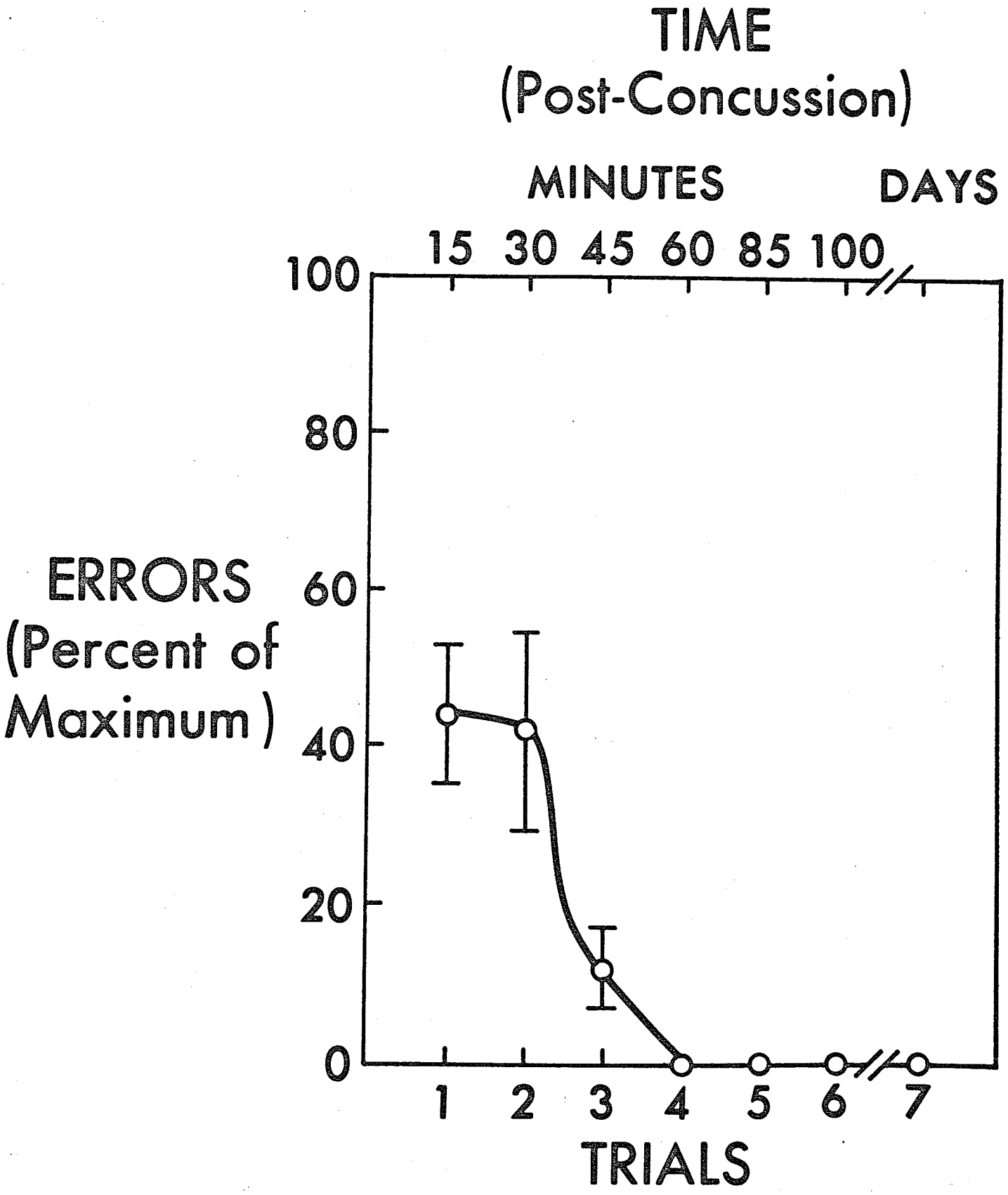

TIME
(Post-Concussion)
MINUTES
DAYS
15 30 45 60 85 100
100
80
60
40
20
0
ERRORS
(Percent of
Maximum)
1 2 3 4 5 6 7
TRIALS

Figure 9

The time required to complete the maze increased dramatically following concussion. Solid circles indicate pre-concussion completion times (with repeated testing on consecutive days). Open circles indicate time required for completion following concussion. Return to baseline values occurred after four or five trials, over about 60 minutes. (n = 12)

*** significant at $p < .001$

* significant at $p < .05$

(Student's t-test)

TIME
(Post-Concussion)

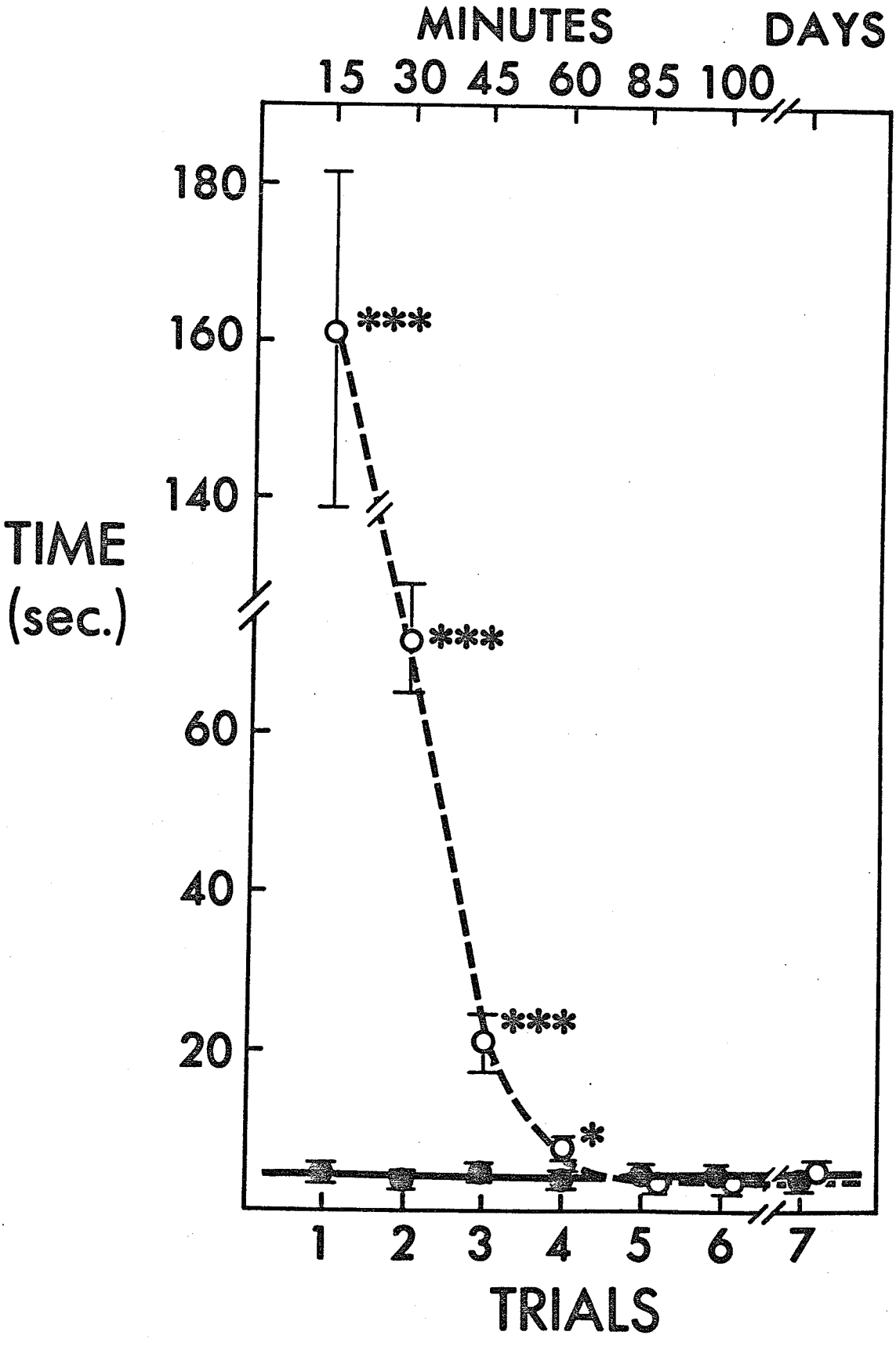
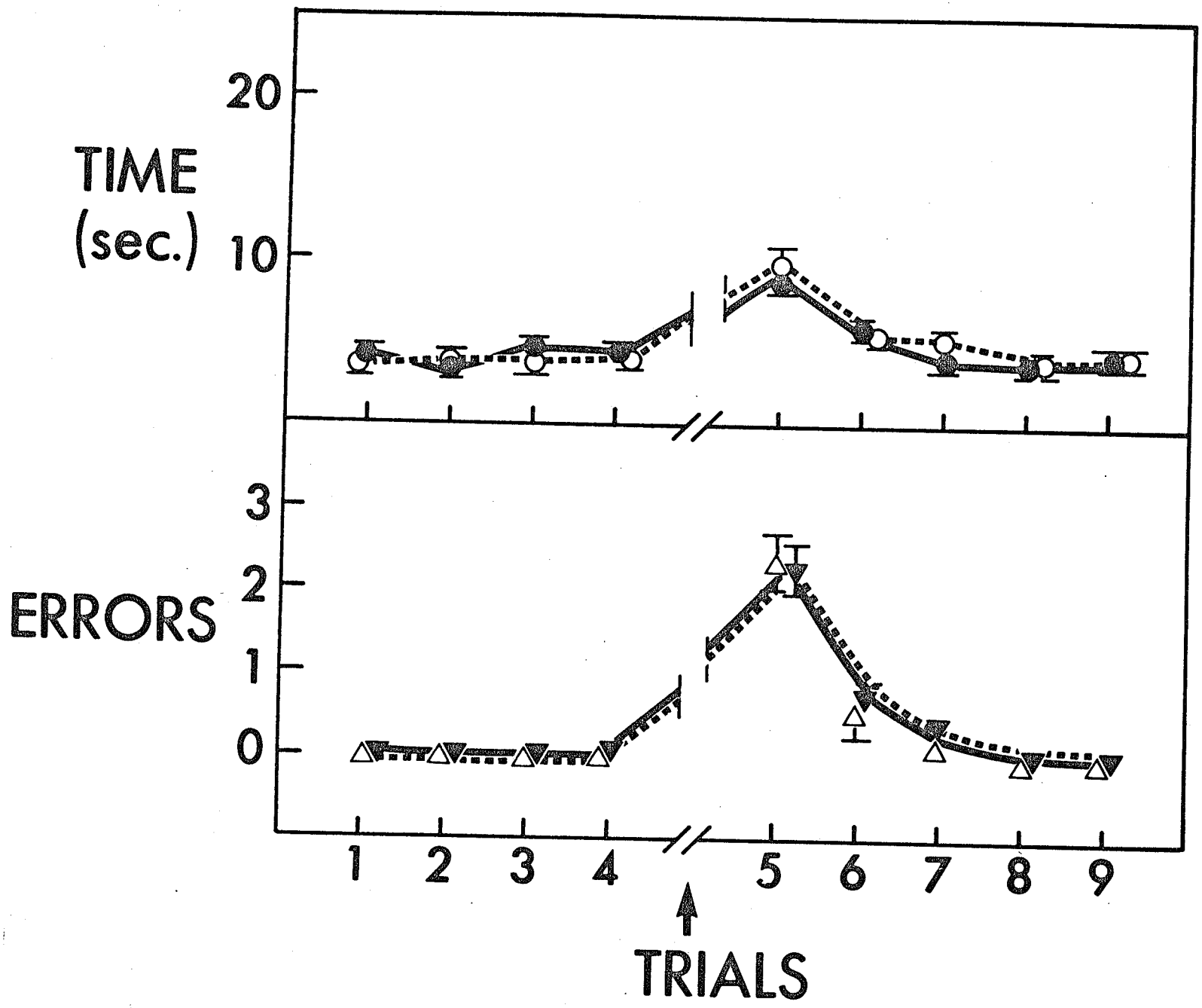


Figure 10

After rats had established an optimal performance in one maze pattern, the maze was changed to a more difficult pattern. This was accompanied by an increase in the number of errors, which returned to 0-baseline after two to three trials. Similarly, there was a transient increase in the time period required to complete (learn) the new maze, which also returned to the baseline. There is no detectable difference between animals tested one to ten days post-concussion (open symbols) and controls (closed symbols). Graph documents data for rats tested one day following concussion. Concussed n = 12
Control n = 15

(Arrow marks time of change to new maze).


TIME
(sec.)
20
10
ERRORS
3
2
1
0
1
2
3
4
5
6
7
8
9
TRIALS

SPECTRAL ANALYSIS OF THE ELECTROENCEPHALOGRAPHIC RESPONSE TO CONCUSSION IN CONSCIOUS RATS

As stated in the introduction, several studies of electroencephalographic (EEG) recordings from concussed experimental animals have been published. These studies have been quoted, and their results discussed. In summary, the main criticism of such studies has been the use of general anesthesia during the experiments. Drugs used in anesthesia preclude any correlation of the EEG response to concussion with the state of consciousness of the animal (since this is clouded before the concussing blow is administered). Also, the anesthetics are able to produce profound changes in the EEG; more commonly the appearance of widespread delta activity or discontinuous recordings of the "burst-suppression type" (Scott, 1976). Moruzzi and Magoun (1949) and Magoun (1963) have shown that anesthetics, such as the barbiturates and ether, may block reticular relays within the brain stem. Obviously then, even if the state of anesthesia could be maintained at the same level--a difficult task when working with small experimental animals--the EEG records would reflect the presence of anesthetic drugs in the preparation. The results of such a study would reflect the combined effects of anesthesia and concussion, which may or may not be greater than the effects of concussion alone.

In order to detect subtle changes in the EEG record--which may not be obvious by visual assessment--and, more importantly, in order to quantitate such changes and analyze them statistically, we employed the technique of spectral analysis. This technique is described in a paper by Havlicek, et al. (1977).

MATERIALS AND METHODS

Thirty-two Sprague-Dawley rats, weighing 200-250 grams were used. Under general anesthesia, with intraperitoneal pentobarbital sodium (35 mg/kg) and chloral hydrate (160 mg/kg), platinum electrodes were inserted into the epidural space through drill holes in the calvarium, being especially careful not to injure or penetrate the dura. Recordings were bipolar, with the electrodes separated 3 mm over the sensorimotor cortex. The electrodes were fixed in place with dental acrylic cement. After electrode insertion, the rats were allowed 7-10 days to recover.

In recording the electroencephalogram, a Grass Model 78 polygraph with 7P511 EEG amplifiers was used (time constant 250 msec.). At a PDP 8/E computer site, additional analog low pass filters were used to avoid aliasing caused by frequencies above 25 Hz (cutoff frequency 25 Hz, roll-off approximately 18 db/ octave). The record was divided into 11 second epochs by "trigger pulse", marked on a separate channel.

The EEG analog signal and the trigger pulse were recorded on a Hewlett-Packard 3960 Instrumental FM tape recorder (15/16 in/sec. tape speed) for further computer processing. The Fast Fourier Transform (FFT) was performed off-line on a PDP 8/E computer, using Rothman's version of the FFT algorithm (Rothman, 1968).

Samples of 10.24 sec. duration for each channel of EEG signal were digitized at a frequency of 100 Hz. The FFT transform for each sample generated a 256 point spectrum per channel between 0.10 and 25 Hz with resolution of 0.098 Hz per point. The frequency spectra data generated were reduced to nine integrated values, each representing one of eight frequency bands, and a total sum (Table 2).

All power spectra information was automatically transferred and stored on magnetic disk for statistical evaluations, which were performed using one-way and two-way analyses of variance, and Duncan's multiple range test.

TABLE 2

DELTA	1	(D1)	=	0.10 - 1.48	Hz
DELTA	2	(D2)	=	1.56 - 3.51	
THETA	1	(T1)	=	3.61 - 5.57	
THETA	2	(T2)	=	5.66 - 7.52	
ALPHA	1	(A1)	=	7.62 - 9.47	
ALPHA	1	(A2)	=	9.57 - 12.50	
BETA	1	(B1)	=	12.60 - 17.48	
BETA	2	(B2)	=	17.58 - 25.0	
SUM				1.56 - 25.0	Hz

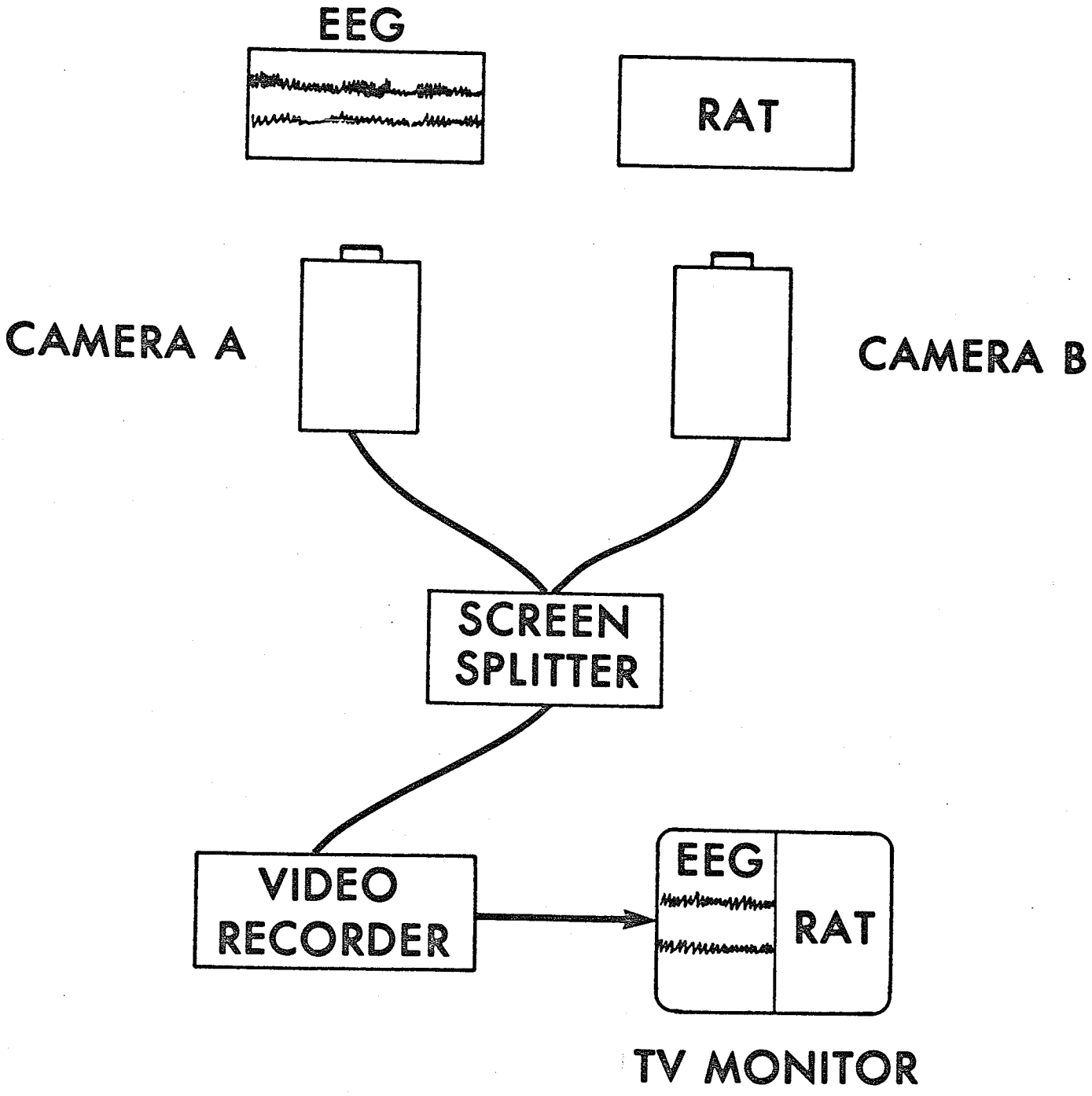
Experiments were performed between 0800 to 1200 hours in an electrically shielded chamber, with a grid floor. The grid floor is extremely useful in the "neurological evaluation" of the rat. It is an excellent, continuing test of coordination. Electroencephalographic recordings were taken for at least 20 minutes prior to concussion, during concussion, and then continued post-concussion, until the EEG returned to normal.

Each experiment was also recorded on video-tape using split-screen image processing. The rat in the shielded cage was recorded simultaneously with the ink-on-paper EEG printout, employing two Panasonic WV341P Television cameras, screen splitter, CCTV monitor, and Omnivision II VHS recorder (Fig. 11). The video records were made at 30 frames per second, and could be replayed in slow motion in order to study the factors involved in the concussion (eg. degree of cranial acceleration), and to correlate any changes in behavior with the instantaneous EEG response.

At the time of concussion, the rat was held manually, conscious and active, under the influence of no medications. Excessive recording artifacts produced by handling the rat at the moment of concussion precluded satisfactory interpretation of the record during this immediate period. However, data were available within five seconds of the blow to the head. The concussion was produced by a blow to the occiput by the previously described padded lead-tipped dart, shot from a spring-loaded pistol.

RESULTS

The resulting concussion was labelled as Stage 1-2 or Stage 3-4. Twenty-seven of the thirty-two rats resumed spontaneous locomotion, usually within 10 to 20 seconds. Initially, this locomotion was inappropriate and uncoordinated. By 10 to 20 minutes following the concussion, the gait, posture, and attitude of these 27 rats could not be differentiated from normal rats. Four of the rats


EEG
RAT
CAMERA A
CAMERA B
SCREEN SPLITTER
VIDEO RECORDER
EEG
RAT
TV MONITOR

remained immobile for 45 minutes. None of the these four rats recovered to its pre-concussion state. One rat died within five minutes of the blow, with an EEG that was completely flat from the moment of impact. The remaining records were continued until they had either recovered, or had been repeated at 24-36 hours and were still depressed. The rats were sacrificed at varying intervals following the concussion (five minutes to seven days). Post-mortem examination was performed at this time, and demonstrated pathology in each rat that did not recover from the blow; cerebral contusion at the site of impact in one animal, mild subarachnoid hemorrhage (mainly in the occipital region) in three animals, and a posterior fossa surface hematoma in one animal (Figure 12). In none of these cases did the EEG return to normal within 24 to 36 hours. These animals were, therefore, excluded from the following analyses by reason of the fact that they had suffered a concussion complicated by structural brain damage. The 27 rats that were concussed and later demonstrated both physical and electrical recovery showed no post-mortem evidence of permanent injury.

Almost one-third of the concussed rats suffered a generalized convulsion most apparent in the hind-limbs. These rats were classed as Stage 4 concussion, since a transient period of apnea invariably accompanied the seizure.

The response to concussion is documented in this series of still photographs taken from consecutive "freeze-frames" of a videotape record of a concussion experiment (Figure 13).

The decrease in EEG amplitude exemplified in Figure 13-h varied with the stage of concussion. For the entire group of rats, the change is evident in Figure 14 A, B, which shows significant depression of the EEG power spectra--of alpha 1 and 2, beta 1 and 2, and theta 1 frequency bands. There is no significant depression of the slow frequencies (theta, delta). In fact, the delta 2 frequency is slightly increased. The decrease in power spectra for the

Figure 12

Photograph of formalin-fixed brain from Rat W-26. Hematoma surrounds the brain stem posteriorly and anteriorly (not shown). The EEG record following experimental head injury in this animal did not recover.

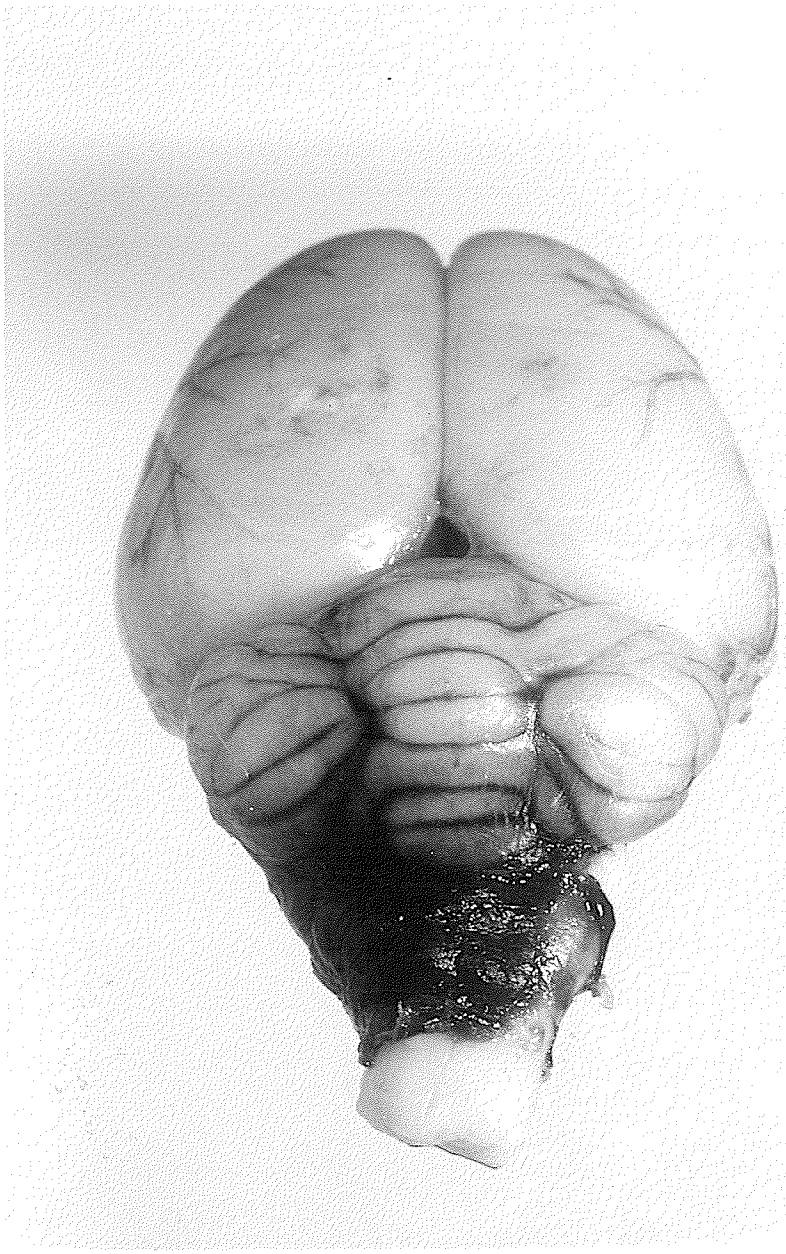


Figure 13

Frame (a) : Conscious rat at rest in recording cage.

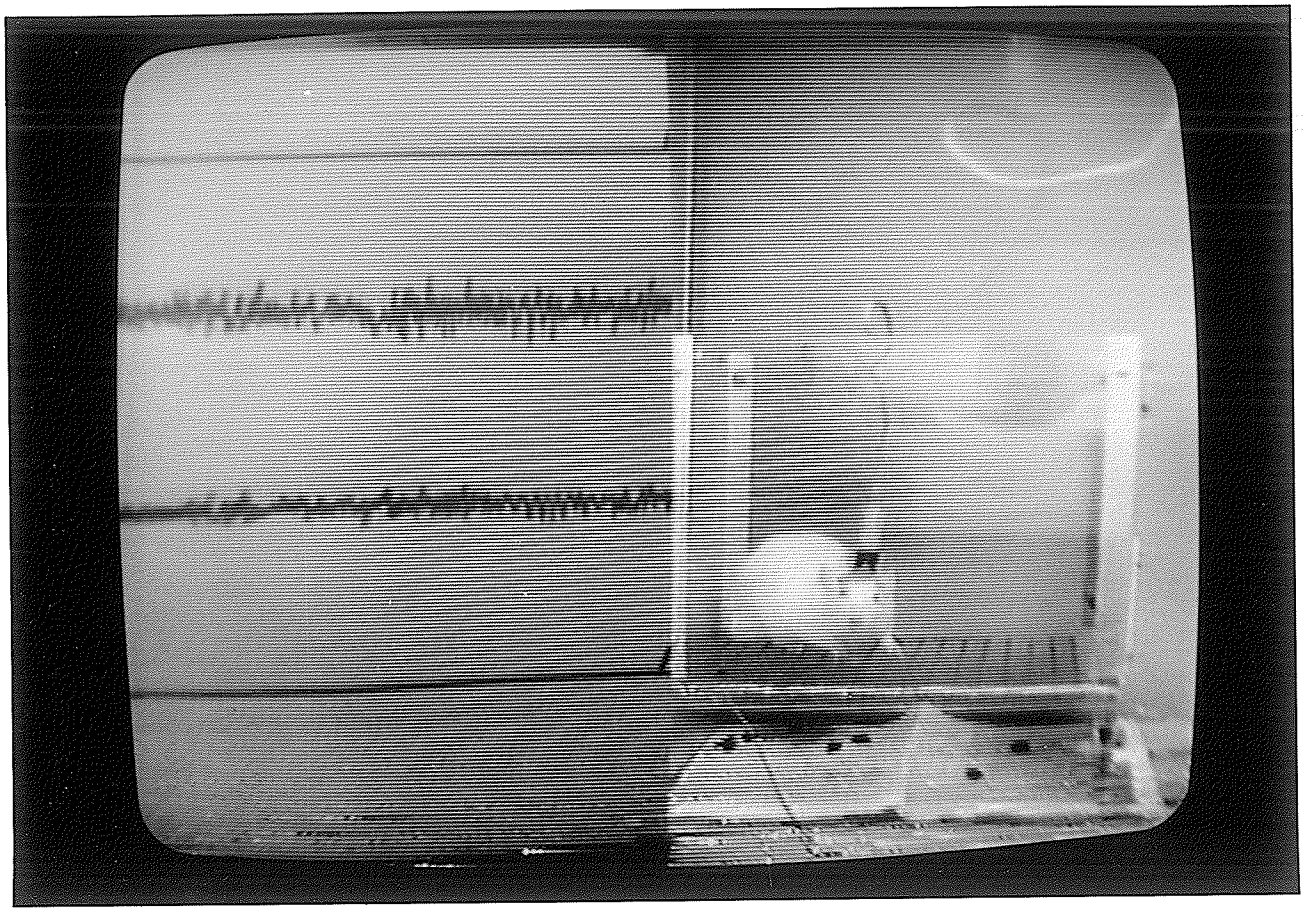
The EEG record on the left is recorded simultaneously with the record of the rat's activity (on the right). EEG record speed = 6 mm/sec. The upper channel recording is from Left Hemisphere electrodes. EEG artifacts in following frames are due to handling the rat outside of the grounded recording cage.

Frame (b) : Conscious rat held manually. Lead-tipped dart (double image) can be seen in Right upper corner. The tip of the dart (arrow) is about to strike the occiput of the rat.

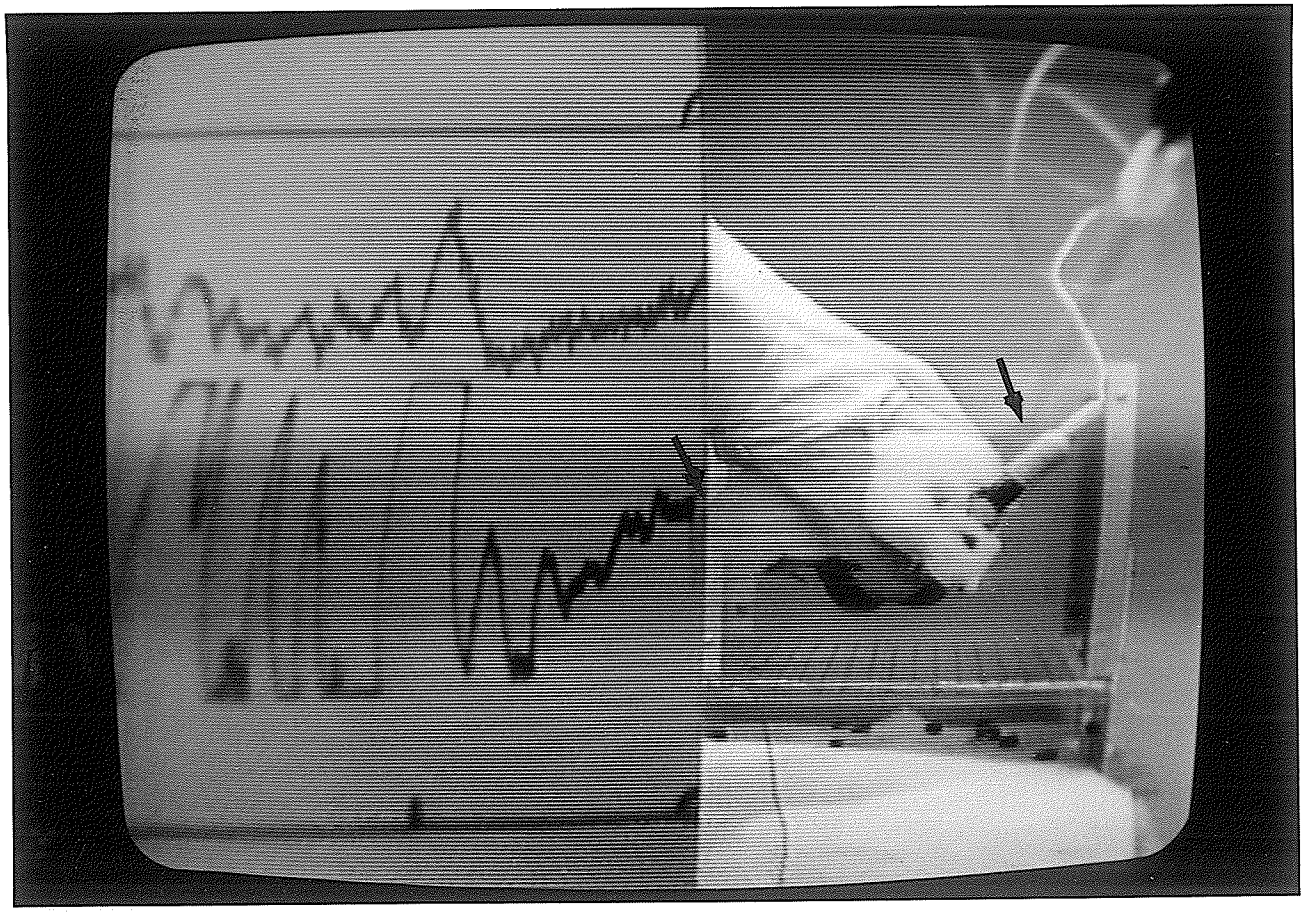
Frames (c-g): Demonstrate the rotational acceleration imparted to the rat's head immediately following the blow to the head. This sequence occurred over a time period of 0.132 seconds. Artifact on the EEG due to contact by the striking object is marked by an arrow in Frame (b).

Frame (h) : Immediately following the blow to the head, the rat demonstrates th physical signs of concussion. It lies prostrate in the recording cage, hypotonic and immobile, with the tongue protruding and the left front limb dangling through the grid floor of the cage. The EEG record on the left side of the screen shows markedly depressed amplitude over both hemispheres.

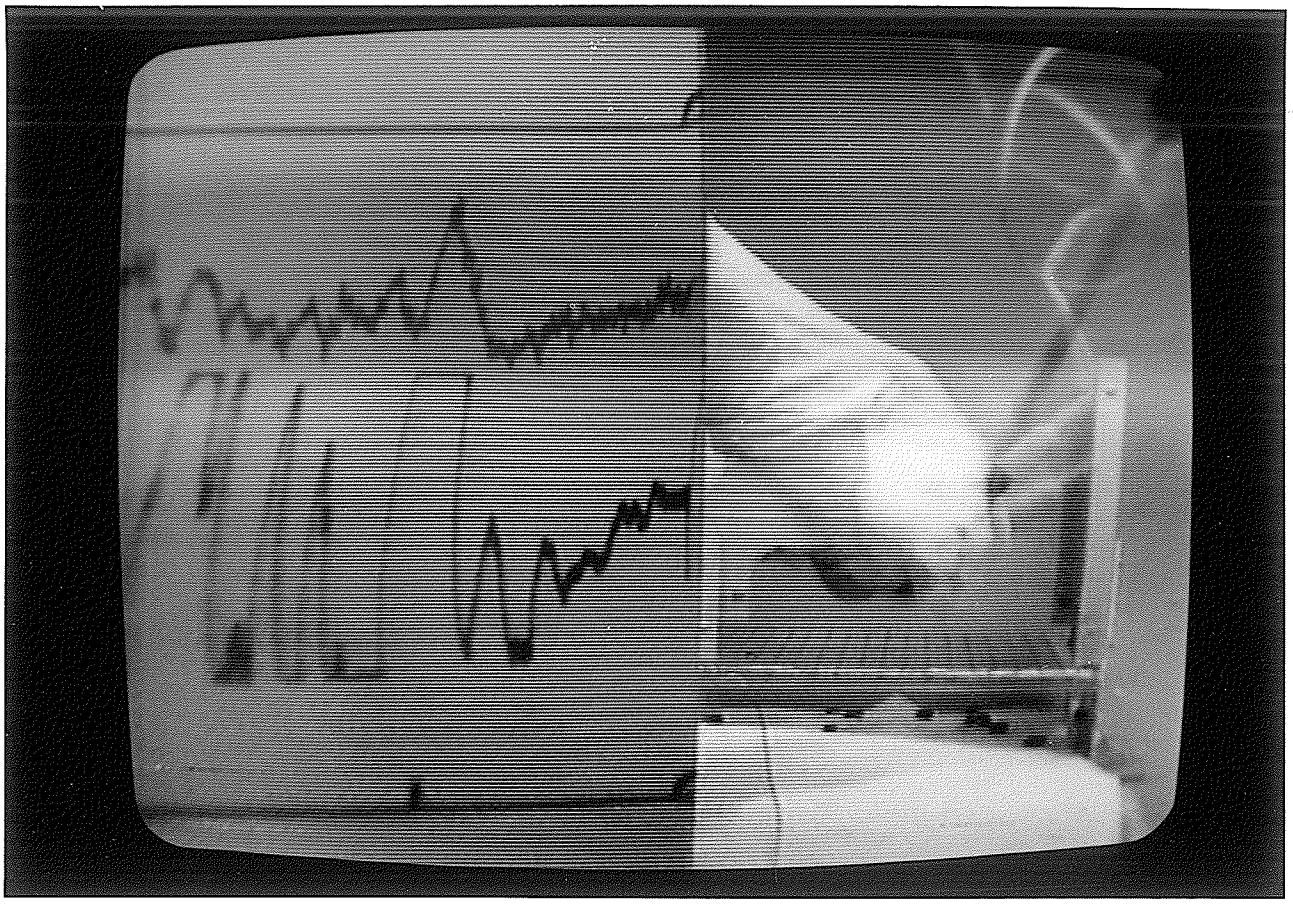
Frame (i) : 1.6 hours following concussion. The EEG has returned to normal an the rat demonstrates normal gait, posture, and attitude.



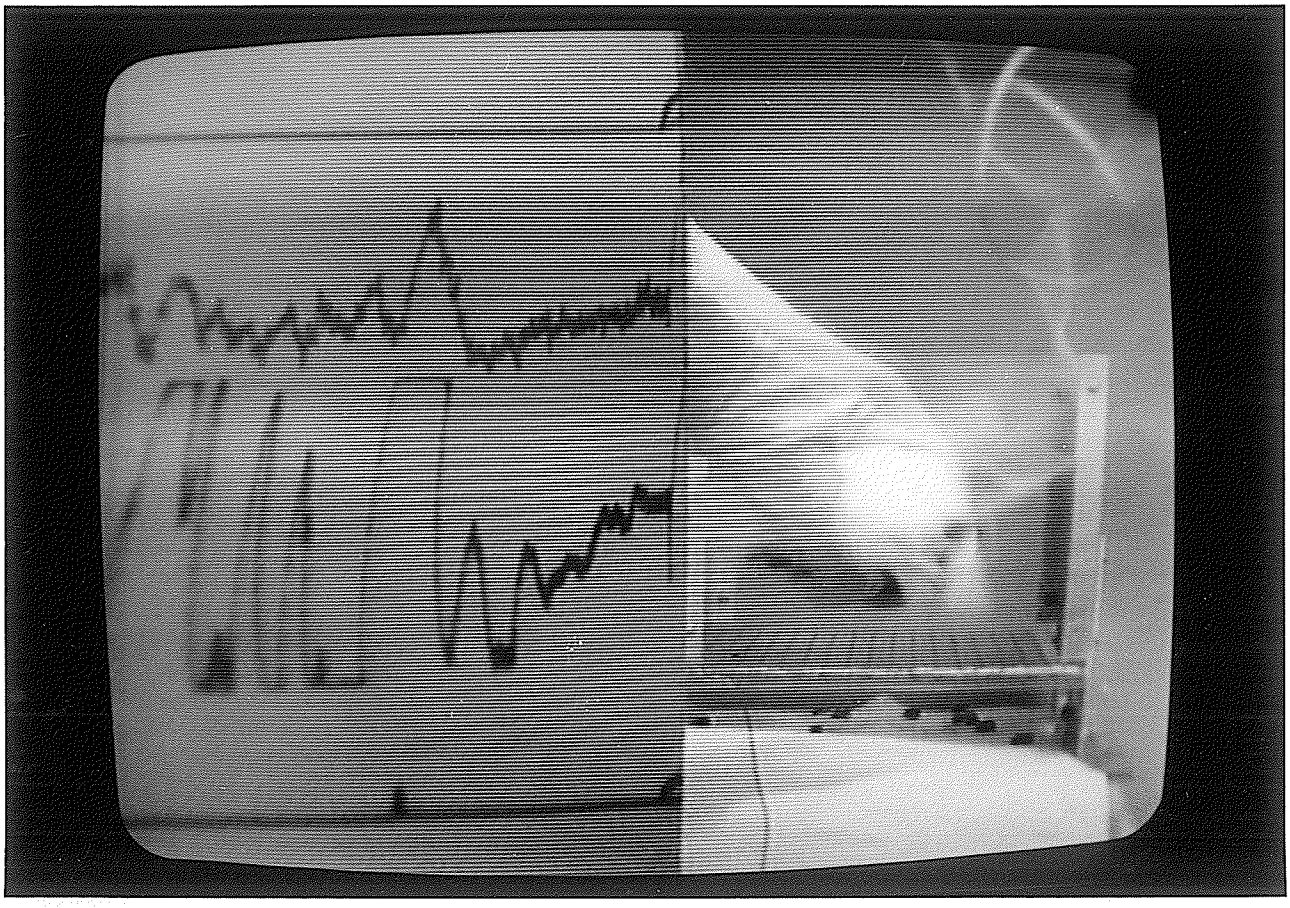
a



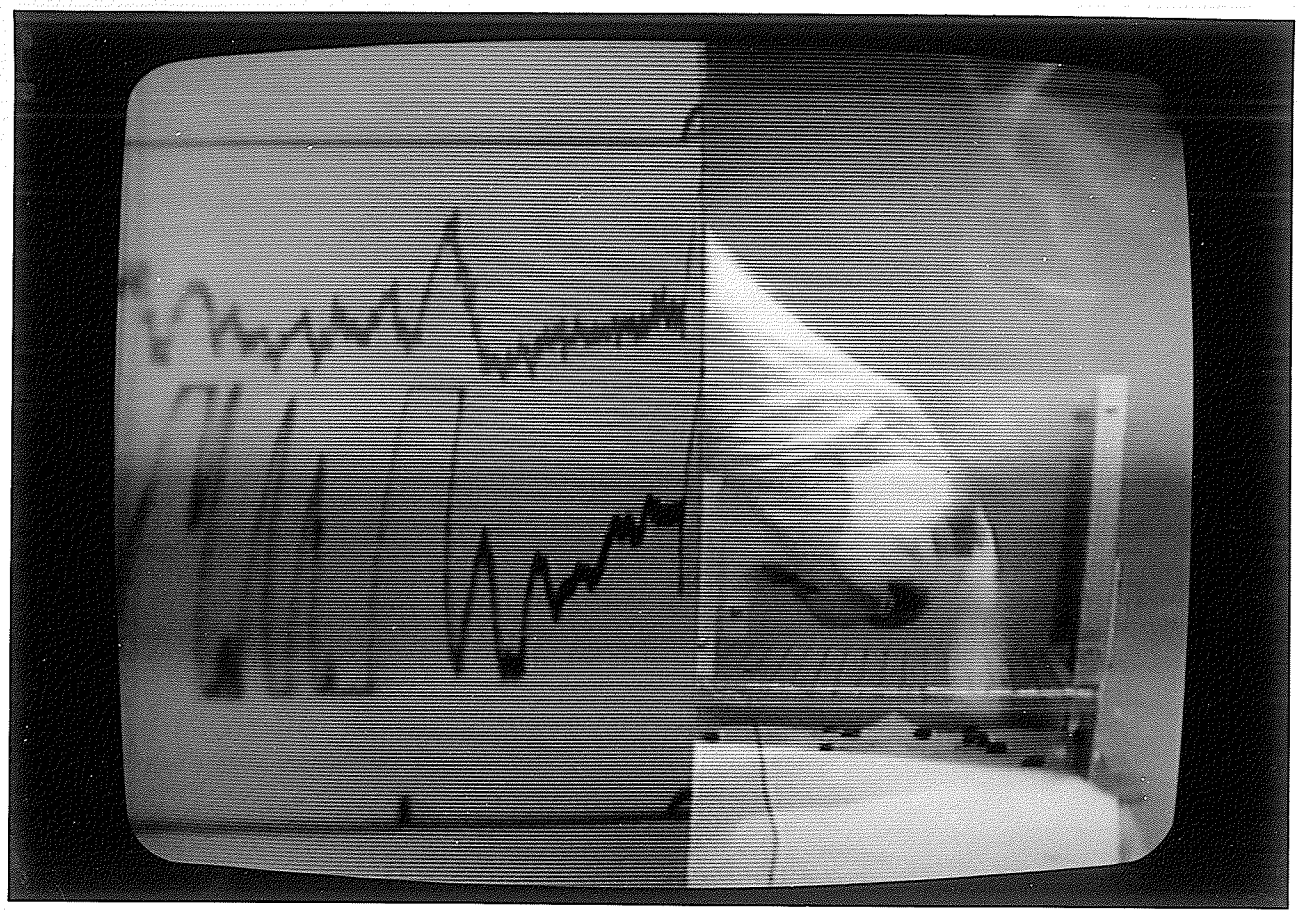
b



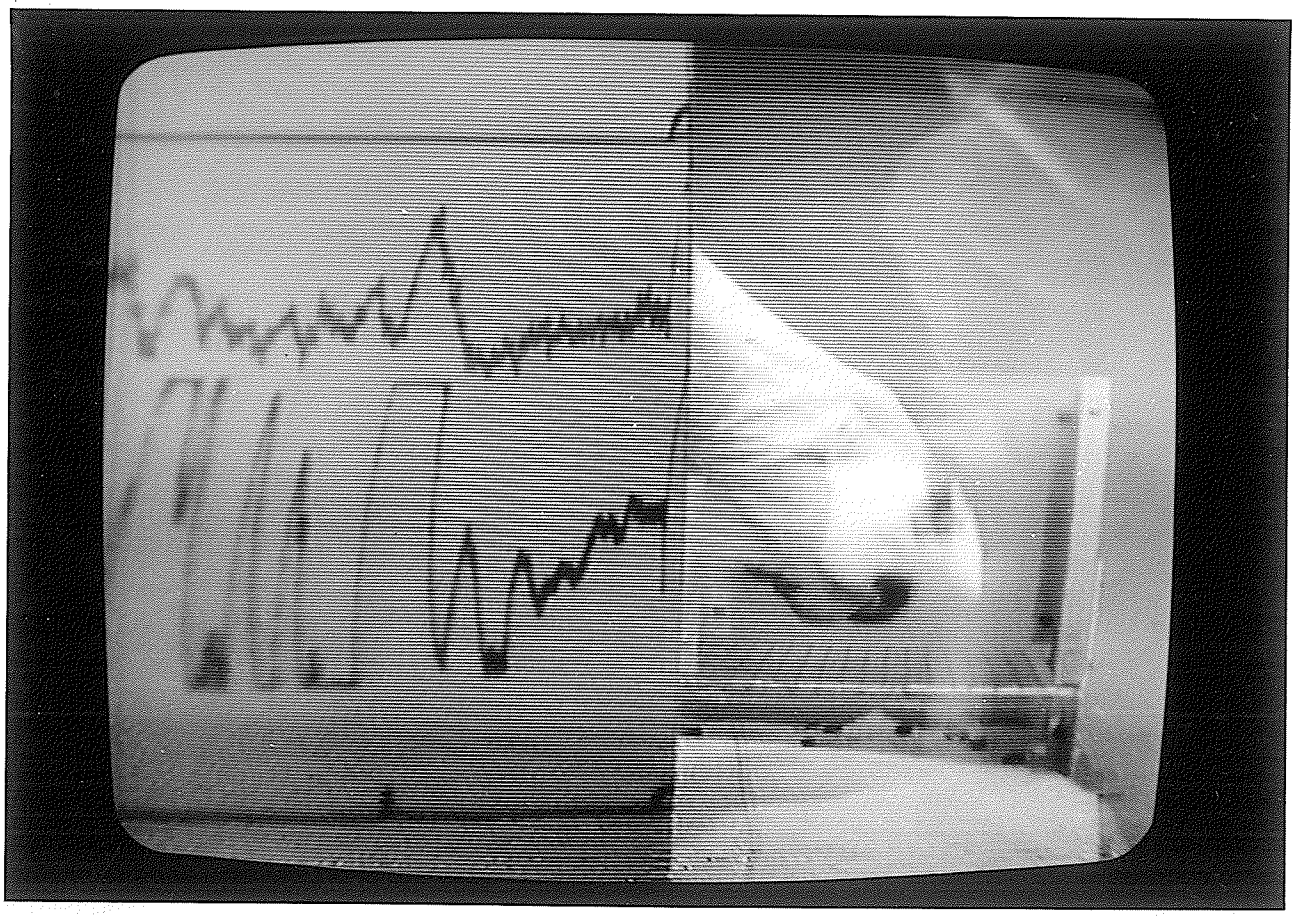
c



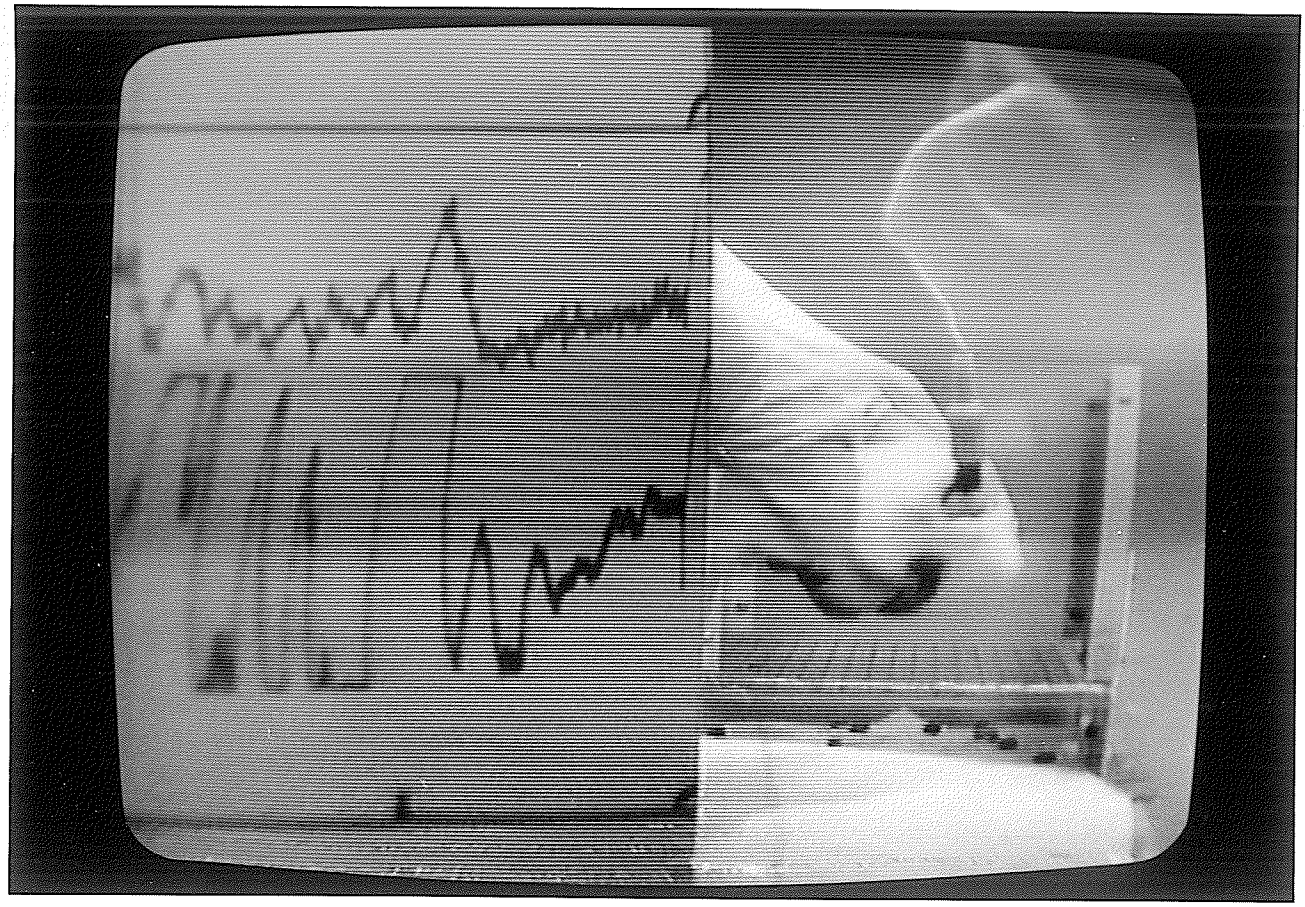
d



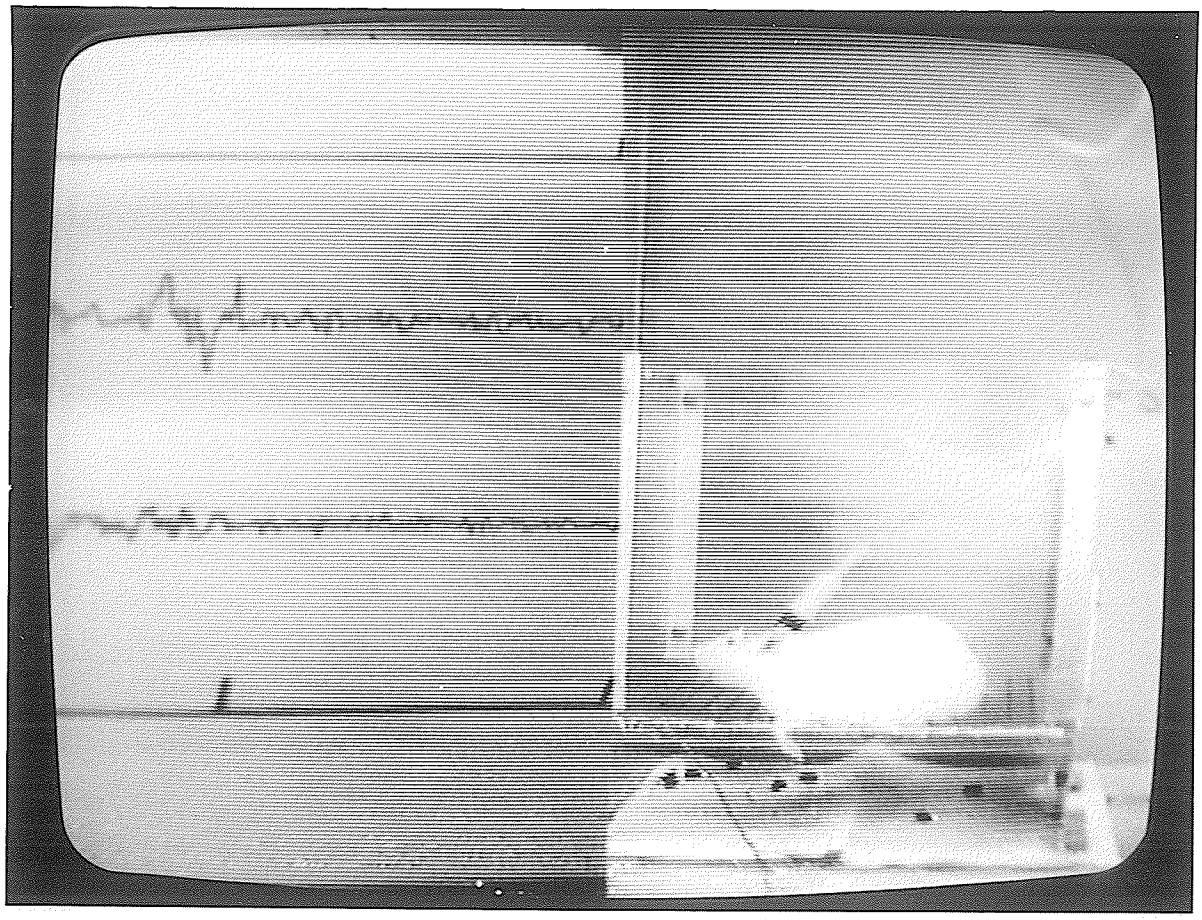
e



f



g



h

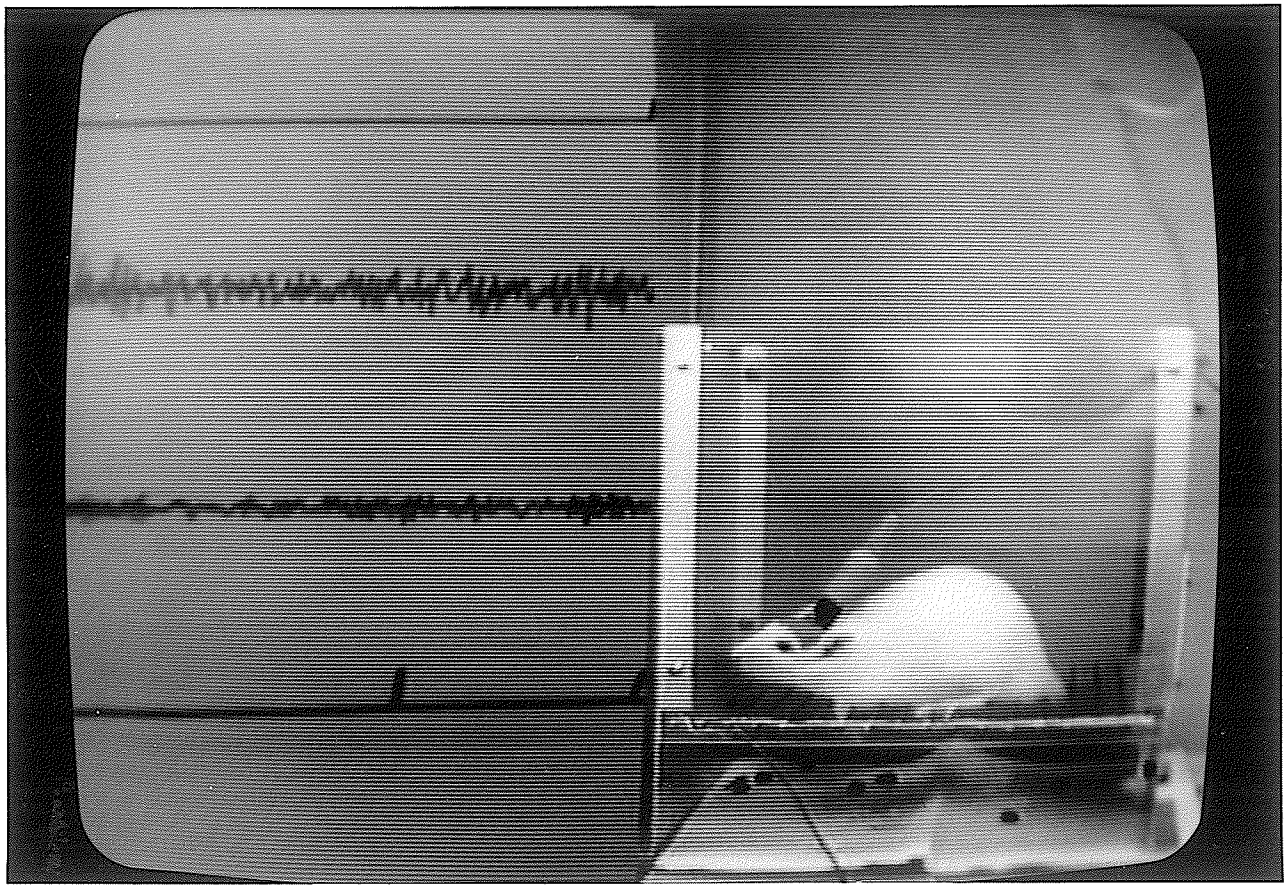


Figure 14 Stages 1-4 of Concussion

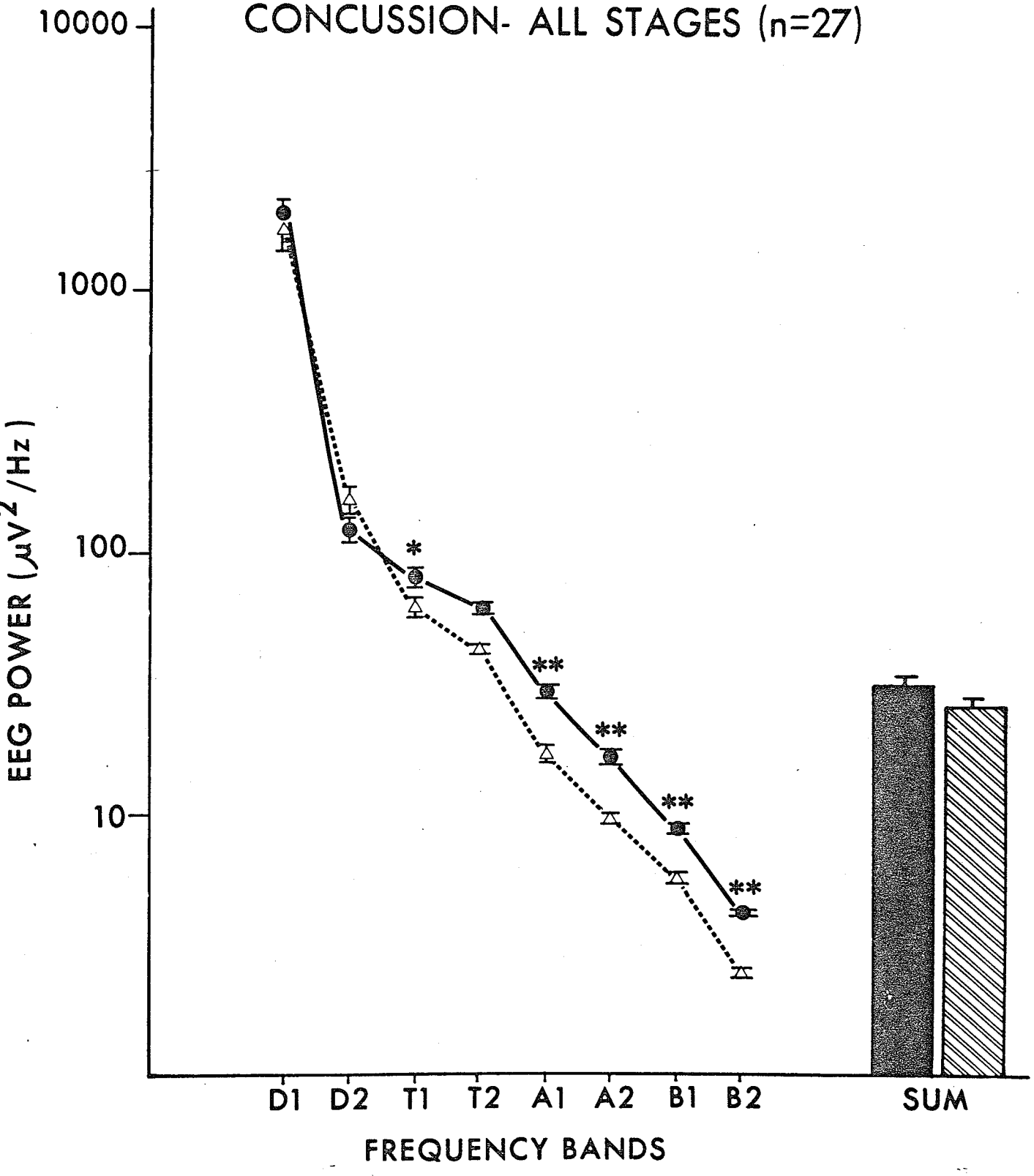
A) EEG power ($\mu V^2/Hz$) plotted on the ordinate (logarithmic scale). Frequency bands plotted on the abscissa. Solid line, pre-concussion record; Interrupted line, post-concussion record. Vertical bars indicate standard error. Following Concussion, there is a decrease in the power spectrum affecting the alpha (A1, A2), beta (B1, B2) and theta (T1) bands.

* significant at $p < .05$

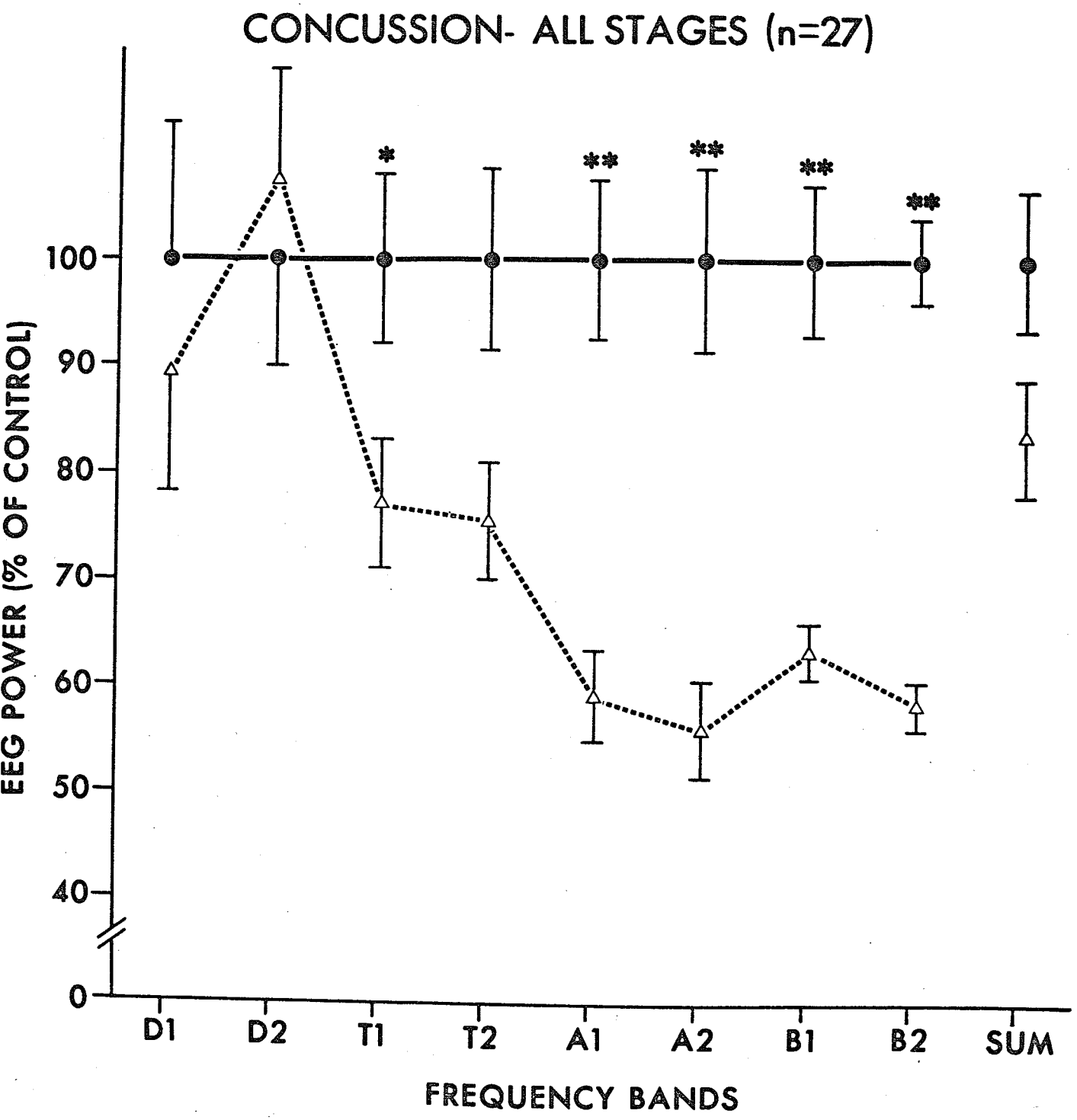
**significant at $p < .01$ (Duncan's multiple range test)

B) Post-concussion record plotted as a percentage of the pre-concussion record. The power spectrum of the Delta 2 band is increased, while other bands are decreased. Solid line: Pre-concussion

Interrupted line: Post-concussion


CONCUSSION- ALL STAGES (n=27)
10000
1000
100
10
EEG POWER (μV^2/Hz)
*
**
**
**
**
D1
D2
T1
T2
A1
A2
B1
B2
SUM
FREQUENCY BANDS

14A


CONCUSSION- ALL STAGES (n=27)
EEG POWER (% OF CONTROL)
100
90
80
70
60
50
40
0
*
**
**
**
**
D1
D2
T1
T2
A1
A2
B1
B2
SUM
FREQUENCY BANDS

14 B

faster frequencies, averages 40 percent.

Fifteen rats were concussed to Stage 1-2, that is, impaired motor activity without respiratory irregularities. The EEG records of these rats demonstrated markedly diminished voltage in the alpha and beta frequencies (25 percent and 37 percent respectively). There was very little change in theta frequencies (10 percent decrease). The delta 2 spectrum was increased by 15 percent (Fig. 15). Twelve rats were concussed to Stage 3-4, that is impariment of consciousness, associated with transient apnea. These records demonstrated more profound depression of the EEG amplitude, now affecting all frequency bands (Fig. 16). The pattern of depression, however, was similar to that observed with Stage 1-2 of concussion in that the amplitudes of the faster frequencies (A1, A2, B1, B2) were depressed much more than the slower frequencies.

The EEG depression reached a nadir at about five minutes following concussion and then began to recover in the 27 rats concussed to Stage 1-2 or Stage 3-4 (Fig. 17, 18).

During the recovery period, the appearance of high voltage slow waves was often witnessed (Fig. 19). The records returned to normal at an average of two hours post-concussion (Figs. 19, 20). In those rats that demonstrated permanent cerebral damage at post-mortem examination, the EEG did not return to normal. In rats recovering from concussion, it is interesting to note that the EEG abnormality persisted longer than any detectable abnormality in the rat's gait, posture, or attitude (i.e. two hours compared to 20 minutes). The recorded EEG abnormalities occurred over both hemispheres, although not always symmetrical to all rats.

DISCUSSION

Studies of craniocerebral injury in non-anesthetized animals of this nature have not been previously recorded. Letcher, et al. (1973) studied the

Figure 15 Stage 1-2 Concussion

A) Following Stage 1-2 concussion, there is a decrease in the power spectrum affecting the alpha and beta frequency bands most noticeably. There is no significant depression of the slower frequencies. In fact, there is a slight increase in the delta 2 power spectrum.

B) The post-concussion data are expressed as percentages of the pre-concussion (control recording).

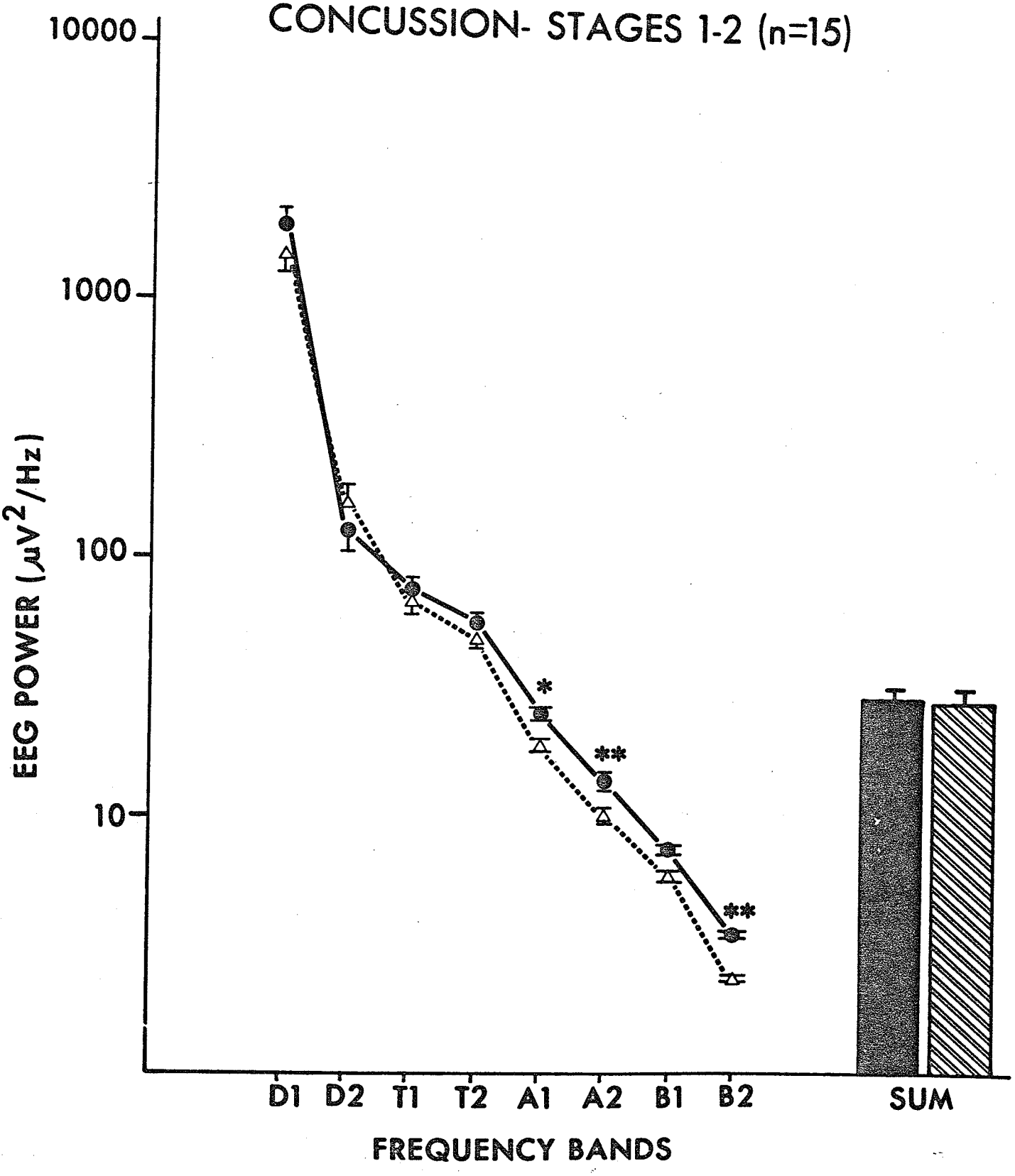
Solid line: Pre-concussion

Interrupted line: Post-concussion

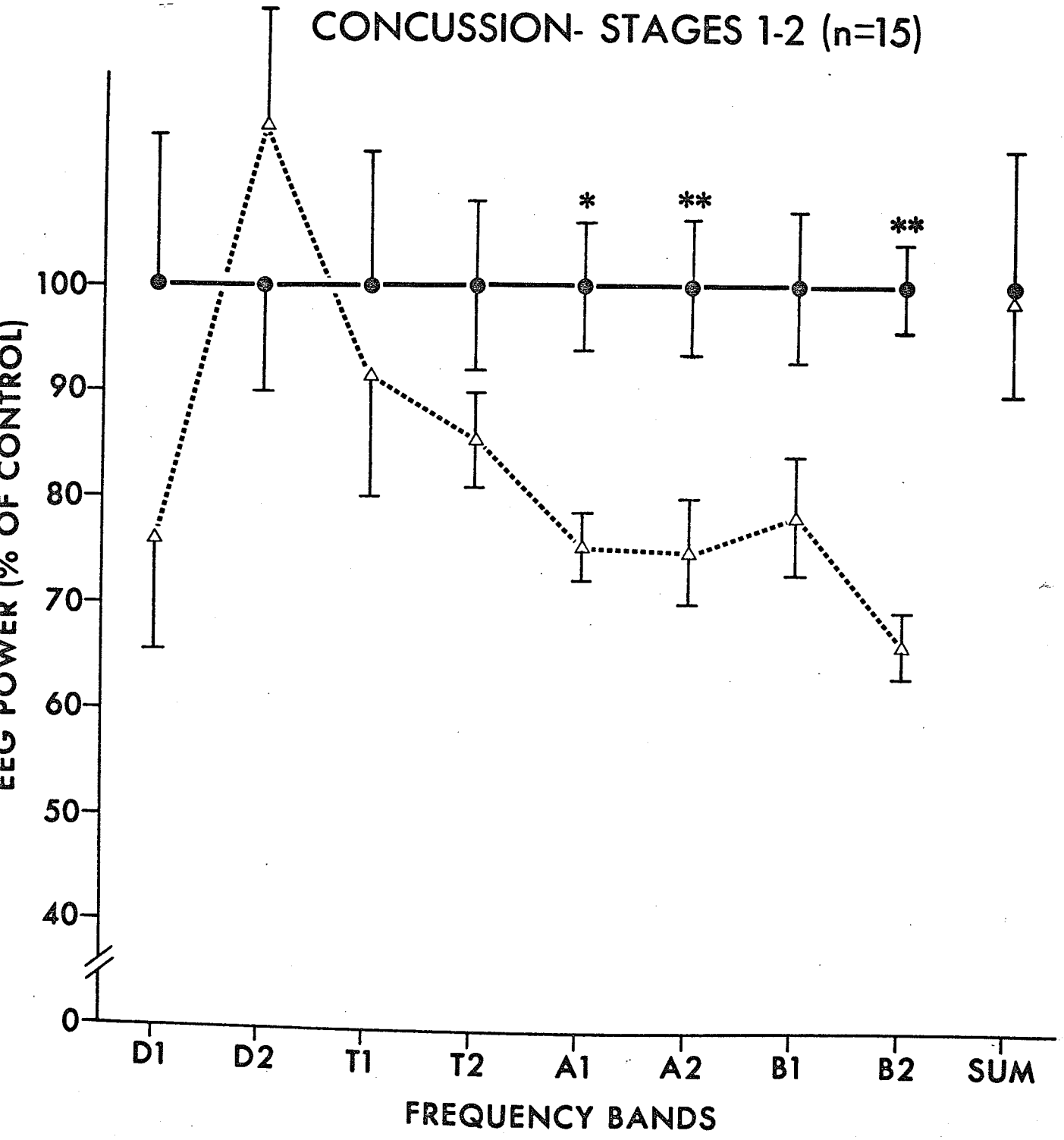
Vertical bars: Standard error

*$p < 0.05$

**$p < 0.01$


CONCUSSION- STAGES 1-2 (n=15)
10000
1000
100
10
EEG POWER (μV^2/Hz)
*
**
**
D1
D2
T1
T2
A1
A2
B1
B2
SUM
FREQUENCY BANDS

15A


CONCUSSION- STAGES 1-2 (n=15)
EEG POWER (% OF CONTROL)
100
90
80
70
60
50
40
0
*
**
**
D1
D2
T1
T2
A1
A2
B1
B2
SUM
FREQUENCY BANDS

15 B

Figure 16 Stage 3-4 Concussion

A) Following Stage 3-4 concussion, the EEG is more profoundly depressed than that observed following Stage 1-2 concussion. This affects all frequency bands. The alpha and beta (A1, A2, B1, B2) frequency bands are again more markedly affected than delta and theta (D1, D2, T1, T2).

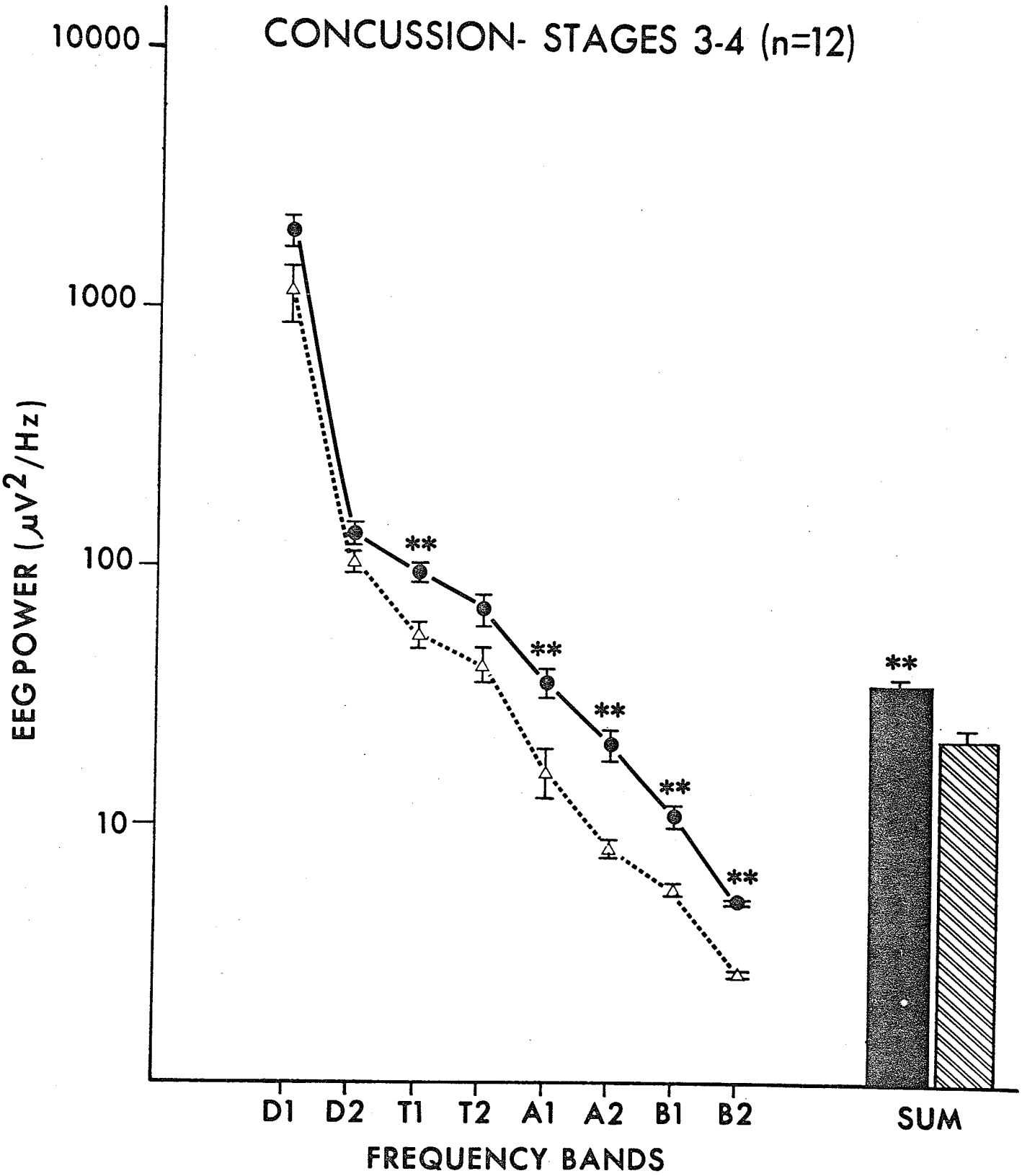
B) Post-concussion record plotted as a percentage of control record. Depression of EEG power varies from 70 percent of control (delta 2) to 43 percent of control (alpha 2) and is more marked than that observed in Stage 1-2 concussion.

Solid line: Pre-concussion

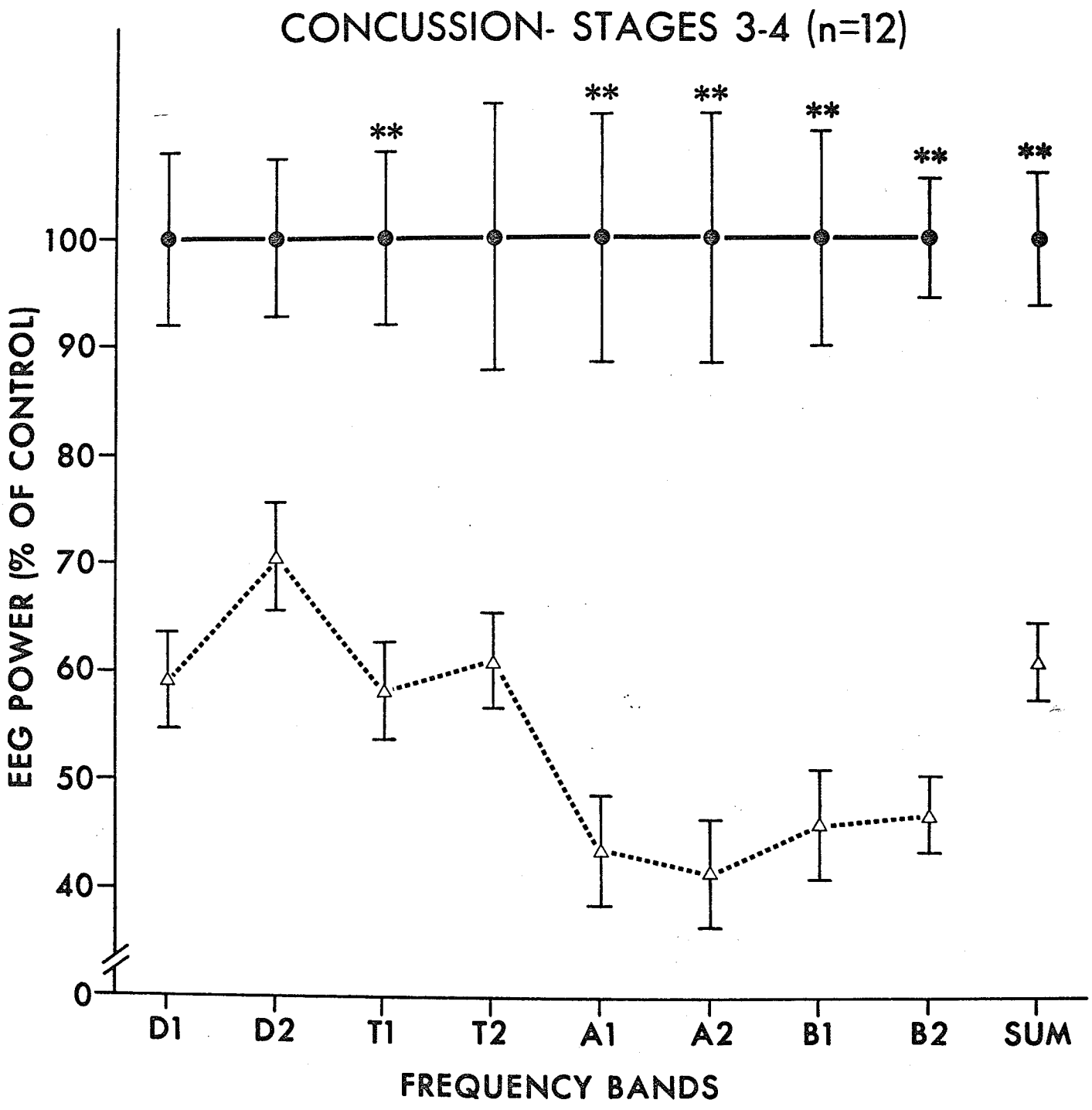
Interrupted line: Post-concussion

*$p < 0.05$

**$p < 0.01$


CONCUSSION- STAGES 3-4 (n=12)
10000
1000
100
10
EEG POWER (μV²/Hz)
**
**
**
**
**
**
D1
D2
T1
T2
A1
A2
B1
B2
SUM
FREQUENCY BANDS

16A


CONCUSSION- STAGES 3-4 (n=12)
EEG POWER (% OF CONTROL)
100
90
80
70
60
50
40
0
**
**
**
**
**
D1
D2
T1
T2
A1
A2
B1
B2
SUM
FREQUENCY BANDS

16 B

Figure 17

1) Control (pre-concussion)

2) Record taken 4.8 minutes post-concussion. There is marked depression of the alpha and beta frequencies, with the appearance of theta rhythm. (Paper speed 6mm/sec. Calibration in right lower corner).

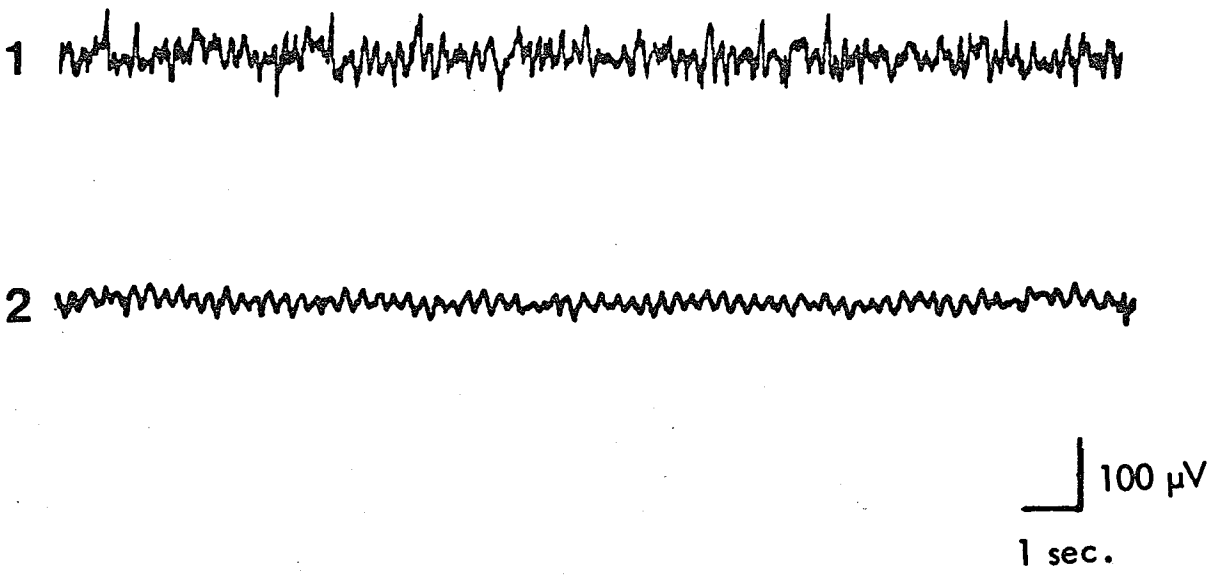

1
2
100 μV
1 sec.

Figure 18

The depression of the fast frequencies is more apparent at a faster recording speed (30 mm/sec).

1) control (pre-concussion)

2) 5 minutes post-concussion.

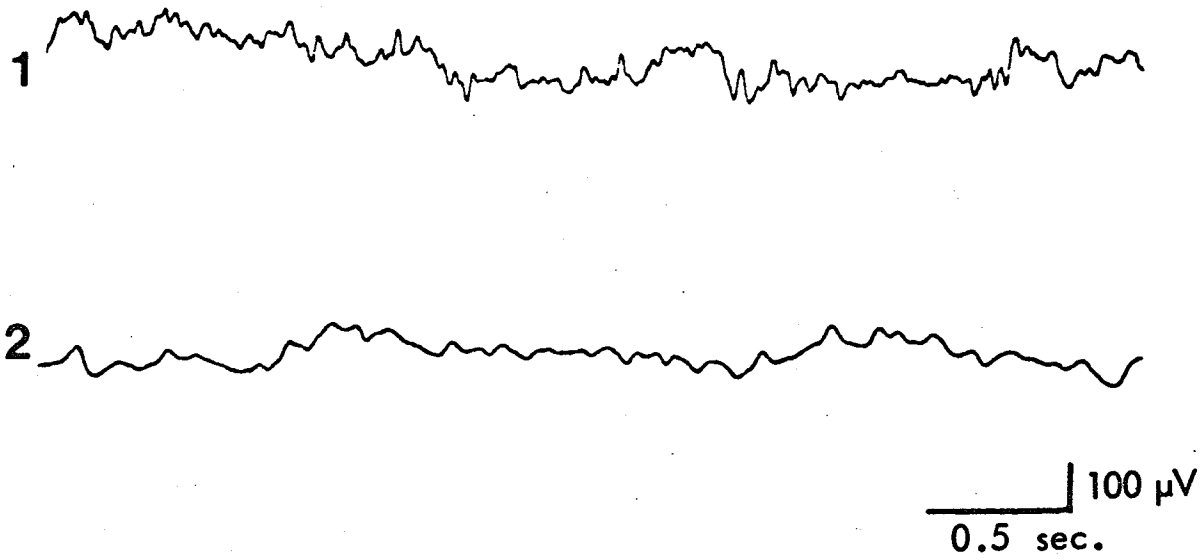
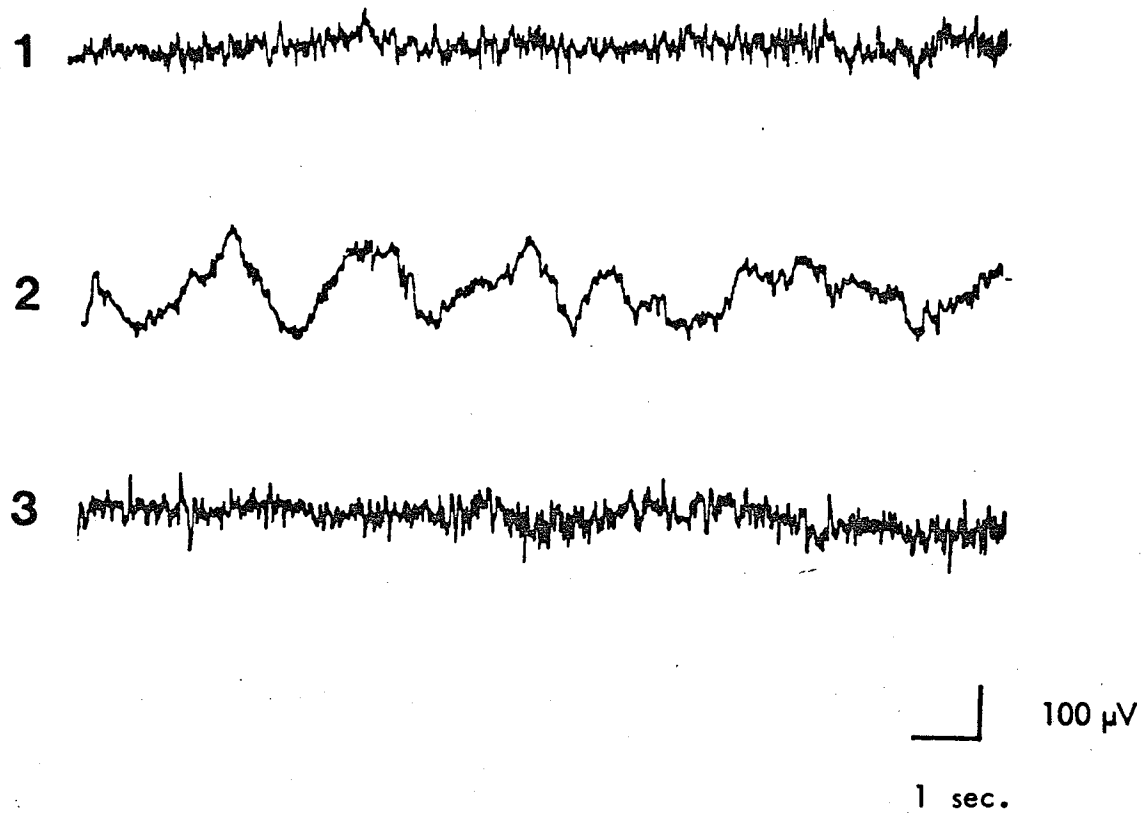

1
2
100 μV
0.5 sec.

Figure 19 Follow-up Record

1) Control (pre-concussion)

2) Record 4.5 minutes post-concussion. Demonstrates decreased voltage in most frequencies, with the appearance of high-voltage slow waves.

3) In this particular case, recovery of the EEG has occurred within 20 minutes of concussion.


1
2
3
100 μV
1 sec.

EEG's of monkeys that were anesthetized 24 hours prior to their experiments, but were alert at the time of impact. Their report discussed the EEG records in only four animals and so could not be subjected to statistical analysis. They observed depression of the EEG at the site of impact, and the delayed appearance of slow waves, similar to our records.

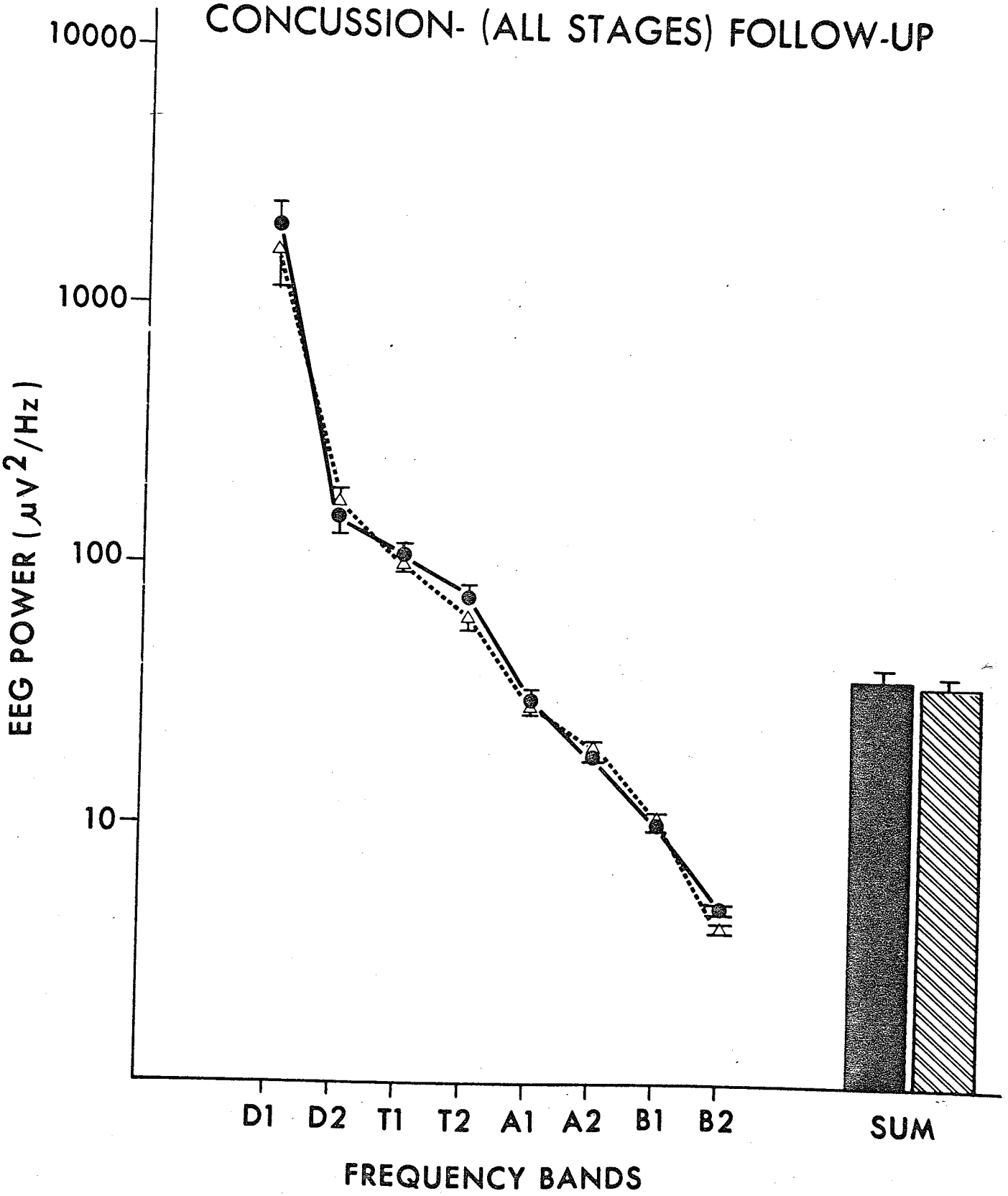
Sullivan, et al. (1976) studied the spectral analysis of EEG data in experimental compression-type brain injury where an impact was administered to a fixed head. Their animals were anesthetized, so that any correlation of EEG activity with change of consciousness was precluded. However, with injuries of 1.9 atmospheres, with apparently no pathological changes, the EEG was depressed in all frequencies. Recovery occurred only to one-half the baseline by the time of sacrifice two hours after the trauma. In these studies, the EEG may have recovered completely if the records had been continued (particularly in view of the absence of anatomical lesions), but the possible cumulative effects of trauma and anesthesia upon the EEG cannot be clarified in such a preparation.

Our studies have the advantage that spectral analysis of the EEG could be performed immediately following trauma and changes observed in the EEG could be correlated not only with changes in consciousness, but also with more subtle changes in behavior, posture, and tone recorded simultaneously on split screen video-tape. The records were continued until the rat and its EEG had recovered to normal in 27 of the 32 experiments. In the remaining five experiments, evidence of permanent cerebral injury ruled out the diagnosis of uncomplicated concussion, and the EEG did not recover completely.

Our records substantiated the importance of dysfunction of the cerebral cortex in the phenomenon of concussion in non-anesthetized animals. This dysfunction is reflected in the markedly decreased power spectrum affecting the faster EEG frequencies (Alpha 1 and 2, Beta 1 and 2) in animals concussed to

Figure 20 Follow-up Record

At two hours following concussion, the EEG power spectrum has returned to control values. (Solid line, pre-concussion; Interrupted line, post-concussion).


CONCUSSION- (ALL STAGES) FOLLOW-UP
10000
1000
100
10
EEG POWER (μV²/Hz)
D1
D2
T1
T2
A1
A2
B1
B2
SUM
FREQUENCY BANDS

Stage 1-2. These same frequencies are affected--to a greater degree--in animals concussed to Stage 3-4. The increase in the delta 2 power spectrum, observed in animals with mild (Stage 1-2) concussion has several possible explanations. Many have suggested that the delta waves may originate from damaged cortical neurons, and so may be recorded from cortical areas in the proximity of tumors, infarcts, or traumatic lesions (Williams, 1941; Laufer, et al. 1954; Rodin, et al. 1965; Scott, 1976). Ward and Clark (1948) have suggested that increased delta activity may be a result of being able to "see" the delta waves originating in the subcortical structures through the "inactivated" cerebral cortex. Guyton (1976) has observed that delta waves are generated in the "isolated cortex" that is created when fiber tracts between the thalamus and cortex are transected. This would indicate that the delta wave occurs intrinsically in the cortex when it is not being "driven" by the subcortical structures. The new appearance of, or increase in the delta spectrum would then suggest that activity in the reticular activating system has fallen to a level too low to maintain normal excitability of the cortex, so that the cortex then becomes its own pacemaker. The use of subcortical electrodes in order to determine the site of origin of the observed delta waves was considered. However, the presence of electrodes within the brain substance at the time of experimental head injury would likely result in the production of cerebral lacerations or hematomas, thereby complicating rather than simplifying EEG interpretation.

The suppression of the power spectra of all eight frequency bands in Stage 3-4 concussion is similar in nature and duration to the phenomenon of cortical spreading depression, first described by Leão (1949). Leão's original experiments produced transient dysfunction of the cerebral cortex in response to several insults, including local trauma to the cortex. The electrical activity which was depressed always re-occurred, with the same characteristics as

before the depression was elicited. Furthermore, the post-trauma EEG was marked by the appearance of slow waves, as we have observed in concussion.

Our observations are quite different from EEG tracings recorded from experimental animals with lesions within the reticular activating system (RAS). The classical appearance of the EEG in such a preparation is one of hypersynchrony (increased amplitude) (Lindsley, et al. 1949; French, 1952; French and Magoun, 1952). Previous theories regarding the pathophysiology of concussion have concentrated upon the role played by dysfunction of the RAS (Foltz, et al. 1953; Foltz and Schmidt, 1956; Jefferson, 1944; Shetter and Demakas, 1979; Walker, et al. 1944; Ward, 1966). Although there is no doubt that small lesions in that region may produce profound disturbances of consciousness (French, 1952; French and Magoun, 1952; Magoun, 1963), the EEG picture of such a lesion is not compatible with the spectral analysis of the EEG following concussion of an alert animal, which we have reported here.

Rowbotham (1961), Ommaya and Gennarelli (1974), and Feiring (1974) have also emphasized the prominent role of cortical dysfunction in traumatic unconsciousness. These authors observed that in both clinical and experimental situations, head injuries which produced loss of consciousness were characterized by lesions predominantly in the cerebral cortex. Only with the most severe injuries were primary brain stem lesions also witnessed. The latter occurred extremely rarely in isolation.

Ommaya and Gennarelli (1974), in their excellent studies, have suggested that the brain is affected centripetally in concussive head injuries; the brain stem being the last structure to be affected, since it is located at the greatest depth from the cortical surface. Mathematical analysis of the behavior of the brain during rotational acceleration also suggests that shear strains and stresses should be greatest in the cortex (Joseph and Crisp, 1971).

It is well recognized that pathology affecting the cerebral cortex diffusely may produce disorders of consciousness (French, 1952; Plum and Posner, 1972). Generalized cortical dysfunction would better explain post-concussion antegrade amnesia than would RAS dysfunction. We have demonstrated that the post-concussion EEG abnormalities persisted for an average of one and one-half hours after the gait, posture, and attitude of the concussed rat had returned to normal. This may be an experimental correlation of post-concussion antegrade amnesia in the athlete. Following a concussion, the athlete's behavior and appearance may be normal, but because of continuing higher cortical dysfunction, new experiences are not committed to memory. Similar temporary memory impairment, persisting after the recovery to normal locomotion and behavior was observed in the concussed rats confronted with a maze that they had learned prior to concussion.

Attempts to explain the phenomenon of concussion based on theories of RAS or cortical dysfunction alone, do not take into full acount the experimental and clinical observations. As Magoun (1963) has pointed out, the functions of the brain stem RAS and the cortex are intimately interdependent. Hence, impairment of either structure is likely to affect the other. Indeed, the unknown cellular substrate of concussion is most likely common to both structures. The fact that the resolution of the EEG abnormalities observed in this report was rapid supports the theory that concussion results from a reversible functional change, rather than from a permanent structural change.

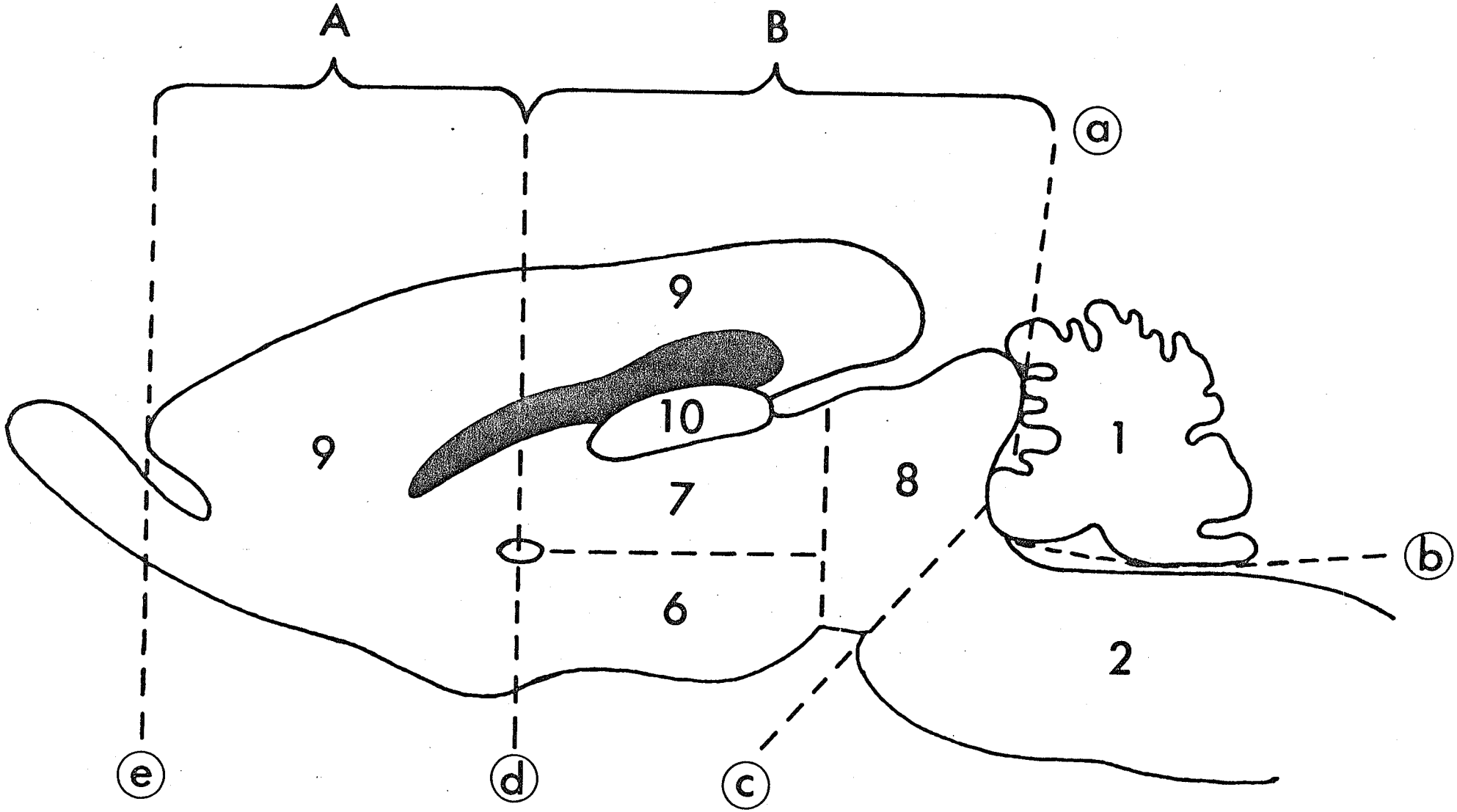
STUDIES OF PUTATIVE NEUROTRANSMITTERS AND RECEPTORS IN EXPERIMENTAL CONCUSSION

A = Radioreceptor Studies

Although there are several controversies surrounding previous experimental work on concussion, it is generally agreed that pressure gradients are produced within the brain substance at the time of impact due to the previously described phenomenon of inertial loading of the brain (Sellier and Unterharnscheidt, 1966; Goldsmith, 1966; Thomas, et al. 1967; Ripperger, 1975). These pressure gradients are associated with the development of shear strains within the cerebral cortex and brainstem (Gurdjian, et al. 1966; Thomas, et al. 1967; Ripperger, 1975). These pressure gradients are associated with the development of shear strains within the cerebral cortex and brainstem (Gurdjian, et al. 1966; Thomas, et al. 1967; Stahlhammar, 1975; Joseph and Crisp, 1971). Based upon the hypothesis that the abrupt imposition of pressure gradients may produce transient perturbations of neuronal membranes, and as a consequence, of neurotransmission, the effect of concussion upon the binding of ligands by receptors involved in opiate, cholinergic, and dopaminergic neural pathways was studied.

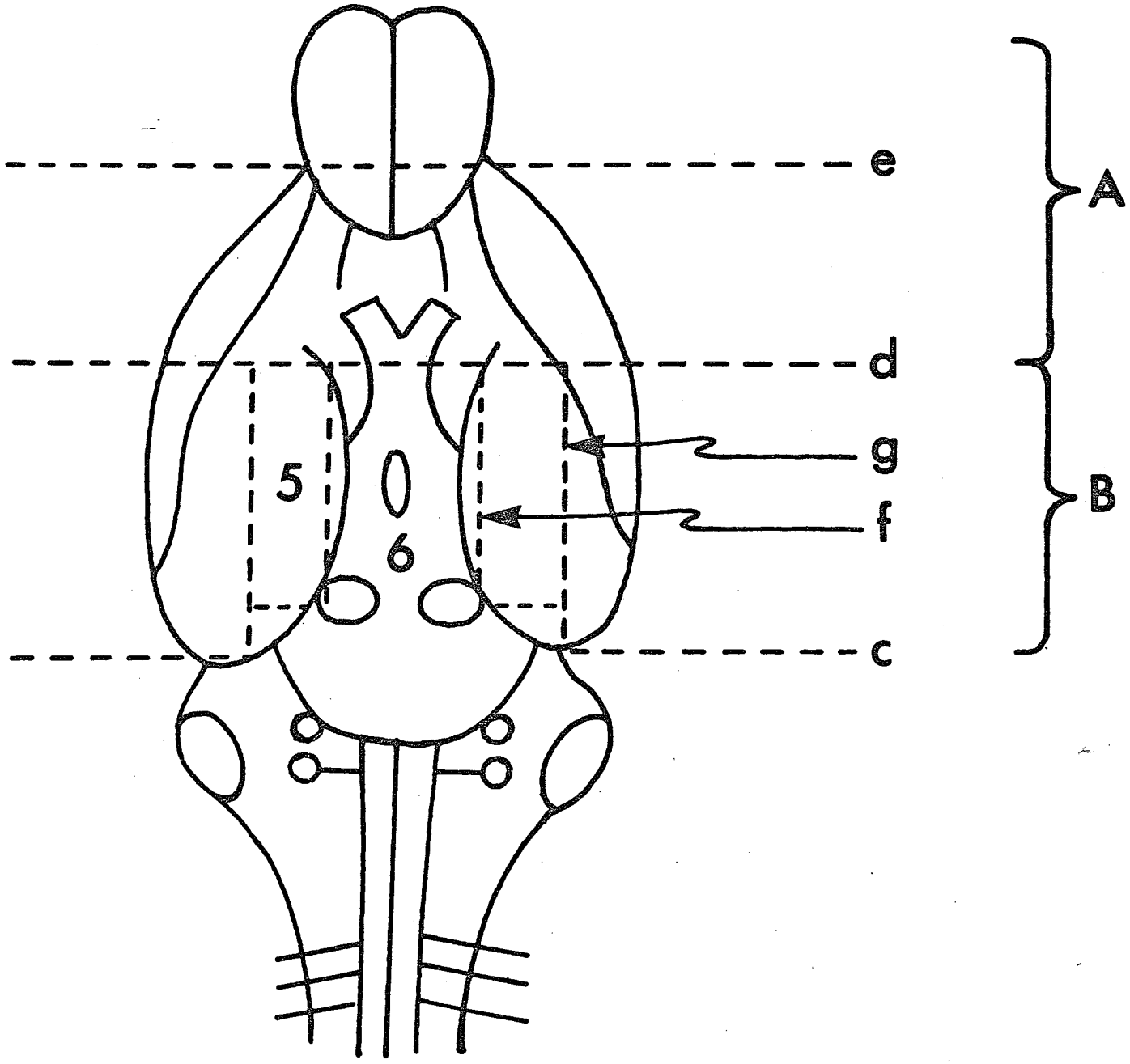
MATERIALS AND METHODS

Sprague-Dawley rats (males 200-250 grams) were used in the study. Only rats concussed to Stage 3 or 4 (i.e. transient loss of consciousness, associated with transient apnea) were studied. Post-mortem examination disclosed no skull fractures or intracranial hemorrhage. Concussed rats were paired with controls that were manually immobilized for the same time period required to produce concussion. Rats were then sacrificed by decapitation within two seconds of concussion, while still unconscious. The brains were quickly removed, dissected according to the modified guidelines of Glowinski and Iversen (1966) and homogenized in cold 0.05 M. Tris-HCl buffer (pH 7.7). Figure 21 outlines the method of dissection employed in this, and subsequent experiments. Table 3 demonstrates the reproducibility of



From Glowinski and Iversen (1966)

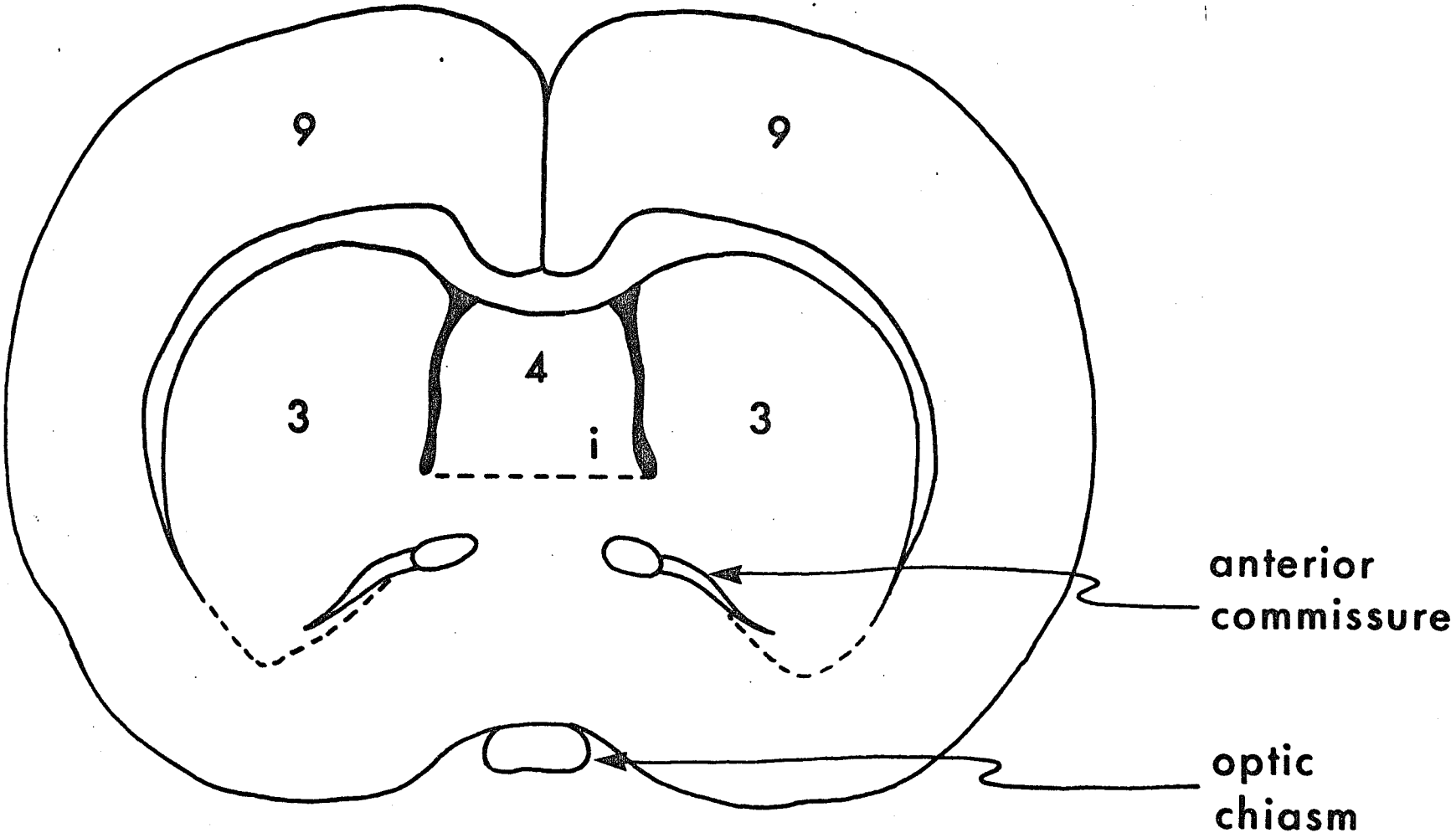
2/ A



From Glowinski and Iversen (1966)

21 B

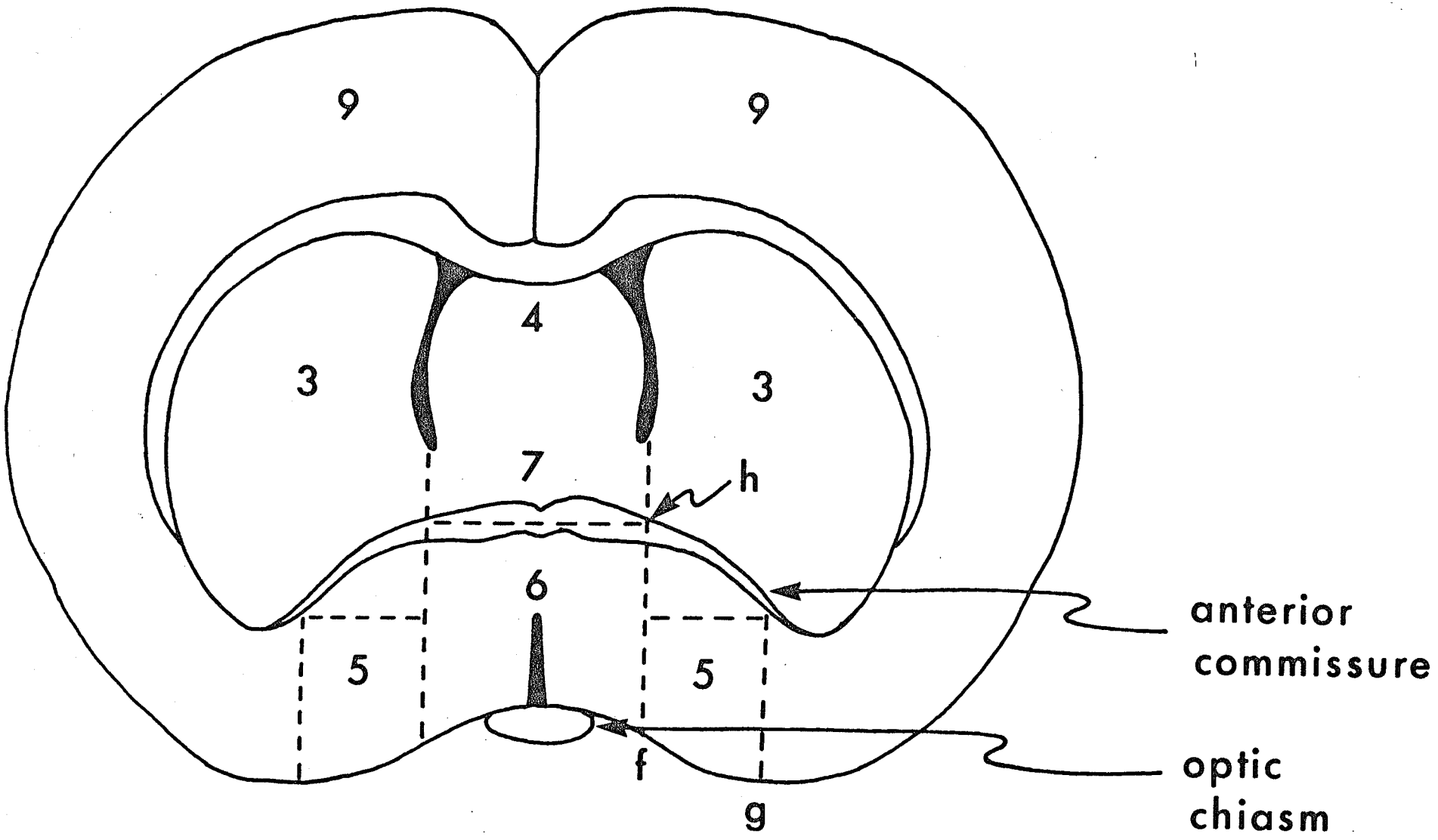
SECTION A



From Konig and Klippel (1967)

21 C

SECTION B



From Konig and Klippel (1967)

21 D

Table 3 - Reproducibility of Dissection Procedures

BRAIN REGION	WET WEIGHT (mg) MEAN ± S.E.M.
1) Cerebellum	245 ± 2.5
2) Hindbrain	223 ± 3.2
3) Corpus Striatum	182 ± 4.3
4) N. Septum	22 ± 1.5
5) Amygdala	34 ± 1.4
6) Hypothalamus	60 ± 1.4
7) Thalamus	44 ± 1.4
8) Midbrain	124 ± 2.2
9) Cortex	7604 ± 72.0
10) Hippocampus	89 ± 2.2
11) Hypophysis	7.2 ± 0.3
	(n = 40)

of the method. The opiate receptor assay was based on the method of Pert and Snyder (1973). Brain regions were homogenized in 30 volumes of cold 0.05 M. Tris-HCl, pH 7.7, and centrifuged for 750,000 g-min. The pellet was re-suspended in Tris buffer and the equivalent of 10 mg. wet weight of brain incubated for 30 minutes at 37 degrees Centigrade in one milliliter containing 2 nM ^{3}H-Naloxone (29 Ci/m-mole, New England Nuclear) and 100 nM NaCl. Each brain region was studied in the absence and presence of 0.1 nM Naloxone in order to determine specific (displaceable) binding. Following the separation of membrane-bound ^{3}H-Naloxone by centrifugation and aspiration of the supernatant, the pellet was dissolved in 0.3 ml. of 2N. KOH at 70 degrees centigrade and 0.2 ml. mixed with scintillation medium for ^{3}H counting. Specific binding was taken as the difference in radioactive counts between samples without excess unlabelled Naloxone and in the presence of excess unlabelled Naloxone.

The muscarinic cholinergic radioreceptor assay was modified from Yamamura and Snyder (1974), similar in principle to the assay described above. The pellet from the brain homogenate was re-suspended in phosphate buffer (50nM Na_3PO_4, pH 7.4) and the equivalent of 10 mg. brain incubated for 30 minutes at 0 degrees centigrade in one milliliter containing 2 nM ^{3}H-Quinuclidinylbenzilate (QNB) (43 Ci/mmole, Amersham Corp.). Each brain region was studied in the presence and absence of 0.1 mM atropine.

The spiroperidol receptor assay (which labels predominantly serotonin receptors in the frontal cortex and dopamine receptors in the striatum) was modified from the technique described by Creese, et al. (1977). The brain homogenate was re-suspended in buffer (50 mM Tris, 120 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% ascorbic acid, pH 7.1) and the equivalent of 10 mg. brain incubated for 20 minutes at 37 degrees centigrade in one milliliter containing 2 nM ^{3}H-spiroperidol (20 Ci/mmole, Amersham). Each region was studied in the presence and absence of 0.1 mM spiroperidol.

Figure 22 ^{3}H-Naloxone Binding in the Brains of Rats Decapitated Two Seconds after Concussion--Experiment 1.

Increased ^{3}H-Naloxone binding was observed only in the hypothalamus. There were no significant changes in other brain regions.

Means and S.E.M. are shown (n = 6)

** $p < .01$ (ANOVA and Duncan's Multiple Range Test)

Open and closed bars represent control and concussed animals respectively.

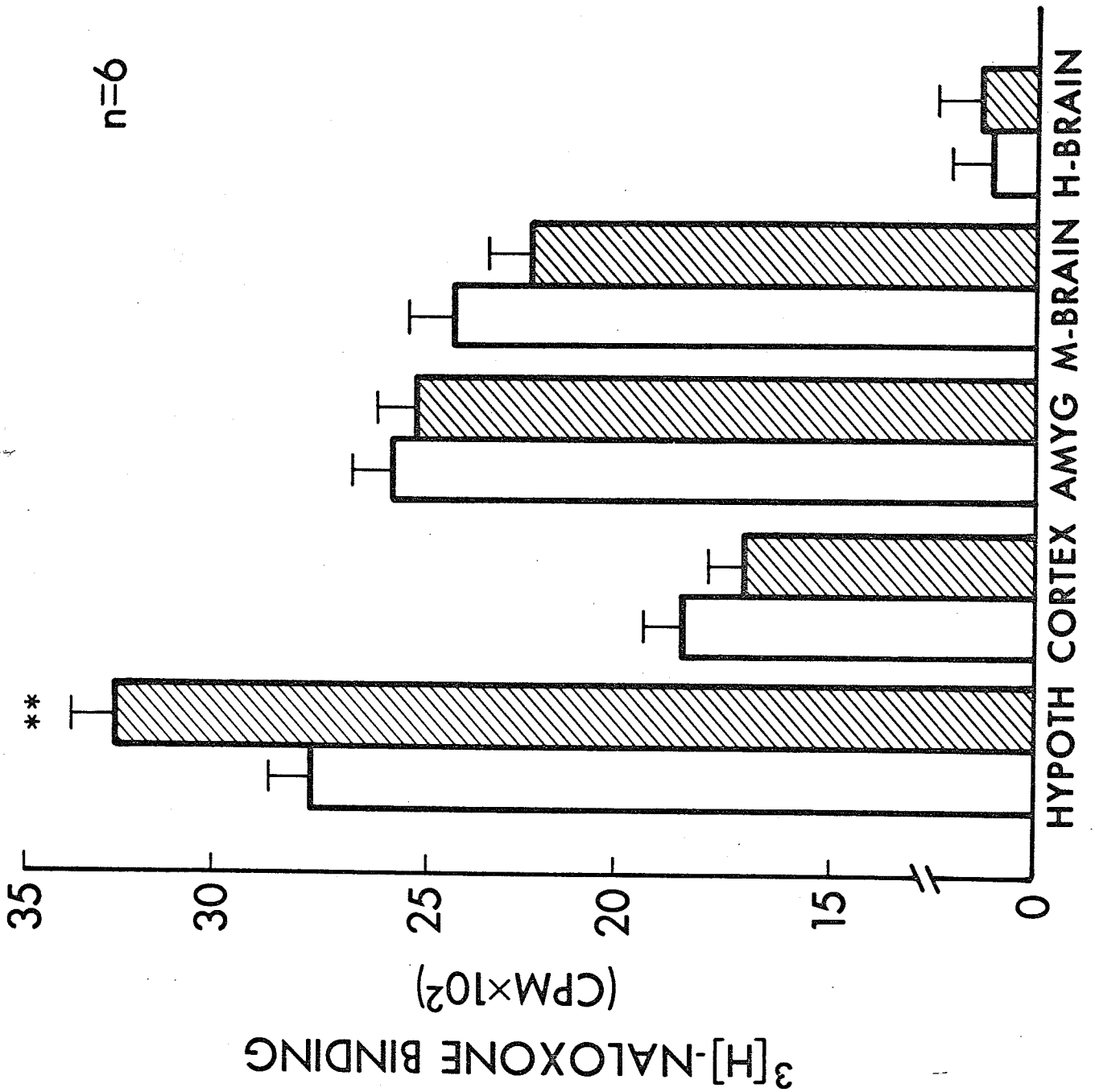

^{3}[H]-NALOXONE BINDING
(CPM×10^2)
35
30
25
20
15
0
**
n=6
HYPOTH
CORTEX
AMYG
M-BRAIN
H-BRAIN

Figure 23 ^{3}H-Naloxone Binding in the Brains of Rats Decapitated Two Seconds after Concussion--Experiment 2.

The experiment was repeated to compare the hypothalamic binding with other brain regions not studied in the first experiment. Again, increased ^{3}H-Naloxone binding was observed only in the hypothalamus. There were no significant changes in other brain regions.

Means and S.E.M. are shown (n = 6)

* $p < .05$ (ANOVA and Duncan's Multiple Range Test)

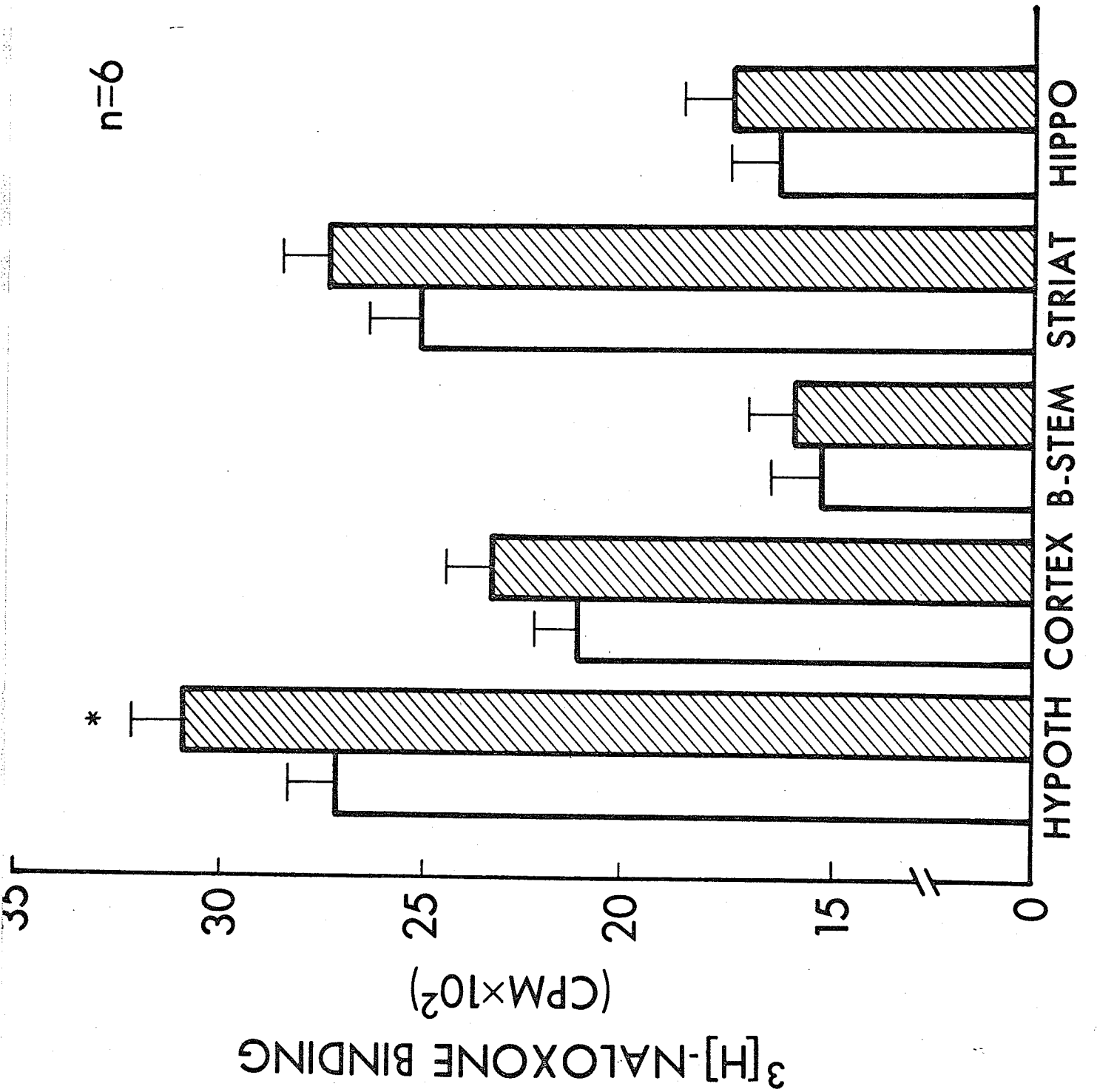

^{3}[H]-NALOXONE BINDING
(CPM×10^2)
35
30
25
20
15
0
*
n=6
HYPOTH
CORTEX
B-STEM
STRIAT
HIPPO

Figure 24 ^{3}H-QNB Binding in the Brains of Rats Decapitated Two Seconds after Concussion

Increased ^{3}H-QNB binding was observed only in the hypothalamus immediately following concussion. There were no significant differences in other brain regions.

Means and S.E.M. are shown

* $p < .05$ (ANOVA and Duncan's Multiple Range Test)

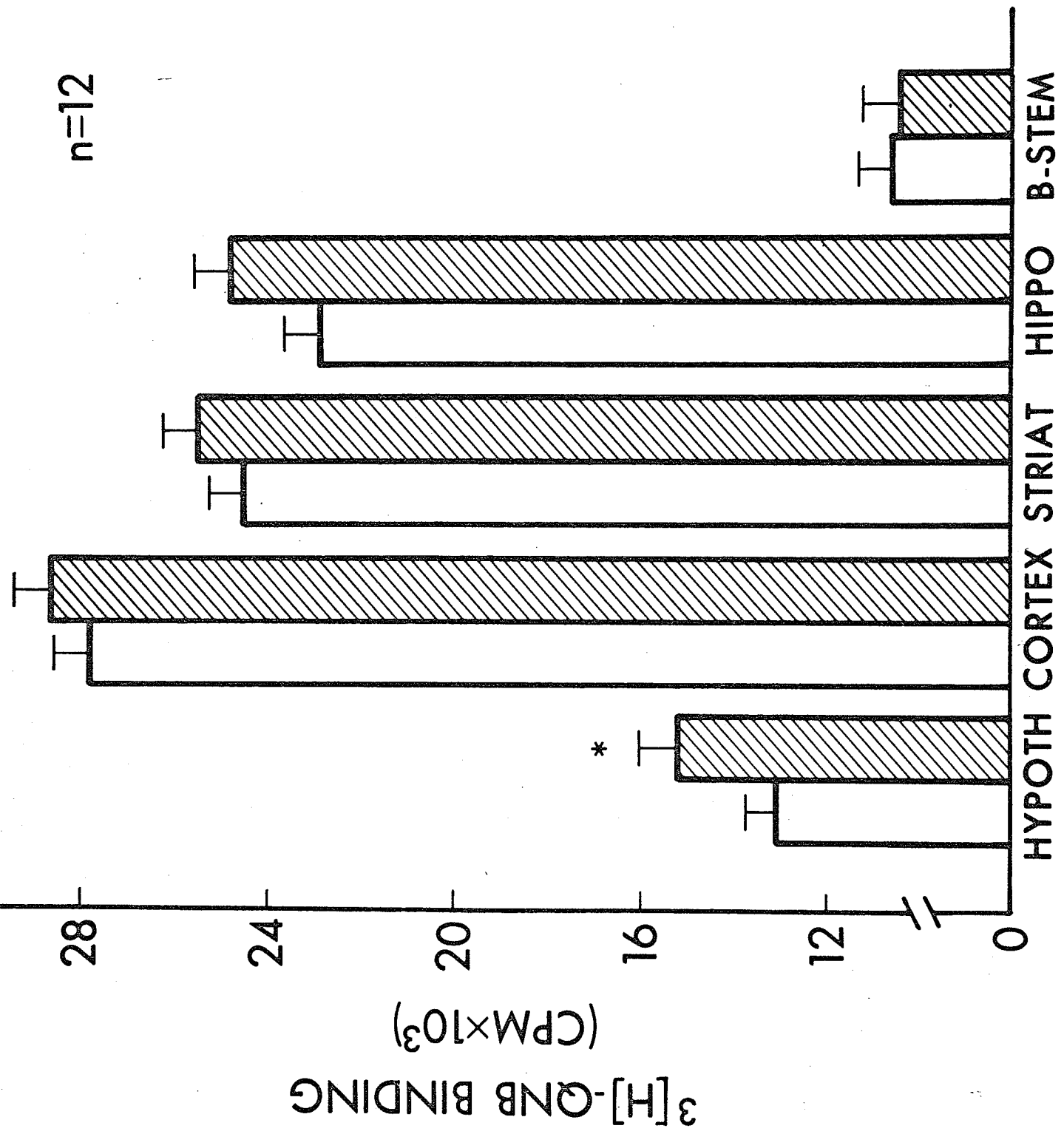

n=12
^{3}[H]-QNB BINDING
(CPM×10^3)
28
24
20
16
12
0
*
HYPOTH
CORTEX
STRIAT
HIPPO
B-STEM

Figure 25 ^{3}H-Naloxone Binding in the Brains of Rats Decapitated Five or Ten Seconds after Concussion

There were no detectable differences in ^{3}H-Naloxone Binding in any of the brain regions studied if the rat was sacrificed after having regained consciousness, at either five seconds (Fig. 25A) or ten seconds (Fig. 25B).

Means and S.E.M. are shown

(n = 12 for each experiment)

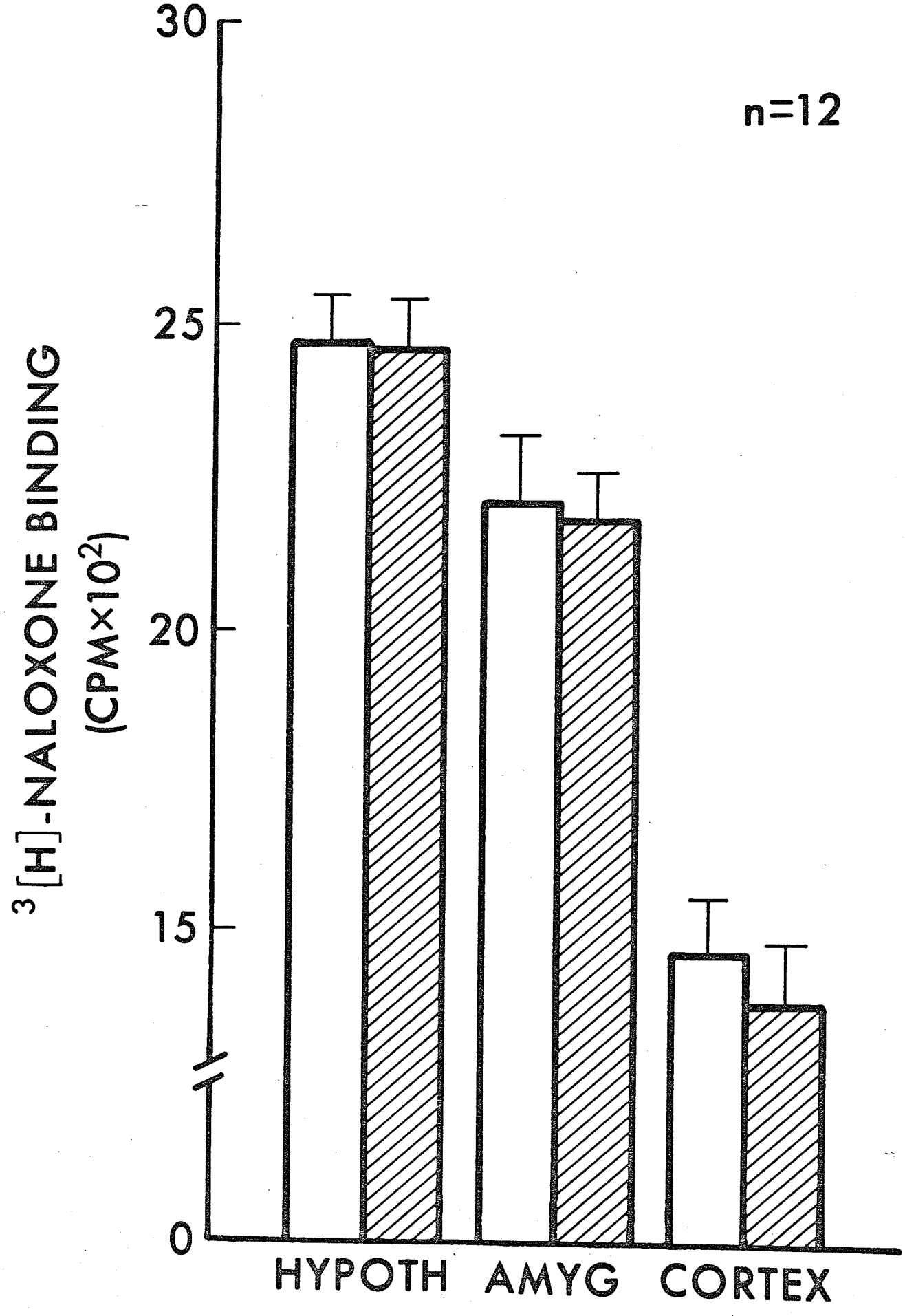

30
25
20
15
0
n=12
^{3}[H]-NALOXONE BINDING
(CPM×10^2)
HYPOTH
AMYG
CORTEX

Figure 26 ^{3}H-Ouabain Binding in the Brains of Rats Decapitated Two Seconds after Concussion

There were no changes in ^{3}H-Ouabain binding in the regions studied. Means and S.E.M. are shown.

(n = 12 for each experiment)

65
60
55
50
0
n=12
^{3}[H]-OUABAIN BINDING
(CPM×10^2)
HYPOTH
CORTEX
STRIAT
B-STEM
HIPPO

Figure 27 ^{3}H-Spiroperidol Binding in the Brains of Rats Decapitated Two Seconds after Concussion

There were no changes in ^{3}H-Spiroperidol Binding in the regions studied. Means and S.EM. are shown.

(n = 12 for each experiment)

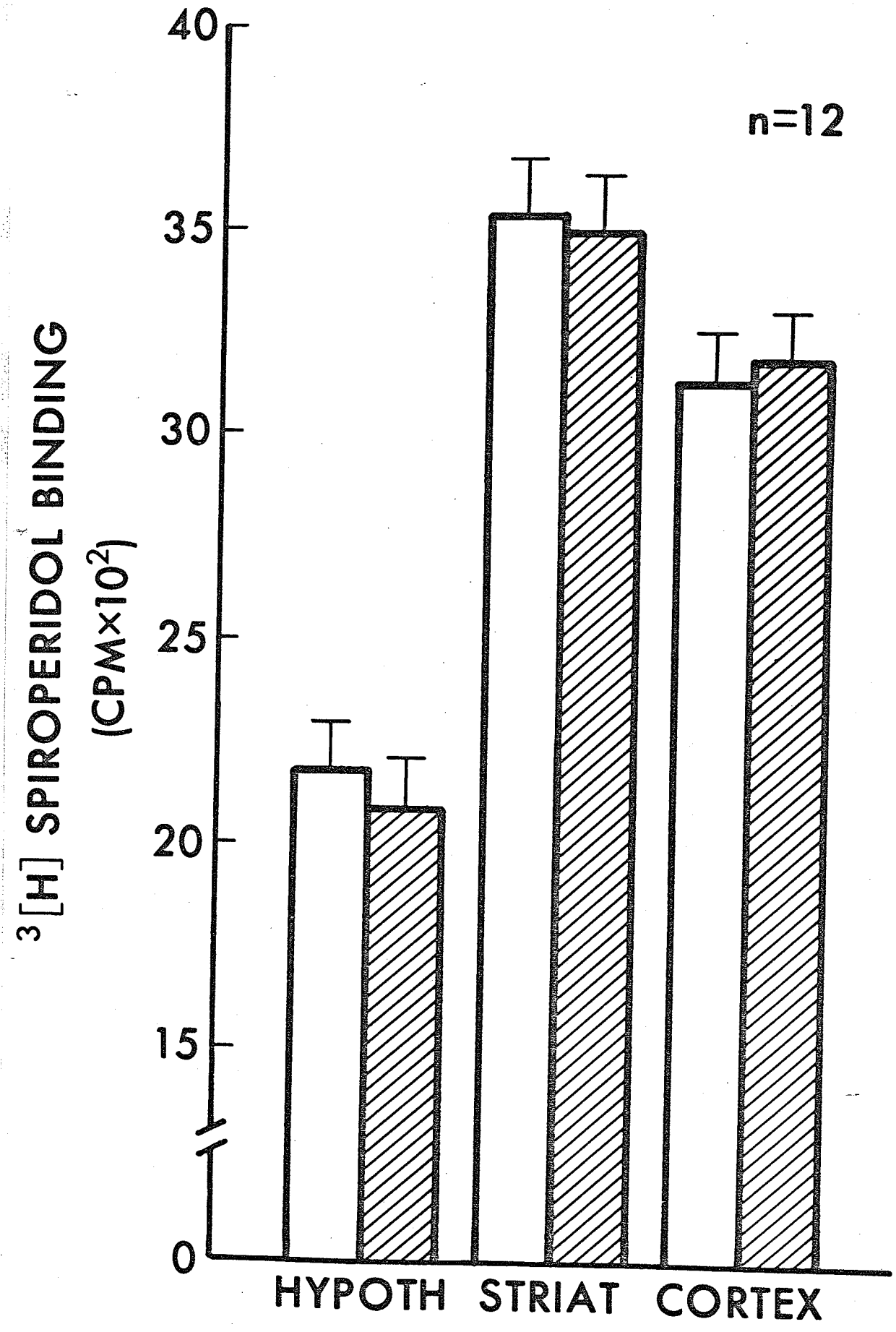

40
35
30
25
20
15
0
n=12
^{3}[H] SPIROPERIDOL BINDING
(CPM×10^2)
HYPOTH
STRIAT
CORTEX

Figure 28 ^{3}H-QNB Binding in Rats Receiving a Concussing Blow to the Head or a blow to the Body

Open bar: control values.

Solid bar: Two seconds post-concussion.

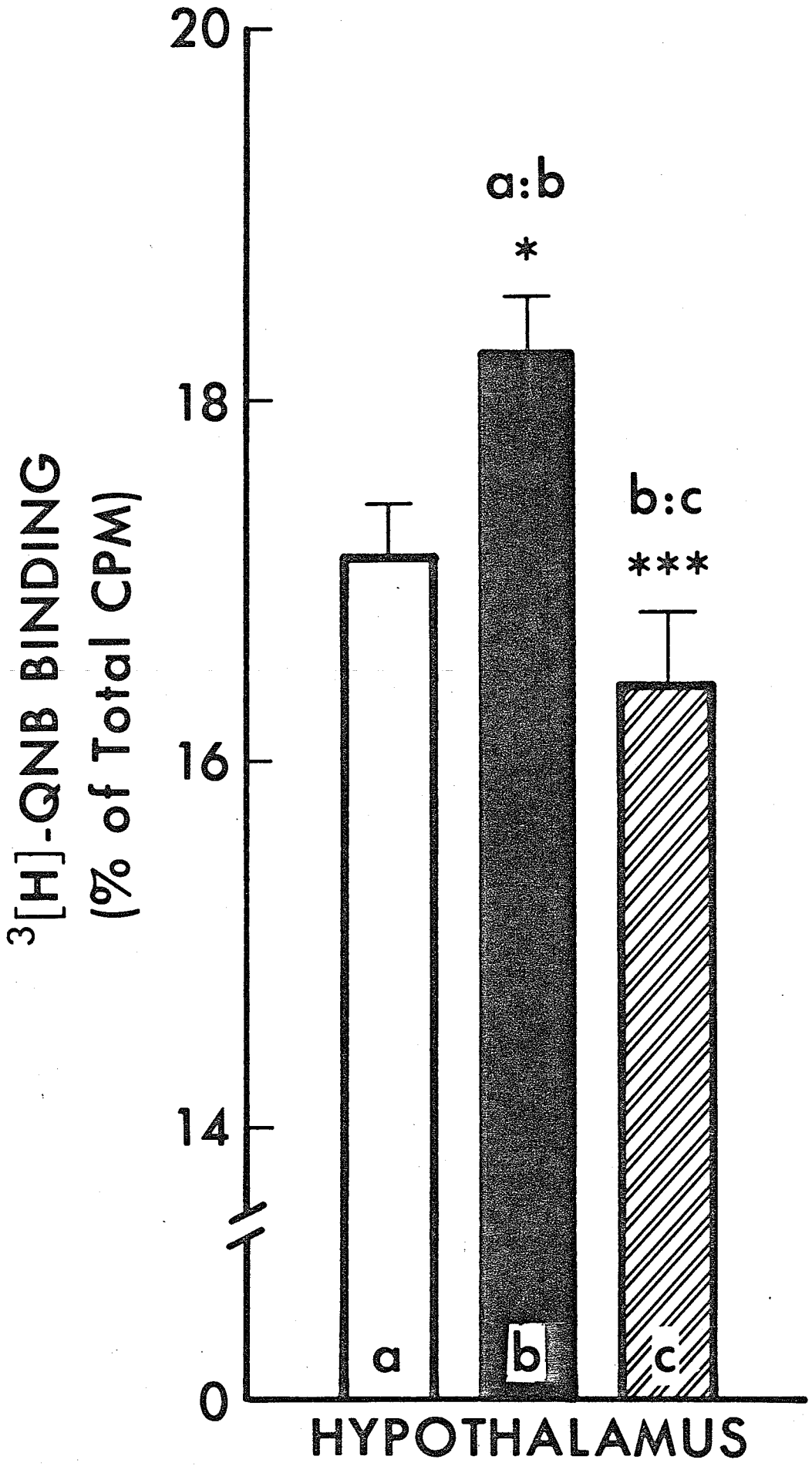
Cross-hatched bar: values in rats subjected to a body blow.

Means and S.E.M. are shown.

(n = 12 in each group)

* Difference between control and concussed significant at $p < 0.05$ (Student's t test)

** Difference between concussed and body blow significant at $p < 0.001$ (Student's t test)


20
18
16
14
0
^{3}[H]-QNB BINDING
(% of Total CPM)
a:b
*
b:c
***
a
b
c
HYPOTHALAMUS

The ouabain receptor assay was modified from the technique of Chow, et al. (1979). The pellet from the brain homogenate was re-suspended in buffer containing 50 mM Tris, 150 mM NaCl, 1.25 $MgCl_2$ and 1 mM ATP, pH 7.4. The equivalent of 10 mg. of brain was incubated for one hour at 37 degrees centigrade in one milliliter buffer containing 2 nM ^{3}H-ouabain (13 Ci/mmole). Each brain region was studied in the presence and absence of 10^{-3}M. ouabain.

RESULTS

Immediately following concussion, significantly increased binding of ^{3}H-naloxone and ^{3}H-QNB was observed, only in the hypothalamus (Figs. 22, 23, 24). When the assay was repeated after the rat had regained consciousness (5 and 10 seconds after the blow), there were no detectable differences in binding (Fig. 25). ^{3}H-Spiroperidol and ^{3}H-Ouabain binding were unchanged in all regions including the hypothalamus (Figs. 26, 27). To exclude the possiblity that the observed changes were non-specific responses to stress, a group of rats received a blow to the body (right flank) instead of to the head (Fig. 28). Following the body blow, in contrast to the enhanced binding in concussed animals, there was a significant decrease ($p < 0.001$) in ^{3}H-QNB binding, suggesting that the alteration in binding in concussed animals was not a non-specific response to stress or trauma.

DISCUSSION

The increased binding of ^{3}H-Naloxone and ^{3}H-QNB suggests that binding of endogenous opiate and cholinergic ligands is decreased immediately following concussion. These changes may have been more dramatic if it had been possible to sacrifice the rats instantaneously at the time of concussion. The apparently decreased binding of endogenous ligands of the opiate and cholinergic receptors may be a result of:

a) a sudden pressure-induced perturbation of the neuronal cell membrane, of which the receptor site is an integral part; this sequence of events may interfere with ligand-receptor binding.

b) An abrupt decrease in the release of the endogenous ligand

or

c) release of a substance which interferes with the binding of the endogenous ligands.

The interpretation of the results is clarified later in the experiments investigating changes in endogenous opiates (endorphins) following concussion. On the basis of these results it is tempting to speculate that, at the time of concussion, mechanical deformation of receptor sites results in diminished affinity for specific endogenous ligands. Within five seconds of the concussing blow, binding and presumably receptor conformation returns to normal.

Clinically, it is well-known that lesions which disturb the integrity of the hypothalamus result in a dramatic impairment of consciousness (Ranson, 1939; French and Magoun, 1952; Martin, et al. 1977). It has been claimed by several investigators that the region of the hypothalamus is an area subjected to the greatest shear strains during the abrupt development of intracranial pressure gradients associated with concussion (Gurdjian, 1953, 1972, 1975; Friede, 1961; Chason, et al. 1966). Hence, it is of interest that disturbances in receptor binding following concussion are detected only in this brain region. Perhaps strains reach a level in the hypothalamus, but not in other brain regions, at which receptor binding is impaired. The transient perturbation of receptor binding, and presumably of neuro-transmission, may explain how neural function is temporarily impaired by concussion with no apparent evidence of brain damage.

B: Effects of Experimental Concussion upon Putative Neurotransmitters - B-Endorphins and Somatostatin

Clinical and experimental studies of concussion strongly suggest that the primary response of the nervous system to this form of trauma is inhibitory. Realizing this, we decided to study the role of the potent putative inhibitory neurotransmitter β-Endorphin. This 31 amino acid peptide derived from the precursor molecule β-lipotropin (93 amino acids) was chosen because its effects upon the nervous system have been thoroughly studied and observed to be predominantly inhibitory. In fact, administration of β-endorphin intracerebroventricularly (ICV) or intraventricularly (IVT) results in behavioral changes very similar to those which follow cerebral concussion. For instance, Bloom, et al. (1976) injected β-endorphin into the lateral ventricles of conscious rats and noted disappearance of corneal reflexes, depression of general motor activity, lack of responsiveness to noxious stimuli, loss of righting reflexes, and with high doses, generalized rigidity. Jacquet and Marks (1976), Guillemin (1976), Goldstein (1976), and Snyder (1977), also noted tranquilization of the injected rat for periods which depended upon the area injected and the dose injected. Segal, et al. (1978) confirmed the development of immobility and decreased locomotion following IVT injection of β-endorphin. Elde, et al. (1978) hypothesized that endogenous opiates, whose cell bodies could be found in the dorsal portion of the nucleus parabrachialis, might have an inhibitory role in controlling respiratory drive. Soon after, Rossier and Bloom (1979) observed respiratory depression after ICV injection of β-endorphin in rats. Havlicek and Friesen (1979) and Havlicek, et al. (1980) demonstrated that high doses of β-endorphin ICV render the experimental rat comatose with absent brain stem reflexes and absent response to noxious stimuli. They likened this condition to a state of general anesthesia. The fact that the changes in behavior noted above could be directly related to the administration of β-endorphin and not merely artifacts of experimental design, was confirmed by their prompt reversal upon injection of the opiate antagonist naloxone.

Most investigators agree that the basal secretion of β-endorphin must be low (Terenius and Wahlstrom, 1978); the reason being that noxious stimuli to a certain extent are very useful in protecting the individual organism. However, in response to injury or acute stress, β-endorphin appears in large amounts in the bloodstream, while the hypothalamus content decreases (Rossier, et al. 1977). (The β-endorphin is secreted in equimolar amounts with ACTH). Such stress is accompanied by analgesia which is partly naloxone-reversible. Several studies have also shown that β-endorphin administered ICV is a powerful epileptogenic agent (see for example, Havlicek and Friesen, 1979; Rossier and Bloom, 1979).

Previous studies in this laboratory have demonstrated that changes in the brain content of the inhibitory peptide β-endorphin may be accompanied by changes in the potent excitory putative neuro-transmitter Somatostatin (SRIF)--in the opposite direction (Havlicek and Friesen, 1979; Kato, et al. 1980; Sundmark, et al. 1980). Hence, we decided to study the brain content of β-endorphin and somatostatin following concussion.

MATERIALS AND METHODS

Experiments were conducted with Sprague-Dawley rats (males, 200-250 grams). Five groups of 12 to 20 rats each were used. Six to ten rats in each group were concussed by the method previously described. Control rats (6-10 per group) were manually immobilized for the time period required to produce concussion. Of the five groups of concussed rats, one group was sacrificed immediately following concussion (within 2.5 to 3.0 seconds). The remaining groups were sacrificed at 5 minutes, 15 minutes, 30 minutes, and 45 minutes following concussion. All rats were sacrificed by microwave irradiation at the power of 2.2 KW for 10 seconds, followed by 10 seconds at 1.1 KW in a Philips microwave oven (Model HN1124) between 0800 and 1000 hours. This method of sacrifice has been shown to result in rapid arrest of enzymatic reactions (Herchl, et al. 1977) and the highest

yield of β-endorphin and somatostatin in brain tissue (Ogawa, et al. 1979; Havlicek and Friesen, 1979).

Following sacrifice, the brains were removed and dissected on ice, following the techniques previously outlined. Each brain region was weighed, homogenized in 0.1 N. acetic acid using a glass tissue grinder (Table 4) and then centrifuged for 30 minutes at 4 degrees centigrade at 3000 RPM. The supernatant was saved; the pellet re-suspended and mixed in a volume of 0.1 N. acetic acid equal to the first extraction volume. The second mixture was re-centrifuged, the supernatant decanted and added to the first extract. The brain extracts were then frozen, to be thawed at the time of radioimmunoassay. The double extraction procedure has been shown to yield higher, less variable values of β-endorphin in brain tissue (Ogawa, et al. 1979).

A) Radioimmunoassay (RIA) for β-Endorphin

The antiserum used in these studies was that of Ogawa, et al. (1979) which binds 50 percent of tracer at the final dilution of 1:50,000. (Antibodies to camel β-endorphin (Peninsula Labs, San Carlos, California) were induced in rabbits after conjugation of β-endorphin to bovine serum albumin (method of Yoshimi, et al. 1978). The antiserum cross-reacts with ovine β-endorphin (100 percent), human β-endorphin (30 percent) and ovine β-lipotropin (50 percent) on an equimolar basis. Gel filtration of brain extracts has demonstrated that 98 percent of "immunoreactive" β-endorphin co-elutes with synthetic β-endorphin; only a very small amount co-elutes with β-lipotropin. Extracts of rat hypophysis and rat brain produced curves parallel to the standard curve (Fig. 29).

β-endorphin (camel) was radioiodinated using the lactoperoxidase method originally described by Thorell and Johannson (1971).

To a test-tube containing 5 μg of camel β-endorphin were added:

1 - 25 μL of 0.4 M. sodium acetate (pH 5.6)

Table 4

Brain Region	Volume of 0.IN Acetic Acid/Extract
Cortex	3.0 ml.
Cerebellum	1.5
Hippocampus	1.5
Striatum	1.5
Midbrain	1.5
Hindbrain	1.5
Nucleus accumbens	1.0
Nucleus Septalis	1.0
Thalamus	1.0
Amygdala	1.0
Hypophysis	1.0

2 - 25 µL of $Na^{125}I$ (1 mCi) (New England Nuclear)

3 - 25 µL of lactoperoxidase (Calbiochem, La Jolla)

4 - 10 µL of 30 percent hydrogen peroxide at 1:15,000 dilution

5 - After 1.5 minutes another 10 µL of 30 percent hydrogen peroxide at 1:15,000 dilution

6 - After 1.5 minutes, the reaction was terminated by the addition of 1 ml. of 2 mM ammonium acetate elution buffer (pH 4.6) and 50 µL of 5 percent BSA.

The reaction products were then separated on a 20 cm x 0.7 cm chromatographic column (Bio-Rad, Mississauga) containing CM-23 cellulose ion exchanger material (Whatman, U.S.A.) at 4 degrees centigrade. As soon as the reaction mixture was placed on the column, collection of 50 drop fractions was begun. Fraction tubes 1-20 contained 3 drops of Trasylol (10,000 K.I.U./ml - Boehringer Ingelheim, Canada) and 3 drops of 50 percent BSA. Fractions 1-7 were eluted with 0.002 M. ammonium acetate (pH 4.6). Fractions 8-30 were eluted with 0.2 M. ammonium acetate (pH 4.6). Fractions 30-60 were eluted with 2 M. ammonium acetate (pH 4.6). The profile of the eluted material is shown in Fig. 30. Sodium phosphate buffer, containing 25 mM EDTA and 5 percent BSA (pH 7.4) was used as the diluent for the reagents. The radioassay was performed as follows:

The immunoactivity of the tracer was determined for each fraction from the second peak of radioactivity. One hundred µL of a 20,000-30,000 cpm dilution was tested for specific binding (Bo/T%) by a double-antibody radioimmunoassay (RIA). The RIA was performed in glass test-tubes (10 x 75 mm) containing:

a) 100 µL. of 1:2 Trasylol solution

b) 100 µL. of phosphate buffer

c) 100 µL. of standard/sample (cβ-endorphin-Beckman) Standards contained 0, 0.1, 0.25, 0.5, 1.0, 1.5, 2.5, 5.0, 10, 25, 50, 100 and 500 ng/ml. (Non-specific binding was determined by a tube containing all of the reaction ingredients

except standard and antibody)

d) 100 μL. of anti cβ-endorphin rabbit serum at 1:10,000 dilution

e) 100 μL. of the tracer (20,000-30,000 c.p.m. in the total count

The above mixture was then incubated at 4 degrees centigrade for 48 hours, following which were added:

f) 100 μL. of sheep anti-rabbit serum (second antibody) at 1:20 dilution

g) 100 μL. of normal rabbit serum at 1:300 dilution

The mixture was incubated for another 24 hours at 4 degrees centigrade following which 500 μL of phsophate buffer were added to each tube. The tubes were then centrifuged at 3000 RPM for 40 minutes at 4 degrees centigrade. The supernatant was carefully decanted and the pellet counted on a Beckman gamma counter (1 minute counts).

In this assay, when using an antibody titer of 1:10,000 in the double antibody test, a satisfactory tracer will usually have more than 40 percent specific binding and less than 10 percent non-specific binding.

Characterization of the RIA for β-Endorphin

1. Sensitivity: The sensitivity of the assay is 0.5 ng/ml. The standard curve is linear from 0.5 ng/ml. to 25-50 ng/ml. Assay sensitivity with half-maximal displacement of tracer is 3-4 ng/ml. (Fig. 29).
2. Specificity: There is no cross-reaction with α-and γ-endorphin, leu-enkephalin, insulin, glucagon, TRH, LH-RH, bombesin, myelin basic protein, growth hormone, prolactin, morphine, or naloxone. There is, however, 50 percent cross-reactivity with ovine β-lipotropin (Lee, 1979: Ogawa, et al. 1979).
3. Inter-assay Variation: ranges from 12 to 17 percent, while the intra-assay variation is 3.8 to 5.5 percent (Lee, 1979).

Figure 29

Standard curve for β-endorphin RIA. Extracts of rat brain produced curves parallel to the standard curve.

Triangles = hypothalamic extracts
(diluted 1:10)

Squares = Hippocampal extracts

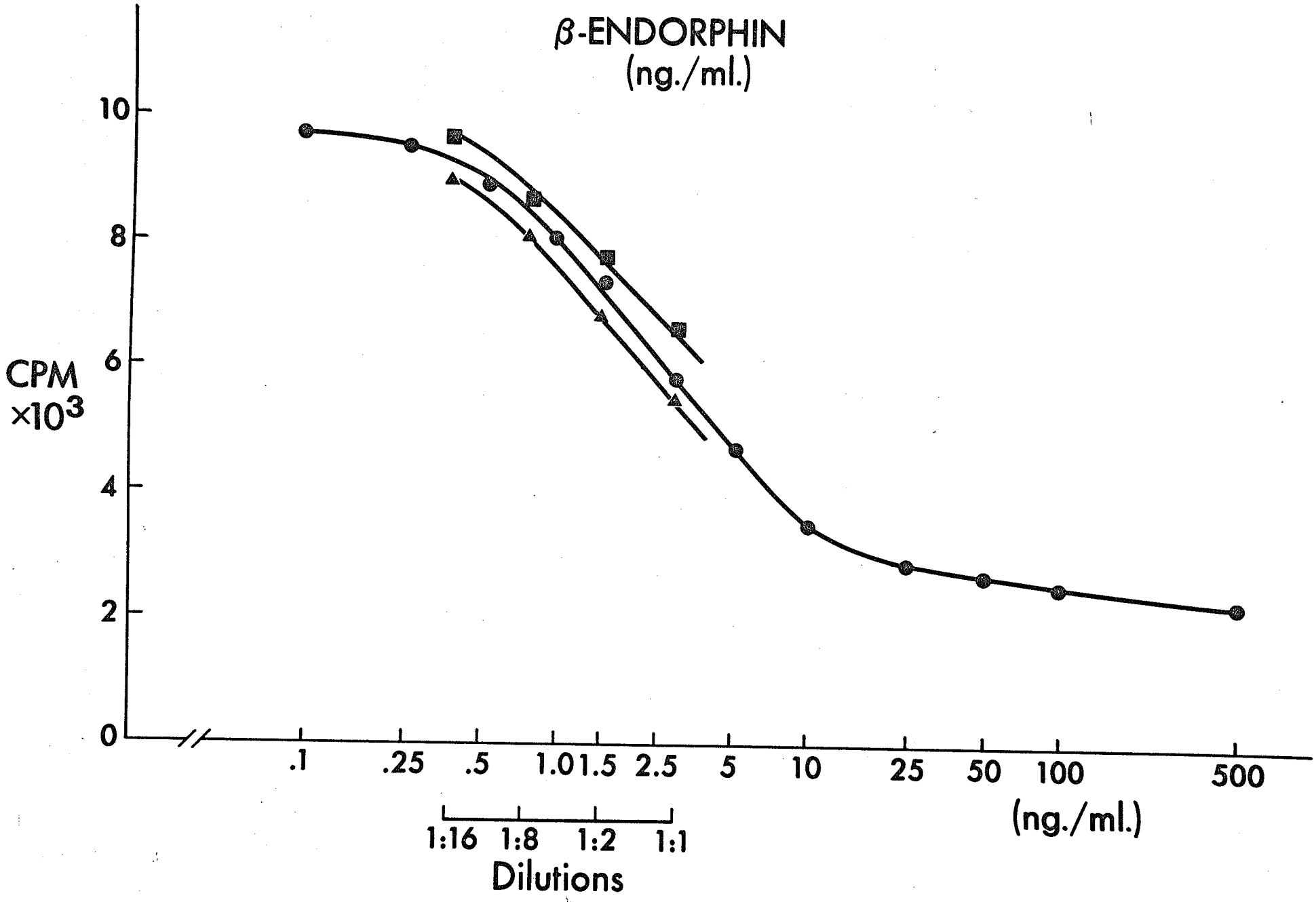
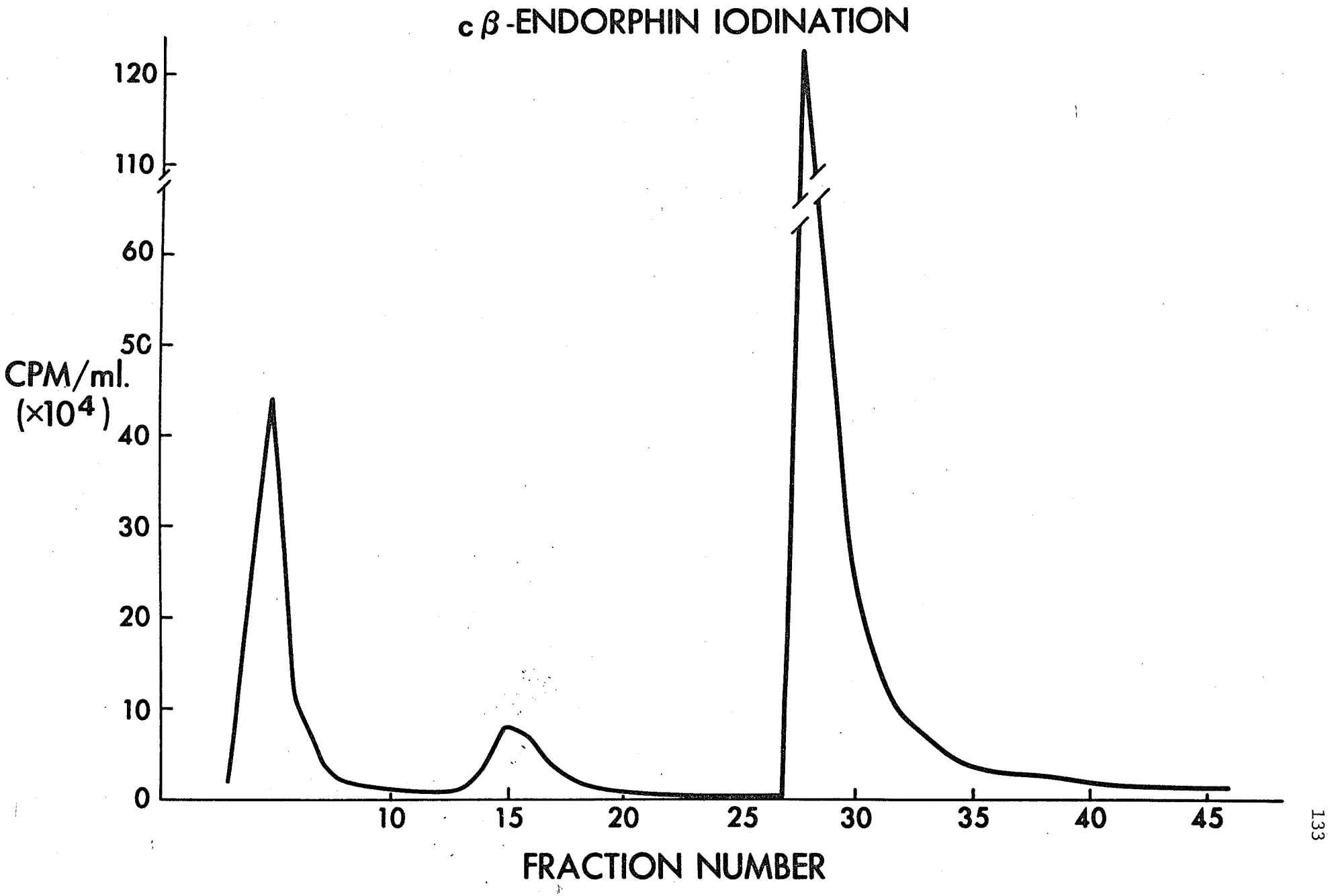

β-ENDORPHIN
(ng./ml.)
CPM
×10³
10
8
6
4
2
0
.1
.25
.5
1.0
1.5
2.5
5
10
25
50
100
500
(ng./ml.)
1:16
1:8
1:2
1:1
Dilutions

Figure 30

Profile of iodinated material eluted from CM23 Cellulose Chromatographic Column after application of mixture of ^{125}I and ^{125}I-β-endorphin. The first peak represents free iodine. The second (small) peak represents damaged tracer. The third peak represents immunoactive β-endorphin tracer.


cβ-ENDORPHIN IODINATION
120
110
60
50
40
30
20
10
0
CPM/ml.
(×10⁴)
10
15
20
25
30
35
40
45
FRACTION NUMBER

B) Radioimmunoassay (RIA) for Somatostatin

Antibodies to somatostatin (Peninsula Labs, San Carlos, California) were induced in rabbits. Tyr^{1}-Somatostatin was radio-iodinated using a method based on that of Epelbaum, et al.(1977).

To a test-tube containing 5 μg of Tyr^{1}-Somatostatin (SRIF) in 5 μL of 0.1 M.acetic acid were added:

1 - 25 μL of 0.4 M sodium acetate (pH 5.6)

2 - 25 μL of $Na^{125}I$ (1 mCi) - New England Nuclear

3 - 25 μL of lactoperoxidase (Calbiochem, La Jolla)

4 - 10 μL of 30 percent hydrogen peroxide at 1:15,000 dilution

5 - After 1.5 minutes another 10 μL of 30 percent hydrogen peroxide at 1:15,000 dilution

6 - 100 μL of sodium azide solution (200 μg/ml.) made up in .002 M ammonium acetate solution (pH 4.6)

7 - 50 μL of 5 percent BSA

8 - 1.0 ml of 0.002 M. ammonium acetate (pH 4.6)

The reaction products were then placed on a 20 cm. x 0.7 cm. chromatographic column containing CM23 ion exchanger material. Collection of 50 drop fractions was begun once the iodinated material had been placed on the column. For fraction tubes 8-20, each tube contained 3 drops of Trasylol (10,000 K.I.U./ml.) and 3 drops of 5 percent BSA. Fractions 1-8 were eluted with 0.002 M ammonium acetate (pH 4.6). Fractions 8-20 were eluted with 0.2 M ammonium acetate. The profile of radioactivity of the eluted fractions is shown in Fig. 31. The immunoactivity of each tube of the second peak of radioactivity was determined by taking 100 uL of a 20,000 30,000 c.p.m. dilution and determining the specific binding (Bo/T%) by RIA. The diluent for the reagents was sodium phosphate buffer containing 25 mM EDTA and 5 percent BSA (pH 7.4).

The RIA was performed in glass tubes (10 x 75 mm) containing:

a) 100 µL of 1:2 Trasylol solution

b) 100 µL of phosphate buffer

c) 100 µL of standard/ sample (Beckman - SRIF) Standards contained 0, 0.1, 0.25 0.5, 1.0, 1.5, 2.5, 5, 10, 25, 50, 100, and 500 ng/ml.

d) 100 µL of rabbit anti-SRIF serum at 1:10,000 dilution

e) 100 µL of tracer (29,000-30,000 c.p.m. in total counts tube)

f) 100 µL of normal rabbit serum at 1:300 dilution

g) 100 µL of sheep anti-rabbit serum at 1:20 dilution

The above contents were mixed and incubated at room temperature overnight. Following this, 500 µL of buffer was added to each tube. The tubes were centrifuged at 3000 RPM for 40 minutes at 40 degrees centigrade. The supernatant was then decanted and the pellet counted in a Beckman gamma counter (1 minute counts).

Similar to the β-endorphin RIA, when using an antibody titer of 1:10,000 in the double antibody test a satisfactory tracer will have more than 40 percent specific binding and less than 5 percent non-specific binding.

Characterization of the RIA for Somatostatin

1. Sensitivity: The sensitivity of the assay is 0.3-0.5 ng/ml. The standard curve is linear from 0.5 to 25-50 ng/ml. Assay sensitivity with half-maximal displacement of tracer is 3.5 ng/ml. (Fig. 32)
2. Specificity: No cross-reactivity has been noted with TRH, LHRH, enkephalin, γ-endorphin, β-endorphin, β-lipotropin, ACTH, glucagon, vasopressin, oxytocin, secretin, prolactin, growth hormone, luteinizing hormone, and follicle-stimulating hormone (Havlicek and Friesen, 1979).
3. Interassay Variation: ranges from 8.1 to 9.6 percent, while intra-assay variation is from 2.3 to 3.8 percent (Table 5).

Table 5 Interassay and Intraassay Variation of Radioimmunoassay for Somatostatin*

	Concentration of Somatostatin (ng./ml.)					
	Same Assay			Different Assay		
	a)	b)	c)	A)	B)	C)
$\bar{x}_s$[1]	15.8%	18.2 %	36.4 %	3.4	0.78	1.9
n	9.0	10.0	9.0	3.6	0.68	1.85
SD[2]	0.6	0.55%	.84%	4.1	0.64	2.0
CV[3]	3.8%	3.0 %	2.3 %	4.0	0.78	1.85
				3.8	0.84	1.7
				3.4		
			$\bar{x}_D$[4] =	3.7	.74	1.86
			SD =	.3	.079	.097
			CV =	8.1%	10.7	9.6%

1 $\bar{x}_s$ = mean % binding of three different samples (a, b, c) measured in the same assay.

2 SD = standard deviation

3 CV = Coefficient of variation ($SD/\bar{x}$)

4 $\bar{x}_D$ = Mean value (ng./ml.) of three different samples (A, B, C) measured in different assays.

*Data kindly supplied by Dr. N. Kato.

Figure 31

Profile of iodinated material eluted from CM23 Cellulose Chromatographic Column after application of mixture of ^{125}I and ^{125}I-tyr-somatostatin. The first peak represents free iodine. The second peak represents immunoactive somatostatin tracer.

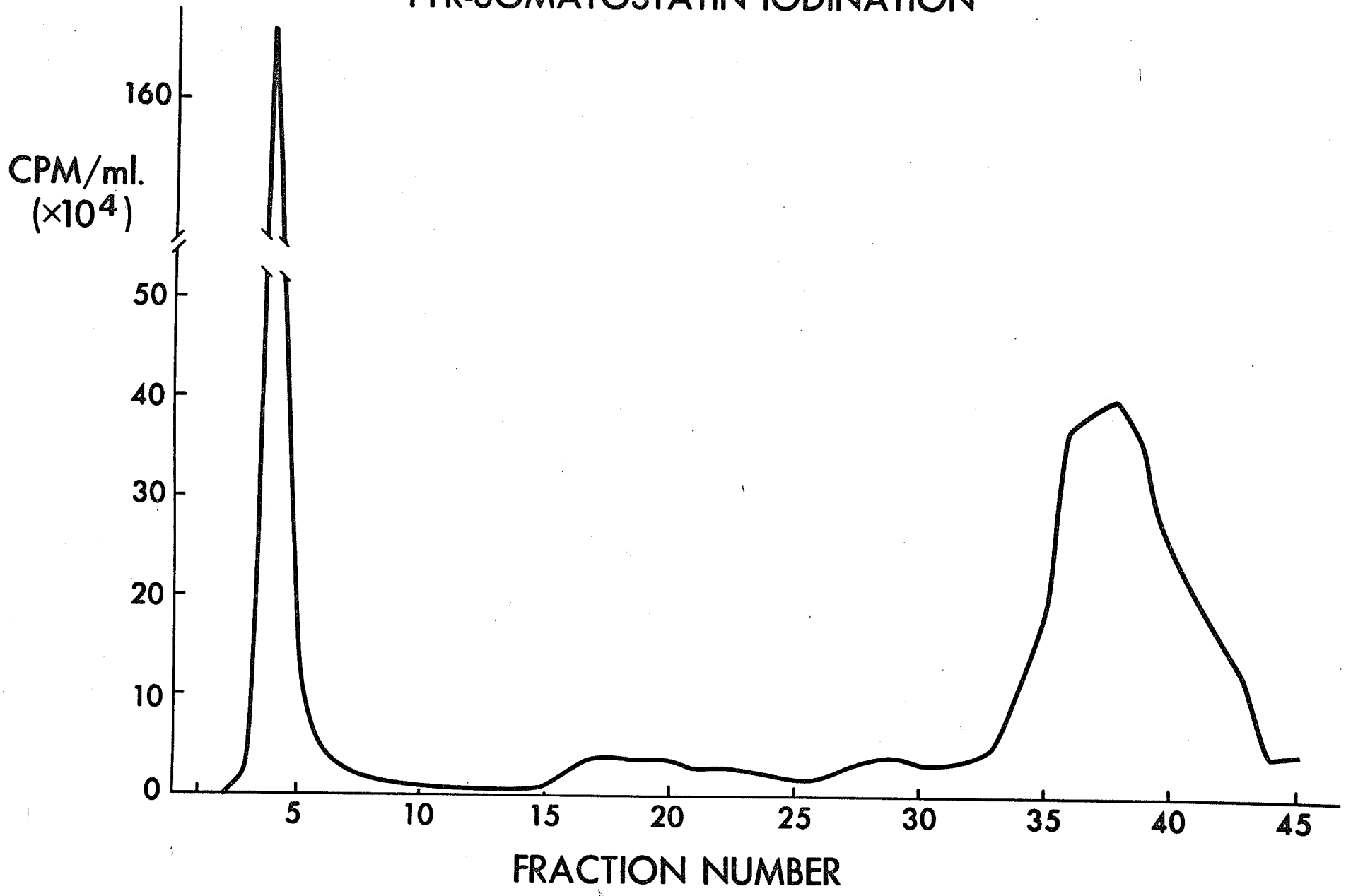
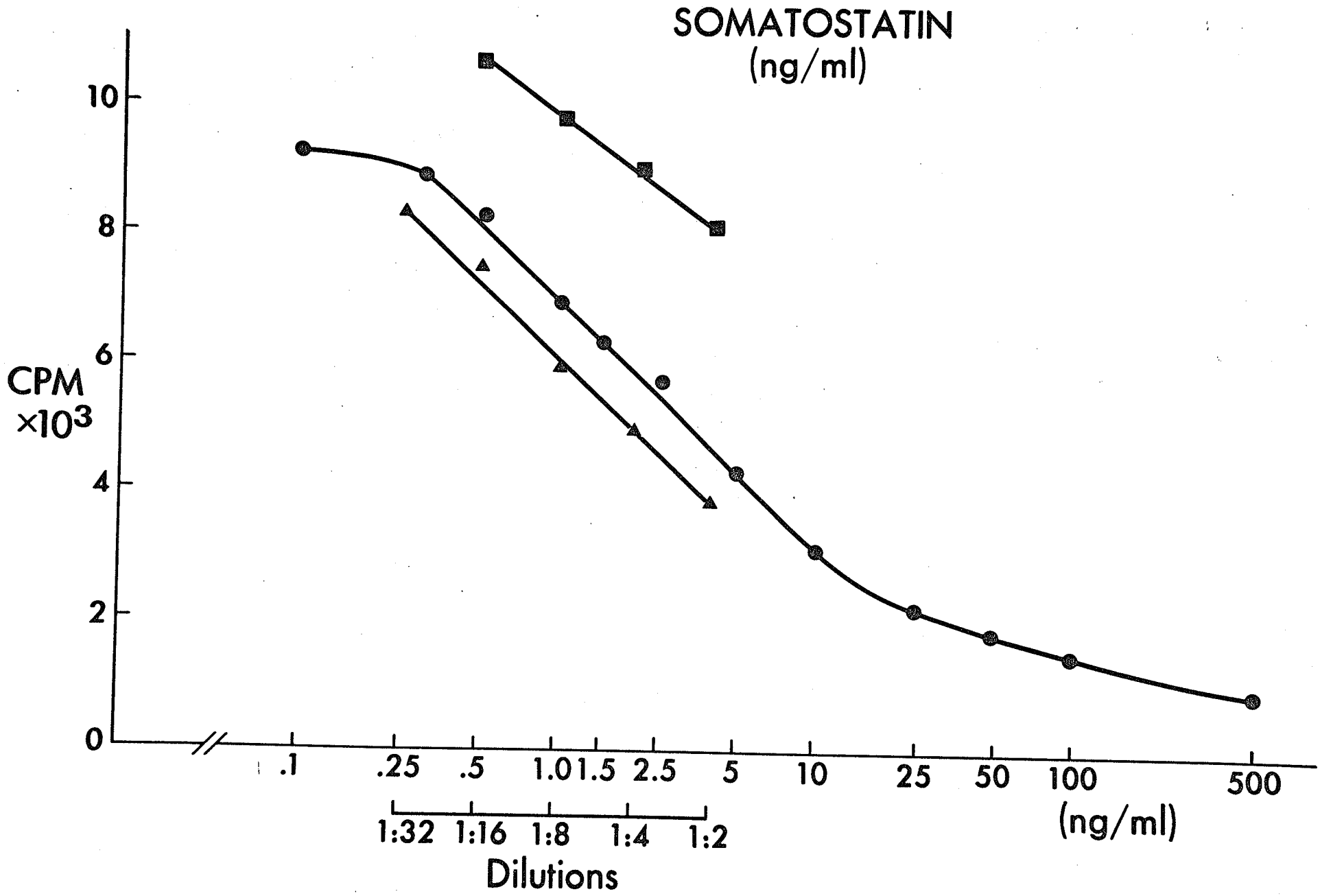

TYR-SOMATOSTATIN IODINATION
CPM/ml.
(×10⁴)
160
50
40
30
20
10
0
5
10
15
20
25
30
35
40
45
FRACTION NUMBER

Figure 32

Standard curve for Somatostatin RIA. Extracts of rat brain produced curves parallel to the standard curve.

Triangles = Hypothalamic extracts

Squares = Cortical extracts


SOMATOSTATIN
(ng/ml)
CPM
×10³
10
8
6
4
2
0
.1
.25
.5
1.0
1.5
2.5
5
10
25
50
100
500
(ng/ml)
1:32
1:16
1:8
1:4
1:2
Dilutions

RESULTS AND DISCUSSION

In the group of animals sacrificed within 2.5 to 3 seconds of concussion there was a significant change in immunoreactive (i.r.) β-endorphin content in the hypothalamus alone, where a decrease to 46 percent of control values occurred ($p < .02$; Student's t test) (Fig. 33A). The content remained lower than normal until 30-45 minutes following concussion, when there was no significant difference from controls. The other area of interest was the hippocampus where, at five minutes following concussion, there was a significant decrease in i.r. β-endorphin content ($p < .02$; Student's t test) (Fig. 33B). Other brain regions demonstrated no significant changes (Fig. 33 C-J).

The observed decrease in β-endorphin content most likely reflects the release of β-endorphin from hypothalamic neurons at the time of concussion. In hypothalamic regions, application of β-endorphin by microiontophoresis or ICV injection depresses neuronal firing (Bloom, et al. (1978). Within five minutes of concussion, neuronal release of β-endorphin in the hippocampus also appears to be increased. The ICV injection or microiontophoresis of β-endorphin into the hippocampus is the only instance in which β-endorphin produces neuronal excitation. In fact, Henriksen, et al. (1977) have demonstrated that at low doses, β-endorphin produces a naloxone-reversible excitatory response which is accompanied by EEG signs of electroconvulsive activity (After 1 to 5 mg. of β-endorphin, these convulsive actions persisted for 3 or more hours).

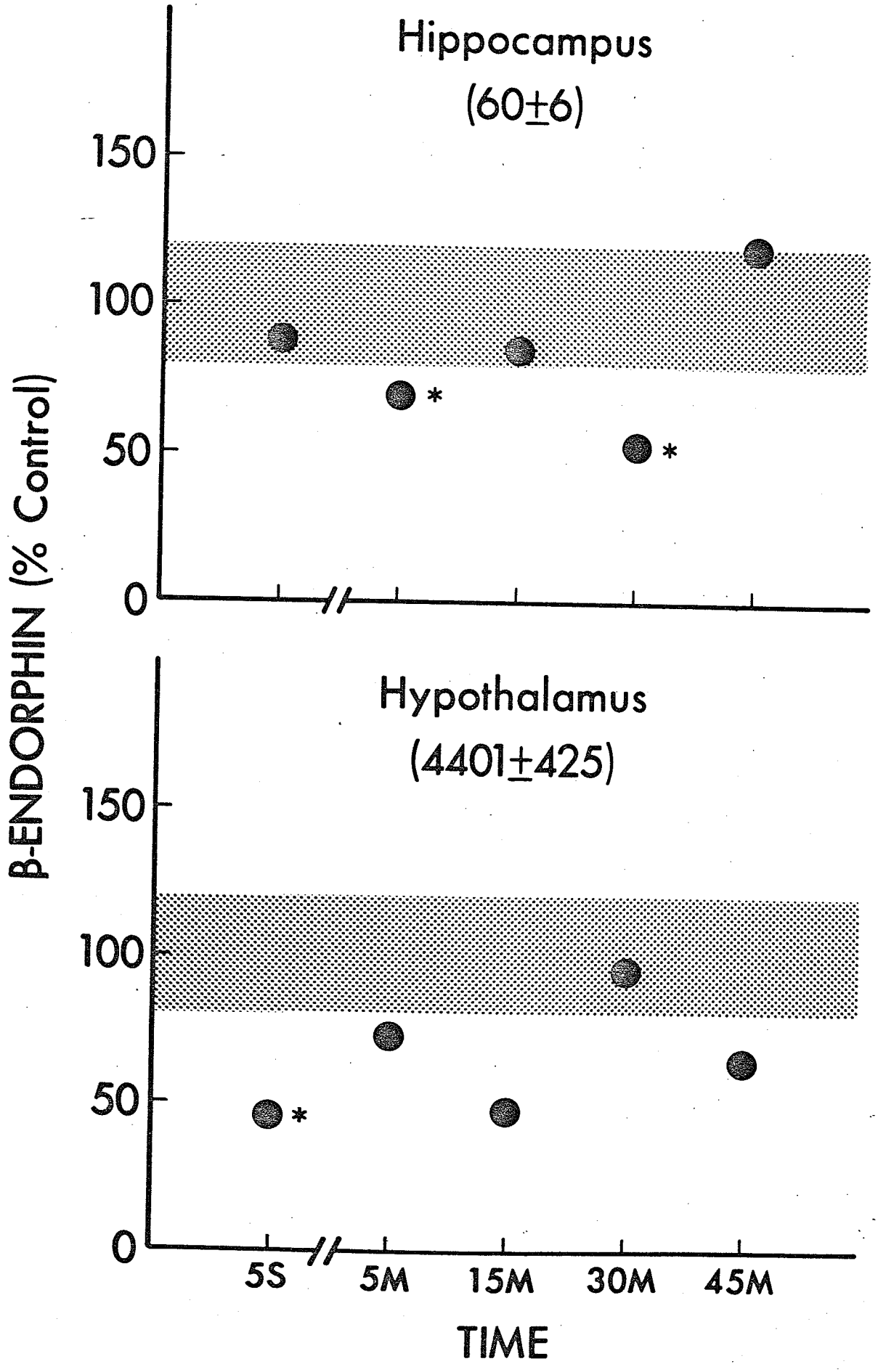
Recalling the previously observed fact that, immediately following concussion the binding of endogenous opiates appears to be decreased in the hypothalamus only, it is tempting to speculate that, although presynaptic stores of β-endorphin are released from hypothalamic neurons by concussion, mechanical deformation of receptor sites results in diminished affinity for the ligand. Within seconds, the receptor sites recover their binding ability and at this time, the increased

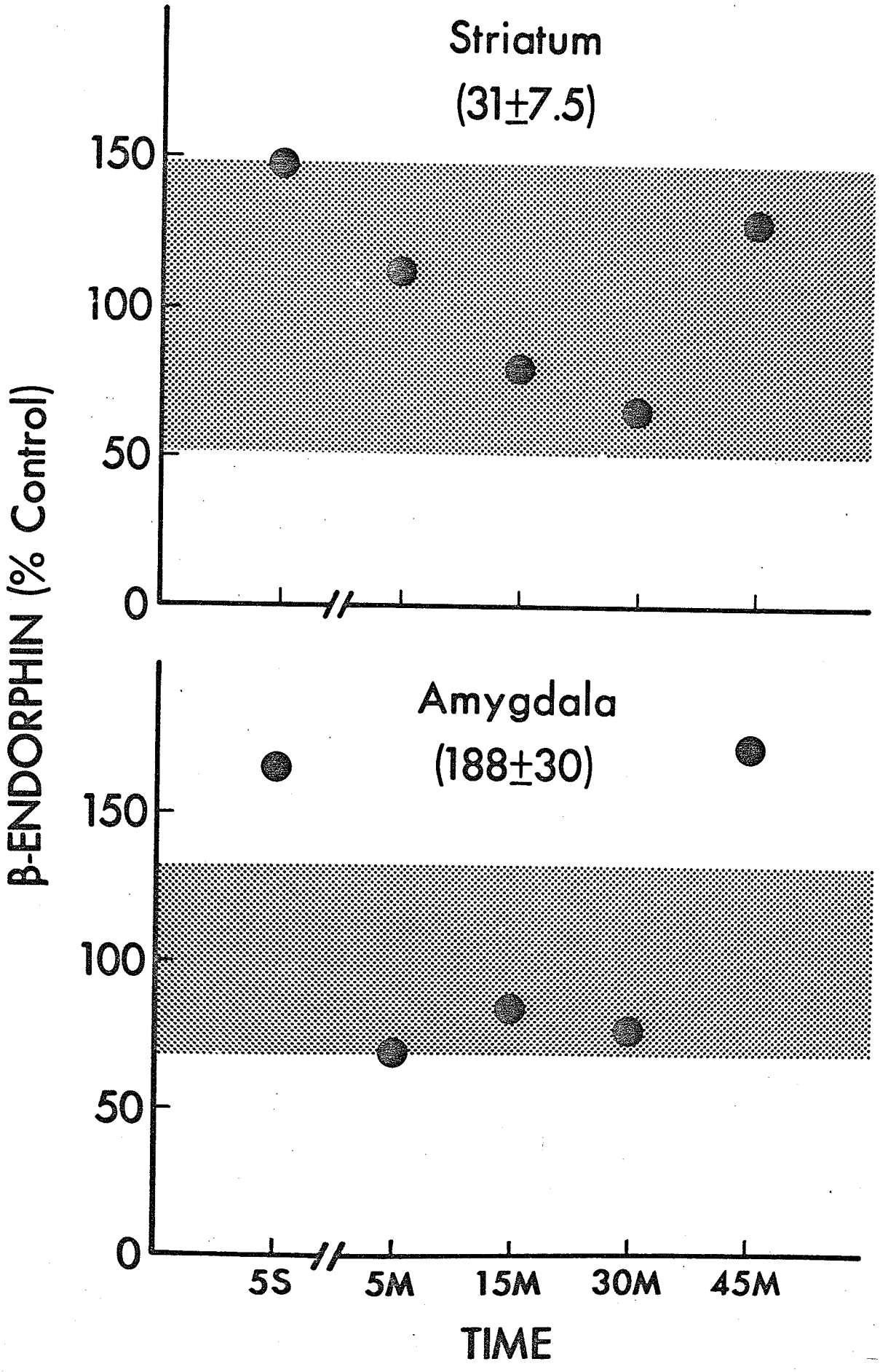
Figure 33 I.r. B-Endorphin Content of Brain Regions Following Concussion

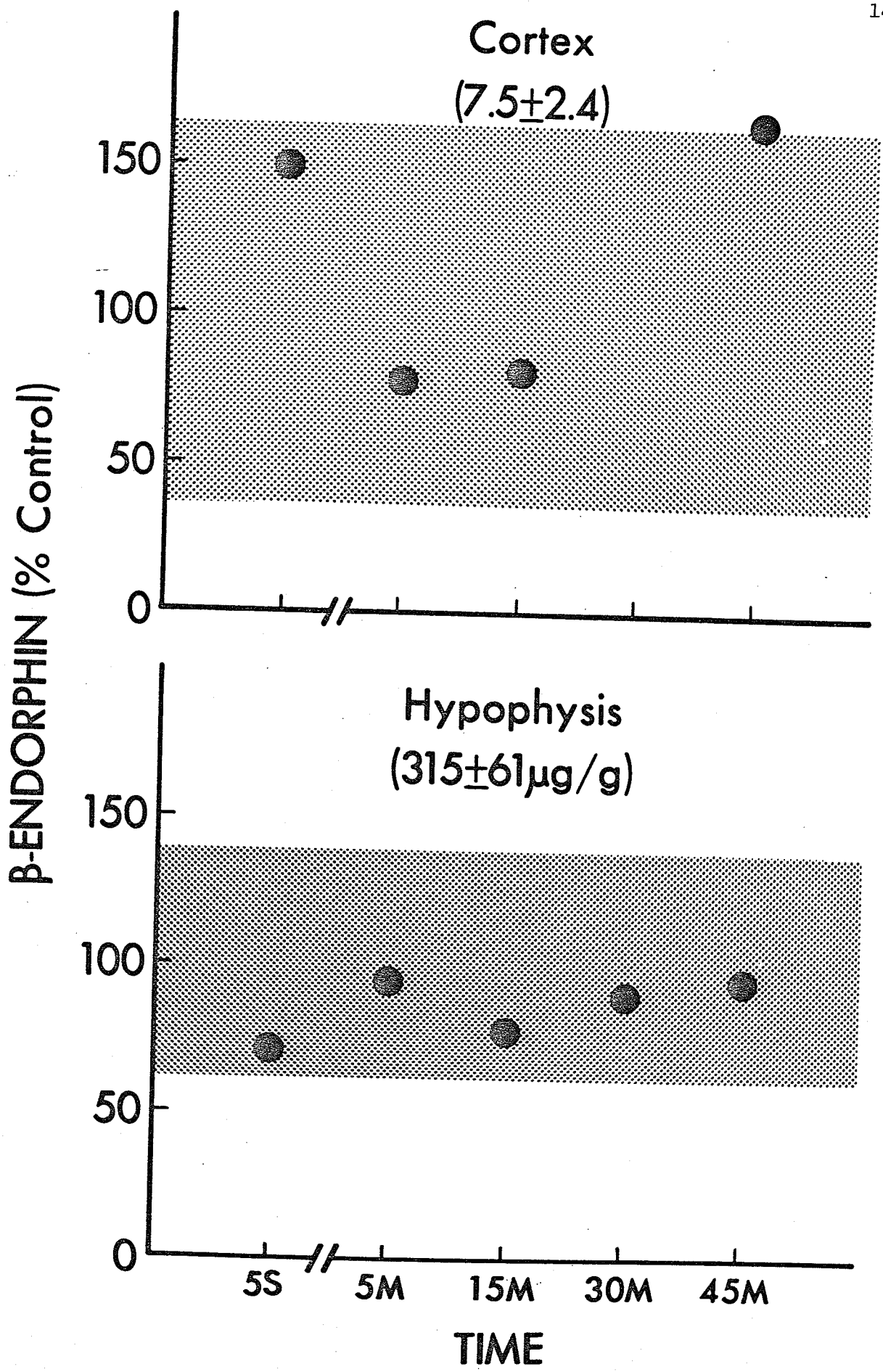
For each brain region, the numbers in brackets indicate the grand mean of control values ± the S.E.M. Units are ng./gm. wet weight. The shaded area on each graph indicates the range of values from two S.E.M.'s below the mean to two S.E.M.;s above the mean. Solid circles indicate the means of six to ten concussed rats at each time interval.

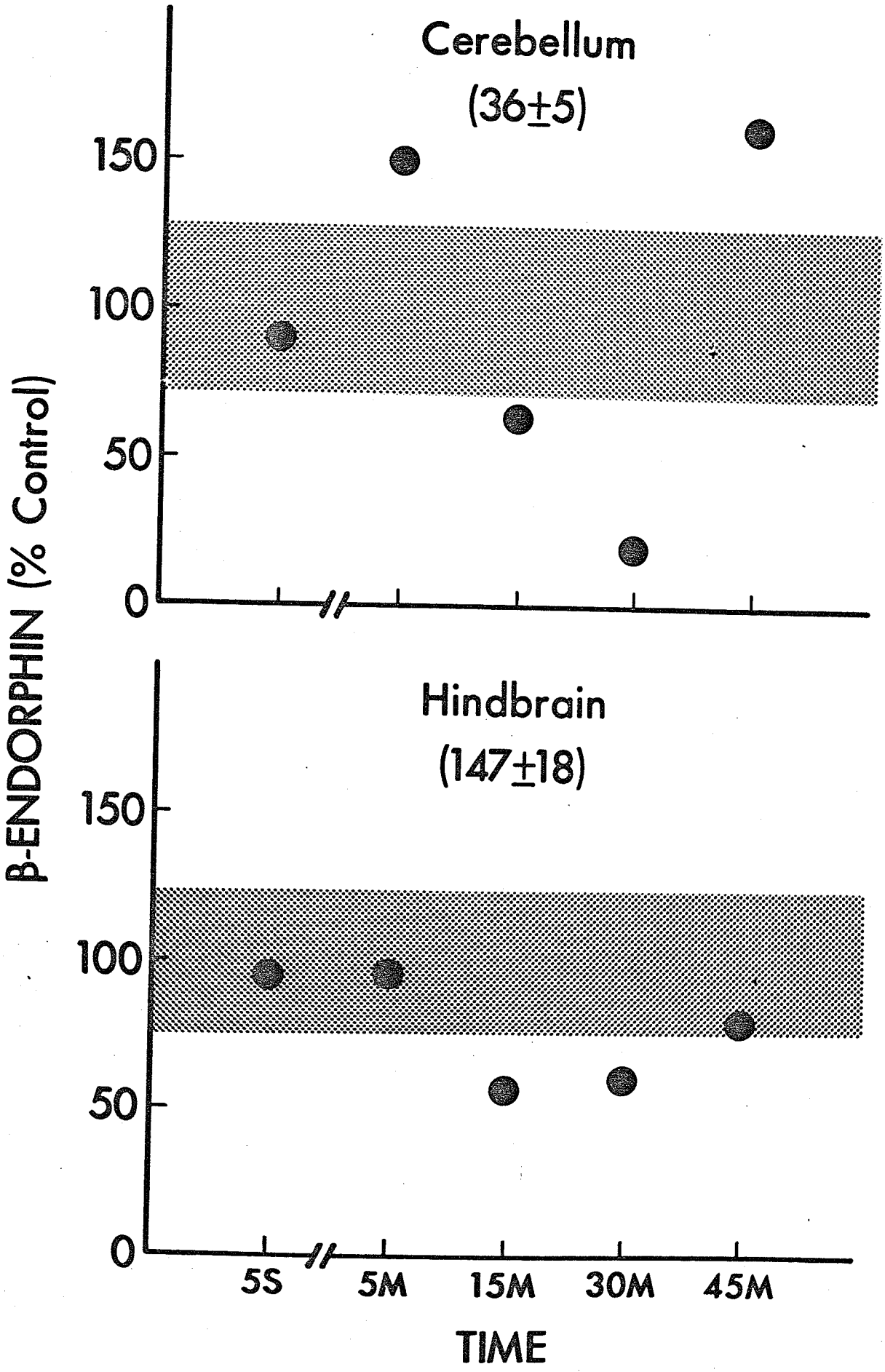
*$p < .02$ (Student's t test)

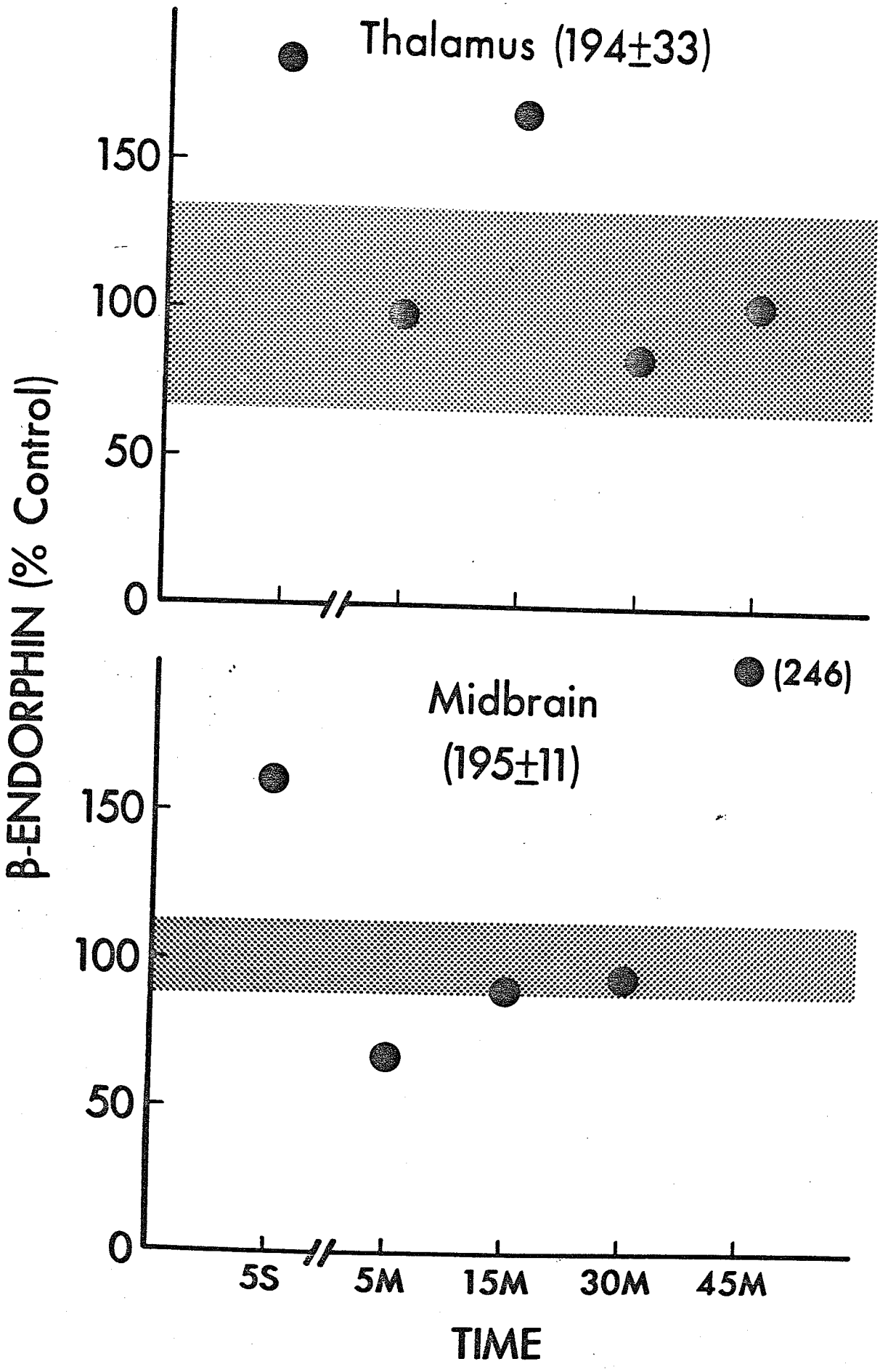
There are significant differences between control and concussed rats only in the hypothalamus and hippocampus.


Hippocampus
(60±6)
150
100
50
0
*
*
Hypothalamus
(4401±425)
150
100
50
0
*
5S
5M
15M
30M
45M
TIME
β-ENDORPHIN (% Control)


Striatum
(31±7.5)
150
100
50
0
Amygdala
(188±30)
150
100
50
0
β-ENDORPHIN (% Control)
5S
5M
15M
30M
45M
TIME


Cortex
(7.5±2.4)
150
100
50
0
Hypophysis
(315±61µg/g)
150
100
50
0
β-ENDORPHIN (% Control)
5S
5M
15M
30M
45M
TIME


Cerebellum
(36±5)
150
100
50
0
Hindbrain
(147±18)
150
100
50
0
β-ENDORPHIN (% Control)
5S
5M
15M
30M
45M
TIME


Thalamus (194±33)
150
100
50
0
(246)
Midbrain
(195±11)
150
100
50
0
5S
5M
15M
30M
45M
TIME
β-ENDORPHIN (% Control)

amount of β-endorphin released produces depression of neuronal transmission in the hypothalamus.

An obvious question is whether or not the findings of decreased hypothalamic β-endorphin content may reflect merely a non-specific response to stress. This seems unlikely in view of the following evidence. Experimental controls were manually immobilized for the same time period required to produce concussion. Such immobilization has been shown to be an effective stimulus to ACTH (and consequently β-endorphin) secretion in rats (deSouza and Van Loon, 1979), and it has been observed in this laboratory that severe immobilization stress results in an increase in the brain content of β-endorphin (Havlicek, unpublished results). In the studies of Rossier, et al. (1977), severe stress (inescapable footshocks at 12/min.) was repeated for periods of 30 minutes or one hour before changes in hypothalamic B-endorphin were observed (i.e. a 19 percent decrease in one experiment and a 26 percent decrease in a second experiment). The dramatic changes recorded here (a 54 percent decrease in B-endorphin within 5 seconds of concussion) are not compatible with either of the above observations.

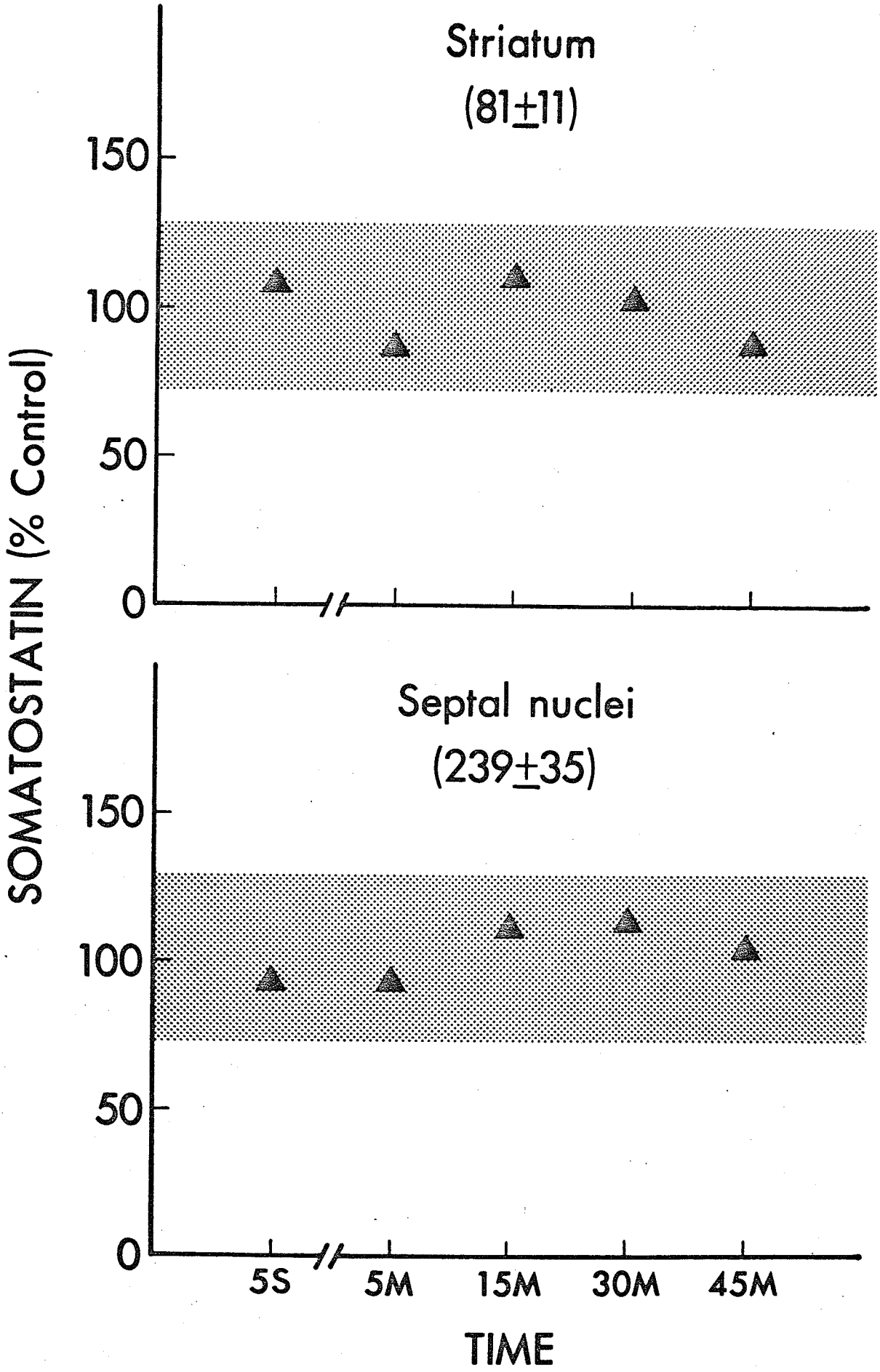
The changes involving the hippocampus are also of interest. The increased release of β-endorphin in this region following concussion may be related to the production of seizures by a concussing blow (half the animals in each group manifested epileptiform activity as one of the physical signs of concussion). This finding certainly warrants further investigation.

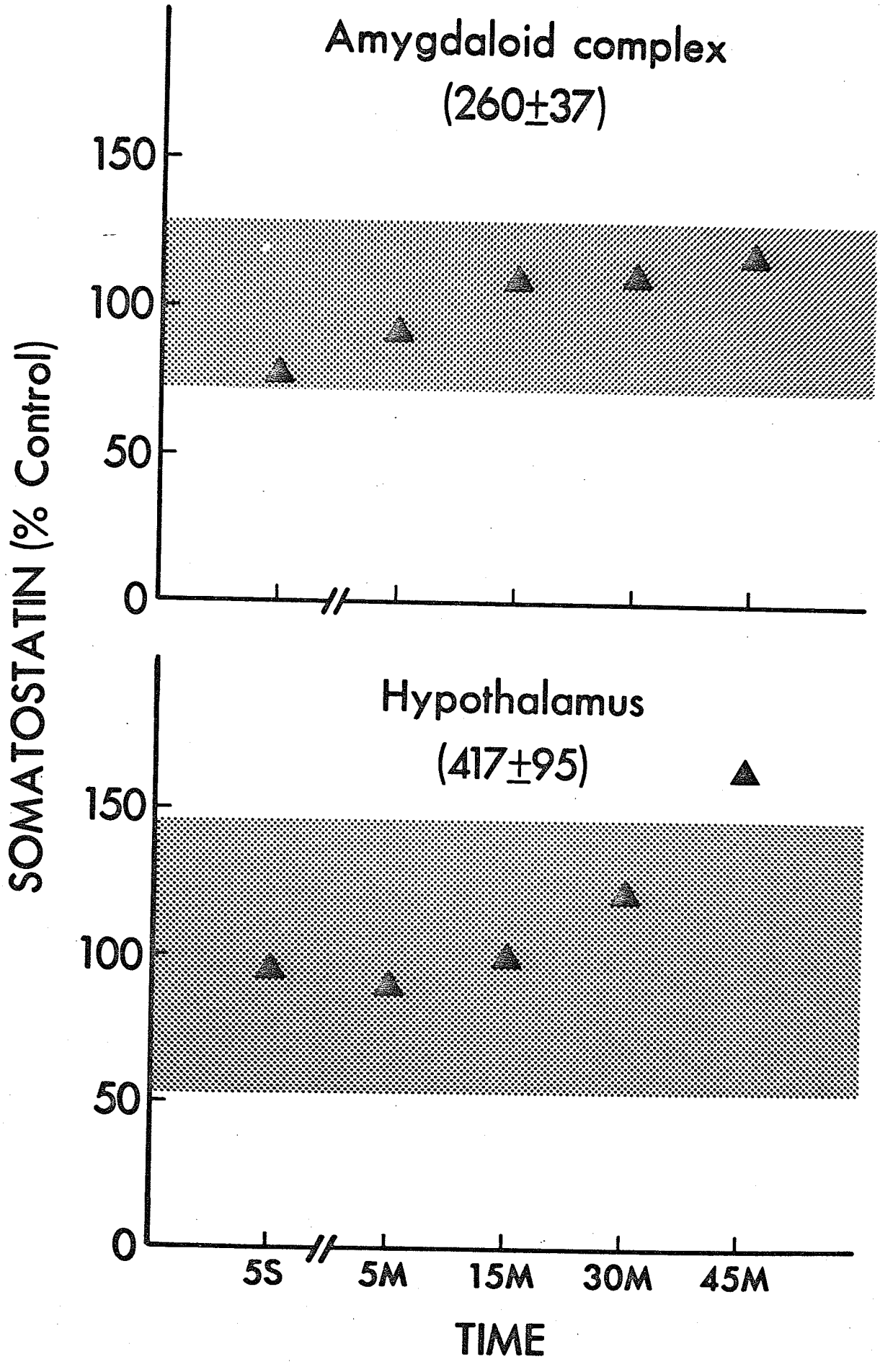
Regarding the putative excitatory neurotransmitter somatostatin, there were no significant changes in content in any of the brain regions studied, at any time period following concussion. Virtually all concentrations of peptide fell within two standard errors of the mean value for each brain region studied (Fig. 34 A-K). If stress were a factor in producing the changes observed in β-endorphin, one would expect to see stress-induced changes in somatostatin also.

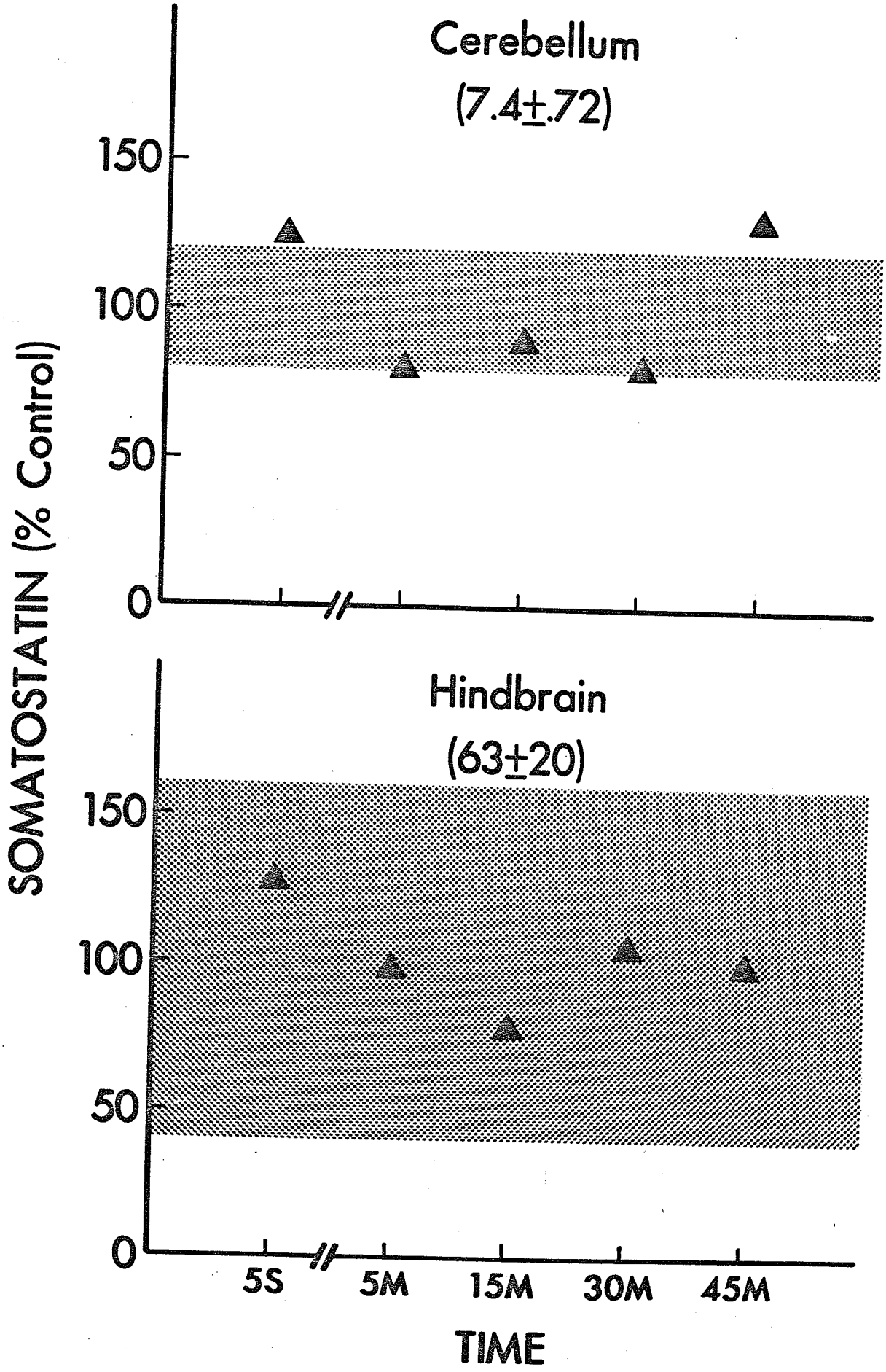
Figure 34 I.r. Somatostatin Content of Brain Regions Following Concussion

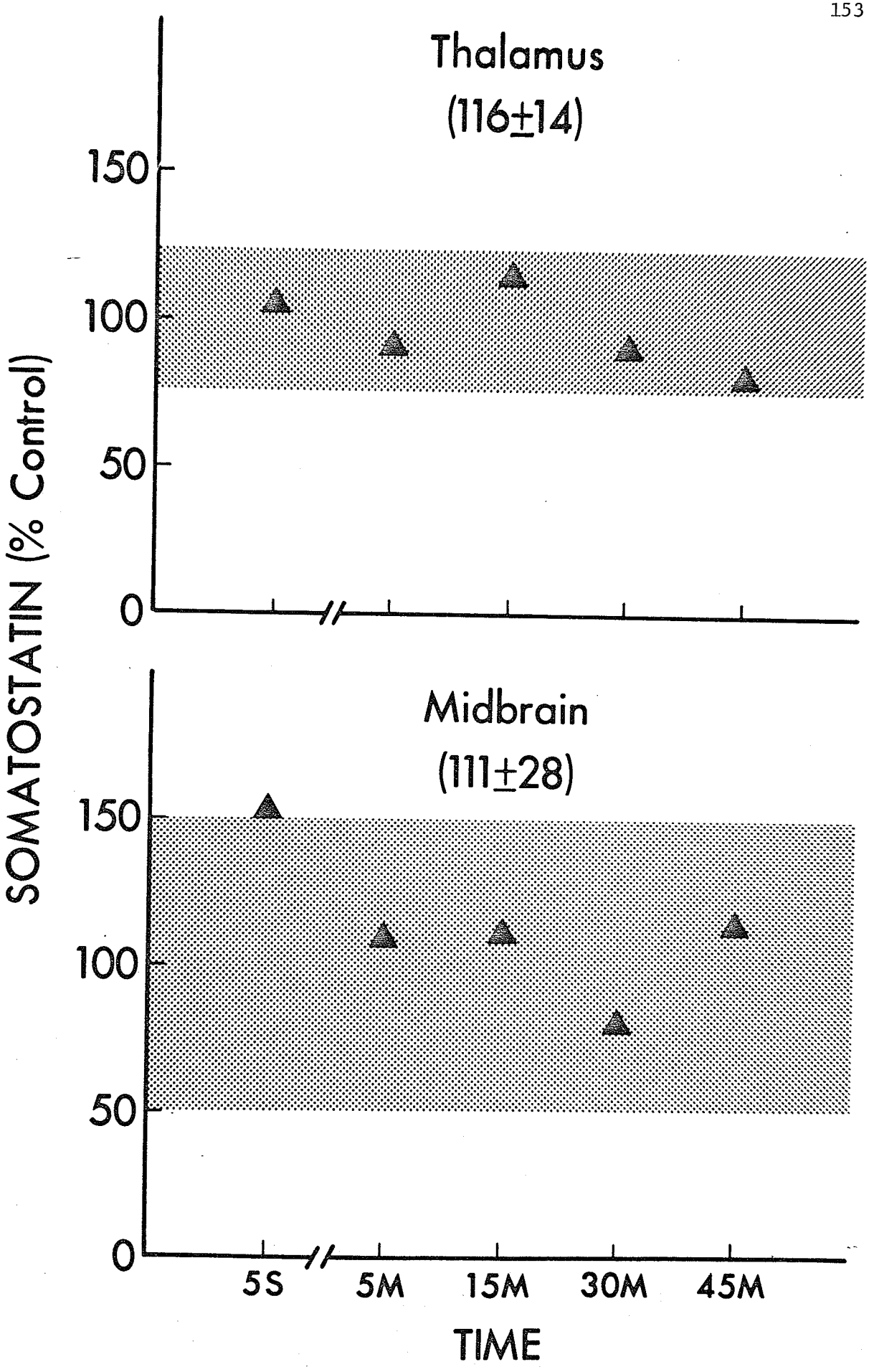
For each brain region, the numbers in brackets indicate the grand mean of control values ± the S.E.M. Units are in ng./gm. wet weight. The shaded area on each graph indicates the range of values from two S.E.M.'s below the mean to two S.E.M.'s above the mean. Triangles indicate the means of six to ten rats concussed at each time interval.

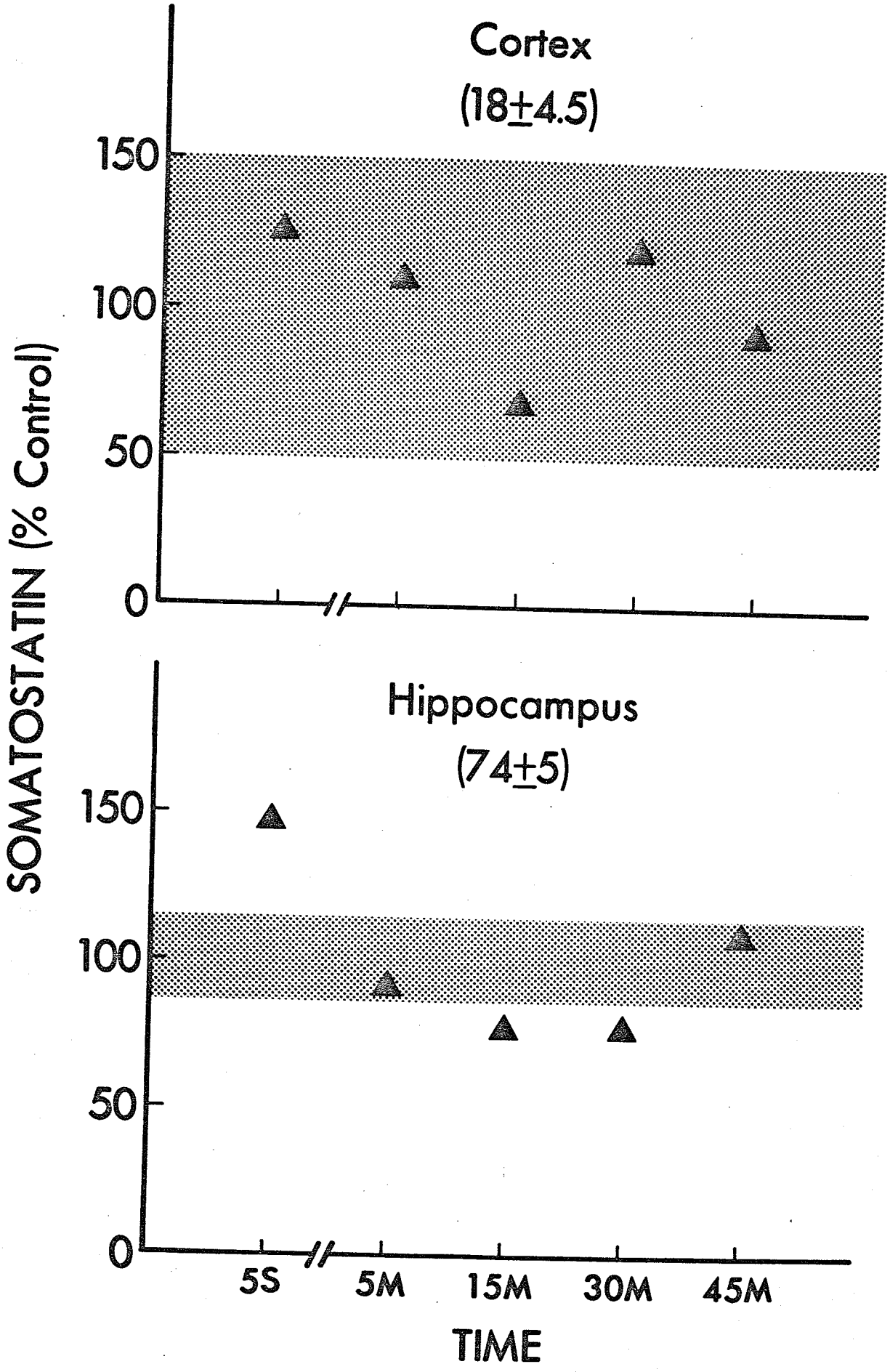
There are no significant differences between control and concussed rats in any brain region.


Striatum
(81±11)
150
100
50
0
Septal nuclei
(239±35)
150
100
50
0
SOMATOSTATIN (% Control)
5S
5M
15M
30M
45M
TIME


Amygdaloid complex
(260±37)
150
100
50
0
Hypothalamus
(417±95)
150
100
50
0
SOMATOSTATIN (% Control)
5S
5M
15M
30M
45M
TIME


Cerebellum
(7.4±.72)
150
100
50
0
Hindbrain
(63±20)
150
100
50
0
SOMATOSTATIN (% Control)
5S
5M
15M
30M
45M
TIME


Thalamus
(116±14)
150
100
50
0
Midbrain
(111±28)
150
100
50
0
SOMATOSTATIN (% Control)
5S
5M
15M
30M
45M
TIME


Cortex
(18±4.5)
150
100
50
0
Hippocampus
(74±5)
150
100
50
0
SOMATOSTATIN (% Control)
5S
5M
15M
30M
45M
TIME

SUMMARY AND CONCLUSIONS

In the experimental investigation of concussion, the use of conscious animals is mandatory in order to be able to correlate the parameters studied with the state of consciousness. Concussion acts as an anesthetic by virtue of the fact that it renders the animal instantaneously unconscious. Concussed animals display no fear or rage on repeated concussion.

Concussion of conscious rats results in a transient impairment of memory, and performance in a previously learned maze. This defect persists for periods longer than the immobility and incoordination also produced by the concussing blow. The behavioral changes following concussion (i.e. impaired maze performance, absence of fear of concussing device) suggest a temporary state equivalent to the post-concussion amnesia observed in the human situation.

Immediately following concussion, there is evidence of decreased binding of the endogenous opiate and cholinergic ligands to their receptors in the hypothalamus. This change appears to be specific in that it is not duplicated by non-concussing trauma, does not affect the other receptors studied (spiroperidol and Ouabain receptors) and occurs only in the hypothalamus. These changes are postulated to be due to mechanical deformation of the receptor sites by the pressure gradients produced at the time of concussion. In support of this hypothesis is the fact that increased hydrostatic pressure has been shown to reverse the effects of narcotic drugs in vivo (presumably by impairing binding) and is well-known to influence the effects of anesthetics on cell membranes (see Tower, 1966 and Johnson and Flagler, 1950; quoted in Tower's paper).

Mathematical analysis of the changes in brain tissue at the time of impact suggest that shear strains produced by this form of trauma are maximal at the cortical surface (see Joseph and Crisp, 1971). However, the presence of significant

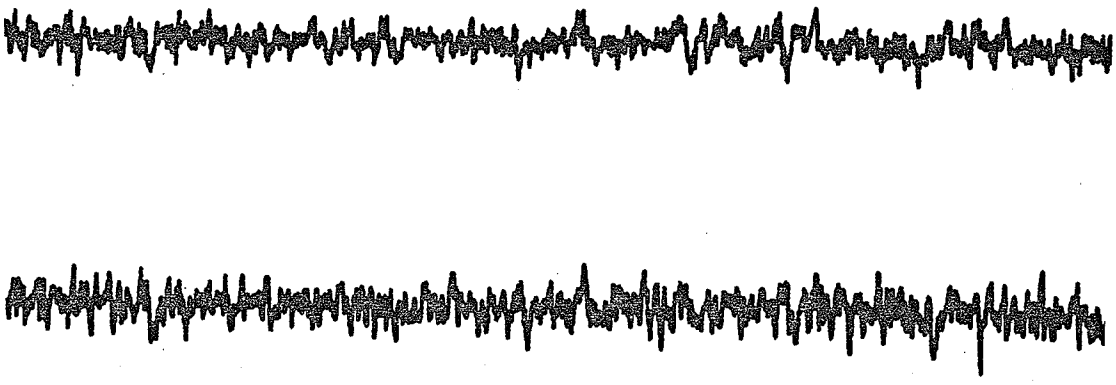
shear strains in the region of the medulla, midbrain, and hypothalamus, (i.e. the region of the craniospinal junction) has been repeatedly discussed (Friede, 1961; Ommaya and Gennarelli, 1974; Gurdjian, 1975). In fact, Ommaya and Gennarelli have postulated that traumatic unconsciousness (concussion) would not be produced until the magnitude of shear strains is large enough to reach the "well-protected mesencephalic part of the brain stem, thus disconnecting the alerting system". The alteration in receptor binding observed here may be the physiological equivalent of the "disconnection" referred to by Ommaya and Gennarelli.

Concussion of conscious rats produces generalized depression of electroencephalographic activity. The depression is most apparent in the faster (alpha and beta) frequencies and is more pronounced with more severe concussion. The power spectrum of the delta frequencies is slightly increased with milder stages of concussion. Rapid recovery of the EEG occurs, incompatible with hemorrhage, edema or structural brain damage. The changes in the EEG post-concussion have been noted to resemble those observed during the phenomenon of spreading depression (Ward, 1966), but the similarity has not been tested further. Modifying the method described by Vyskocil, et al (1972), we implanted cannulas in the right frontal region of Sprague-Dawley rats (males 150-175 gms.) also implanted with bilateral chronic epidural EEG electrodes. The cannulas were placed stereotaxically so that when an injection cannula was inserted into the implanted cannula, it would rest at a depth of 0.5 mm. to 1 mm. below the cortical surface. The animals were allowed to recover from anesthesia before experiments were performed. Employing this model 1.5 to 2.5 µl. of a 10 percent KCl solution were injected via the cannula in seven conscious mobile rats. In each rat, the response was a dramatic depression of EEG activity, visible only over the injected hemisphere, beginning within 60 seconds of the injection (fig. 35, 36) and accompanied by a dense contralateral hemiplegia). The EEG recovered usually within one-half to one hour, always within two to three hours.

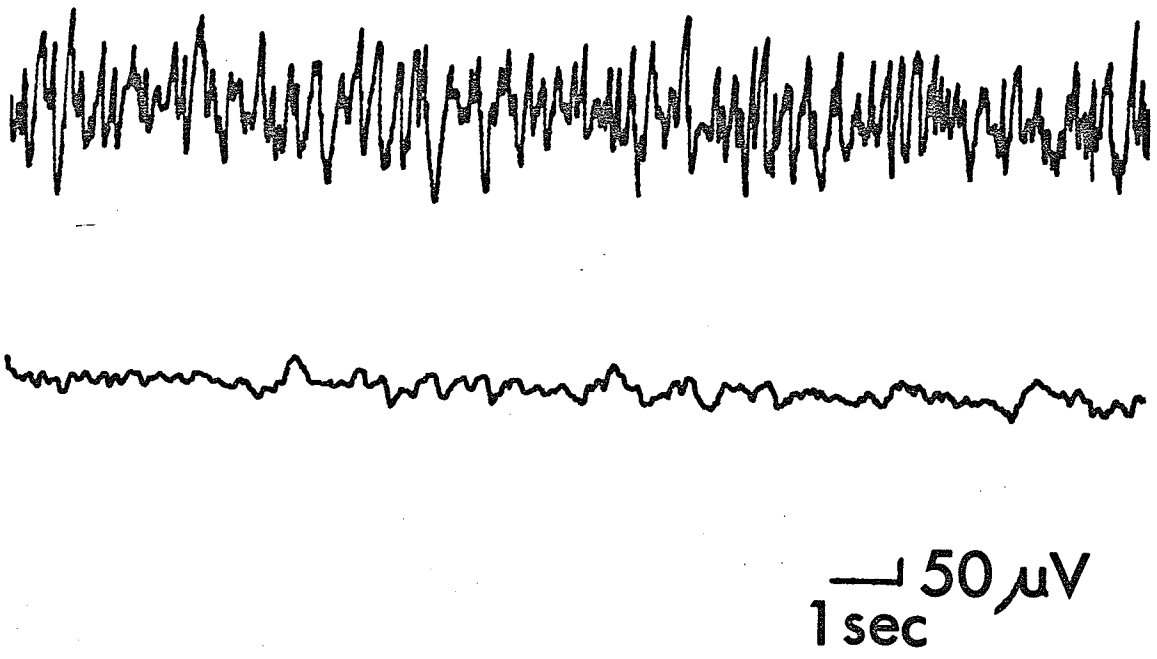
Figure 35 EEG During Spreading Depression

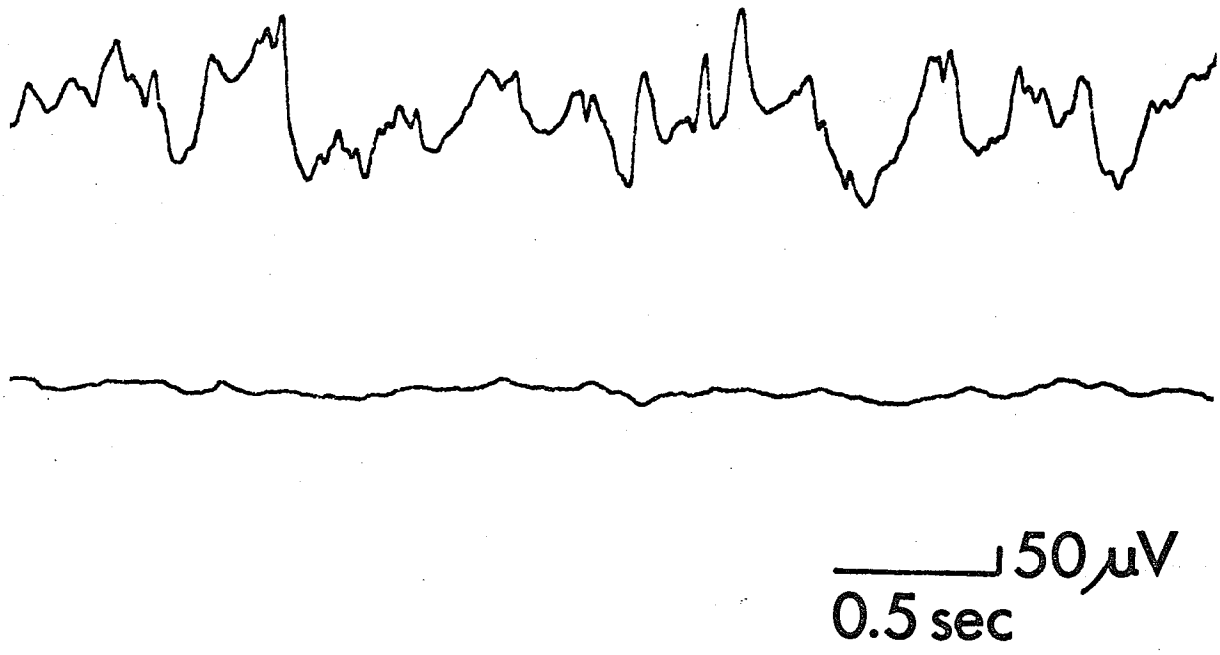
A) Control recording. The lower channel represents right hemisphere electrodes; the upper channel, the left hemisphere electrodes.

B) Following injection of 1.5 μL of 10 percent KCl into the right frontal subarachnoid space, the EEG activity of the right hemisphere demonstrates marked, reversible depression.
(Calibration in right lower corner).



35 a


50 µV
1 sec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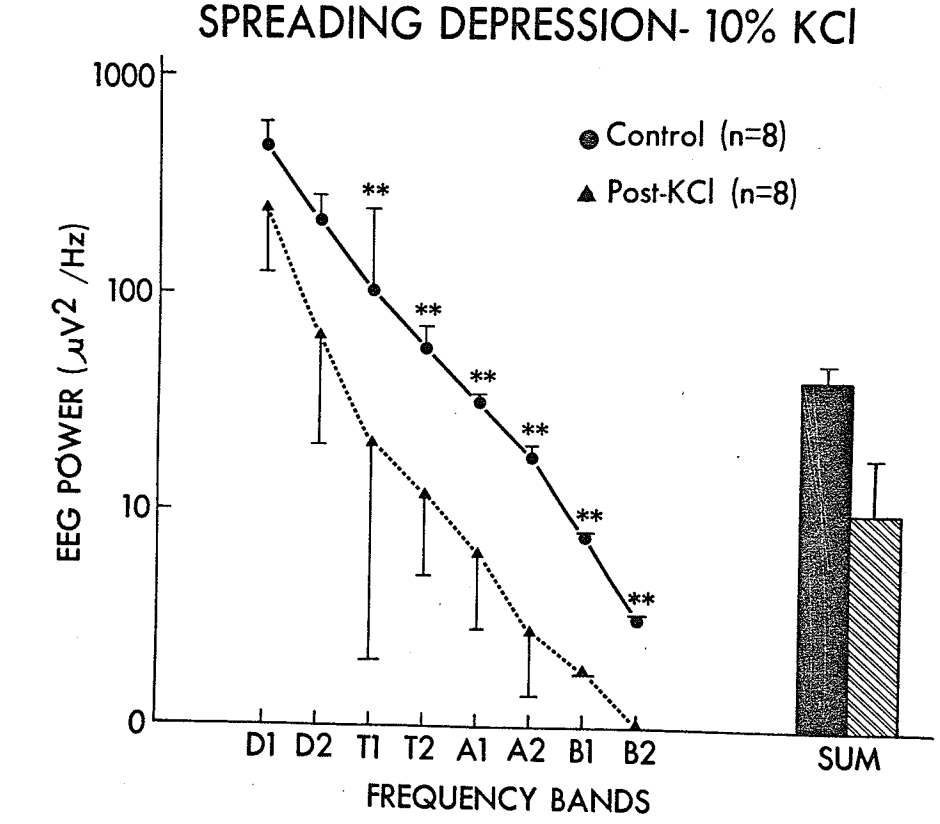
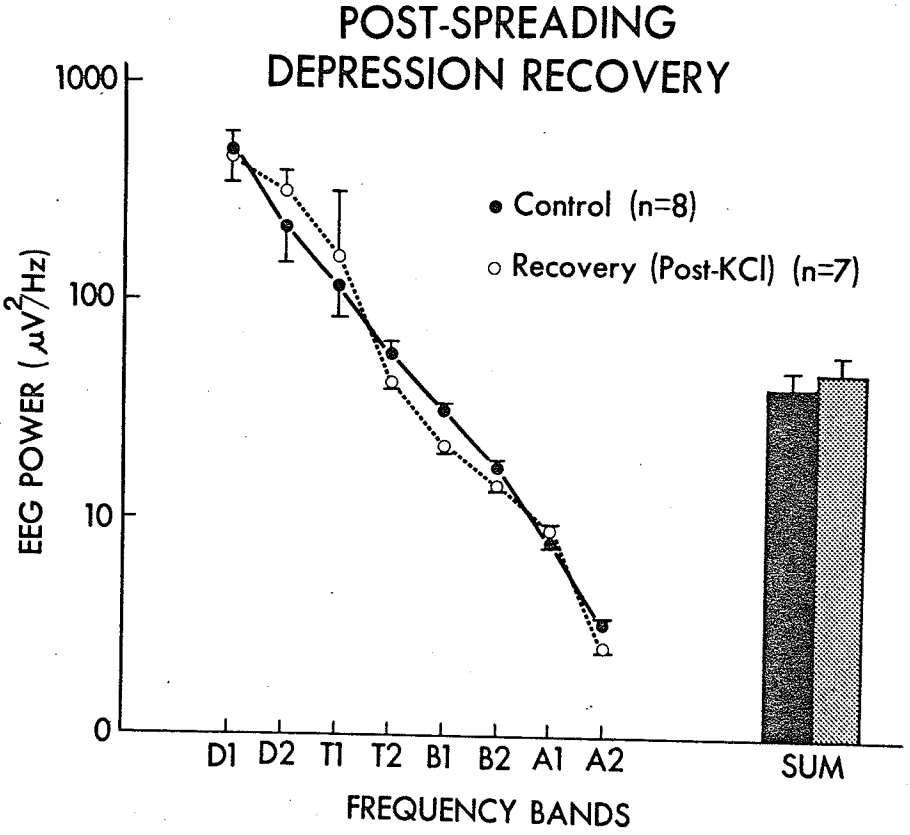
0.5 sec

Figure 36 Spectral Analysis of the EEG During Spreading Depression

The power spectrum demonstrates generalized depression, which recovers in less than two hours following the administration of 10 percent KCl solution supracortically.


SPREADING DEPRESSION- 10% KCl
1000
100
10
0
EEG POWER (μV2 /Hz)
Control (n=8)
Post-KCl (n=8)
**
**
**
**
**
**
D1 D2 T1 T2 A1 A2 B1 B2
SUM
FREQUENCY BANDS

a


POST-SPREADING
DEPRESSION RECOVERY
1000
100
10
0
EEG POWER (μV2/Hz)
Control (n=8)
Recovery (Post-KCl) (n=7)
D1 D2 T1 T2 B1 B2 A1 A2
SUM
FREQUENCY BANDS

b

Controls injected with a 10 percent NaCl solution demonstrated no change in the EEG.

Hence the quality and time course of the EEG changes was observed to be very similar following either concussion or potassium-induced spreading depression. Our EEG findings, especially when compared with the phenomenon of spreading depression, substantiate the fact that the phenomenon of concussion is characterized by significant cortical dysfunction in addition to previously emphasized dysfunction of the reticular activating system. Perhaps the cortical dysfunction is more important than previously recognized. For instance, if dysfunction of the reticular activating system were the overwhelming contribution to the clinical picture, one would expect to see the typical EEG hypersynchrony produced by a lesion in that area (Fig. 37).

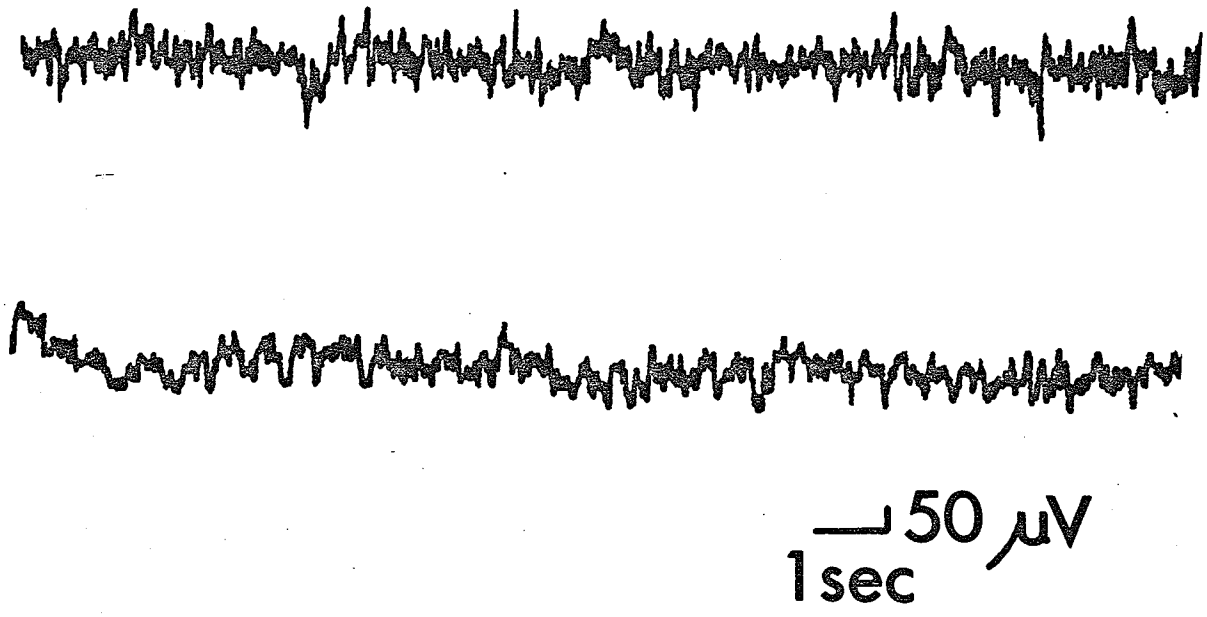
Although eventually reversible, the depression of the EEG power spectrum persists after the concussed rat has regained normal posture, gait and attitude. This may be the experimental correlate of post-concussion amnesia in the human; following concussion the person regains normal appearance and mobility, but because of continuing higher cortical dysfunction, is unable to store recent experiences in the form of memories (see, for example Yarnell and Lynch, 1970, 1973).

Immediately following concussion, there is a significant decrease in the hypothalamic content of the potent inhibitory neuropeptide β-endorphin, suggesting a sudden release of this peptide from hypothalamic neurons at the time of concussion. The levels of the peptide return to values within normal limits at 30 to 45 minutes post-concussion. This may be interpreted as being a protective reflex secretion, similar to that observed following stress (Rossier, et al. 1977). The secretion of this peptide at the time of concussion may contribute to the observed depression of neural function (in particular consciousness) or may be merely an epiphenomenon of concussion.

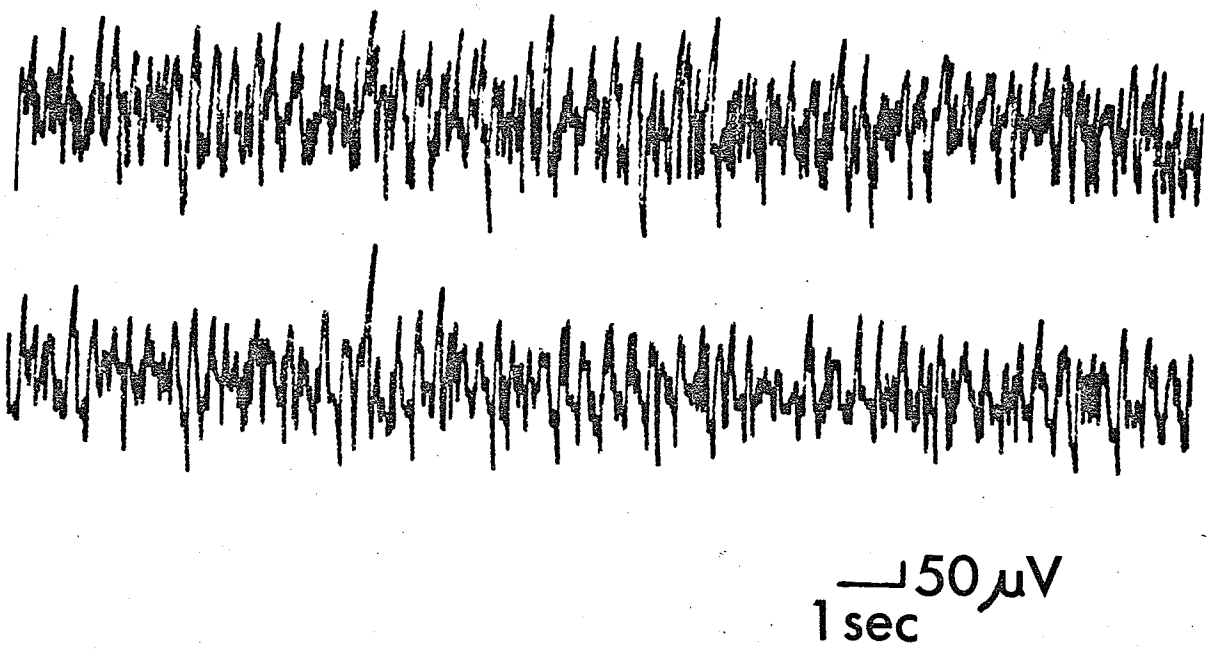
Figure 37 EEG During Interruption of the Reticular Activating System

A) Control recording. Lower channel - right hemisphere. Upper channel - left hemisphere.

B) In this case, marked bilateral hypersynchrony of the EEG is noted. The reticular formation had been effectively blocked by the intraperitoneal administration of Nembutal (35 mg./kg.). A similar picture was observed following stereotaxically - guided injection of 0.5 to 1 µL. of 2 percent Xylocaine directly into the rostral reticular formation of conscious, mobile rats.



a



b

The hippocampal content of β-endorphin was observed to be decreased significantly at 5 and 30 minutes following concussion, suggesting release of β-endorphin from neurons in the hippocampus. This is the only area in which the administration of β-endorphin results in predominantly excitatory effects, with epileptiform EEG changes. This observation may relate to the frequent observation of seizures as a manifestation of concussion in the conscious rat. In view of the above results, it is interesting that, prior to the current explosion in peptide research, Barker (1976) predicted that disturbances in the metabolism of peptides or their receptors could lead to "altered, and possibly pathologic behavior".

SOME IDEAS FOR FUTURE RESEARCH IN EXPERIMENTAL CONCUSSION

Study of single neuron preparations. Eccles (1972) has been a proponent of the theory that the phenomena of memory and learning depend upon neuronal connectivity, and more importantly, the "plasticity" of synaptic transmission. Recently, Droz (1975) and others have demonstrated that when axonal transport is temporarily blocked, nerve conduction is also reversibly blocked. Krems (1942) has shown a partially reversible reduction in the action potentials of isolated periopheral nerves when they were concussed by an air blast. Concussion of a peripheral nerve, or ideally, of an isolated axon would be an excellent model in which to study the phenomenon of reversible neural dysfunction.

Brain extracellular potassium concentration at the time of concussion. High levels of extracellular potassium have been shown to be the etiology of the phenomenon of spreading depression (Leão, 1944, 1947; Grafstein, 1956). The similarities between concussion and spreading depression have also been noted (Ward, 1966). Vyskocil, et al. (1972) have described a potassium electrode suitable for measurement of brain extracellular postassium, but too fragile to employ in experimental concussion. It is tempting to speculate that release of potassium from neurons might result in a temporary depolarizing block and resultant reversible dysfunction of cerebral neurons.

Will naloxone administration alter the behavioral effects of concussion? If any of the physical effects of concussion are naloxone-reversible, it might be concluded that the observed changes in the endorphins and the opiate receptor are contributory to the clinical picture, rather than merely epiphenomena. A study of this nature would be difficult to control, however, because of the marked variability in response to a concussing blow, observed both clinically and experimentally.

Changes in cerebral metabolism. These have been studied extensively, but the reports are incomplete in some aspects, and controversial in others. The technique of Sokoloff, as described by Shinohara, et al. (1979) of measuring cerebral metabolism by following the uptake of ^{3}(H)-2-d-deoxyglucose might successfully demonstrate changes in cerebral metabolism following concussion, and, more specifically, outline whether the changes are more dramatic in cortical or subcortical structures.

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