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THE ACUTE EFFECTS OF GAMMA-HYDROXYBUTYRATE ON SLEEP AND TONIC IMMOBILITY IN THE RABBIT

by

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Roger Godbout was born in Québec, Canada in 1955. He received his primary and secondary education in Québec City. He obtained a B.A. with Honours in Psychology from the University of Ottawa in 1977.

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ABSTRACT

Gamma-hydroxybutyrate (GHB) is a metabolite of gamma-aminobutyric acid which occurs naturally in mammalian brain. The largest concentrations of GHB have been observed in the extrapyramidal system, mainly in the caudate nucleus and nigro-striatal pathway. Administration of high doses of GHB is reported to be followed by increased concentrations of dopamine (DA) and acetylcholine (ACh) in these regions, in association with a decrease in the firing rate of DA and ACh neurons and motor activation. Low doses of GHB are reported to be hypnotic and even to facilitate paradoxical sleep (PS). The mechanism of this phenomenon is still obscure, but is hypothesized to involve the pontine reticular formation.

Tonic immobility (TI) is a natural and reversible behavior characterized in the rabbit by the absence of movement, a rigidity of limb musculature and relative unresponsiveness. Pharmacological studies in the rabbit during TI have associated an increased activity of DA and ACh neurons with a decrease in TI duration. TI and PS have been reported to share behavioral (motoric inactivation and relative unresponsiveness), neuroanatomical (ponto-medullary reticular formation), neurophysiological (ACh and noradrenaline neuronal activity) characteris-

tics. PS and TI could thus be expected to positively covary.

GHB (at 25, 50, 100, 150, 200, 300, 500, 750, and 1,000 mg /kg) along with two saline treatments (SL I = 300 mg/kg; SL II = 1,000 mg/kg) were administrated i.v. to three White and two Brown New Zealand male rabbits in an attempt to reproduce effects on the sleep-waking cycle reported in other species. Latencies to slow wave sleep (SWS) and PS onset were not diminished and time spent in the drowsy stage (D), SWS and PS were not increased relative to saline treatments at any of the dose levels. However, W was decreased at 1,000 mg/kg relative to 100 and 200 mg/kg, and SWS was increased at 1,000 mg/kg relative to 200 mg/kg. Moreover, PS was abolished at 750 and 1,000 mg/kg. Analysis of electromyographic (EMG) results revealed that the 750 mg/kg dose of GHB was followed by increased motoric activation relative to SL I and 300 mg/kg doses. Over all treatment levels, W was found to exhibit the highest EMG levels of activity of all stage and state comparisons. No differences were observed between samples (15 minutes each) of EMG levels of activity taken 2, 60 and 105 minutes after injection.

In a separate experiment designed to study the effects of GHB on TI, GHB (at 300 and 650 mg/kg) and a saline

solution (SL = 475 mg/kg) were administrated i.v. to a second group of New Zealand White rabbits (n=5). Comparisons were made between these conditions and a non-injection session (BL) according to a latin square design. TI was induced just as the session was initiated, and re-induced 40 and 120 minutes later. No differences in TI durations or in frequencies of unsuccessful attempts to induce TI were observed across conditions or time blocks. However, GHB (at any dose) was observed to be followed by longer TI durations relative to the non-drug sessions.

In both parts of the study, an alteration of electroencephalographic patterns consisting of the appearance of slow, high amplitude waves in cortical and subcortical leads occurred at doses > 500 mg/kg. This activity, termed G-SW, was superimposed on otherwise characteristic D or SWS patterns and became progressively more intense with higher doses. Such EEG patterns could not be observed in W episodes preceding TI induction at 0 and 40 minutes after injection of 650 mg/kg, but completely replaced W after 120 minutes. At all times, the EEG patterns of TI at 650 mg/kg were replaced by G-SW.

The major conclusions emerging from the results of this study are that:

1) The postulated sleep inducing or PS enhancing

properties of GHB were not supported;

2) The hypothesis that increasing doses of GHB progressively depresses the ascending reticular activating system was verified at doses > 150 mg/kg;

3) High doses (750 mg/kg) of GHB are associated with motoric activation while the highest dose (1,000 mg/kg) are followed by motoric and behavioral depression;

4) TI is facilitated by GHB at 300 and 650 mg/kg;

5) TI is not dependent upon a specific EEG pattern, rather motor components are most critical to TI;

6) PS and TI are not directly related, and relationships which do exist may be related more to tonic, rather than phasic, events.

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INTRODUCTION

Gamma-hydroxybutyrate (GHB) is a recently identified substance which is present naturally in brain and is thought to be a metabolite of gamma-aminobutyric acid (GABA). GHB has been reported to exhibit a two-fold dose-related effect on behavior: at low doses, GHB has been reported not only to have general hypnotic effects, but also sleep-specific effects, i.e., enhancement of paradoxical sleep (PS). At higher doses, GHB administration is followed by excitatory effects on the central nervous system (CNS), particularly the motor system. GHB is thought to work its hypnotic effects through action on the pontine reticular formation, and its excitatory effects on the dopaminergic system and basal ganglia, both of which are known to play a significant role in motor control.

The present study examines the effects of GHB on two behaviors in the rabbit, i.e., sleep and tonic immobility (TI). Comparisons have been made between TI and PS in the past, and it has been noted that these states share certain behavioral, electroencephalographic (EEG), neuroanatomical and neurochemical characteristics, the most outstanding of which is the reported inhibition of motor activity during both conditions. The present

study was undertaken to provide a further comparison of PS and TI by examining variations in these states subsequent to the administration of GHB. In addition, the study will provide new information regarding dose-related effects of GHB on sleep-wakefulness states and EMG levels of activity in the rabbit.

The literature review will cover fundamental aspects of the dependent variables, namely the sleep-wakefulness cycle and TI, including neurophysiological, neurochemical, electrophysiological and behavioral characteristics. Literature pertinent to the utilization of GHB as an independent variable will then be presented. Based on these reviews, the rationale for the present investigation will be stated, followed by the formulation of hypotheses.

REVIEW OF LITERATURE

1.1 Sleep and wakefulness

1.1.1 Neurophysiology of sleep

Since the identification of the cyclic occurrence of rapid eye movement periods during sleep (Aserinsky and Kleitman, 1953), sleep research has gone through an extraordinarily fertile period of investigation. The discovery of two distinct sleep states, slow wave sleep (SWS) and paradoxical (PS) or rapid eye movement (REM) sleep, in humans and then in lower mammals (Dement, 1955; 1958) was followed by investigations attempting to identify physiological mechanisms underlying each of the two states. Early studies using stimulation and gross lesioning techniques generally circumscribed structures responsible for the control of sleep to the brainstem reticular formation (see Moruzzi, 1972). Subsequently, structures specifically involved in the generation of the sleep states have been localized to more discrete areas including the raphe nuclei, the region of the nucleus locus coeruleus and the gigantocellular tegmental field.

Initial investigations involving extensive lesions of the raphe nuclei in cats suggested a critical role for this system in the triggering and maintenance of

SWS (Jouvet, 1967; Jouvet, Bobillier, Pujol and Renaud, 1967; Jouvet and Renaud, 1966). However, recent results in rats with more precise lesions (Juvancz, 1980; Mouret and Coindet, 1980) suggest instead that the post-lesion effects resulted from the interruption of fibers of passage.

A more complex situation emerges with respect to PS, and two different models of PS generation have been proposed. One model, elaborated by Jouvet and his collaborators and based on lesions as well as unit recording studies, suggests that the nucleus locus coeruleus (LC) of the ventrolateral pontine reticular formation is the triggering zone for PS (Jouvet, 1969; Sastre, Sakai and Jouvet, 1979). However, evidence for LC as an executive generator of all aspects of PS is inconsistent (Henley and Morrison, 1974).

Another model has been proposed by Hobson, McCarley and collaborators and termed the "Reciprocal Interaction Model" (Hobson, McCarley and Wyzinski, 1975; Vivaldi, McCarley and Hobson, 1980). This model primarily involves the neurons of the gigantocellular tegmental field (FTG) of the brainstem reticular formation as executive elements of PS. According to the model, these cells receive inhibitory inputs from the dorsal raphe nuclei and LC. Again, however, some con-

tradictory results tend to diminish the apparent cohesiveness of the model. For example, Hobson and McCarley's studies were done in restrained animals, and FTG cells were later reported to be highly active during active wakefulness, thus invalidating the proposed specificity of these neurons to PS (Siegel and McGuinty, 1977). Moreover, destruction of FTG cell bodies with kainic acid does not seem to alter normal PS manifestation (Sastre et al., 1979).

The elaboration of a comprehensive neurophysiological model for sleep-stage interactions continues to be a challenge, but it is now generally accepted that sleep is not regulated through centers, but through a variety of interacting neuronal and biochemical systems (Jouvet, 1972; Steriade and Hobson, 1976). Indeed, activity of all the neuronal structures explored above is modulated by the presence of various neurotransmitters. Their mode of action with respect to sleep and the above proposed models will be briefly reviewed in the next subsection.

1.1.2 Neurochemistry of sleep

Neurophysiological processes alone could not account for many characteristics of sleep, such as the periodicity of PS or the long-lasting PS rebound (up to

several days) after selective PS suppression (Jouvet, 1972). Consequently, neurochemical processes, with more extended temporal dimensions, were invoked to explain these phenomena. Mapping of the monoamine system in the central nervous system (CNS) by Dahlström and Fuxe (1964) significantly advanced pharmacological studies of behavior by giving an "anatomical dimension" to neurochemical pathways.

In sleep research, investigations have subsequently attempted to identify not only the "centers of sleep", but also their neurohumoral components. Three neurochemical substaces, namely serotonine (5-HT), synthesized in the raphe nuclei, noradrenaline (NA), synthesized primarily in LC neurons, and acetylcholine (ACh), whose site of synthesis is not localized, have been most extensively investigated with respect to sleep mechanisms.

A critical role for <u>serotonine</u> as a SWS promoting agent (Brodie and Shore, 1957; Jouvet, 1969; Jouvet et al., 1966; Ursin, 1972) or as a PS inhibitor (Pujol, Keane and Jouvet, 1978; Steriade and Hobson, 1976; Vivaldi et al., 1980) has often been challenged (Gillin, Mendelson, Sitaram and Wyatt, 1978; Mouret and Coindet, 1980; Weitzman, Rappaport, McGregor and Jacoby, 1968). However, 5-HT is thought to play a significant role in

sleep-wake cycle modulation in mammals (Grouillēr, Nioche, Barailler, Roche and Pacheco, 1980) since specific manipulations of 5-HT synthesis or degradation consistently effect variations in sleep expression (Jouvet, 1972). Furthermore, studies suggest a modulatory role for serotonergic agents on phasic PS events, i.e., ponto-geniculo-occipital (PGO) spikes (Delorme, Froment and Jouvet, 1966a; Jacobs, Henrickson and Dement, 1972).

Noradrenaline has been hypothesized as having an executive role in the appearance and maintenance of PS desynchronized EEG activity and reduced muscle tonus (Jouvet, 1972; Stern and Morgane, 1974). However, several neuropharmacological studies have failed to support such a specific relationship, and it has consequently been suggested that NA is not essential to PS (Ramm, 1979), but rather has an interactive relation with executive elements by contributing to the modulation of their activity (Steriade and Hobson, 1976).

The importance of acetylcholine in sleep mechanisms has been stressed in both the LC/Raphe model of Jouvet (1969) and the Reciprocal Interaction model of McCarley and Hobson (1975a, 1975b). Pharmacological evidence, based on cholinergic agonist or antagonist administration and direct ACh injection in the CNS

(Domino and Stawinski, 1971a, 1971b; George, Haslett and Jenden, 1964; Hernandez-Peon, O'Flaherty and Mazzuchelli - O'Flaherty, 1967; Jouvet, 1962; Silberman, Vilvadi, Garfield, McCarley and Hobson, 1980; Van Dongen, 1980), as well as measurements of ACh cortical release during sleep (Jasper and Tessier, 1971), have linked ACh specifically to tonic events of PS (muscle atonia, EEG desynchronization) as well as to phasic events (vestibular-eye movement interaction) (Pompeiano, 1972). Moreover, ACh has been related to behavioral depression (Karczmar, 1979) as well as catalepsy (Ahtee and Kääriäinen, 1974) -- features that may resemble tonic behavioral inhibition seen in PS. ACh has also been observed to inhibit SWS cortical spindle activity (8-12 Hz) triggered by perfusion of dopamine (DA) in the caudate nucleus (Hall and Keane, 1975). ACh thus seems to be part of the intricate neurotransmitter interaction involved in the sleep-wake cycle (Karczmar, 1978a, 1978b; Roth and Bunney, 1976).

The interaction of neurochemical systems in the CNS has been extensively investigated (see Karczmar, 1978a). Not only do neurotransmitters share certain common enzymes, such as tyrosine hydroxylase for NA and DA synthesis, or monoamine oxydase (MAO) for mono-

amine catabolism (Jouvet, 1972), but specific relationships between neurotransmitters have been established. With respect to sleep, 5-HT seems to have an inhibitory effect on ACh (Pujol et al., 1978; Harza, 1971) and ACh is thought to have a facilitatory effect on NA neurons (Vivaldi et al., 1979; Jacobs and Jones, 1978). Moreover, it is suggested that ACh, rather than NA, is the primary neurochemical responsible for the atonia observed in PS (Van Dongen, Broekkamp and Cools, 1978). In fact, both major models of sleep mechanisms agree that an interactive intervention of cholinergic and aminergic mechanisms is critical in the triggering and maintenance of PS.

1.1.3 Sleep stages: general behavioral and electrographic characteristics in the rabbit

The rabbit, like other mammals, exhibits predictable alterations of electrographic patterns correlated with behavioral states of wakefulness, drowsiness and sleep -- the latter being subdivided into SWS and PS. Behaviorally, sleep in the rabbit generally resembles that in other mammals, i.e., the animal remains immobile except for brief arousals marking state transition. The rabbit can often sleep with eyes partially opened, and, during SWS, the head can be held erect, despite reduced responsivity at this time relative to

wakefulness.

Electrographically, sleep in the rabbit is also generally comparable to that in other mammals (see Faure, 1962, 1963; Kawakami, Negoro and Teresawa, 1965; Kawakami and Sawyer, 1962; Khazan and Sawyer, 1963; Narebski, Tymicz and Lewosz, 1969; Roldan and Weiss, 1963; Thoman, Waite, Desantis and Denenberg, 1979; Weiss and Roldan, 1964). Sleep onset is normally characterized by a progressive slowing of EEG activity (3-7 Hz), both in cortical and subcortical leads, relative to waking. Cortical electrodes also record a low level of spindle activity that later on becomes more prominent, along with a more sustained slowing of the EEG. Concomitant with normal electrographic activity, monopolar leads referred to nasal bone often record a 2-3 Hz respiratory rhythm. Following this drowsy or sleep onset EEG activity, SWS occurs. In both stages, i.e., drowsy and SWS, the electrooculogram is generally stable and the electromyogram (EMG) shows a decrease in muscle activity levels that correlates well with the behavioral quiescence mentioned above. Usually within 20-60 minutes after sleep onset rabbits enter the paradoxical phase of sleep which is characterized by cortical low voltage fast activity with a prominent theta component, while respiratory

rhythms in monopolar leads referred to nasal bone have disappeared. EOG recordings show rapid eye movements bursts, and an incomplete decrease in EMG amplitude occurs (Pivik, Sircar and Braun, 1981). Generally, the animal will awaken (W) after the PS episode and the cycle will be reinitiated.

1.2 Tonic immobility

1.2.1 Behavioral and electrographic properties of tonic immobility

TI is a reversible and naturally occurring state present in a variety of species ranging from coelenterates to mammals (Crawford, 1977; Gilman and Marcuse, 1949; Hoagland, 1928; Ratner, 1958) which is increasingly difficult to elicit in higher mammals (Svorad, 1957).

TI is thought to represent either an innate fear reaction (Gallup, 1974; Gallup, Nash, Donegan and McClure, 1971; Woodruff, 1976) or a hypnotic state of sedation (Draper and Klemm, 1967) which, however, is different from the human "hypnotic trance" (Lerner, 1962). TI induction is usually performed by rapid dorsoflexion accompanied by a short period of restraint (Silva, Estable and Segundo, 1959).

In the rabbit, TI is characterized behaviorally by

an absence of movements (motoric inactivation), a loss of the righting reflex, a rigidity of limb musculature and relative unresponsiveness, even if eyes may remain open (Carli, 1968, 1969a; Klemm, 1966b). No characteristic variations in heart rate or blood pressure have been reported (Carli, 1974).

During electroencephalographic recordings, neocortical leads in rabbits show an arousal pattern, but during more extended TI episodes a slower, more synchronized EEG accompanied by spindles may appear (Carli, Lefebvre, Silvano and Vierucci, 1976; Silva et al., 1959). If the episode is sufficiently prolonged, periods of PS may even be observed (Harper, 1971; Hoskovec and Svorad, 1969).

1.2.2 Neurophysiology of tonic immobility

Lesion studies have shown that the cortex, cerebellum, 8th nerve, labyrinth, thalamus, hypothalamus and hippocampus are not necessary for TI induction, since absence of their respective influences do not modify the TI response in the rabbit (Carli, 1969b, 1971; McBride and Klemm, 1969; McGraw and Klemm, 1969). Transection of the neuraxis above the superior colliculus did not abolish the TI response either. Since units in pontine nuclei, FTG and medullary reticular

formation have shown increased firing during TI (Klemm, 1969a), it has been hypothesized that during TI there is a descending inhibitory influence originating from the ponto-medullary reticular formation upon spinal motoneurons (Carli, 1969b, 1971, 1977; Klemm 1969a, 1969b, 1971a, 1971b; McBride and Klemm, 1969; McGraw and Klemm, 1969; Prestrude, 1977).

1.2.3 Neurochemistry and pharmacology of tonic immobility

Few attempts have been made to relate TI to a specific neurotransmitter.

The first neurochemical group assayed for TI-related action was the catecholamines (CA). Davis (1963) found that chlorpromazine (CPZ) (a potent CA receptor blocker) increased TI duration in the rabbit. These results were later confirmed by Klemm (1965a) who, in addition, observed no difference between EEG recordings following CPZ administration with or without TI induction. Schaeppi and Rubin (1965) also observed increased TI duration with CPZ in the rabbit, but noted a greater variability in the response. Other CA antagonists, e.g., alpha-methyldopa (a synthesis blocker) and reserpine (a storage inhibitor), are also reported to facilitate the TI response in the rab-

bit (Scotti de Carolis, Carruyo and Longo, 1969). Wallnau, Carwike and Dewey (1979) reported that haloperidol, which specifically blocks DA receptors, increased TI duration in the chicken. In the same study, administration of a DA receptor stimulating agent (apomorphine) was reported to elicit the opposite effect. The NA uptake blockers imipramine and amphetamine (also a NA efflux stimulator) were observed to inhibit the TI response too (Maser and Gallup, 1974; Scotti de Carolis et al., 1969). It seems then, that an increased activity of CA neurons is related to shorter TI durations whereas CA antagonists may facilitate the response.

The involvement of ACh in TI remains obscure. In fowl, scopolamine (a cholonergic receptor blocker) decreases TI duration and physostigmine (which inhibits the ACh breakdown by cholinesterase) facilitates the TI response (Thompson 1977; Thompson Piroch, Gallen and Hatton , 1974; Woodruff, Hatton, Frankl and Meyer, 1976). However, the reverse is observed in guinea pigs and rabbits (Hatton, Woodruff and Meyer, 1975; Woodruff et al., 1976).

Regarding 5-HT, only studies dealing with the administration of parachlorophenylalanine (PCPA: a 5-HT synthesis blocker) have been reported. In the chicken, a 5-HT blockade mechanism is thought to yield an inhibi-

tory effect on TI since PCPA decreases TI duration in this species (Hicks, Maser, Gallup and Edson, 1975; Maser and Gallup, 1974; Wallnau and Gallup, 1979). However, PCPA showed no effects when injected in rabbits (Scotti de Carolis et al., 1969).

Morphine and morphine-like substances (beta-endorphine and met-enkephaline) have also been assayed to evaluate the possible involvement of TI in the pain mechanism. Results do not contradict such an assumption, i.e., both types of substances in the chicken, and morphine in the rabbit are consistent in increasing TI duration (Bloom, Segal, Ling and Guillemin, 1976; Davis, 1963; Hicks et al., 1975; Olson, Kastin, Lahoste, Olson and Coy, 1980; Peters and Hughes, 1978; Wallnau and Gallup, 1979). Such results, however, are not conclusive since administration of naloxone alone (an opiate receptor blocker) does not have any effects on TI duration (Galeano, Morcos, Cloutier, Desmarais and Beaudry, 1978; Wallnau and Gallup, 1979).

The study of the neurochemistry of TI is relatively new and there is a paucity of research in this area. Accordingly, more investigation into this field is needed to define mechanisms and parameters of TI.

1.3 Paradoxical sleep and tonic immobility: a comparison

Besides behavioral similarities such as motoric inactivation and relative unresponsiveness (Carli, 1968, 1969a; Lievens, 1960; Sidis, 1908), there is other evidence suggesting that the comparison between TI and PS is a legitimate one. For example, both states are natural and spontaneously occurring and both share neuroanatomical and neurophysiological characteristics that are well-enough defined to suggest a common base. Furthermore, lesion studies in the rabbit (Carli, 1969b, 1971) and in other species (McBride and Klemm, 1969) have indicated that brainstem areas essential to the expression of TI are similar to those shown to be necessary for the elaboration of PS, i.e., the ponto-medullary reticular formation (Jouvet, 1972; Jones, 1979). Polysynaptic as well as monosynaptic reflexes of both extensors and flexors are also reported to be depressed during TI and PS in the rabbit (Carli, 1968, 1969a). Moreover, Morrison (1979) compared PS and TI on the basis that both could be an exaggeration of the orienting reflex. Changes in levels of EMG activity have also been reported to be a feature common to both states (Carli, 1969z; Galeano, Leung, Robitaille and Roy-Chabot, 1979; Pivik et al., 1981;

Van Reeth, 1963). Finally, major neurotransmitters (ACh and NA) are involved in the mediation of both states.

In other respects, however, these states differ markedly. For example, episodes of PS are relatively predictible since they follow a natural cyclicity. That is not the case for TI, which must be triggered by the appropriate stimulus from the environment. Although both states are characterized by desynchronized cortical EEG activity, PS, but not TI, is accompanied by the appearance of rapid eye movement bursts and EMG twitches (Klemm, 1966; Carli, 1969a, 1969b). Also, hippocampal activity during PS is of higher frequency than that during TI (Harper, 1971), and, whereas there is a widespread increase in CNS unit activity during PS (Steriade and Hobson, 1976), such increases have been reported to occur exclusively in the ponto-medullary reticular formation during TI (Klemm, 1969, 1971).

1.4 Gamma-hydroxybutyrate: state and event correlates

GHB is a short chain fatty acid in mammalian brain thought to be a metabolite of GABA (Bessman and Fishbein, 1963; Doherty, Hattox, Snead and Roth, 1978; Roth, 1970, 1976). Across various species examined, endogenous GHB has been found mainly in hippocampus,

midbrain/diencephalon and cerebellum, with the highest concentrations in the caudate nucleus (Doherty and Roth, 1976; Doherty et al., 1978; Roth, 1970, 1976). One study assayed GHB levels in the CSF during the sleep-wake cycle (Tabakoff and Radulovacki, 1976) and reported higher levels during wakefulness; unfortunately, nothing was done to differentiate PS from SWS in this analysis.

GHB is described as a non-toxic and non-addictive hypnotic (Mamelak, Escriu and Stokan, 1977; Vickers, 1969), which is also considered as an anesthetic (Muyart and Laborit, 1977) and a parasympatheticomimetic (Lund, Humphries and Virtue, 1965).

Administration of GHB has been reported to be followed by increased concentration of ACh and DA in whole brain and brainstem, with highest levels in the extrapyramidal system (mainly caudate nucleus and nigro-striatal pathway) (Doherty et al., 1975; Gessa, Vergiu Crabai, Boero, Cabonu and Camba, 1966; Giarman and Schmidt, 1963; Muyart and Laborit, 1977; Roth, 1976; Roth, Salzman and Nowyck, 1978; Roth and Suhr, 1970; Sethy, Roth Walters, Marini and von Woert, 1976; Stock, Magnusson and Andén, 1973). These increased concentrations are thought to be related to a two-fold mechanism effecting a decrease in the firing rate of

cholinergic and dopaminergic fibers and an increased turnover of these substances (Andén, Magnusson and Stock, 1973; Roth, Walters and Aghajanian, 1973; Sethy et al., 1976). A compensatory increase in the synthesis of the neurotransmitter follows the termination of transmitter release and accompanying decrease in nerve impulse transmission.

In addition to effects on DA and ACh, GHB is reported to have contradictory and nonsignificant effects upon levels of NA and 5-HT (Gessa et al., 1968; Roth and Suhr, 1970). Despite its origin, it also appears that CNS effects of GHB are undoubtedly unrelated to changes in GABA levels (Jouvet, 1972; Roth and Giarman, 1969; Walters and Roth, 1977).

Studies of GHB blood and CSF levels in monkeys after i.v. administration of doses ranging up to 1,000 mg/kg have shown a peak in serum levels at around 40 minutes post-injection and a maximum in CSF after 2-3 hours. Traces were still detectable in serum and CSF 24 hours after injection, while the electrical recordings returned to normal within 18 hours (Snead, Yu and Huttenlocher, 1976).

Two types of "behavioral-electroencephalographic dissociations" (Wikler, 1952) have been reported after

administration of GHB. In some studies, behavioral depression after administration of this substance has been accompanied by spike discharges indicative of epileptiform activity (Snead et al., 1976); conversely, awake and responsive human subjects have been reported to display theta and delta rhythms for 10 to 20 seconds following GHB administration (Metcalf, Emde and Stripe, 1966; Yamada, Yamamoto, Fujiki, Hishikawa and Kaneko, 1967). These paradoxical phenomena are not well understood and have not been replicated (Broughton and Mamelak, 1979, 1980). It has been proposed that they could result from differences in methodology, e.g. doses, rate and route of administration, paired with species differences (Delay, Deniker, Perrier, Ginestet, Sempé and Verdeaux, 1965; Metcalf et al., 1966; Lettieri and Fung, 1979).

GHB has recently been used in the treatment of narcolepsy, a sleep disorder characterized by irresistible sleep attacks usually accompanied by loss of muscle tone (Broughton and Mamelak, 1979, 1980; Mamelak et al., 1977). GHB has been reported to be associated with the following effects on night sleep in humans: increased slow wave sleep, decreased stage I, decreased amounts of short sleep episodes, reduced fragmentation and increased duration of PS periods, and

reduced latency to onset of PS. Thus, unlike synthetic hypnotics, GHB does not suppress but rather facilitates PS, and its withdrawal shows no rebound effects but a return to pretreatment levels instead (Mamelak et al., 1977). This leads to a most interesting aspect of GHB, i.e., its sleep-inducing effect.

Since 1961, GHB has successfully been used to induce sleep. This effect occurs within a few hundred seconds following administration in various species including chickens (Hayashi, 1967), mice (Rizzoli, Agosti and Galzigna, 1969), rats (Godschalk, Dzoljik and Bonta, 1977), cats (Heidt, Schlör, Buss and Stock, 1975), dogs (Hayashi, 1965) and humans (Appia, 1967; Hoes, Vree and Guelen, 1980). Most interestingly, it has also been reported to induce true PS in cats in a dose range varying from 50 to 100 mg/kg i.p. and from 150 to 200 mg/kg i.v. (Delorme, Riotte and Jouvet, 1966b; Jouvet, Cier, Mounier and Valatx, 1961; Matsuzaki, 1968; Matsuzaki and Tagaki, 1967; Matsuzaki, Tagaki and Tokizane, 1964; Stock, Heidt, Buss and Schlör, 1978; Vern and Hubbard, 1971). The sleep inducing property of GHB has been hypothesized to be related to a critical concentration of the substance in CSF (Hayashi, 1967; Snead et al., 1976) -- an hypothesis that would favour an intra-CNS locus of action

for GHB hypnotic effects. In fact, this last hypothesis is substantiated by studies in cats with caudal pontine haemorrhage, midpontine lesions, pre-pontine decerebration and pons isolation (Delorme et al., 1966b; Jouvet et al., 1961; Matsuzaki, 1968). PS could still be induced by GHB only in preparations exhibiting intact nuclei reticularis pontis oralis and/or caudalis, i.e., areas that are recognized as being critical for the normal appearance of PS (see 1.1.1).

GHB doses exceeding hypnotic levels are reported to effect an increase in motoric activation (Matsuzaki, 1968; Stock et al., 1978) that can be related to epileptoid episodes (Godschalk, Dzoljik and Bonta, 1976; Snead, 1978). This observation can be related to the fact that DA and ACh within the basal ganglia are known to be involved in the modulation of both normal and abnormal motor activities (Cools, Hendricks and Korten, 1975). Since it has been observed that GHB inhibits DA and ACh transmission in the caudate nucleus (see above), and since the caudate nucleus is known to exert inhibitory control over motor activity (Villablanca, Marcus and Olmstead, 1976), a positive, but non-linear, relationship between GHB and levels of motoric activation may exist.

There are only two studies on the effects of GHB administration in rabbits (Schneider, Thomalske, Trautmann, Smolarz and Sabbagh, 1963; Winter and Samson, 1956) and they do not include analyses of effects on sleep-wake cyclicity and EMG levels of activity. In one study (White and Samson, 1956), a single dose-level of 500 mg/kg i.v. was used, and a "sleep-like state" with slow waves and spindle activity was observed. In the other study (Schneider et al., 1963), although several i.v. dose-levels were used, the ranges within dose groups used to describe EEG effects were so wide that no specific statement regarding dose-related effects could be made. However, an interesting progressive dose-related model relating the effects of GHB on the EEG was proposed in which it was hypothesized that at lower doses (70-500 mg/kg) the substance yields an early functional depression of thalamo-caudo-cortical structures during which slow activity (2-3 Hz, 500 Uv) appears even though the animal still responds to noxious stimulation. At higher doses (600-1,200 mg/kg), a progressive depression of the ascending reticular activating system was hypothesized since animals would not respond to stimulation. It is only at the higher doses (> 1,000 mg/kg) that the reticulo-spinal (motor) systems are depressed.

The literature review suggests that GHB facili-

tates PS, even if the reported effects are inconsistant with activation of putative sleep mechanisms (decreased firing of cholinergic fibers, no noticeable effects on NA or 5-HT...). It is also known that GHB acts on the dopaminergic system in the same manner as do TI facilitators (i.e., decreased activity), even though the action on ACh (another putative TI modulator)is paradoxical and contradictory. The facilitatory effect of GHB on motoric activation at higher doses in the rabbit has also been noted (Schneider et al., 1963).

The present study has four main goals: first, to systematically examine the reported hypnotic property and PS enhancing effect of GHB in a species not yet evaluated in that respect; second, to measure the effects of GHB on quantified motor activity: third, to assay the effects of GHB on TI; and finally, fourth, based on obtained results, to examine the relationship that is hypothesized to exist between PS and TI.

1.5 Hypotheses

The present study will examine variations in sleep patterns, levels of motoric activation and TI following GHB administration. It is hypothesized that:

1) Low doses (25-300 mg/kg) will facilitate the

occurrence of sleep, particularly of PS (earlier onset and/or greater duration);

2) Higher doses (500-750 mg/kg) will evoke motoric activation (higher EMG levels), more wakefulness and less sleep, while the highest dose (1,000 mg/kg) will be associated with motoric depression;

3) TI duration will be facilitated by lower GHB doses and decreased by higher doses (300 amd 650 mg/kg respectively);

4) In view of the literature presented, PS and TI will covary in a positive manner.

METHODOLOGY

2.1 Subjects and surgical procedures

Ten adult New Zealand male rabbits (eight White and two Brown) weighing 3.6-4.2 kg at the time of surgery were used in this study. They were divided into two groups of five rabbits each, one of which was used in the study of the effects of GHB administration on electrographic (sleep-waking cycle) parameters, and the other was used to examine the effects of GHB administration on the TI response.

Animals were food deprived during twelve hours preceding surgery. Thirty minutes before surgery, chlorpromazine (Largactil, 0.4 mg/kg, i.m.) was administrated as a pre-anesthetic sedative. The animal was shaved over the head and neck surface to expose areas in which electrodes were to be implanted. Sodium pentobarbital (nembutal) diluted 1:1 with distilled sterile water was then injected i.v. through the marginal ear vein (0.5 ml/kg). Upon loss of the crossed extensor pinch reflex, surgery commenced and sodium pentobarbital was then delivered as required to maintain this depth of anesthesia. A mineral corticosteroid (Decadron, 0.25 mg/kg, i.m.) was injected each three hours to counterbalance the stress-induced depletion of this substance.

Animals were implanted for chronic EEG recordings

over right and left central motor cortex with a pair of stainless steel flat-ended screws (# 0-80). Similar screws were implanted bilaterally for eye movement (EOG) recordings in the supraorbital bone ridge and in the nasal bone for the reference (indifferent) electrode. Three to four multistrand, teflon-coated stainless steel wires were placed deeply into the deltoid neck muscle for muscle activity (EMG) recording.

Following surgery, Penicillin (Penlong-S, 0.4 ml/kg, i.m.) was administrated, and an antibiotic powder (Dicloxacillin) was sprinkled over the wound before closing with sterile catgut sutures.

2.2 Holding and recording environments

Holding and recording areas were maintained on a 12 hour light-dark schedule, with the light period extending from 7 a.m. to 7 p.m. Food and water were provided ad lib and all animals were held in individual cages and regularly checked for infections. During the experimentation period, only ear mites and snuffles (common cold) were detected and were promptly treated with medicated mineral oil (Hexamite) and an antibiotic (Tetracycline, dissolved in drinking water), respectively. The holding area was kept at a temperature of 23° C and 54% humidity.

Animals were recorded individually in cages lighted at an intensity of 100 Lux, sound dampened and electrically shielded. Connections from the cage to the polygraph were made through an ensemble of cables, counterweight boom and swivel commutator (Airflyte Electrocannular Slipring Assembly, model no. CAY 675-24) to ensure free movement of the animal in the recording cage. Recordings were taken for two consecutive hours between 9:00 and 17:00 hours. Each animal was habituated for at least 20 hours to the recording cage before experimental data were collected.

2.3 Polygraph settings

A post operative period of 1 week was allowed before recording sessions began. Electrographic recordings were generally done using bipolar derivations. Signals were amplified and filtered on a Beckman R 612 eight-channel polygraph as follows: EOG -- high frequency filter, 30 Hz, low frequency filter, 0.5 Hz; EEG -- high frequency filter, 30 Hz, low frequency filter, 1.5 Hz; EMG -- high frequency filter, 100 Hz, low frequency filter, 5 Hz. All channels were preamplified (x .01) and filtered for 60 Hz noise activity.

EMG signals were led to a resetting integrator

which effected full-wave rectification and conversion of these rectified EMG voltages to digital pulses. These pulses were displayed together with other electrographic data on polygraph paper write-out. Prior to recordings, system noise was determined by shorting EMG leads and counting number of resets over a period of one minute. To determine the performance curve of the apparatus, various voltage and frequency combinationsproduced by a function generator (Hewlett-Packard, 3303 Function generator) were processed through the system. Variations in amplification factors used in this study were maintained within the linear response range for the apparatus determined by this procedure.

2.4 Methodology: sleep-wake recordings

Three White and two Brown New Zealand rabbits were used in this part of the study. Nine different doses (25, 50, 100, 150, 200, 300, 500, 750 and 1,000 mg/kg) of GHB (Gamma OH, Laboratoires Egic, Paris; prepared in ampuls of saline solution, at a concentration of 200mg/ml, with a ph of 7.9) and two different volumes of .09 NaCl (saline) solution (SL I and SL II) were separately administrated i.v. in the marginal ear vein following the sequence presented in Table 1.

Table 1

Sequence of GHB administration
for the sleep-wake cycle recordings,
with date and time of administration

	25	50	100	150	200	SL I	300	500	1,000	SL II	750
Rabbit 1	01/04 14:40	04/04 15:02	10/04 13:20	12/04 15:50	15/04 10:29	17/04 11:01	22/04 10:52	25/04 14:42	01/05 10:15	04/05 12:18	03/06 13:50
Rabbit 2	02/04 12:45	09/04 10:58	11/04 10:30	14/04 10:36	16/04 10:44	19/04 09:46	24/04 14:58	30/04 11:39	03/05 14:40	12/05 13:55	26/06 15:55
Rabbit 3	02/04 17:10	11/04 16:45	13/04 13:40	15/04 13:15	18/04 10:04	21/04 10:20	23/04 15:58	28/04 10:27	02/05 13:11	05/05 11:31	14/06 14:38
Rabbit 4	03/04 16:56	09/04 13:35	11/04 14:07	14/04 13:15	16/04 14:38	18/04 13:56	22/04 14:15	26/04 15:35	01/05 14:28	04/05 09:37	03/07 15:08
Rabbit 5	03/04 13:00	09/04 17:33	12/04 17:13	14/04 16:00	16/04 17:23	18/04 16:18	23/04 10:47	12/05 10:18	29/04 13:14	04/05 15:15	*

* This rabbit died during a previous trial, following a 1,500 mg/kg dose of GHB

Since it was reported that 80-90% of labeled GHB is excreted into exhaled air and 10-20% is excreted in urine within 24 hours after either oral or intraperitonial administration (Hoes et al., 1980; Whitehead and Virtue, 1965), at least 48 hours elapsed between dose injections in order to allow complete elimination of the injected substance from the body. Moreover, tolerance to hypnotic effects of GHB following repeated use has not yet been demonstrated (Mamelak et al., 1977; Vickers, 1969). The saline volumes delivered were equal to a 300 mg/kg dose of GHB (SL I) and to a 1,000 mg/kg dose of GHB (SL II). Immediately after injection, the subject was connected to the cable arrangement described above, and electrographic recordings were initiated for two consecutive hours. The time interval from end of injection to start of recording was noted.

2.5 Methodology: TI assessment

Five White New Zealand rabbits were used in this part of the study. TI testing was conducted under the following conditions: three drug levels, one saline (SL) level (corresponding in volume to mean amount of GHB levels) and one baseline (BL) session (no injection). The author was solely involved in every phases

of this part of the study since experienced and skillful manipulations were required to assure cohesiveness in the various experimental procedures involved; therefore, blindness of experimental conditions was discarded as a control. Testing was performed according to a latin sqare design (see Table 2).

Table 2

Sequence of GHB administration
for the TI experiments,
with date and time of administration

	BL	SL	300	650	1,000
Rabbit 1	10/11 11:05	19/11 11:40	12/11 12:40	05/11 12:55	21/11 12:05
Rabbit 2	05/11 13:25	14/11 10:45	07/11 11:15	12/11 15:15	10/11 15:30
Rabbit 3	10/11 11:20	05/11 16:05	14/11 11:50	07/11 16:00	12/11 13:45
Rabbit 4	12/11 09:30	10/11 12:20	05/11 16:30	14/11 14:15	07/11 12:30
Rabbit 5	03/12 10:05	27/11 11:45	01/12 10:50	29/11 08:55	25/11 10:00

Each session was divided into three time blocks: immediately post-injection, 40 minutes post-injection and 120 minutes post-injection. The baseline session followed the same temporal distribution. Each block consisted of five individual trials, with two minute intervals between the end of one trial and the beginning of the next. To induce TI, the rabbit was dorsoflexed and placed in a V-shaped trough, as described by Klemm (1966). The duration of each TI episode was timed with a stopwatch and extended from the moment of complete release of restraint (which lasted five seconds) to spontaneous righting by the rabbit. If a trial failed, i.e., if the animal righted himself within five seconds after release from restraint, the intertrial period was reduced to one minute. After five trials, the animal was returned to his cage until the next time block.

2.6 Sleep stages: definitions and scoring criteria

Electrographic recording analyses were based on 30 second epochs. Composite criteria for stage determinations used in this study were: <u>wakefulness</u> (W) -- low voltage fast, mixed frequency EEG, activated EMG, sporadic EOG activity; <u>drowsy</u> (D) -- increased EEG amplitude relative to W, EMG levels similar to relaxed

W, and rare EOG activity; slow wave sleep (SWS) -- predominantly slow, high amplitude EEG with frequent spindles, EMG activity stable but reduced relative to W and D, rare EOG activity; paradoxical sleep (PS) -- low voltage fast, mixed frequency EEG, presence of regular theta activity, transient, non-sustained reductions of nuchal EMG activity relative to other stages, phasic EMG activity, isolated and clustered bursts of rapid eye movements.

Frequency counts of quantified EMG activity were made from the paper write-outs and then paired with independently scored stage determinations made by two individuals blind with respect to injection condition (agreement > .85). Discrepancies were resolved by mutual agreement.

2.7 Data analysis

The effects of GHB on sleep-wakefulness patterns during two hours of electrographic recordings yielded two groups of data: one related to the distribution of various sleep stages during the period of recording and the other generated by the analyses of EMG activity.

The time elapsed from the end of injection to the onset of a particular sleep stage was used as the onset latency for that particular stage. Proportions of the

total recording time attributed to each stage were also calculated in percentage and were used as the proportion of total recording time for that stage. These two dependent variables were computed for all eleven treatment levels and analysed using a univariate analysis of variance for repeated measures. Post hoc analyses were performed according to the Dunn's method, using a $p < .05$ criterion.

The analysis of EMG activity was performed by counting integrated EMG resets per 30 second interval. Reset counts were not calculated for the entire recording period but were done on three fifteen minute samples distributed at the beginning, middle and end of each recording session. EMG resets counts were computed according to dose, time block and sleep stage. Along with SL I treatment, GHB doses of 100, 300, 500, 750 and 1,000 mg/kg were used in the univariate analysis of variance for repeated measures. Post hoc analyses were based on the Dunn's method ($p < .05$).

Data from the portion of the study dealing with the effects of GHB on TI were analysed for the four treatment levels, namely baseline, saline, 350 and 650 mg/kg of GHB. A trial at 1,000 mg/kg was also intended, but subjects were so sedated that no state comparable to TI could be induced. Mean TI durations and number

of failed trials were calculated independently for each cell and were analysed using the Friedman two-way analysis of variance test.

RESULTS

3.1 Effects of GHB on sleep-waking cycle patterns

Characteristic stage transitions are presented in Figures 1 to 5. Subcortical tracings (aimed at the lateral geniculate nuclei, bipolar recordings) are included in the figures, and although this activity was not essential in the present study, information from these tracings helped in stage determinations.

Baseline values obtained at both SL I and SL II levels (Table 3) agree with previously reported values from data gathered for similar time periods (Faure, 1963). Apparent discrepancies between proportions reported for W and D + SWS in this study compared to those of Kawakami and Sawyer (1962), Narebski et al. (1969) and Thoman et al. (1979) come from the fact that these recordings were done on a 24 hours basis, thus including a period when the rabbit usually exhibits predominantly W (Narebski et al., 1969).

Figure 1: Transition from waking (W) to drowsiness (D). Abbreviations for Figures 1 to 5 are: EOG: electrooculogram; EEG Cort.: cortical EEG; EEG Subcort.: subcortical EEG; EMG: nuchal EMG; INT. EMG: integrated EMG. Vertical arrows indicate the moment of transition from one state to another. Vertical calibration marks = 100 µv.

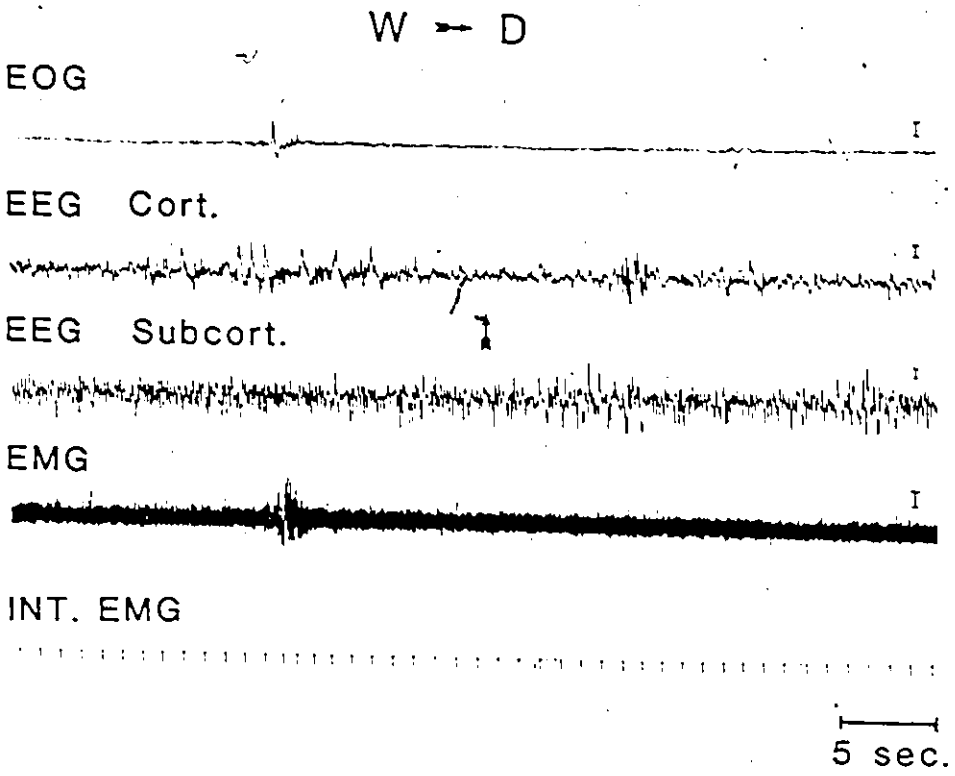

W → D
EOG
EEG Cort.
EEG Subcort.
EMG
INT. EMG
5 sec.

Figure 2: Transition from drowsiness (D) to slow wave sleep (SWS).

D → SWS

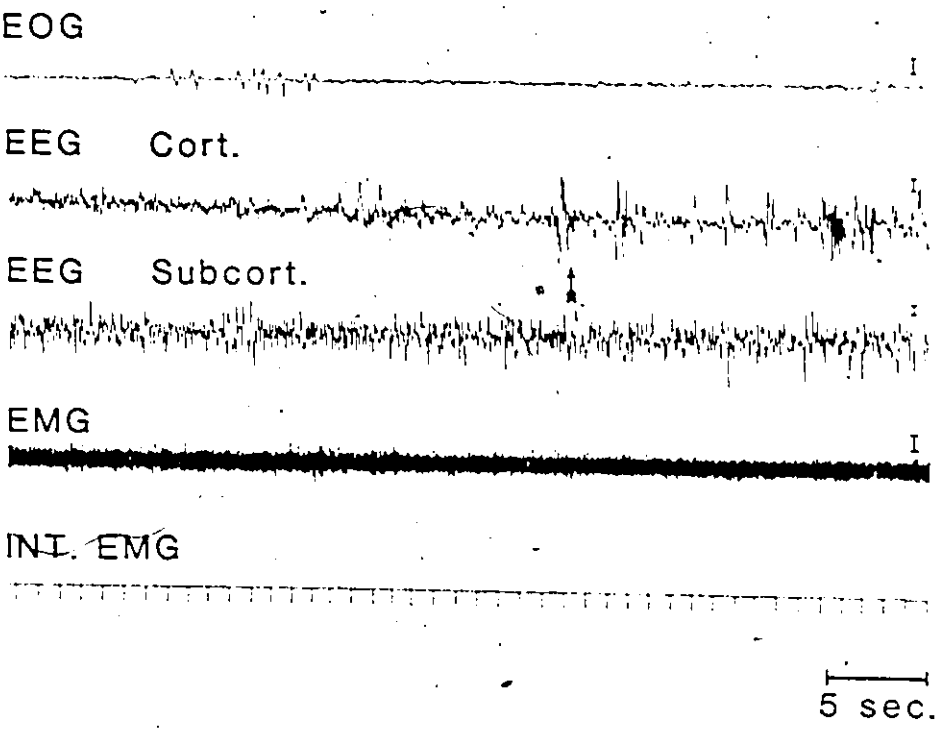


Figure 3: Transition from slow wave sleep (SWS) to paradoxical sleep (PS).

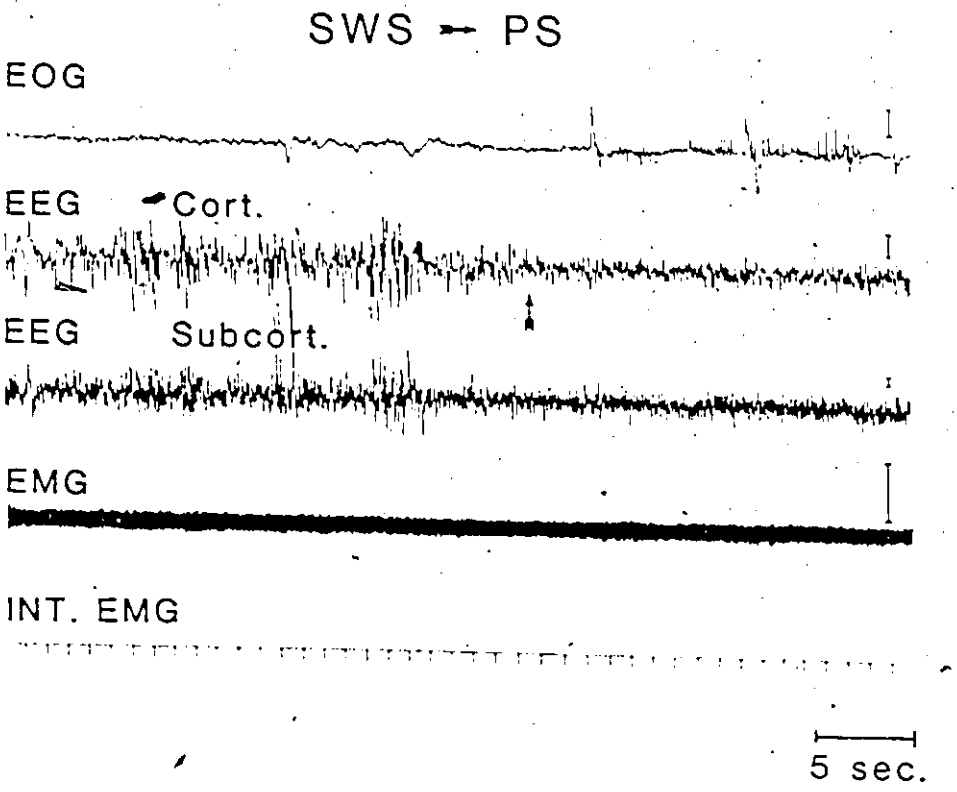

SWS → PS
EOG
EEG Cort.
EEG Subcort.
EMG
INT. EMG
5 sec.

Figure 4: Transition from slow wave sleep (SWS) to waking (W).

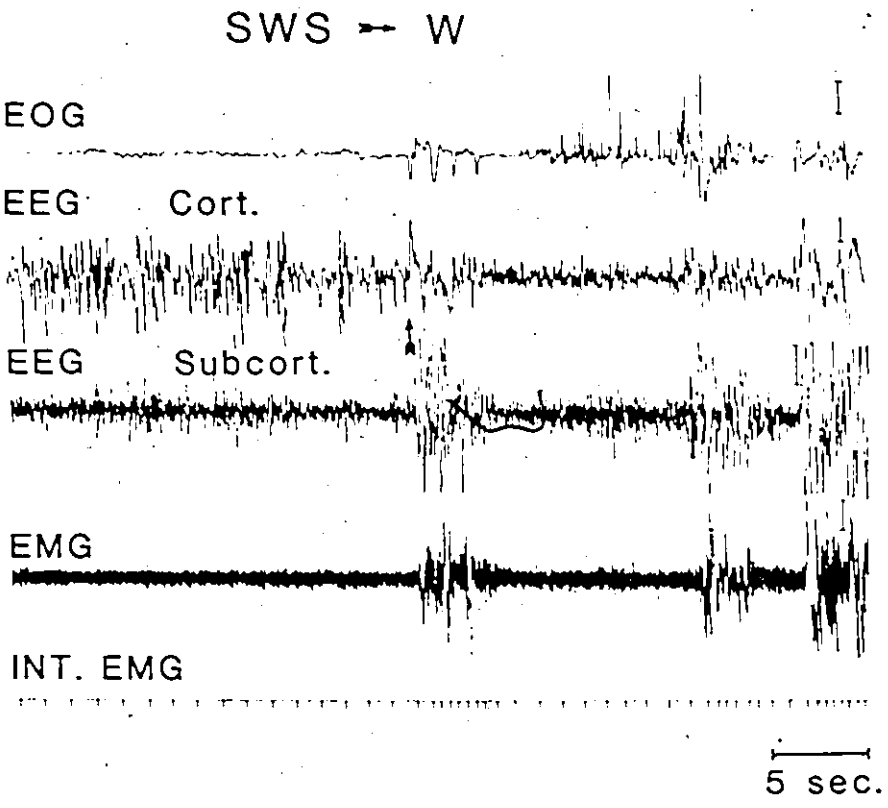

SWS → W
EOG
EEG Cort.
EEG Subcort.
EMG
INT. EMG
5 sec.

Figure 5: Transition from paradoxical sleep (PS) to waking (W).

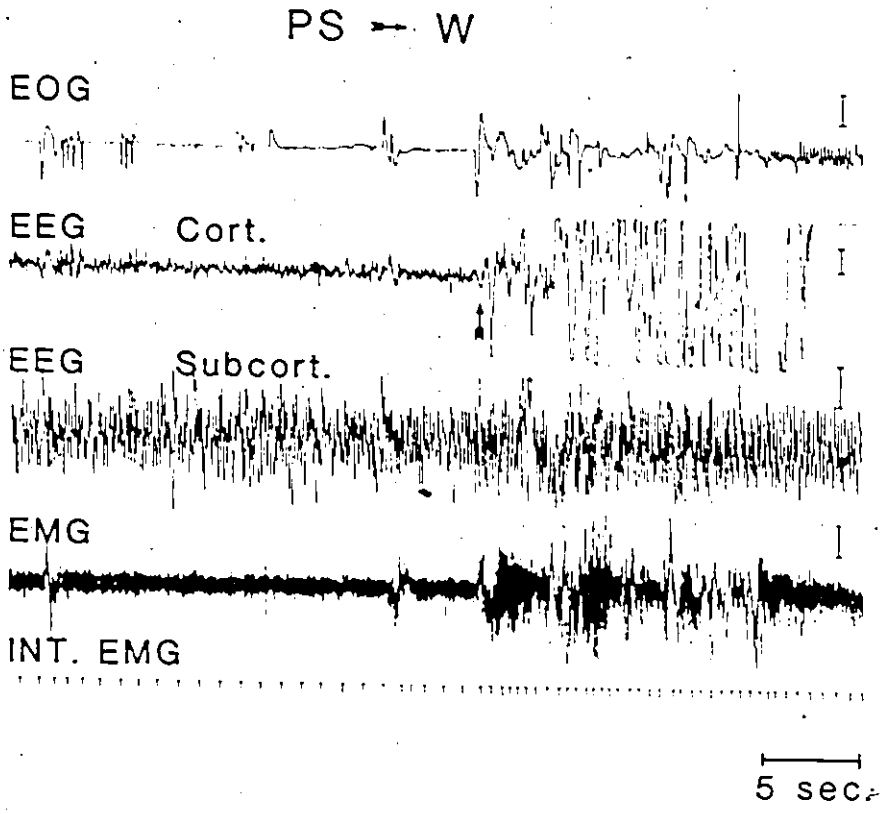

PS → W
EOG
EEG Cort.
EEG Subcort.
EMG
INT. EMG
5 sec.

Table 3

Baseline proportions (%) of total recording time for each stage of the sleep-waking cycle

	This study SL I	This study SL II	Faure, 1963	Kawakami and Sawyer, 1962	Narebski et al., 1969	Thoman et al., 1979
W	27.5	25.4	23.0	60.1	71.3	56.0
D + SWS	69.8	73.3	60.0	36.7	25.5	29.7
PS	2.7	1.3	7.0	2.7	3.1	3.3

Mean latencies to sleep stages (D, SWS, PS) were calculated at each treatment level and results from this analysis are presented in Appendix A and illustrated in Figure 6. One-factor univariate analysis of variance with repeated measures was performed (as for every following analysis) for each stage with post hoc analyses based on the Dunn's method (all at $p < .05$).

Only the latency to PS was significantly affected ($p < .08$) and post hoc tests showed that only doses of 750 and 1,000 mg/kg were associated with a greater latency since PS was completely absent at these dose levels. The reduction of PS onset latency at 150 mg/kg did not reach statistical significance.

Data concerning the proportions of total recording time for each stage of the sleep-waking cycle according to GHB doses used are summarized in Appendix B and illustrated in Figure 7. The amount of D was not affected by any of the treatment levels, but effects were observed for W ($p < .05$), SWS ($p < .07$) and PS ($p < .1$). SL treatments were not associated with any significant differences in proportions when compared to various GHB doses. However, post hocs showed that a dose of 1,000 mg/kg significantly increased proportions of W when compared to 100 and 200 mg/kg, and of SWS when compared to 200 mg/kg. Still, the most obvious effect was on PS

which was completely abolished at 750 and 1,000 mg/kg. Again, there was an increase of PS at 150 mg/kg which did not reach significance.

Figure 6: Mean latencies (min.; ± standard error: SE) to stage onset across doses. All recording sessions are of two hours duration and began with a W epoch.

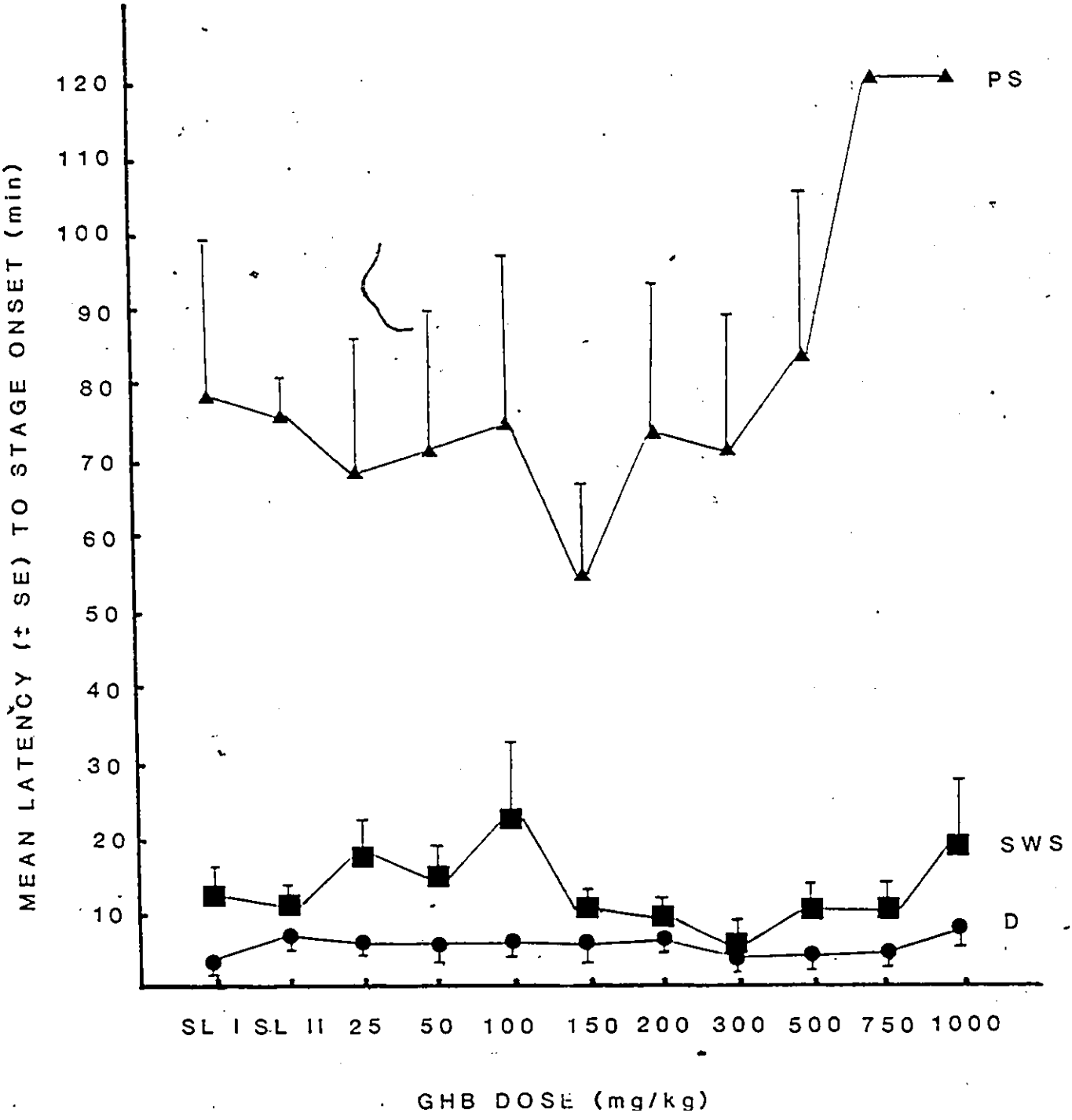
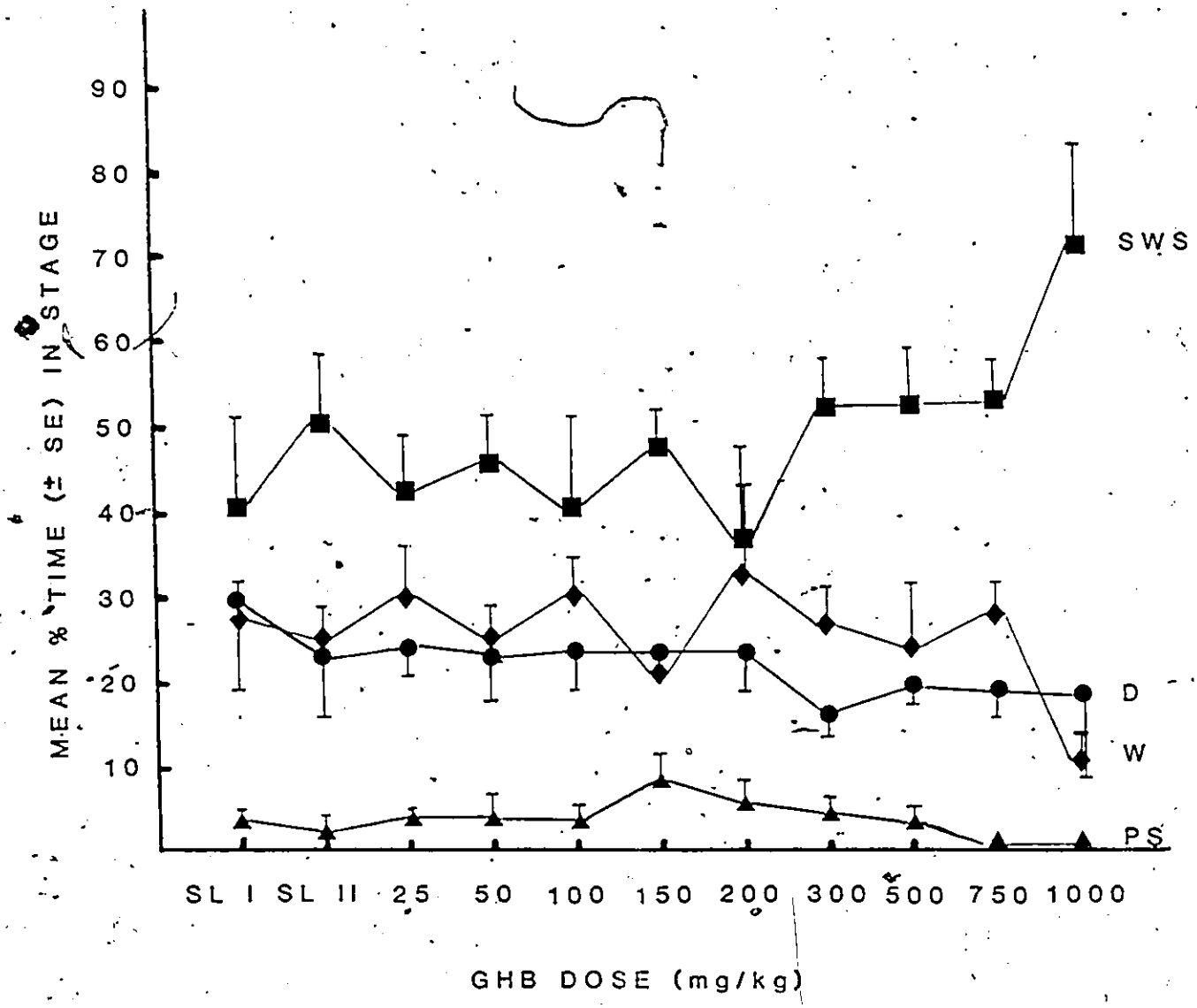

MEAN LATENCY (± SE) TO STAGE ONSET (min)
120
110
100
90
80
70
60
50
40
30
20
10
PS
SWS
D
SL I
SL II
25
50
100
150
200
300
500
750
1000
GHB DOSE (mg/kg)

Figure 7: Mean proportions (± standard error: SE) of total recording time (%) for sleep-waking stages across doses. No PS was observed at 750 and 1,000 mg/kg GHB.


MEAN % TIME (± SE) IN STAGE
90
80
70
60
50
40
30
20
10
SWS
D
W
PS
SL I
SL II
25
50
100
150
200
300
500
750
1000
GHB DOSE (mg/kg)

3.2 Effects of GHB on behavior and EEG patterns during the sleep-waking cycle

Post-GHB behavioral changes were not noted at doses lower than 300 mg/kg. At that level, some diminution in alertness was observed in the majority of animals in both experimental groups. Animals were less active and manipulations were facilitated. In the 650-750 mg/kg dose range, all animals exhibited these features and at 1,000 mg/kg, they were more sedated and even TI could not be induced immediately after injection since the animals were flaccid. No cortical spike discharge or gross bodily movements were detected at any dose, although at times some twitching activity was present in the EMG tracings.

At the lower and mid-range dose levels (up to 500 mg/kg), GHB-related effects on patterns of EEG activity were not evident. However, at 750 mg/kg, an alteration in EEG occurred in most animals with variable latency (Table 4), and at 1,000 mg/kg the effect was present in all animals. This drug-related variation consisted of the occurrence of high amplitude (250-500 μv), slow (2-4 Hz) waves which became progressively more concentrated at the 1,000 mg/kg dose-level (Figure 8). This activity, termed GHB-slow wave (G-SW), was superimposed on otherwise characteristic D or SWS EEG patterns.

Coincident with behavioral arousal from G-SW episodes, these EEG patterns would disappear and desynchronized patterns characteristic of W would reappear.

Table 4

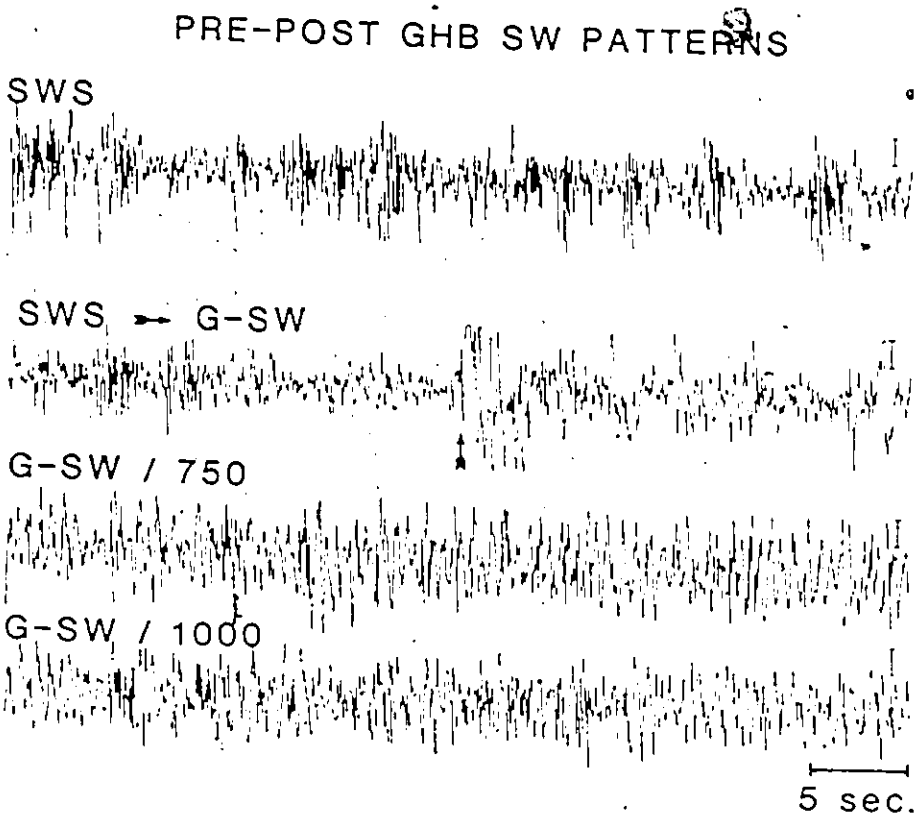
Onset latencies to G-SW activity (min.)

	750 mg/kg	1,000 mg/kg
Rabbit 1	none	19
Rabbit 2	13	22
Rabbit 3	35*	17
Rabbit 4	32	48
Rabbit 5	**	56

* ended 4 minutes before termination of the session

** Rabbit 5 died before the 750 mg/kg session

Figure 8: Slow wave activity associated with GHB administration. GHB-slow waves (G-SW; 250-500 μV, 2-4 Hz waves) appeared at dose levels of 750 (G-SW/750) and 1,000 (G-SW/1,000) mg/kg. The initial onset (vertical arrow) of this activity is shown in the second tracing (SWS -> G-SW), and characteristic patterns at 750 and 1,000 mg/kg are illustrated.


PRE-POST GHB SW PATTERNS
SWS
SWS → G-SW
G-SW / 750
G-SW / 1000
5 sec.

3.3 Effects of GHB on EMG levels of activity

EMG resets counts were analysed according to dose (SL I, 100, 300, 500, 750 and 1,000 mg/kg), sleep wakefulness stage (W, D, SWS and PS) and time block (first, middle and last 15 minutes of total recording period). Significant variations on EMG levels were noted only for dose levels ($p < .006$) and sleep-waking stages ($p < .000001$) thus stressing the fact that the effects were comparable across the three time blocks. These results, collapsed across time for the five animals, are illustrated in Figure 9 and 10. Post hoc tests on dose levels revealed that rabbits injected with 750 mg/kg of GHB displayed significantly increased EMG levels of activity when compared with either SL I or 300 mg/kg levels. Post hoc tests on EMG resets counts across sleep-waking stages showed an increased EMG level of activity in W when compared with either D, SWS and PS. Analysis of dose and stage interactions did not reach significance.

Figure 9: Quantified EMG activity levels (means and standard errors) across GHB doses. Data are based on 45 minutes of recording per rabbit per dose.

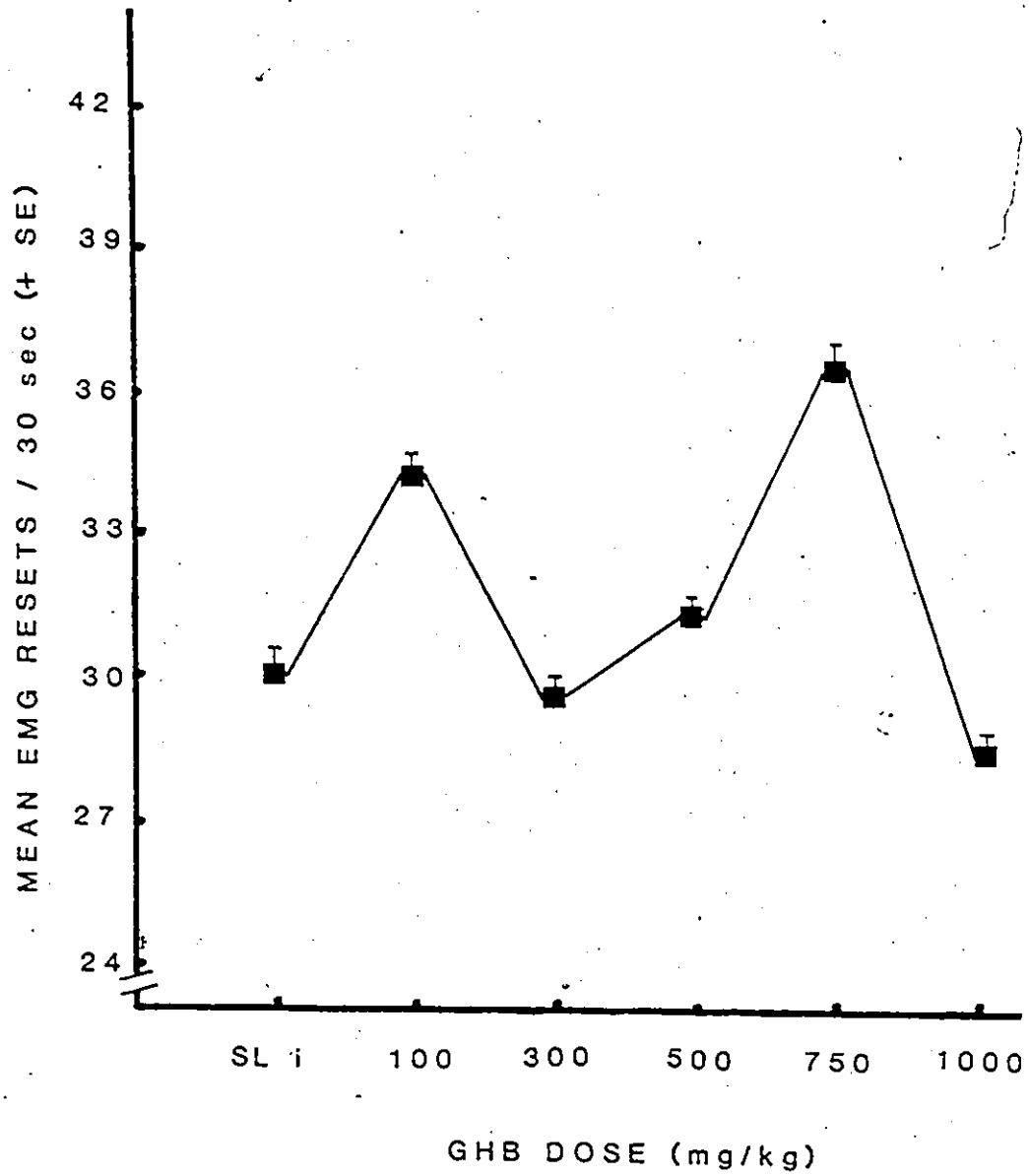
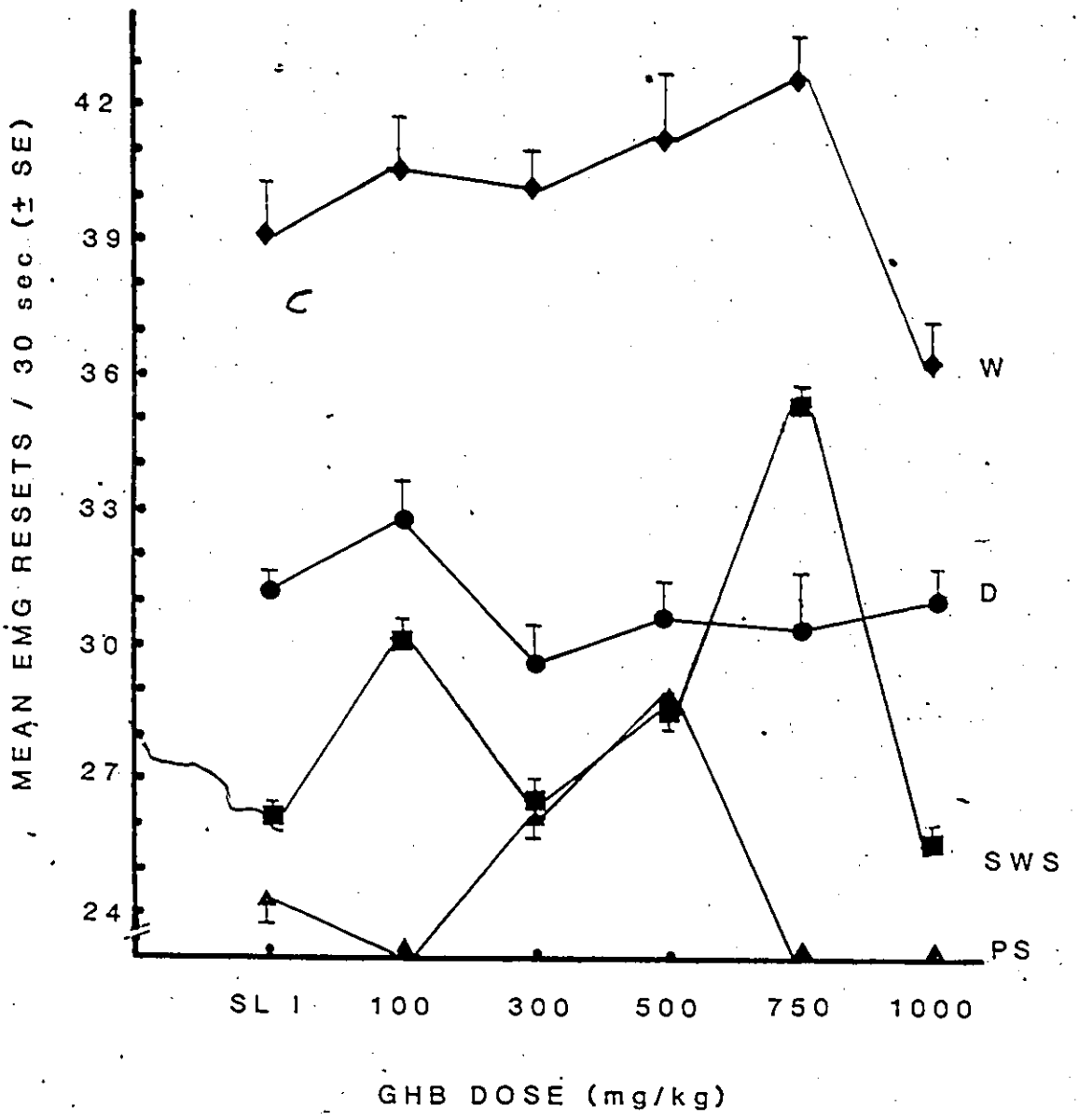

MEAN EMG RESETS / 30 sec (+ SE)
42
39
36
33
30
27
24
SL i
100
300
500
750
1000
GHB DOSE (mg/kg)

Figure 10: Mean levels (± standard errors) of quantified EMG activity across doses for each stage. Note that at 100 mg/kg, no PS epoch was scored in any 15 minute sample used, even though it was present at other times following the same dose.


MEAN EMG RESETS / 30 sec (± SE)
42
39
36
33
30
27
24
W
D
SWS
PS
SL I
100
300
500
750
1000
GHB DOSE (mg/kg)

3.4 Effects of GHB on TI

Behaviorally, rabbits exhibited the normal characteristics of TI during BL, SL, 300 and 650 mg/kg trials, i.e., the animals were immobile, unresponsive, and rigidity of limb musculature could be observed; eyes could be either open or closed, and the animals could exhibit unsuccessful attempts to right themselves. Respiratory rates, calculated from five second samples visually determined, were variable within episodes and did not differ between experimental treatments. Except on one occasion where micturition occurred after the first trial at 300 mg/kg, micturition or defecation did not occur during the experimental sessions.

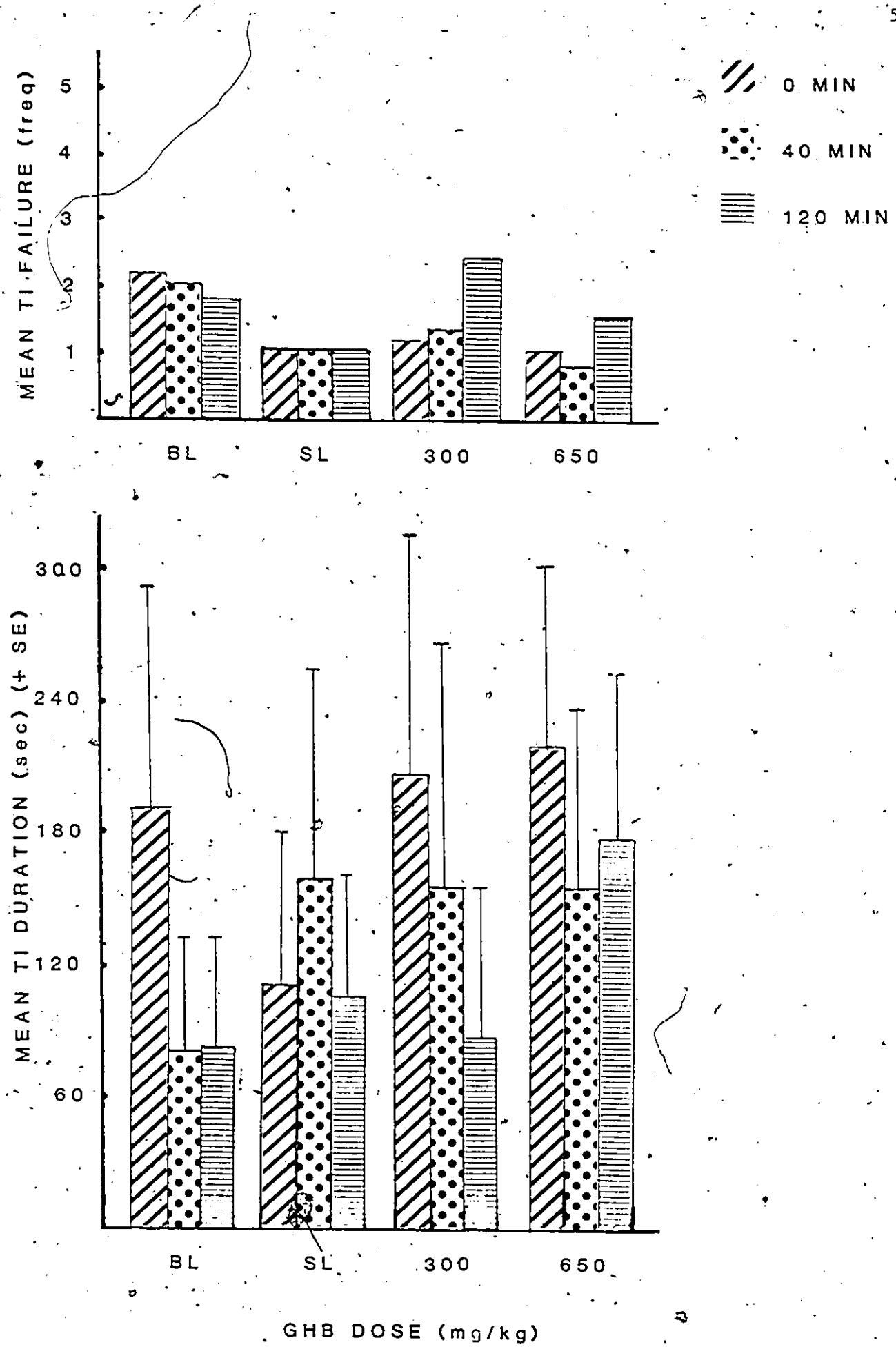
No TI could be induced immediately after injection of 1,000 mg/kg of GHB since the normal characteristics of TI were not observed in any animal. Instead, the rabbits were flaccid and eyes were always closed. A very slow respiratory rate was prominent and no unsuccessful attempts at righting were noted. For this reason, these sessions were discarded and were not included in the data analyses.

For each time block (0, 40 and 120 minutes after injection) at each treatment level (BL, SL, 300 and 650 mg/kg of GHB), three measures of TI were obtained: duration of the TI episode, frequency of failure (un-

successful TI induction), and electrographic recordings. The latter recordings were taken in a subsample of rabbits following administration of 650 mg/kg GHB after the experimental schedule was completed. This was done to avoid possible confounding effects of recordings on the experimental treatment.

Since the assumption of homogeneity of the variance was not satisfied, mean TI durations and failure frequency differences between doses and time blocks were analysed using the non-parametric Friedman two-way analysis of variance test. Results illustrated in Figure 11 are summarized in Appendix C.

Figure 11: Frequency of TI induction failures and mean durations (sec.; + standard errors: SE) of TI episodes across conditions as a function of three time intervals after injection.


0 MIN
40 MIN
120 MIN
MEAN TI FAILURE (freq)
5
4
3
2
1
BL
SL
300
650
MEAN TI DURATION (sec) (+ SE)
300
240
180
120
60
BL
SL
300
650
GHB DOSE (mg/kg)

While no significant differences in mean duration or failure frequency was found between time blocks or dose levels, a trend ($p < .1$) toward lenghthening of TI episodes was observed at GHB dose levels of 300 and 650 mg/kg relative to non-GHB (BL and SL) conditions. Baseline electrographic recordings during TI were characterized by the absence of marked EEG change, i.e., 150 Uv, 8-9 Hz activity was maintained, and a reduction in nuchal EMG activity (see Figure 12). Representative tracings from one subject for the three time blocks following 650 mg/kg presented the following patterns: in the minute following injection (Figure 13), normal EEG activity in W shifted upon TI induction to a high voltage, slow activity pattern (4-5 Hz, 250-275 Uv) without spindle activity as described in 3.2; at 40 minutes (Figure 14), TI induction was followed immediately by EEG patterns resembling G-SW (250 Uv, 4 Hz) and spindle activity appeared within a minute; after 120 minutes (Figure 15), EEG before and after TI induction was similar and was comparable to G-SW and to patterns observed during TI after 0 and 40 minutes.

Figure 12: Transition from W to TI in an untreated rabbit. For Figures 13 to 16: EOG: electrooculogram; EEG Cort.: cortical EEG; EEG Subcort.: subcortical EEG, aimed at right hippocampus and referred to right motor cortex; EMG: nuchal electromyogram; INT. EMG: integrated electromyogram. Vertical arrows indicate the moment of state transition. Vertical calibration marks = 100 µV.

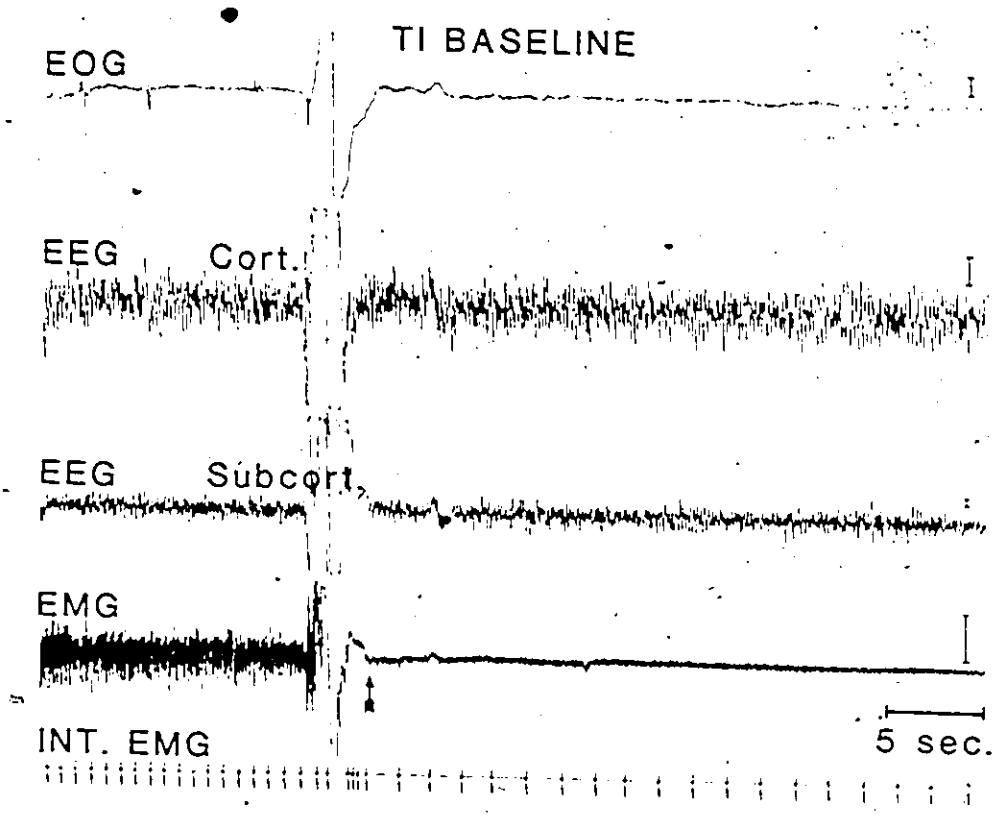

TI BASELINE
EOG
EEG Cort.
EEG Subcort.
EMG
INT. EMG
5 sec.

Figure 13: Transition from W to TI immediately following 650 mg/kg of GHB.

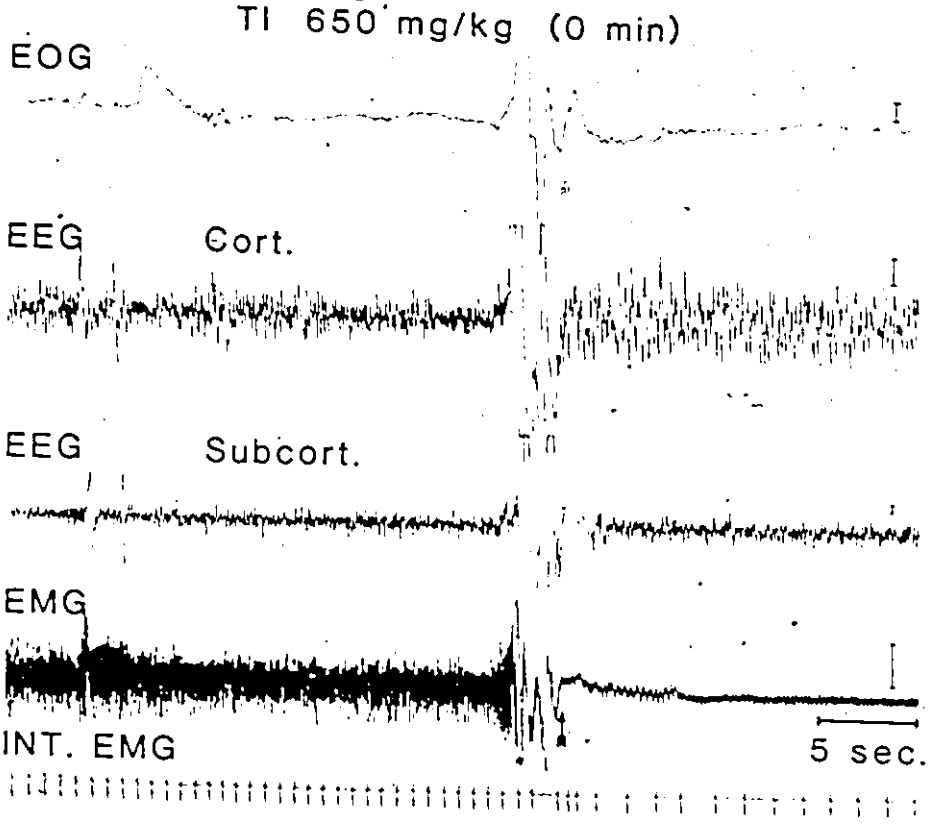

TI 650 mg/kg (0 min)
EOG
EEG Cort.
EEG Subcort.
EMG
INT. EMG
5 sec.

Figure 14: Transition from W to TI 40 minutes after 650 mg/kg of GHB.

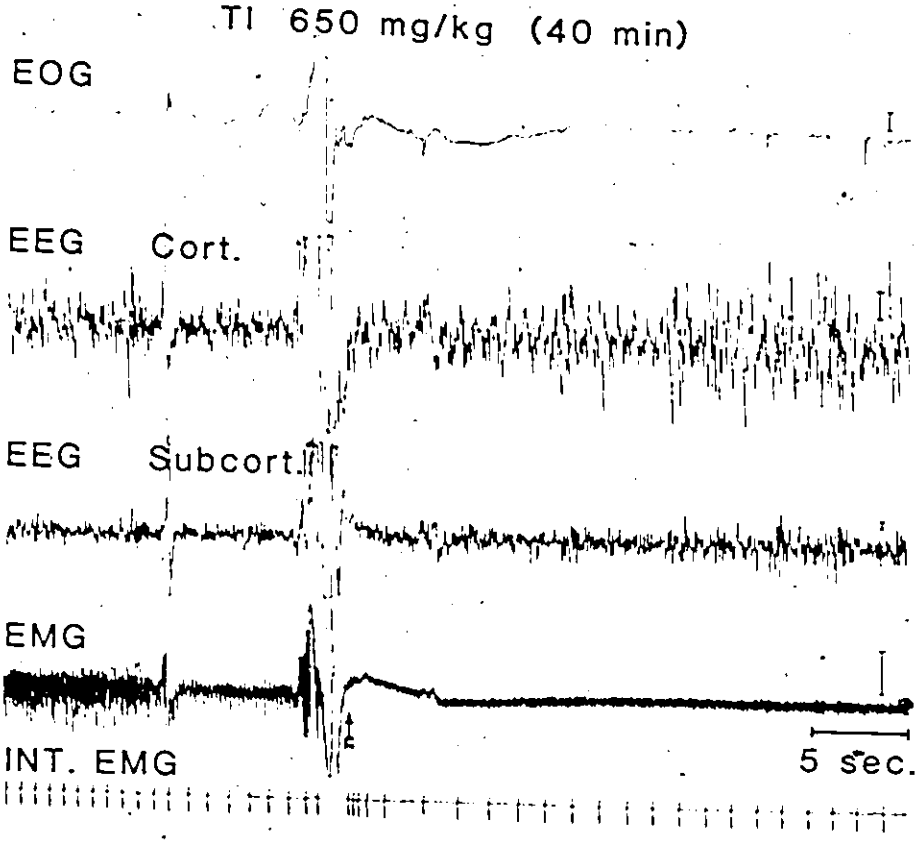
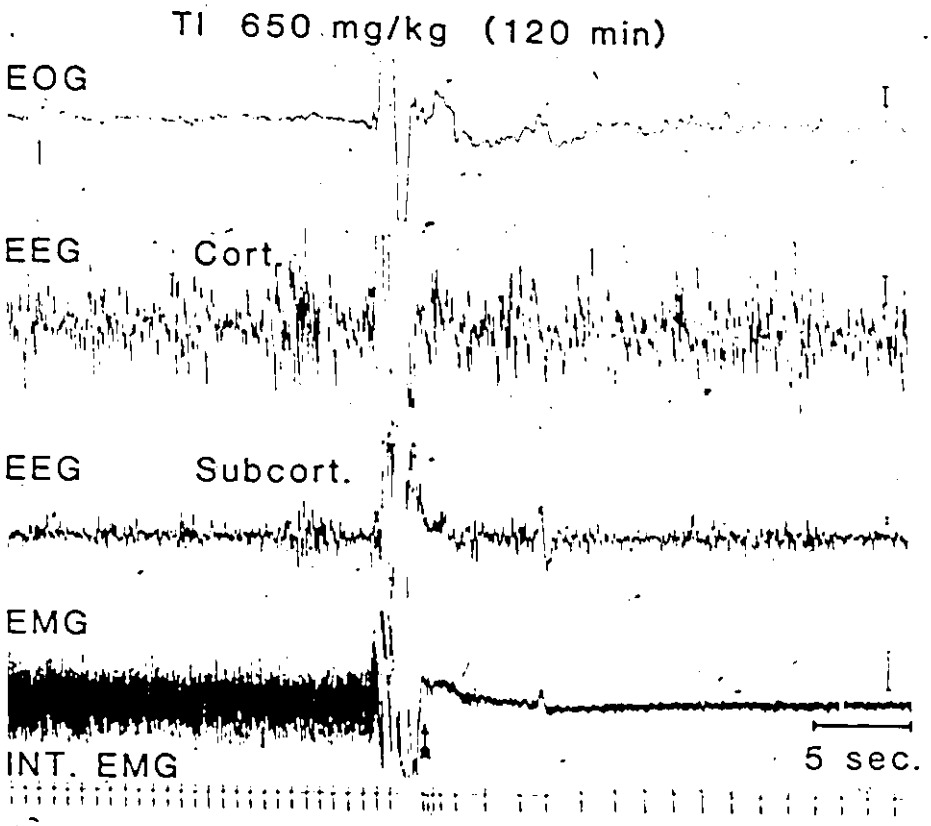

Tl 650 mg/kg (40 min)
EOG
EEG Cort.
EEG Subcort.
EMG
INT. EMG
5 sec.

Figure 15: Transition from W to TI 120 minutes after 650 mg/kg of GHB.


TI 650 mg/kg (120 min)
EOG
EEG Cort.
EEG Subcort.
EMG
INT. EMG
5 sec.

DISCUSSION

The sleep-wake cycle pattern results of this study failed to support the postulated sleep inducing or PS enhancing effects of GHB, i.e., latency to onset of SWS or PS was not diminished at any of the dose-levels used. Moreover, no differences were found between time spent in W, D or SWS across GHB dose levels relative to the saline control levels. However, it was observed that PS was abolished at high GHB doses (750 and 1,000 mg/kg). In view of these largely negative results, the possibility that these effects resulted from uncontrolled confounding variables must be examined.

Two possible sources of such experimental bias are: recording time (duration and time of day) and stress factors. Perhaps the recording periods were too brief to detect GHB-related effects or perhaps circadian factors (time of day) dampened certain effects, e.g., reduced the tendency to sleep or to experience certain sleep stages. Since Snead et al. (1976) reported a maximum concentration of GHB in serum within 40 minutes and in CSF within two hours at a dose of 500 mg/kg, two hours seems to be a recording period adequate to include time intervals during which maximal drug related effects should be occurring; moreover, other state and event correlates discussed in

section 1.4 (e.g., changes in DA and ACh activity, behavioral depression, motoric activation, EEG alterations) have always been reported to occur well before two hours post-injection.

The possible influence of circadian factors on sleep patterns was well controlled by scheduling sessions between 9:00 and 17:00 hours, a period that should maximize occurrence of sleep in general and PS in particular (Narebski et al., 1969).

It is known that the rabbit is a very sensitive animal and that both sleep patterns and TI are influenced by stress. Possible sources of stress in this investigation were injection procedures and volume of substance injected. To control for these variables, two saline controls were included, corresponding respectively to GHB doses of 300 and 1,000 mg/kg, and results with such treatments were similar to reported control values in the rabbit (see 3.1).

Other potentially stressing factors such as accommodation to recording environment and recovery from surgery were controlled for by allowing generous time periods preceding experimental recordings. Thus, the results of the present study cannot be readily attributed to these methodological shortcomings or contaminating factors.

Closer examination of the data shows that W decreas-

ed at 1,000 mg/kg when compared to 100 and 200 mg/kg, and that SWS values at 1,000 mg/kg were enhanced relative to those at 200 mg/kg. These observations, together with the consideration that PS was abolished at 750 and 1,000 mg/kg, hint at an effect of GHB on patterns of the sleep wake cycle. Figure 7, which presents data on duration of stages across GHB dose levels, is interesting in this respect: at a dose of 200 mg/kg and higher, there is a progressive, although statistically nonsignificant, increase in SWS durations, as well as a progressive decrease in PS duration which begins at 150 mg/kg. It thus seems that effects of GHB become apparent from around 150 mg/kg and increase with dose. Such a progressive tendency of W to decrease and SWS to increase support the hypothesis formulated by Schneider et al. (1963) that increasing doses of GHB progressively depress the ascending reticular activating system (ARAS). From the present results, we can hypothesize that this depression of the ARAS is noticeable only relative to an activating effect observed at lower doses (150-200 mg/kg). The depressive phase of such a bipolar effect could not be accounted for by an exhaustion of ARAS neural elements following overstimulation since the postulated activating phase did not reach a level that could permit such an overreaction, i.e., W and SWS were not

different from baseline. A possible explanation of the observed bipolar effect could lie in the epileptogenic properties of GHB at very high doses (Snead, 1976). However, not much effort has been devoted to this issue as related to the present problem.

The possibility that G-SW obscured the EEG tracings and contaminated reliability of stage determination should be considered. However, since G-SW consistently appeared only after SWS was initiated, and since in one case (rabbit no. 1, at 750 mg/kg) no G-SW was observed although D and SWS onset latencies were the shortest of the group, the SWS enhancing effect cannot be accounted for by an artifactual effect of G-SW.

An EEG phenomenon comparable to G-SW was reported by Longo (1966), White and Boyajy (1960) and Wikler (1952) following atropine administration, but enough dissimilarities exist to suggest that the phenomenon they reported is different from the G-SW described in the present study. For example, atropine is known to induce spindle bursts (8-12 Hz) along with delta activity and this pattern was observed to persist through W (Jouvet, 1972; Longo, 1966). This was not the case for GHB in the present study since EEG activity desynchronized upon W, except at 120 minutes after administration of 650 mg/kg. Even then, no spindle activity

was observed during W. Behaviorally, Longo (1966) proposed that the state induced by atropine should be termed "confusional" as opposed to full sleep or waking as observed in the present study. Moreover, Karczmar (1979) stressed the fact that atropine is not accompanied by behavioral depression or sleep, which was the case with GHB.

The hypothesis related to motoric activation in the present study stated that doses of 500-750 mg/kg would evoke higher EMG levels while 1,000 mg/kg would be associated with motoric depression. The results of the quantitative analysis of EMG activity after 500-750 mg/kg partly support this hypothesis and contradict the suggestion by Schneider et al. (1963) regarding the proposed early intervention of GHB in thalamo-caudo-cortical structures, since a significant increase in EMG resets counts, possibly reflecting the depression of the motoric inhibitory system centralized in caudate nucleus, was not observed before 750 mg/kg. The sudden decrease observed at 1,000 mg/kg gives clear support to the hypothesis stated above and may reflect the mechanism proposed by Schneider et al., 1963 about a late depression of the reticulo-spinal system (responsible for activation of spinal motoneurons).

The fact that, regardless of dose, W was always found to exhibit higher EMG activity levels relative to other stages, is in accordance with previous findings; however, it is uncommon to observe, as in the present study, no tonic reduction in EMG activity during PS. This phenomenon has been previously observed in the rabbit (Tabushi and Himwich, 1969; Pivik et al., 1981) and artifactual influences such as electrical interference, recording procedure or differences in strains of the species have been discarded as possible explanations for the phenomenon; rather, a less powerful tonic inhibitory system was hypothesized for the rabbit, and such a proposition is not contradicted by the present results.

TI analyses revealed many important results, among which is the interesting fact that the stress induced by the administration procedure (injection) of the saline solution did not alter significantly the ease of induction or duration of the TI trials relative to the baseline control, even if a trend could be observed. This might be explained by the fact that insertion of the needle was not a sufficiently stressful experience to the rabbits, but a more important observation is that the additional manipulations brought about by the injection procedures did not contaminate the results.

The observation that GHB is followed by longer TI episodes, regardless of wich of the two experimental doses was used (contrary to the dose specificity expected) supports the reported effect of DA and ACh activity antagonists which are known to facilitate TI duration. It has to be noted, however, that the ease of induction of TI, reflected by the amount of failed trials, was not influenced by administration of GHB. It was also shown that TI is a phenomenon which is not dependent upon electrocortical activity since the behavioral manifestations of this state were induced and maintained despite obviously different EEG patterns. The fact that S-SW appeared during TI at a time when it was not yet noticeable on the W tracings also illustrates this absence of relationship between the state and its concomitant EEG activity and suggests a different underlying mechanism. Furthermore, since it was impossible to induce TI after administration of 1,000 mg/kg GHB, a dose hypothesized to depress the reticulo-spinal system, it is likely that a motor component, rather than a cortical one, is most critical to TI. It seems that TI induction stimulates the mechanism that triggers S-SW and thus makes the two phenomena related somehow, but more definite identification of such a mechanism is still obscure.

The information gathered on TI and PS during this investigation leads to the conclusion that PS and TI are not obviously and readily related. This is supported by the following facts: 1) PS has specific EEG characteristics while TI does not; 2) effects of GHB on TI and PS durations are not similar: TI is facilitated by 300 and 650 mg/kg while comparable dose levels present a progressive inhibitory effect on PS; more precisely, TI persists, and is even facilitated, whereas PS is abolished at comparable high doses (650 and 750 mg/kg respectively).

This last comment regarding high doses of GHB is of particular interest when associated with the observation that the EMG level of activity was increased with a comparable dose (750 mg/kg). These results suggest that high motoric activation may differentially influence PS and TI durations. The underlying mechanism of this proposed relationship is unclear but a tentative explanation will be presented for the purpose of discussion.

It was observed that comparable doses of GHB (low enough not to modify EMG level of activity) had a facilitatory effect both on PS (at 150 mg/kg) and TI (at 300 mg/kg). However, it is possible that the motoric activation associated with high doses of GHB (750 mg/

kg) is antagonistic to and overrides the "triggering mechanism" of PS. Such a mechanism has been hypothesized by Jouvet (1969, 1972) to be composed of phasic events (just preceding PS episodes (isolated, non-clustered PGO spikes, EMG phasic drops) and to be followed by the PS "maintenance mechanism", which would be composed mainly of the tonic events of PS (EMG atonia, EEG desynchronization). Since TI is a tonic state induced by external stimulation, it does not require an endogenous "triggering mechanism" and it is thus hypothesized that TI may sustain higher EMG levels of activity. This proposed model would support the hypothesis that inhibitory components of PS are weak in the rabbit (Pivik et al., 1981).

It can be concluded that even if the relationship between PS and TI could not be clearly stated from the present results, a common ground to the two states could not be ruled out since PS and TI tended to react in the same direction (facilitation) to low doses of GHB (150 and 300 mg/kg, respectively). However, the specificity in the process of induction of the two states is clearly a major difference and a possible source of difficulty when the two behaviors are manipulated for the purpose of comparison. It is apparent from the present study that PS and TI may possibly

share more than behavioral correlates, and the nature of this interaction is worthy of further study.

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Appendix A

Mean latencies to each stage of the sleep-waking cycle (min.)

	SL I	SL II	25	50	100	150	200	300	500	750	1,000
D	4.3	7.1	6.4	6.2	6.2	5.8	6.3	4.3	4.8	4.9	7.5
SWS	12.2	11.1	16.9	15.1	20.7	10.1	9.4	6.7	10.8	10.6	18.5
PS	79.4	75.9	68.6	70.6	74.3	54.2	73.1	71.2	83.4	120.0	120.0

Appendix B

Proportions of total recording time (%)
for sleep-waking stages across doses

	SL I	SL II	25	50	100	150	200	300	500	750	1,000
W	27.48	25.44	30.42	26.48	30.50	21.36	33.76	26.72	24.78	27.82	10.32
D	29.04	22.74	24.62	24.44	24.62	23.32	23.56	16.70	19.94	19.22	18.18
SWS	40.76	50.64	42.04	45.26	41.88	47.62	37.18	52.26	52.42	52.92	71.52
PS	2.72	1.32	3.30	3.82	3.00	7.70	5.52	4.60	2.88	0.00	0.00

Appendix C

TI mean durations (min.)

	BI	SI	300	650
Rabbit 1	00.617	00.592	00.728	02.649
Rabbit 2	00.346	00.307	00.433	00.315
Rabbit 3	03.533	00.336	10.000	01.497
Rabbit 4	04.787	06.493	06.098	06.932
Rabbit 5	00.117	02.686	00.326	02.344

TI failures (frequencies

	BI	SI	300	650
Rabbit 1	4	3	3	1
Rabbit 2	0	3	2	7
Rabbit 3	13	7	14	8
Rabbit 4	0	0	0	0
Rabbit 5	13	2	6	2