THE UNIVERSITY OF CALGARY

Electrophysiological and Neurochemical Effects of Hydrogen Sulphide

by

Barbara Skrajny

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Electrophysiological and Neurochemical Effects of Hydrogen Sulphide" submitted by Barbara Skrajny in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

The neurotoxic effects of high concentrations of hydrogen sulphide (H_2S) are well documented; however there are limited data on chronic exposure to low levels of this toxicant. The objective of this thesis was to determine the effects of low levels of H_2S on hippocampal electroencephalographic activity (theta) in correlation to changes in monoamine and amino acid levels.

It was demonstrated that exposure to low levels of H_2S produced an increase in total power of hippocampal theta activity in a dose dependent manner. Repeated exposure resulted in a cumulative effect on hippocampal theta that required 2-3 weeks for complete recovery. It was also shown that exposure to 100 ppm of hydrogen sulphide altered monoamine and amino acid levels in rat hippocampus, frontal cortex and cerebellum. It is postulated that the observed neurochemical and electrophysiological effects may be relevant to neurological symptoms described for humans following hydrogen sulphide intoxication.

ACKNOWLEDGEMENTS

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DEDICATION

This thesis is dedicated to my husband Andrzej

and to my parents Halina and Kazimierz

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1.0 INTRODUCTION

1.1 Overview

The unpleasant odour and potential toxicity of hydrogen sulphide (H_2S) were noted early in human history. The first publication about its toxicity was published in 1713 by the father of occupational medicine Bernardo Ramazzini (Ramazzini, 1713). In his book titled *De Morbis Artificum (Diseases of Workers)*, Ramazzini described a painful inflammation of the eyes which was common among the cleaners of privies and cesspits. He hypothesized that the eye irritation was caused by some volatile acid released in the cleaning process (Ramazzini, 1713). Hydrogen sulphide itself was discovered later in the same century by Carl Wilhelm Scheele, a chemist from Sweden (Smith, 1989).

Animal experimentation on the effects of hydrogen sulphide began during the end of the 19th century. In 1863 Hoppe-Seyler (Smith, 1989) reported that passing a stream of pure hydrogen sulphide through a blood sample caused a formation of a greenish pigment, which he called sulphemoglobin. This observation led to the hypothesis that hydrogen sulphide was a blood poison analogous to carbon monoxide. In 1938 Belgian physiologists, the Heymans, (Smith, 1989) discovered that sulphide, cyanide and azide stimulate the chemoreceptors in the carotid body, and produce an increase in respiration. It was later demonstrated that

hydrogen sulphide as well as cyanide are potent inhibitors of cytochrome oxidase (Nicholls, 1975; Nicholls et al., 1976). Many experiments have been inspired by the analogy of hydrogen sulphide to hydrogen cyanide (Peterson, 1977; Wever et al., 1975), however, it has been demonstrated that the two chemicals may be toxicologically different, e.g. hydrogen sulphide has a greater tendency to produce pulmonary edema than cyanide (Beauchamp et al., 1984). Recently it was suggested that H_2S acts via mechanisms other than inhibition of cytochrome oxidase e.g. inhibition of monoamine oxidase (Warenycia et al., 1989b) or disruption of membrane lipids (Beck et al., 1983). Despite such a long history, the toxicology of hydrogen sulphide is still not fully understood, nor are the mechanisms of its toxicity or recovery.

1.2 Physical and Chemical Properties of Hydrogen Sulphide

Hydrogen sulphide is a gas, heavier than air with a density of 1.5392 g/litre at 0°C and atmospheric pressure. It burns in air with a pale blue flame and can be explosive when mixed with air in limits of 4.3% to 46% by volume (Beauchamp et al., 1984). H_2S can undergo two different types of oxidation reactions (Beauchamp et al., 1984); in the presence of oxygen, H_2S burns forming sulphur dioxide (equation 1.1), whereas with insufficient oxygen conditions it yields elemental sulphur (equation 1.2).

$$2H_2S + 3O_2 \rightarrow 2H_2O + 2SO_2 \qquad (\text{eq.1.1})$$

$$2H_2S + O_2 \rightarrow 2H_2O + 2S \qquad \text{(eq. 1.2)}$$

H_2S is soluble in both aqueous and organic solvents such as alcohol, ether and glycerol. Because of its high lipid solubility, it can easily penetrate biological membranes. H_2S has a low boiling point (-60.8°C) and a low evaporation energy (4.46 cal/mol) (Beauchamp et al., 1984).

In aqueous solution, H_2S dissociates in two stages. The first dissociation results in the formation of hydrosulphide ion (equation 1.3) whereas dissociation of the second proton yields the sulphide ion (equation 1.4) (Beauchamp et al., 1984).

$$H_2S \rightarrow H^+ + HS^- \qquad \text{(eq. 1.3)}$$

$$HS^- \rightarrow H^+ + S^- \qquad \text{(eq. 1.4)}$$

The pK_a for the first dissociation step is 7.04, and 11.96 for the second stage. At physiologic pH (i.e. pH = 7.4) about one-third of H_2S exists in the undissociated form, and two-thirds as the HS^- ion. (Beauchamp et al., 1984).

1.3 Emission Sources of Hydrogen Sulphide: Exposure

Environmental H_2S is derived from both natural and industrial sources. It is one of the principal compounds involved in the natural cycle in the environment (National Research Council, 1979). H_2S results from bacterial anaerobic decomposition of both plant and animal proteinaceous material (Copper et al., 1976; Sittig, 1975), and occurs in natural gas deposits such as "sour gas", sewers, volcanic gases and

sulphur springs. Approximately 100 million tons of H_2S are released into the atmosphere annually from natural sources. Although the majority of atmospheric hydrogen sulphide is from natural origins (Beauchamp et al., 1984), human industrial activities are also a significant source of this gas. H_2S is also a by-product of a variety of industrial processes such as petrochemical refineries, natural gas plants, pulp and paper mills, viscose rayon producers and food processing plants. It was estimated that three million tons of H_2S a year were of industrial origin (Urone, 1976). Atmospheric concentration of this pollutant ranges from 0.00007 ppm (0.0001 mg/m^3) in urban areas to 0.07 ppm (0.01 mg/m^3) in areas near industrial sites (Beauchamp et al., 1984). Much higher concentrations of hydrogen sulphide have been measured during accidental release; for example, following the accidental release of H_2S in Poza Rica, Mexico in 1950 (McCabe and Clayton, 1952), the concentrations of H_2S were estimated to be in range 1000-2000 ppm (1400-2800 mg/m^3).

Several occupations are at high risk to exposure of high concentrations of hydrogen sulphide. According to the standards established by the National Institute for Occupational Safety and Health (NIOSH) the concentration of H_2S at the work place should not exceed 10 ppm (14 mg/m^3) for an 8 hour working shift (NIOSH, 1977). It is well known, however, that hazardous exposures to high concentrations do occur accidentally. In the pulp and paper industry, workers

have been exposed to significant concentrations of H_2S (Goyer, 1990). These concentrations correlate to the specific production processes and can be as high as 35 ppm (49 mg/m^3), for example, in batch digester areas during manual wood loading (Goyer, 1990). Concentrations of hydrogen sulphide following accidental exposure in a work place can range from 100 ppm (140 mg/m^3), in which a worker lost consciousness while sawing ebonite boards (Brown, 1969), to as high as 12000 ppm (17000 mg/m^3) in a case of a truck driver who died while cleaning the tank used for transporting industrial waste (Simson and Simson, 1971).

1.4 Uptake, Distribution and Elimination of H_2S

The lung is the primary route of entry of H_2S into the body (Beauchamp et al., 1984)). Histochemical procedures have shown that inhaled H_2S is distributed to the brain, liver, kidneys, pancreas and small intestine (Voigt, 1955). The metabolism of hydrogen sulphide can be divided into three different pathways: (1) oxidation to sulphate; (2) methylation and (3) reaction with metallo- or disulphide-containing proteins (Beauchamp et al., 1984). It has been demonstrated in a number of studies that oxidation to sulphate and subsequent excretion by the kidneys is the major metabolic and excretory pathway (Dziewiatkowski, 1945). In his study, Dziewiatkowski (Dziewiatkowski, 1945) demonstrated that after oral administration of [^{35}S]-barium sulphide to rats, 50% of the

administered dose was excreted by the kidneys within 24 hours. The metabolites found in urine were sulphate or ethereal sulphate derivatives. Approximately 10% was excreted in the faeces and only 5% of the original dose remained in various tissues (Dziewiatkowski, 1945). The mechanisms of oxidation are not completely known, but it has been proposed that this metabolic pathway is enzymatically catalyzed by sulphide oxidase (Der-Garabedian, 1945 a and b). The sulphide oxidase activity has been found to be preferentially associated with the liver mitochondrial fraction (Barthomelew et al., 1980).

The second detoxification route is methylation of hydrogen sulphide to methanethiol and dimethylsulphide which are considered to be less toxic than H_2S. This reaction is catalyzed by thiol S-methyltransferase (Weisiger and Jakoby, 1980). The highest activity of this enzyme has been found in intestinal mucosa and liver (Weisiger and Jakoby, 1980).

The third biotransformation reaction of hydrogen sulphide is a combination with alkali metals present in metalloenzymes such as cytochrome oxidase. This reaction is regarded to be largely responsible for the toxic action of this gas (Beauchamp et al., 1984).

1.5 Toxicology of Hydrogen Sulphide

1.5.1 General Toxicological Considerations

Hydrogen sulphide is often referred to as a broad-spectrum toxicant because it affects most of the organ

systems. Mucous membranes and tissues with a high oxygen demand, however, are the most susceptible to the toxic action of this agent. Although the mechanisms of its toxicity are not completely understood, it is believed that hydrogen sulphide is a potent cytochrome c oxidase inhibitor (Nicholls, 1975; Wever et al., 1975) even more potent than cyanide (Smith et al., 1977). It has been reported that cytochrome c oxidase is inhibited upon exposure with sulphide *in vitro* (Nicholls et al., 1976; Wever et al., 1975) and recently it has been demonstrated that exposure of rats to sublethal concentrations of H_2S (50-400 ppm; 70-560 mg/m^3) produced significant depression in the activity of cytochrome c oxidase and succinate oxidase in the mitochondrial fraction of lung tissue *in vivo* (Khan, et al. 1990). It has been proposed that hydrogen sulphide binds to ferric ion of cytochrome oxidase and thus inhibits this terminal enzyme of electron transport (Beauchamp et al., 1984). This results in cessation of energy production followed by cell death. Other studies (Beck et al., 1983) suggest that another mechanism of acute poisoning may be the disruption of critical disulphide bonds of membrane lipids which causes rapid neurological dysfunction and respiratory paralysis.

Hydrogen sulphide toxicity is concentration dependent. At low concentrations it produces local inflammatory and irritative effects on moist membranes of the eye and the respiratory system (Beauchamp et al., 1984). At higher

concentrations it can cause systemic intoxication that may lead to death (Smith et al., 1979). The most common reported toxic effect of this toxicant, eye irritation, is often referred to as "gas eye" resulting from exposures to 10 ppm (14 mg/m^3) or even 5 ppm (7 mg/m^3) (Elkins, 1950). "Gas eye" is manifested by pain, lacrimation and photophobia. A more severe form of this condition may lead to keratoconjunctivitis associated with vesiculation of the epithelium or even corneal ulceration that may cause permanent impaired vision (Beasley, 1963; Michal, 1950).

Hydrogen sulphide exerts an irritant action throughout the entire respiratory tract (Beauchamp et al., 1984). At low concentrations (50 - 200 ppm; 70 - 280 mg/m^3) it causes rhinitis, pharyngitis, laryngitis and bronchitis (Milby, 1962). Exposure to high concentrations (200 - 300 ppm; 280 - 420 mg/m^3) has been associated with haemorrhagic pulmonary edema (Burnett et al., 1977). Another complication following exposure to H_2S is development of pneumonia, which has been related to the inhibitory effect of this gas on alveolar macrophages (Rogers and Ferrin, 1981). It has recently been reported that a population living downwind from a natural gas refinery exhibited an increase of respiratory symptoms, such as coughing, wheezing and dyspnea, compared to people living in a control reference area (Dales et al., 1989).

Animal studies have demonstrated that exposure to 1 (1.4 mg/m^3), 10 (14 mg/m^3) and 100 ppm (140 mg/m^3) for 8 hours per

day for 5 weeks had no effect on baseline measurements of airway resistance, dynamic compliance, tidal volume or minute volume (Hulbert, 1989). Histologic examinations of trachea and lungs following high level inhalation (100-500 ppm; 140-700 mg/m^3), revealed increased permeability of the mucosa to dextran in a dose related fashion (Yang, 1991) and abnormalities in pulmonary surfactant activity (Green et al., 1991). Other studies have shown that inhalation of 400 ppm (560 mg/m^3) induced necrosis and exfoliation of respiratory and olfactory mucosal cells (Lopez et al., 1988).

1.5.2 Neurotoxic Effects of Hydrogen Sulphide - Effects on the Human

The neurotoxic effects of the exposure to high concentrations of hydrogen sulphide are well documented. Neurological symptoms of the exposure to concentrations about 500 ppm include sudden fatigue, convulsions, unconsciousness, and respiratory failure that may lead to death (Reiffenstein et al., 1992). Exposure to higher concentrations (1000 ppm) result in rapid collapse and respiratory paralysis (Beauchamp et al., 1984). After resuscitation, victims may exhibit motor disturbances, such as ataxia, tremor, and rigidity (Tvedt et al., 1991) as well as psychiatric disturbances including hallucinations and memory problems (Tvedt et al., 1991). It has also been reported that exposure to high concentrations of hydrogen sulphide causes prolongation of P-300 event-related

potentials latency (Wasch et al., 1989). The P-300 latency test is a useful measure in the detection of cognitive dysfunction, even if routine neurological examination results are normal (Goodin et al., 1983). This test is prolonged in patients with dementing illness. Chronic exposure to low levels of hydrogen sulphide have also been associated with numerous neurophysiological and neuropsychological effects. Among the most common symptoms are nervousness, drowsiness, poor memory, headache and sleep disturbances (Milby, 1962). A recent report (Gaitonde and Sellar, 1987) described a case of a 20 month old child who developed encephalopathy following exposure to H_2S at approximately 0.6 ppm (0.85 mg/m^3). The previously healthy child exhibited gross truncal ataxia, choreoathetosis, and dystonia. Computed tomograms of the brain showed low density areas in both basal ganglia and surrounding white matter. All the clinical symptoms and tomogram abnormalities ceased spontaneously in 10 weeks.

1.5.3 Neurotoxic Effects on Experimental Animals

More than 60 years ago Schmitt and Beck (Schmitt and Beck, 1930) reported that sodium sulphide (a volatile salt of H_2S) could alter nerve conduction in a frog nerve-muscle preparation. In 1966, Lund and Wieland showed that exposure of three rhesus monkeys to 500 ppm (700 mg/m^3) of hydrogen sulphide for 22 minutes produced necrosis of the cerebral cortex, reduction in Purkinje cells and focal gliosis (Lund

and Wieland, 1966). Since that time many other studies have been conducted in order to investigate the possible site of action of H_2S on the nervous system.

It has been reported that acute i.p. treatment of adult male rats with sodium bisulphide in a dose two times higher than a lethal dose for 50% of the animals (twice the LD_{50} dose) increased the concentrations of alanine, aspartate, GABA, glutamate, glycine and taurine selectively in the brain stem, whereas no changes were detected in other brain areas (Kombian et al., 1988). These data correlate with a previous report by the same authors that sodium sulphide is selectively taken up by the brainstem (Warenycia et al., 1989a). In contrast, chronic exposure to low levels of H_2S (less than 75 ppm; 105 mg/m^3) during the critical phase of development (5th day of gestation until day 21 after birth) depressed the concentrations of aspartate, glutamate and GABA in the rat cerebrum (Hannah et al., 1989).

It has also been demonstrated that a high dose of sodium sulphide (twice the LD_{50} dose) increased norepinephrine (NE) and epinephrine (EP) levels in rat hippocampus and striatum (Warenycia et al., 1989b). In the brainstem, levels of all three catecholamines (NE, EP, dopamine) as well as 5-hydroxytryptamine were increased. These changes were attributed to inhibition of monoamine oxidase (Warenycia et al., 1989b). Similar effects have been observed during chronic exposure to low levels of H_2S during development (Skrajny et

al., 1993). Following the exposure to 75 ppm (105 mg/m^3), both serotonin and norepinephrine levels were increased in the cerebellum as well as the frontal cortex.

In vitro neuronal preparations have been used as models to study the mechanism of action of hydrogen sulphide. The rat hippocampal preparation is of particular interest because it may relate to the memory losses that have been described in survivors of H_2S poisoning. In the experiment by Baldelli and coworkers (Baldelli et al., 1989 and 1990) hippocampal CA1 neurons have been studied using intracellular recordings in the current-clamp mode. Hippocampal slices were superfused with CSF solution containing 27-200 μM NaHS (Baldelli et al., 1990). NaHS caused a concentration-dependent membrane hyperpolarization and reduction in membrane resistance. Upon washout, a further hyperpolarization was noted. Extracellularly recorded population spikes and EPSP field potentials as well as intracellular EPSPs revealed a depression of synaptic transmission produced by NaHS (Baldelli et al., 1989). It was suggested that the reduction in membrane resistance was probably due to activation of Na^+/K^+ ATPase whereas hyperpolarization was the result of the opening of K^+ channels (Baldelli et al., 1990). The type of potassium channels involved, however, remains to be determined. Pharmacological studies have excluded involvement of the "delayed rectifier" potassium channel, the fast transient voltage dependent "A" current, the neurotransmitter-regulated

"M" channel, or the Ca^{2+}-activated nonspecific ion channel (Baldelli et al., 1990). The involvement of the ATP sensitive potassium conductance is also unlikely, since injection of ATP did not alter sulphide-induced hyperpolarization (Baldelli et al., 1990); however, the mechanism of action of this channel per se is not well understood.

Similar changes of membrane excitability have been demonstrated in hippocampal slices during anoxic or hypoxic conditions (Krnjevic and Xu, 1990). Brief periods of anoxia in hippocampal CA1 neurons result in hyperpolarization. In addition a decrease in resistance and excitability, as well as depression of synaptic potentials (especially inhibitory postsynaptic potentials) can also occur (Leblond and Krnjevic, 1989). The effects are not fully blocked by any of the potassium channel antagonists, including cesium, 4-aminopyridine (4-AP), tetraethylammonium (TEA), quinine and apamine (Leblond and Krnjevic, 1989). It has been postulated that anoxic conditions lead to a rise of cytosolic-free Ca^{+} and increase in the opening of the Ca-activated potassium channels (Krnjevic and Xu, 1990). Since hydrogen sulphide is a potent inhibitor of cytochrome c oxidase, it can be postulated that the electrophysiological effects observed in CA1 neurons following treatment with sulphide are simply due to anoxic conditions caused by the inhibition of the oxidative metabolism. Pharmacological studies (Baldelli et al. 1990), however, revealed that hydrogen sulphide acts by other than

metabolic inhibition. The hyperpolarization induced by sulphide application was found to be sensitive to TEA, whereas potassium channels involved in anoxic changes were not blocked by TEA. The effects of sulphide and anoxia then, however similar, are probably achieved by different cellular mechanisms (Reiffenstein et al., 1992).

It has been reported that dorsal raphe neurons respond to NaHS exposure in a manner similar to that of hippocampal CA1 cells; hyperpolarization following the application of NaHS, and further hyperpolarization after washout of the sulphide (Kombian et al., 1993). In some dorsal neurons, a blockade of hyperpolarization with Ba^{2+} and Cs^{+} revealed an underlying depolarizing current (Kombian et al., 1993).

In order to study the effect of hydrogen sulphide on tetrodoxin (TTX)-sensitive Na^{+} channels, the patch-clamp method was used on murine neuroblastoma cells (clone N1E-115 derived from sympathetic ganglia) (Warenycia et al., 1989c). NaHS alone failed to alter the TTX-sensitive Na^{+} channels; however, in combination with taurine or cysteic acid it inhibited the channels completely and reversibly. Both taurine and cysteine alone did not affect these sodium channels.

The crayfish receptor has been utilized as another model to study hydrogen sulphide neurotoxicity (Roth and Hannah, 1989). Using extracellular recording techniques, the effects of sodium sulphide, sodium bisulphide and gaseous hydrogen sulphide were studied on the physiological (stretch) and

chemically-induced firing activity of the neuron. It was observed that sulphide salts altered the discharge of the sensory neuron in a complex time-dependent manner. Sulphide caused an initial block of action potentials (APs), followed by a prolonged enhancement of AP amplitude, and another inhibition after washout of sulphide. This complex action of sulphide on a sensory neuron strongly suggests that multiple sites and mechanisms of action may be involved.

In summary, hydrogen sulphide appears to produce its neurotoxic effects via several different mechanisms. It may hypopolarize neurons by opening potassium channels; reduce the membrane resistance due to the activation of Na^+ K^+/ATPase; and alter the neurotransmitter content in various brain regions in both adult and developing animals.

1.6. Hippocampal Theta Activity as a Model to Study Hydrogen Sulphide Neurotoxicity.

To further the studies of hydrogen sulphide neurotoxicity, the effects of this gas were examined on the in vivo rat hippocampal theta activity. Theta activity is the most characteristic electroencephalographic (EEG) activity of the hippocampus recorded *in vivo* (Bland, 1986). It has been related to such behavioral states as arousal, anxiety, awareness, sensory input, learning and memory. Since many of these properties appear to be relevant and significant to the state of H_2S poisoning, the hippocampal formation may prove to

be an ideal electrophysiological model system for the study of H_2S neurotoxicity. Furthermore, the hippocampus is well characterized both morphologically and electrophysiologically (Lopes da Silva et al., 1990; Amaral and Witter, 1989, O'Keefe and Nadel, 1978)

The in vitro hippocampal slice preparation has been used successfully to study the mechanism of action of hydrogen sulphide (Baldelli et al., 1989, 1990). Furthermore it was recently proposed as a model for the study of neurotoxicity in general (Fountain et al., 1990). The slice preparation offers many advantages: multiple tests of a variety of neuronal properties can be performed using a single slice; it preserves the classic trisynaptic neuronal circuit, and offers an easy access for electrophysiological, pharmacological and toxicological manipulation. It also presents several disadvantages. For example the *in vitro* slice preparation is deprived of afferent inputs, as well as efferents connecting the hippocampal formation with other structures of the brain.

The *in vivo* system provides several advantages over the hippocampal slice preparation. The whole animal preparation allows the use of inhalation exposure, which in the case of hydrogen sulphide, is the major route of intoxication. Inhalation exposure preserves the major site of entry (lung) as well as its distribution to different organs, and allows for biotransformation and elimination. The presence of the intact blood brain barrier is another factor that must be

taken into account while studying the neurotoxic effects of xenobiotics (Douglas and Doyle, 1991). This preparation also allows the investigation of the effects of chronic exposure.

Electroencephalographic studies have been recognized as a valuable tool in neurotoxicology (Fox et al., 1982). It has been postulated that EEG activity may be indicative of specific aspects of behaviour, thus, changes in EEG should reflect the alteration in behaviour and vice versa (Fox et al., 1982). The study of hippocampal EEG, and "theta activity" in particular, offers the additional advantage of relatively direct extrapolation to dysfunction of learning, memory and other behavioral and cognitive processes (Grastyan, 1959; Vanderwolf, 1988). Thus it is proposed that the hippocampal theta activity in the freely moving animal may be used as an valuable test to investigate the neurotoxic effects of hydrogen sulphide as well as other toxicants.

1.7 Anatomy and Electrophysiology of the Hippocampal Formation

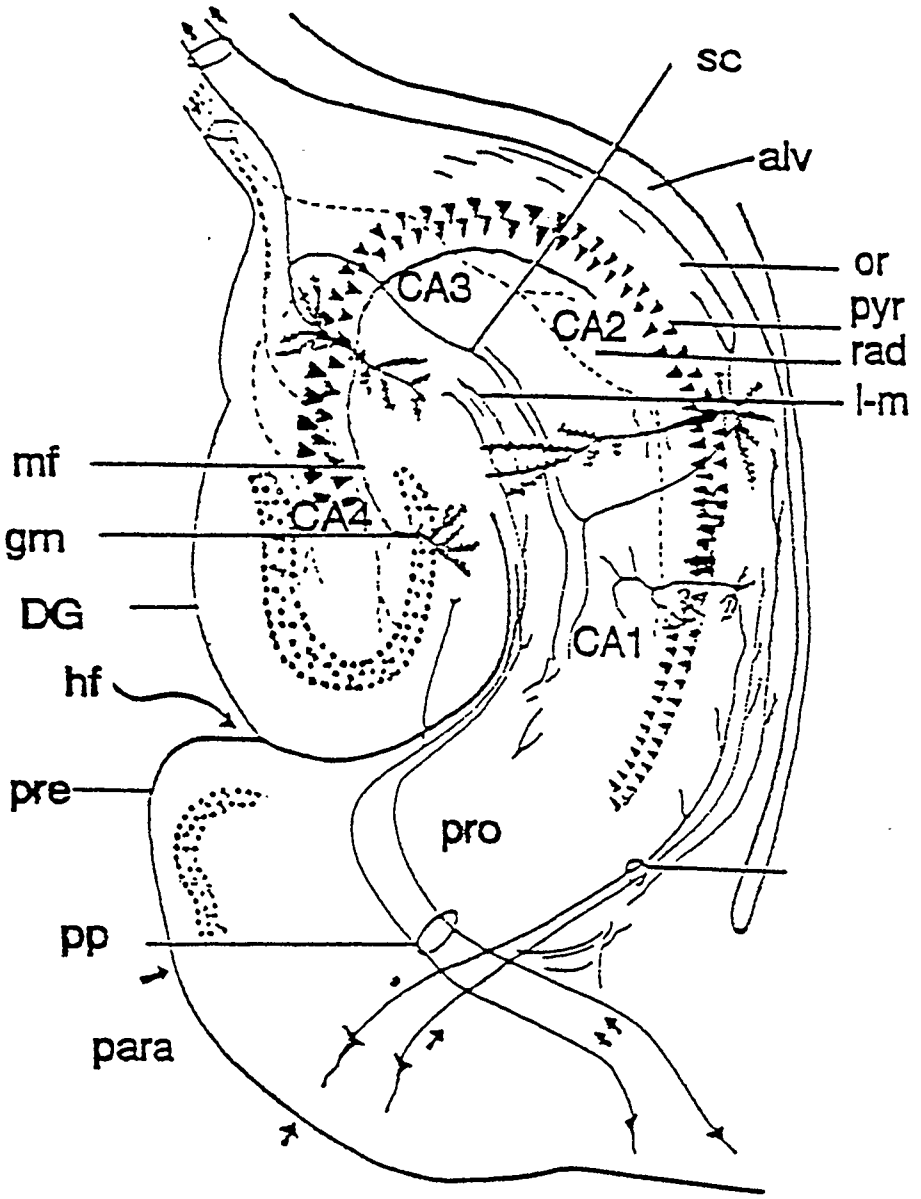
1.7.1. Anatomy of the Hippocampal Formation.

The hippocampal formation occupies a large portion of the rat forebrain. It consists of four cortical regions: dentate gyrus (fascia dentata), hippocampus proper (Ammon's horn), the subicular complex and entorhinal cortex (Amaral,1989). A schematic of the hippocampal formation is presented in Figure 1. Based on the classical studies of Ramon y Cajal (Cajal, 1955) several internal layers of the hippocampus proper can be

differentiated (Lopes da Silva et al., 1990): 1) the stratum pyramidale (pyr) contains cell bodies of the pyramidal cells; 2) stratum oriens (or) is comprised of basal dendrites of pyramidal cells, numerous polymorph cells and the collaterals of the axons of CA3 pyramidal cells; 3) the alveus (alv) contains axons of the pyramidal cells and incoming fibres; 4) stratum radiatum (rad) is composed of proximal segments of the apical dendrites, sparse cell bodies and several fiber systems of which the most important are the Schaffer collaterals (sc); 5) stratum lacunosum contains collaterals of the pyramidal cells and some fibres extrinsic to the hippocampus; 6) stratum moleculare consists of fibres as well as the distal segments and terminal branches of the apical dendrites. Some authors combine the two last layers into the stratum lacunosum-moleculare (l-m). The final layer is the epithelial zone, which forms the lining of the ventricular surface of the hippocampus (Lopes Da Silva et al., 1990).

From the subiculum border, following the curvature of the structure, there is a notable gradation in size of the pyramidal cells and the configuration of their apical dendrites. Based of these morphological observations Lorente de No (Lorente de No, 1934) subdivided the hippocampus proper into four fields: CA1, CA2, CA3 and CA4. The CA4 region is often referred to as the hilar region of the dentate gyrus. In the terminology of Ramon y Cajal (Cajal, 1955), two subfields of hippocampus proper are differentiated: regio

Figure 1. A schematic diagram of the intrahippocampal connections. Included are: pyramidal neurons (CA1 to CA4), dentate granule neurons (grn), Schaffer collaterals - sc, mossy fibers - mf, alveus - alv, perforant path - pp, stratum oriens - or, stratum pyramidale - pyr, stratum radiatum - rad, stratum lacunosum-moleculare -l-m, dentate gyrus - DG, hippocampal fissure - hf, presubiculum - pre, parasubiculum - para, prosubiculum - pro. (Adapted from Isaacson, 1982)


sc
alv
or
pyr
rad
l-m
CA3
CA2
CA1
CA4
mf
gm
DG
hf
pre
pro
pp
para

superior that corresponds to the CA1 region and regio inferior that corresponds to CA2 and CA3 regions (Cajal, 1955).

The dentate gyrus consists of three layers: the granule cell layer (grn), molecular layer containing apical dendrites of the granule cells and their afferents, and hilar region which is characterized by the presence of polymorph neurons (Lopes da Silva et al, 1990).

The subicular complex is divided into three subdivisions: presubiculum, subiculum and parasubiculum. The subiculum consists of a molecular layer and pyramidal layer. The entorhinal cortex, particularly in rodents, is generally divided into lateral and medial areas (Lopes da Silva et al., 1990).

1.7.2. Major Circuits of the Hippocampal Formation

Anatomical (Blackstad et al., 1970; Hjorth-Simonsen and Jeune, 1972) and electrophysiological (Andersen et al., 1971) evidence suggest that the internal circuitry of the hippocampus is organized in a lamellar fashion. A lamella includes: mossy fibres (mf), Schaffer collaterals (sc), and CA1 neurons in the alveus (alv). The mossy fibres are the axons of the dentate granule cells. The fibres gather together in the hilus and terminate on both neurons in the polymorphic zone of the dentate hilus (Amaral, 1978) and the proximal dendrites of the CA3 pyramidal cells. There are about five fold fewer pyramidal cells in the CA3 region than there are

granule cells in the dentate gyrus (Boss et al., 1987). Since each mossy fibre terminates on about 10 - 15 pyramidal cells (Claiborne et al., 1986), each CA3 pyramidal cell is innervated by approximately 50 - 100 granule cells. The neurotransmitter at mossy fibre synapses appears to be glutamate or aspartate (Lopes da Silva et al., 1990). Axon collaterals of the CA3 pyramids, known as Schaffer collaterals, distribute to the CA1 region. These axons synapse in the stratum radiatum with the apical dendrites of CA1 pyramidal cells. Glutamate or aspartate is also the most likely neurotransmitter at these synapses (Lopes da Silva et al., 1990). The Schaffer collaterals also distribute to the subiculum; and in turn, both the CA1 and the subiculum contribute to form the fornix. The fornix carries the output of the hippocampus to various structures. Fibres from the pyramidal fields project to the septum and the contralateral pyramidal fields (Nauta and Feirtag, 1986). The rest of the fornix arising from the subiculum, project to the nucleus accumbens, mammillary body, and the anterior nucleus of the thalamus (Nauta and Feirtag, 1986).

The intrinsic connections of the hippocampus also exhibit a longitudinal organization by which various lamellae are interconnected (Amaral, 1978). In addition a large number of interneurons are present within all fields of the hippocampus (Swanson et al., 1978). These interneurons may interact with many principal neurons along both transverse and longitudinal

axes of the hippocampus (Swanson et al., 1978).

A major afferent input to the hippocampal formation arises from the entorhinal cortex. The fibres of this pathway are mainly from layers II and III of the entorhinal cortex and reach the molecular layer of the dentate gyrus by way of the perforant pathway (Schwartz and Coleman, 1981). The entorhinal projection to the hippocampal formation most likely utilizes glutamate or aspartate as the neurotransmitter (Di Lauro et al. 1981, and White et al. 1977), however somatostatin, enkephalin and cholecystokinin may also be present in this pathway (Lopes da Silva et al, 1990).

The second major source of hippocampal afferents is from the septum (Chandler and Crutcher, 1983; Monmaur and Thomson, 1983). The two largest inputs from the medial septum/ vertical limb of the diagonal band of Broca (MS/vDBB) are cholinergic and GABAergic in nature (Smythe et al., 1992). Two types of fibres projecting from the MS/vDBB to the hippocampus have been described: type I with thick coarse fibres (Freund and Antal, 1988) and large terminal boutons and type II which are delicate thin fibres with numerous *en passant* varicosities (Frotscher and Leranth, 1985). Type II fibres appear to originate from cholinergic Ms/vDBB cells and terminate in both granule and pyramidal cells of the hippocampal formation (Frotscher, et al., 1989). Type I fibres have been identified as GABA-ergic and innervate hippocampal inhibitory interneurons (Freund and Antal, 1988).

1.8. Hippocampal Theta Rhythms.

The hippocampal formation is capable of producing a sinusoidal waveform termed theta rhythm (theta) or rhythmical slow wave activity (RSA). Depending on the species and the behavioral condition, the frequency of this waveform can range from 3 to 12 Hz (Green and Arduini, 1954; Green and Rawlins, 1979; Vanderwolf, 1969). In human electroencephalography (EEG), theta refers to activity in the 4-7 Hz band. Although the frequency of the hippocampal RSA extends beyond this range, the term theta is commonly used to describe this activity. Hippocampal theta rhythm has been described in a number of small mammals including rats, rabbits, guinea pigs, gerbils, cats and dogs (Bland, 1986). A similar rhythm has been found in non-human primates (Stewart and Fox, 1991); however, the prevalence and behavioral correlates of theta in primates is at present not well defined.

In the absence of theta rhythm, two other patterns of hippocampal EEG occur: large amplitude irregular activity (LIA) and small amplitude irregular activity (SIA).

Saul and Davis (1933) first described theta or "action currents" recorded from the hippocampus. Five years later, Jung and Kornmuller (1938) demonstrated that stimulation of peripheral nerves resulted in rhythmical slow wave activity in the hippocampus of rabbits (Jung and Kornmuller, 1938). In 1954, Green and Arduini suggested that hippocampal theta may be a "specialized paleocortical arousal reaction" (Green and

Adruini, 1954). Vanderwolf (1967) was first to correlate hippocampal theta with observable behaviour and stated that hippocampal theta activity "may be a reflection of the activity of some complex circuitry which appears necessary for voluntary movement".

1.8.1 Characteristics of Theta

On the basis of pharmacological and behavioral studies, two types of theta activity have been distinguished: type 1 and type 2 theta (Table 1). Type 1 theta has a frequency of 6 - 12 Hz, is abolished by some anaesthetics, resistant to atropine sulphate and is correlated with voluntary movements such as walking, running, swimming, rearing, jumping and manipulation of objects with the forelimbs (Bland, 1986). Type 2 theta has a frequency of 4 - 9 Hz, is unaffected by most anaesthetics, abolished by atropine and occurs during immobility in response to sensory stimuli (Bland, 1986). It has been well established that type 2 theta is mediated by muscarinic cholinergic receptor activation. In 1975 Kramis et al. demonstrated that type 2 theta was abolished by injection of atropine sulphate but not by atropine methyl nitrate which does not cross the blood-brain barrier (Kramis et al., 1975). Later it was demonstrated that the anticholinesterase agent, eserine or physostigmine, elicited type 2 theta (Vanderwolf, 1975). Further support for the cholinergic nature of type 2 theta was obtained by intraventricular injections of

Table 1. Properties of type 1 and type 2 hippocampal theta activity.

	TYPE 1 THETA	TYPE 2 THETA
Occurrence	voluntary movements	alert immobility
Frequency	6 - 12 Hz	4 - 9 Hz
Generators	CA1 & DG	CA1 & DG
Mediation	serotonergic	cholinergic
Pharmacology	atropine resistant	atropine sensitive

hemicholinum-3, a compound that blocks the transport system by which choline accumulates in the terminal of cholinergic fibres (Robinson and Green, 1980). Hemicholinum-3 produced a severe attenuation of type 2 theta, and subsequent systemic injection of choline restored the activity (Robinson and Green, 1980).

The neurochemical system responsible for type 1 theta activity is still unknown, although serotonin appears to be a prime candidate (Vanderwolf and Baker, 1986). There is clear evidence that reserpine reduces the effectiveness of atropine-resistant input that produces hippocampal type 1 theta (Leung, 1984). It has also been demonstrated that treatment with p-chlorophenylalanine (PCPA) and atropine attenuate or eliminate type 1 theta activity, however PCPA alone has little effect on this activity (Vanderwolf and Baker, 1986). Furthermore it has been demonstrated that central serotonergic stimulation produced an increase in motor activity, and this was correlated with an increase in atropine-resistant hippocampal theta activity (Robertson et al., 1991).

1.8.2 Generators, Topography and Cellular Mechanisms of Theta Generation.

In the earlier studies on hippocampal theta activity, the CA1 pyramidal region was regarded as a primary zone of theta generation (Green and Arduini, 1954; Petsche and Stumpf, 1960). However, in the 1970s, Winson and Bland (Winson, 1974;

Bland et al., 1975) demonstrated that there was a second generator of theta activity located in the stratum moleculare of dentate gyrus. Unit recordings obtained from single cells in the hippocampal formation of urethane-anaesthetized rats revealed the existence of a population of cells which discharge in relation to simultaneously recorded hippocampal field activity. Two major classes of cells have been differentiated: theta-on which are more active during theta, and theta-off cells which are more active during LIA (Colom and Bland, 1987). In addition, the rate of theta-on cell discharge increases linearly with increasing theta frequencies, while theta-off cell discharge rate increases linearly with decreasing theta frequencies (Colom and Bland, 1987). Theta-on cells are blocked by administration of atropine sulphate and activated by cholinergic agonists (Bland and Colom, 1988). Intracellular recordings suggest that pyramidal and granule cells are theta-on cells (Munoz et al., 1990; Nunez et al.,1987). Pyramidal cells constitute a more heterogenous group - some are complex spike cells, while others are theta-on cells. The identity of theta-off cells still remains to be determined, although GABA-ergic interneurons are prime candidates (Bland and Colom 1989).

Despite extensive investigation, the neuronal mechanisms of hippocampal theta activity are not well understood. It has been demonstrated that during theta rhythm, both CA1 pyramidal and dentate granule neurons exhibit rhythmic membrane

potential oscillations that are phase-locked to the extracelluarly recorded theta activity in those regions (Munoz et al., 1990; Leung and Yim, 1986; Fox et al., 1980). Fujita and Sato (1964) suggested that intracellular theta rhythm of CA1 pyramidal cells was primarily composed of excitatory postsynaptic potentials (EPSPs), and that inhibitory postsynaptic potentials (IPSPs) were not involved. Contrary to these findings, Fox et al. (1980) provided data suggesting that IPSPs were important to the genesis of intracellular theta rhythm in CA1 neurons. Recently, Nunez et al. (1990) have provided evidence supporting Fuita and Soto's original conclusion. They found that chloride or cesium ion diffusion had no effect on intracellular theta, whereas hyperpolarizing current injections increased the amplitude of the recording activity.

It is well documented that input from the medial septum/vertical limb of the diagonal band of Brocca (MS/vDBB) plays an important role in the generation of hippocampal theta activity. The work of Green and Arduini (1954) suggested that a pathway arising in the reticular formation and projecting to the hippocampus via the septum is responsible for producing theta activity. Furthermore, several studies have demonstrated that the presence of theta activity relies on the integrity of the septum (Sainsbury and Bland, 1981; Bland et al., 1984). In addition, rhythmic cells in the medial septum/diagonal band complex have been identified that discharge in synchrony with

hippocampal theta activity (Petsche et al., 1962; Gaztelu and Buno, 1982). It has been postulated (Rawlins et al. 1979; Sainsbury and Bland, 1981) that the septum serves as a "pacemaker", and rhythmic discharges, originating in the medial septal region, are responsible for the production of theta in the hippocampal formation. *In vitro* studies of carbachol-induced "theta" activity in hippocampal slices (Konopacki et al. 1987) do not support the septal "pacemaker" hypothesis. These studies demonstrate that a rhythmic input is not critical for generating hippocampal "theta" activity; carbachol-induced generator mechanisms intrinsic to the hippocampal formation can produce rhythmical slow waves (Konopacki et al. 1987). However, important inputs for modulating that activity originates in medial septum (Smythe et al. 1991). Further support for medial septum involvement was demonstrated by the study that showed that acetylcholine release in the hippocampus was enhanced by septal stimulation (Dudar, et al., 1979). Later work demonstrated that neurons which exhibited slow activity are cholinergic (Griffith and Matthews, 1986) while neurons with rhythmic high frequency activity are probably GABA-ergic in nature (Smythe et al, 1992). Recently, Smythe and et al. (1992) suggested that both cholinergic and GABA-ergic efferent activity to the hippocampus is essential for theta generation. Cholinergic projections provide the excitatory drive of hippocampal theta-on cells, while GABA-ergic projections act to reduce the

overall level of inhibition by inhibiting hippocampal GABA-ergic interneurons (theta-off cells) (Smythe et al., 1992)

In addition, it has been postulated that alpha$_2$ adrenergic receptors possess a modulatory role in the production of type 1 and type 2 theta (Heynen and Sainsbury, 1991; Sainsbury and Partlo, 1991 and 1993). Direct infusion of norepinephrine or an alpha$_2$ agonist, detomidine, attenuated electrical stimulation-induced theta in the urethane-anaesthetized rats (Heynen and Sainsbury, 1991). In freely moving rats, detomidine produces a virtually continuous type 2 theta, while type 1 theta is attenuated (Sainsbury and Partlo, 1991). It has been hypothesized that hippocampal noradrenergic innervation from the locus ceruleus may play an inhibitory role on mechanisms underlying the production of type 2 theta. The administration of an alpha$_2$ agonist, such as detomidine, may also cause a release of ACh at the hippocampus via inhibition of noradrenergic fibres (Sainsbury and Partlo, 1991).

1.9 Hypothesis

It is hypothesized that subchronic exposure to non-lethal levels of hydrogen sulphide alters hippocampal theta activity in CA1 and dentate gyrus regions recorded in the freely moving animal.

1.10 Objectives

The objectives of this thesis were:

(1) to determine the threshold effect of H_2S exposure on the hippocampal theta activity in freely moving rats

(2) to establish a dose-response relationship of H_2S on the total power of hippocampal theta activity; exposure paradigm to be the same for all concentrations of H_2S

(3) to determine whether repeat exposures to H_2S result in a cumulative effect on the total power of hippocampal theta activity

(4) to determine whether H_2S exposure affects type 1 (movement related) or type 2 (immobility related) theta

(5) to establish whether the effects of H_2S exposure on hippocampal theta activity are reversible or irreversible

(6) to determine neurotransmitter levels in the hippocampal region following exposure and relate changes to alteration of electroencephalographic activity

(7) to evaluate the usefulness of hippocampal theta activity as an electrophysiological parameter for the study of neurotoxic agents.

2.0 METHODS

2.1 Subjects

Male Sprague-Dawley rats weighing between 200 - 250 grams were supplied by the University of Calgary Biosciences Vivarium. The animals were housed in separate plastic cages and maintained on a 12 hour light-dark cycle with *ad libitum* food and water except during the exposure to H_2S.

2.2 Surgical Procedure

Rats were anaesthetized with Somnotol (sodium pentobarbital 65mg/cc, at doses of approximately 50 mg/kg; MTC Pharmaceuticals, Cambridge, Ontario), placed in a stereotaxic frame and the plane between bregma and lambda was levelled to horizontal. An uncoated tungsten wire placed in the cortex served as the indifferent electrode. Tungsten wire insulated with Kynar (vinylidene fluoride resin; The Plastic Dept., Pennwalt Corporation, Philadelphia) and tip electrolytically etched in saturated potassium nitrate solution, served as the recording electrodes. Coordinates for electrode placement were: 3.4 mm posterior from bregma, 1.8 mm lateral from midline and respectively 1.8 mm for CA1 and 2.7 mm for dentate placement ventral to dural surface. The indifferent and two recording electrodes were held in place with dental acrylic affixed to stainless steel screws (M 1.6 x 0.35 x 3 mm; Spae-Naur Inc., Kitchener, Ontario) threaded into the skull. Rats were allowed to recover from the surgery for at least one week prior to exposure.

Five rats were implanted with neocortical electrodes. Coordinates for neocortical placement were: 2.0 mm posterior from bregma, 2.5 mm lateral from midline and 1.0 ventral to dural surface. Each of these rats had one electrode implanted in dentate gyrus of the hippocampus and one in neocortex.

2.3 Exposure

Rats were exposed to hydrogen sulphide concentrations (100, 75, 50 or 25 ppm) 3 hrs/day for five consecutive days. The exposure took place in an acrylic environmental chamber that was designed to permit continuous observation of the animal and recording of hippocampal EEG during the exposure. Filtered room air was drawn through the chamber with a pump and mixed with certified H_2S (1990 ppm of H_2S in nitrogen; Linde, Union Carbide Canada Limited). The mixture was passed through an orifice plate to measure flow rate, then through a diffuser in the top of the chamber. An air flow of 11 litre/min was sufficient to allow a complete chamber replacement every three minutes. The volume of the H_2S-nitrogen mixture did not exceed 5% of the total volume of the air drawn into the chamber. The concentration of H_2S was continuously measured using a digital GFG monitoring system (model GMA-100; GFG Gas Electronics, Inc., St. Louis, Mo.). Control exposures to nitrogen were carried out since the H_2S used in this study was balanced in nitrogen. The flow rate of nitrogen was chosen to be the same as for the mixture of H_2S/N_2 during the exposure to the highest concentration of hydrogen

sulphide (100 ppm). The paradigm and conditions of the exposure were maintained as described above.

2.4 Recordings

Recordings of hippocampal theta activity were made with a Grass model 7D Polygraph with Wide Band A.C. EEG Pre-amplifier model 7P5B (Grass Medical Instruments Corporation, Quincy, Mass.). Filter settings were: 1 Hz for the half amplitude low filter setting and 35 Hz for the half amplitude for the high filter setting. The signals from the polygraph were amplified 10 times and displayed on a digital oscilloscope (Textronix, Beaverton, Oregon). All signals were stored on video cassette using a NeuroData interface (Model DR-484; Neuro Data Instruments Inc., New York, NY) and VCR (RCA) for subsequent computer analysis. During recording, the behaviour of the animal was continuously observed and comments by the investigator were stored on tape using the voice channel.

2.5 Experimental Design

Control exposures with nitrogen/air mixture were carried out for five animals. Rats were exposed three hours a day for five consecutive days.

Three other groups of 5 animals each were exposed to 25, 50 or 75 ppm of hydrogen sulphide (the paradigm of the exposure was described above in paragraph 2.3). Six animals were treated with 100 ppm H_2S for five days; five were allowed to recover for a month, and then a second trial of exposures

to the same concentration of hydrogen sulphide was carried out. Three rats were treated with an i.p. injection of 50mg/kg atropine sulphate (Sigma Chemical Co., St. Louis, Mo.) prior to the last exposure to 100 ppm H_2S.

2.6 Experiment With Detomidine.

Four rats were treated with 5mg/kg of detomidine (Dormosedan, detomidine hydrochloride 10mg/ml solution; SmithKline Beecham, Mississauga, Ontario). A 30 minutes of baseline testing prior to drug administration and three hours of control recording with detomidine were carried out for each animal. Rats were then exposed to 100 ppm H_2S three hours a day for five consecutive days beginning a day after the control with detomidine. Two more injections of detomidine were given for each rat: prior to the first and the last exposure to H_2S.

2.7 Spectral Analysis of Hippocampal EEG

Ten-minute recordings of EEG activity prior to H_2S exposure (control) and following 1, 2 and 3 hours of exposure were made. Representative forty-second samples of walking and immobility chosen for each time sample were subjected to power spectral analysis. Data from the VCR tape were inputted into a Comtex personal computer via an A/D board (Data Translation 2801). These data were digitized at a sampling rate of 102.4 Hz to fulfill Nyquist criterion, and divided into 2.5 second

epochs. For each epoch, the power spectral density function was computed using computer software developed in this laboratory written in Turbo Pascal 5.0 using Asyst 3.0 Fast Fourier Transform algorithms. Four measures were obtained from each spectrum: the total power (V^2/Hz), the peak frequency (Hz), percent of the total power in a window of 4 - 12 Hz and noise level. Total power represented the area under the curve from 0 - 25 Hz. Peak frequency (Hz) was the frequency which had the greatest amount of power in that epoch. Noise level was determined by measuring the power of the signal at 4 points (20 - 21 Hz, 21 - 22 Hz, 22 -23 Hz and 24 - 25 Hz) and calculating a mean for those four points.

2.8 Power Spectral Analysis: Theory

Power spectral analysis is a mathematical method of describing a waveform. It is a sensitive tool for detecting periodicity within the waveforms as well as determining the relative energy content of the periodicities. Detailed description and mathematical considerations of this method have been presented elsewhere (Dumermuth, 1987; Cooper et al., 1980). In this thesis only basic principles of power spectral analysis will be presented.

The process of power spectrum estimation may be divided into several stages. The first stage, known as sampling, is the conversion of a continuous (analog) signal into discrete, quantized (digital) samples. Sampling must be done at a rate

at least twice that of the highest remaining frequencies to satisfy the Shannon-Nyquist Theorem (Hinrichs, 1987).

After sampling and conversion of the sampled elements the data are subjected to analysis. The theoretical basis for the Fourier transform is Fourier's expansion theorem according to which any signal can be approximated by a sum of uncorrelated sine and cosine functions with different amplitudes and frequencies (Cooper et al., 1980). EEG activity is considered a random nonperiodic signal. Its instantaneous values cannot be predicted at any given time and it does not behave in certain, defined patterns, thus it is believed to have a period which approaches infinity. Such a signal cannot be precisely determined but can only be described by statistical reference values, e.g., mean of variance. There are two ways of calculating power spectra for EEG signals. The first method is based on the calculation of the autocorrelation function, (the time-mean of the product $f(t + \tau)$ by $f(t)$) and then calculating the power spectral density function according to Weiner's theorem by applying a Fourier transform to the autocorrelation function (for detailed mathematical consideration see Bendat and Piersol, 1966 or Lopes da Silva, 1987). Another way to calculate the power spectral density function for random nonperiodic signals is to square the Fourier transform (Cooper et al., 1980). In the present study the second procedure was employed to calculate the power

spectral density function of hippocampal EEG in freely moving rats.

The power spectral density function represents the unit change in power per unit change in frequency. It can also be described as average variance of a complex wave as a function of frequency (Findeiss et al., 1969). A power spectral density curve in turn provides graphic representation of the relative power content of the various frequency components in the signal; the total energy of the signal is proportional to the area under the curve over the segment length being analyzed, the amplitude and the area under the peaks are directly related to the energy content of the periodicity (Findeiss, et al., 1969).

2.9 Histological Confirmation of Electrode Placement

Upon completion of the experiments, rats were deeply anaesthetized with Somnotol (sodium pentobarbital; MTC Pharmaceuticals, Cambridge, Ontario) and perfused with physiological saline to flush the blood from the brain and then with 10% formalin. Brains were carefully removed and stored in 30% glucose-formalin solution. The fixed tissue was embedded in O.C.T. (embedding medium for frozen tissue specimens, Canlab) and 40 μM sections were cut in a microtome (model 500 OM, Microm GmbH, Heidelberg, Germany). Sections were mounted on glass, gelatine-coated slides and stained with cresyl violet. Placement of the electrode was determined by

locating post-electrode tracks.

2.10 Neurotransmitter Analysis

Twenty four rats were exposed to 100 ppm of hydrogen sulphide for 3 hours/day for five consecutive days in a 90 L acrylic environmental chamber. A maximum of 12 animals were placed in each chamber. The paradigm and conditions of the exposure were described above (2.3). Control animals were treated under similar conditions without H_2S, one treated group (n=12) and one control group (n=12) were exposed simultaneously in two identical chambers. The air flow (30 L/min) was sufficient to produce a complete chamber replacement every three minutes. After the last exposure, rats were anaesthetized with halothane, decapitated and brains rapidly removed. Three brain regions were dissected: hippocampus, frontal cortex and cerebellum. The tissue was placed in precooled 2-methylbutane and stored at -70°C until assays were performed.

2.10.1 Monoamine Assay

Norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) were measured using high-performance liquid chromatography (HPLC) with electrochemical detection. Tissue samples were weighed while still frozen and prepared according to the method described by Mefford (1981).

The chromatographic system consisted of a Perkin Elmer series 4 pump, BAS LC-4B electrochemical detector, and Vista

402 integrator. For 5-HT and 5-HIAA determinations the solvent (0.05 M KH_2PO_4 buffer, pH 4.0, containing 0.6 M of hexane sulphonic acid and 15% v/v methanol) was pumped at 1.2 ml/min through a C-18 (15 cm) Novapack reverse-phase analytical column. 5-HT and 5-HIAA were electrochemically detected in a thin layer cell with glassy carbon electrode operated at 0.7 V relative to an Ag-AgCl reference electrode. Separation of catecholamines was obtained with 0.05 M KH_2PO_4 buffer, pH 4.5, containing 70 mg/l dodecyl sulphonic acid, 100 mg/l EDTA and 20% (v/v) methanol and a C-18 phenyl (15 cm) Novapack reverse-phase analytical column (flow rate 1.2 ml/min). The applied potential was 0.6 V. The amounts of monoamines were calculated with reference to internal standards.

2.10.2 Amino Acid Assay

Selected amino acids, aspartic acid (Asp), glutamic acid (Glu), glycine (Gly), threonine (Thr), alanine (Ala), GABA and taurine (Tau), were quantified using pre-column derivatization with o-phthaldialdehyde (OPT) and isocratic separation of OPT-derivatives using a reverse-phase HPLC-electrochemical detection system. Tissue samples were weighed and homogenized in 0.5 ml water-methanol mixture (1:1) and 25 μl of internal standard. The homogenate was centrifuged (10000 rpm for 5 minutes) and 100 μl of supernatant was derivatized with 160 μl of OPT-reagent for 20 minutes at room temperature. OPT-reagent was obtained by adding 54 mg of o-phthaldialdehyde to 1 ml ethanol, 1 ml sodium sulphite (1M) and 18 ml disodium

tetraborate (0.1 M, pH 9.5). This reagent was stable at room temperature for several weeks. The amino acid derivative mixture was diluted and injected into the HPLC system. The mobile phase (flow rate 1.0 ml/min) consisted of 0.05 M phosphate buffer, pH 6.8, containing 300 μL/l 0.4 M tetrabutylammonium hydroxide and 18% (v/v) methanol. Proper separation of OPT-derivatives was obtained using a Perkin Elmer C-18 3 μM (5 cm) reverse-phase analytical column. Amino acid derivatives were detected with glassy carbon electrode operating at 0.85 V versus a Ag/AgCl reference electrode.

All chemicals used for the assays were HPLC grade. Standards were obtained from Sigma Chemical Co., St. Louis, Mo.

2.11 Statistical Analysis

Statistical analysis were performed using GraphPad Instat (Graphpad Software V2.01, San Diego, CA).

The neurochemichal data were analyzed using nonparametric two-tailed Mann-Whitney test. Differences were accepted as statistically significant at $p < 0.05$. All data are expressed as means ± S.E.M.

The statistical significance of EEG data was established using ANOVA and Dunnett Multiple Comparison test as well as Tukey-Kramer Multiple Comparison test. Differences were accepted as significant at $p < 0.05$. All data were expressed as means ± S.E.M..

3.0 RESULTS

3.1 Control

The volume of H_2S-nitrogen mixture during the exposure at the highest concentration of sulphide (100 ppm) did not exceed 5% of the total volume of the air drawn through the chamber. Control exposures to nitrogen were carried out in order to exclude the possibility that the effects observed during exposures to H_2S/N_2 mixture were caused by anoxic conditions which might have occurred in the chamber.

The control exposure to nitrogen/air mixture did not result in any changes in the spontaneous hippocampal EEG activity or in the behaviour of the animal. Hippocampal theta activity occurred only during the performance of type 1 voluntary movements. The immobility and automatic behaviour such as grooming or chewing were accompanied by large amplitude irregular activity (LIA). The example recordings of hippocampal EEG activity from dentate gyrus and CA1 recorded during walking and immobility are presented in Figures 2 and 3. Power density spectra of hippocampal EEG activity recorded during walking and immobility are presented in Figure 4 and 5 respectively. The total power of hippocampal theta activity during the control exposure was not significantly changed in both dentate gyrus and CA1 (Figure 6). The peak frequency of hippocampal theta activity also was not altered (Figure 7). It was in the range of 7.24 ± 0.19 Hz (S.E.M) at day 1 of control to 7.48 ± 0.07 Hz at day 2 of control for dentate gyrus and

Figure 2. Example recordings of hippocampal EEG activity recorded from dentate gyrus of a single animal during walking and immobility prior to exposure (upper trace) and following day 1 and day 5 of the control exposure to nitrogen/air mixture.

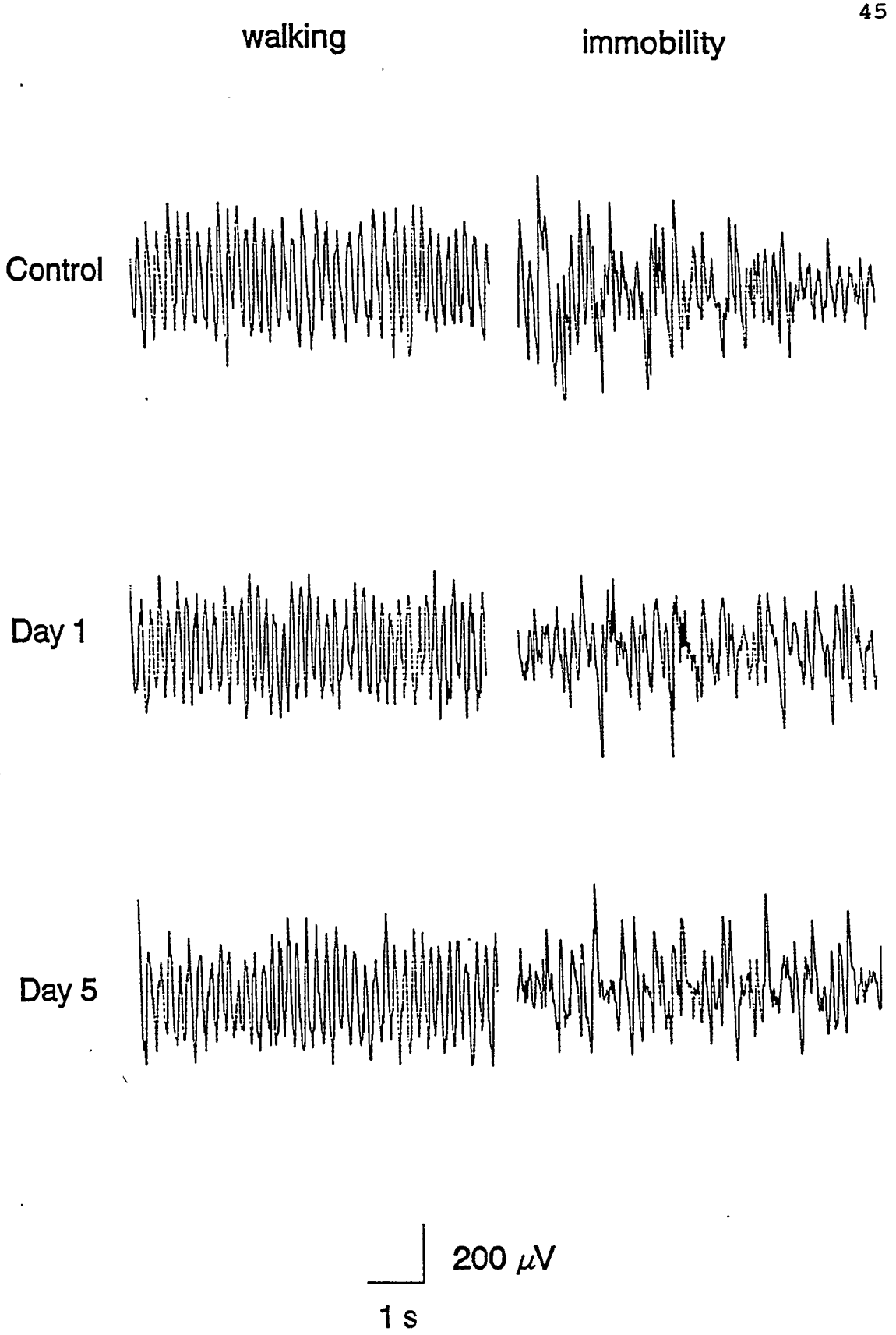

walking
immobility
Control
Day 1
Day 5
200 μV
1 s

Figure 3. Example recordings of hippocampal EEG activity recorded from CA1 of a single animal during walking and immobility prior to (upper trace) and following control exposure to nitrogen/air mixture on day 1 and 5.

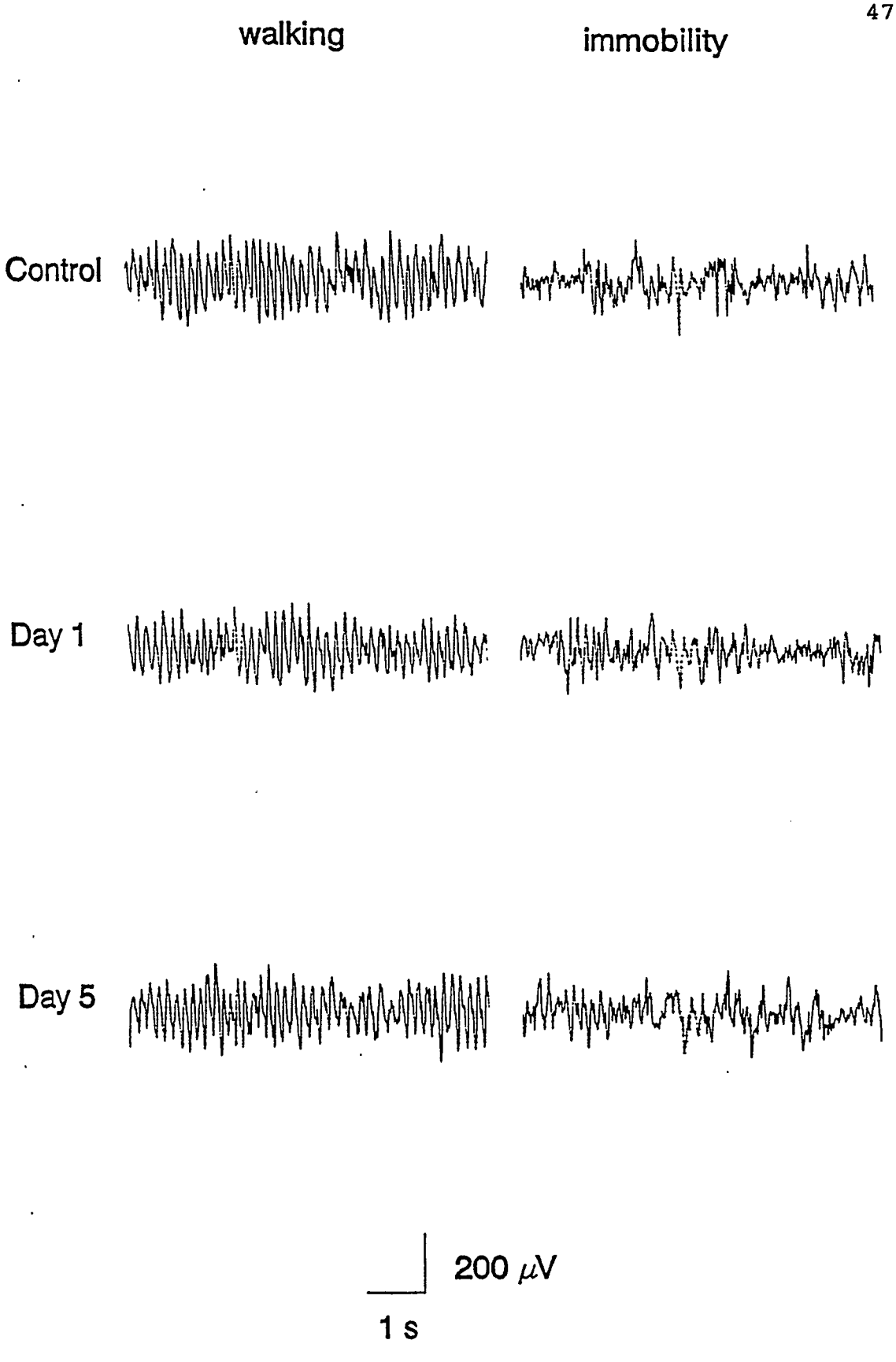
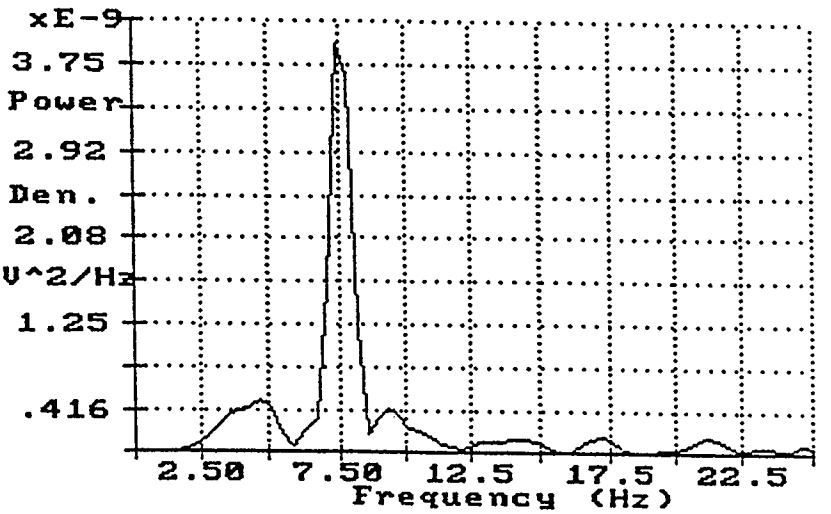
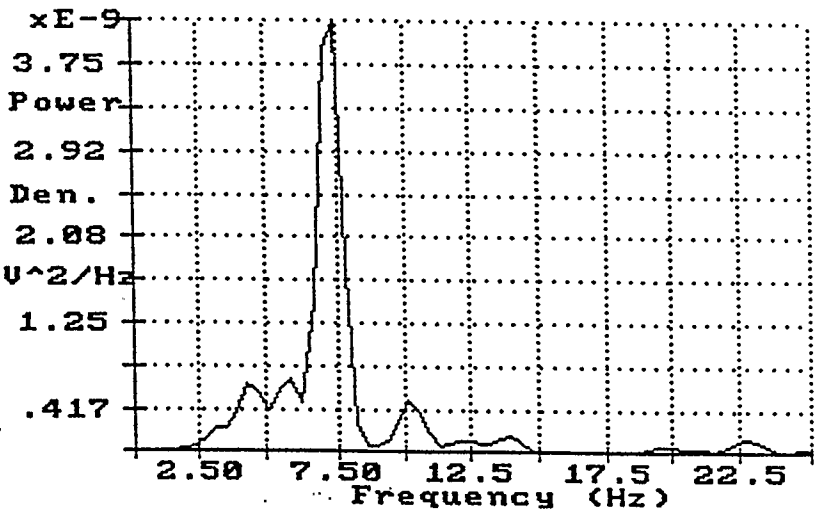

walking
immobility
Control
Day 1
Day 5
200 μV
1 s

Figure 4. Example power spectra of hippocampal theta activity recorded from dentate gyrus of a single animal recorded during walking prior to and following control exposure to nitrogen/air mixture on day 3 and 5.

CONTROL



DAY 3



DAY 5

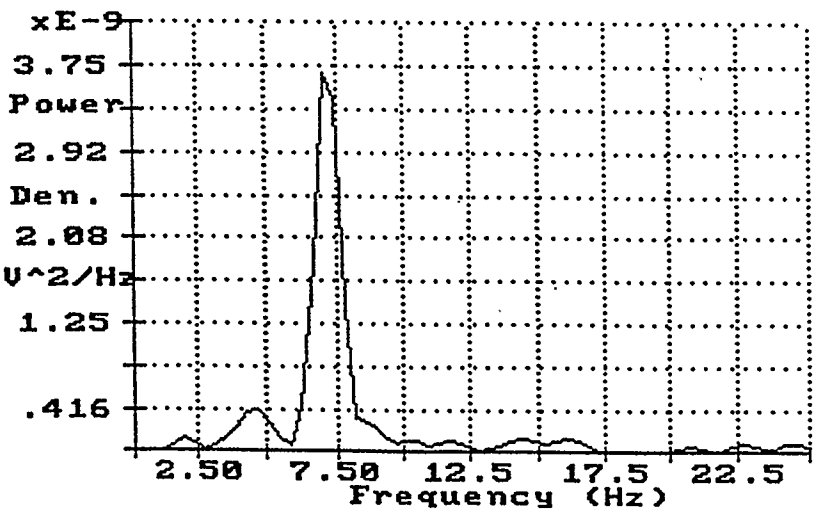
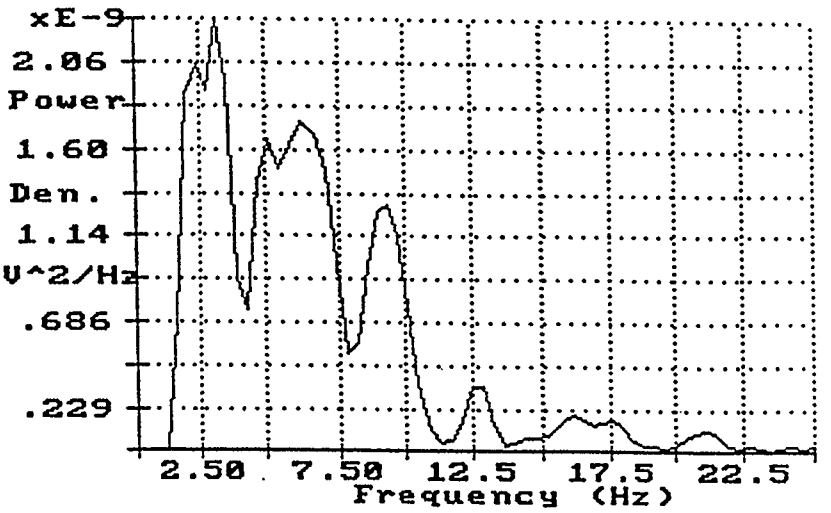
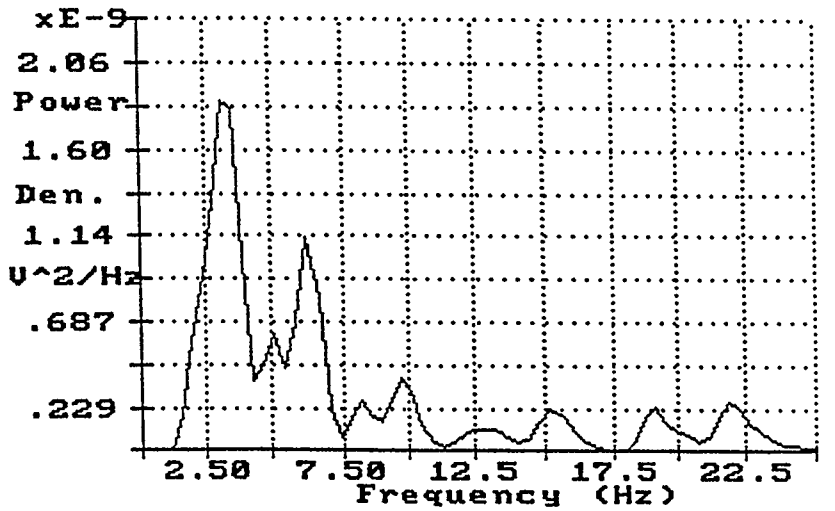


Figure 5. Example power spectra of hippocampal EEG activity (LIA) recorded from dentate gyrus of a single animal during immobility prior to exposure and following day 3 and 5 of the control exposure to nitrogen/air mixture.

CONTROL



DAY 3



DAY 5

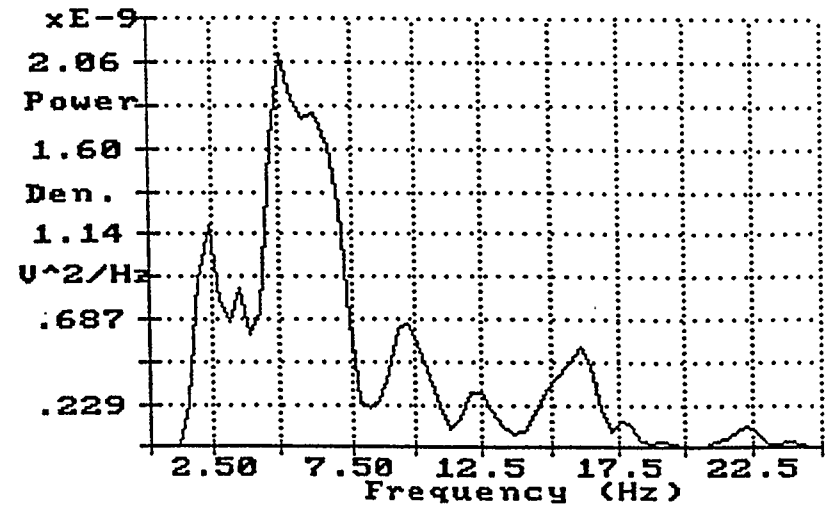
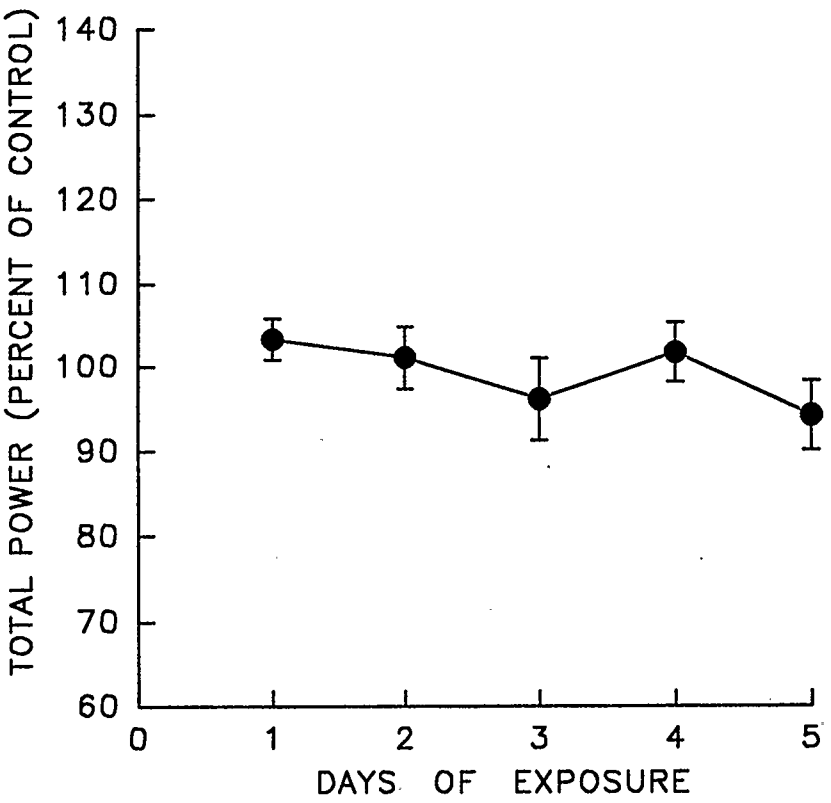
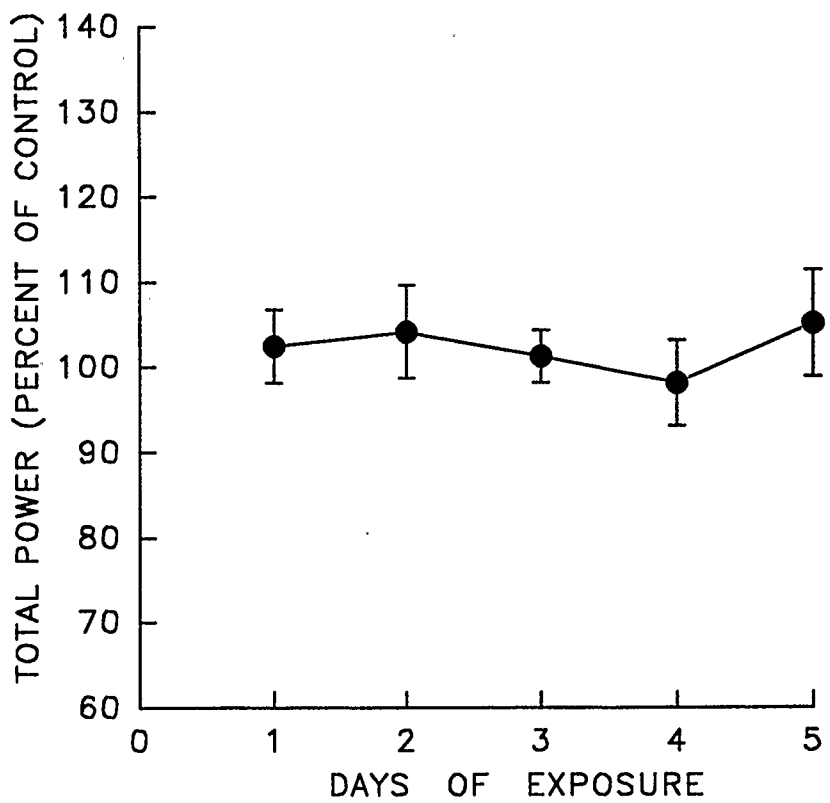


Figure 6. Plots of total power of hippocampal theta activity recorded from dentate gyrus (a) and CA1 (b) regions during the control exposure to nitrogen. Total power represents the area under the power spectral density curve. Each point represents the average ± S.E.M of at least 5 animals.

a)


TOTAL POWER (PERCENT OF CONTROL)
140
130
120
110
100
90
80
70
60
0
1
2
3
4
5
DAYS OF EXPOSURE

b)


TOTAL POWER (PERCENT OF CONTROL)
140
130
120
110
100
90
80
70
60
0
1
2
3
4
5
DAYS OF EXPOSURE

6.89 ± 0.28 Hz to 7.56 ± 0.15 Hz for CA1. The percentage of total power in the frequency window of 4 - 12 Hz during walking was in the range from 74.29 ± 2.32% to 78.4 ± 1.69% for dentate and from 68.06 ± 1.89% to 76.56 ± 2.30% for CA1, whereas during immobility it was in the range from 55.78 ± 3.23% to 59.77 ± 3.09% for dentate gyrus and from 49.63 ± 3.12% to 61.18 ± 2.77% for CA1 (Figure 8).

3.2 Exposure to 100 ppm of Hydrogen Sulphide

3.2.1 Single Exposure to 100 ppm of Hydrogen Sulphide

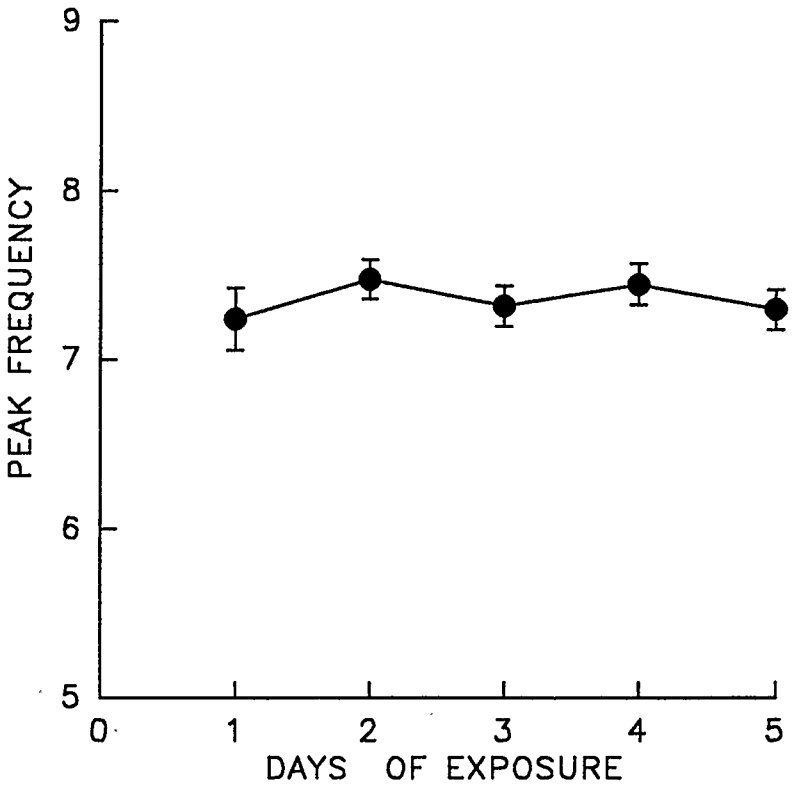
A single exposure to 100 ppm of hydrogen sulphide resulted in gradual increase in amplitude of the hippocampal theta activity (Figure 9) with the highest effect occurring at three hours of exposure. The amplitude of this activity did not return to the control level even the next day after the exposure, however it dropped slightly. Similar effects were observed in both dentate gyrus and CA1 regions.

3.2.2 Repeated Exposure to 100 ppm of Hydrogen Sulphide

Repeated exposure to 100 ppm of hydrogen sulphide resulted in a cumulative increase in amplitude of hippocampal theta activity (Figures 10 and 11). The amplitude of LIA occurring during immobility remained unchanged. Exposure to 100 ppm of H_2S did not alter the basic behaviour-EEG correlation; theta activity always occurred during voluntary movements, large amplitude irregular activity (LIA) was

Figure 7. Plots of peak frequency of hippocampal theta activity recorded from dentate gyrus (a) and CA1 (b) regions during the control exposure to nitrogen/air mixture. Each point represents the average ± S.E.M. of at least five animals.

a)


PEAK FREQUENCY
9
8
7
6
5
0
1
2
3
4
5
DAYS OF EXPOSURE

b)

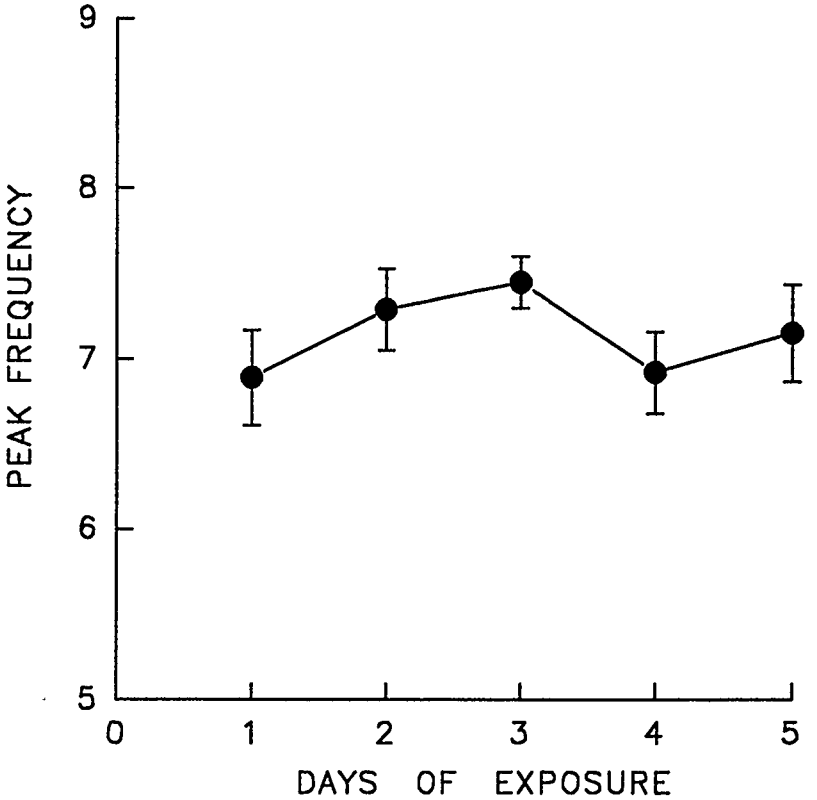
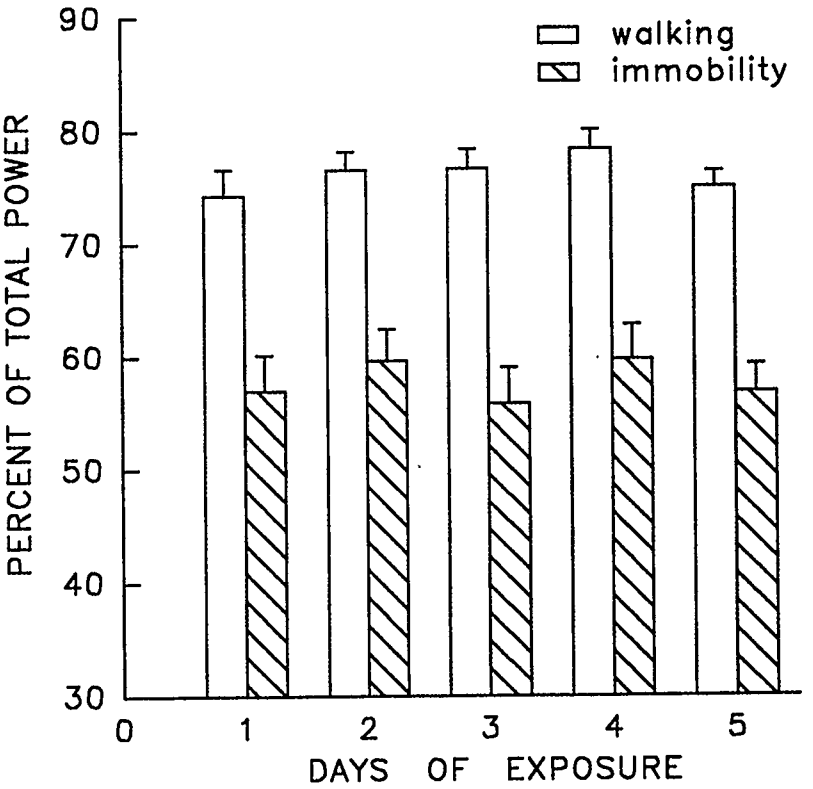

PEAK FREQUENCY
9
8
7
6
5
0
1
2
3
4
5
DAYS OF EXPOSURE

Figure 8. Histograms of the percentage of total power in frequency window of 4 - 12 Hz calculated for the recordings made from dentate gyrus (a) and CA1 (b) regions during the control exposure to nitrogen/air mixture. Each bar represents the average ± S.E.M. of at least five animals.

a)


walking
immobility
PERCENT OF TOTAL POWER
90
80
70
60
50
40
30
0
1
2
3
4
5
DAYS OF EXPOSURE

b)

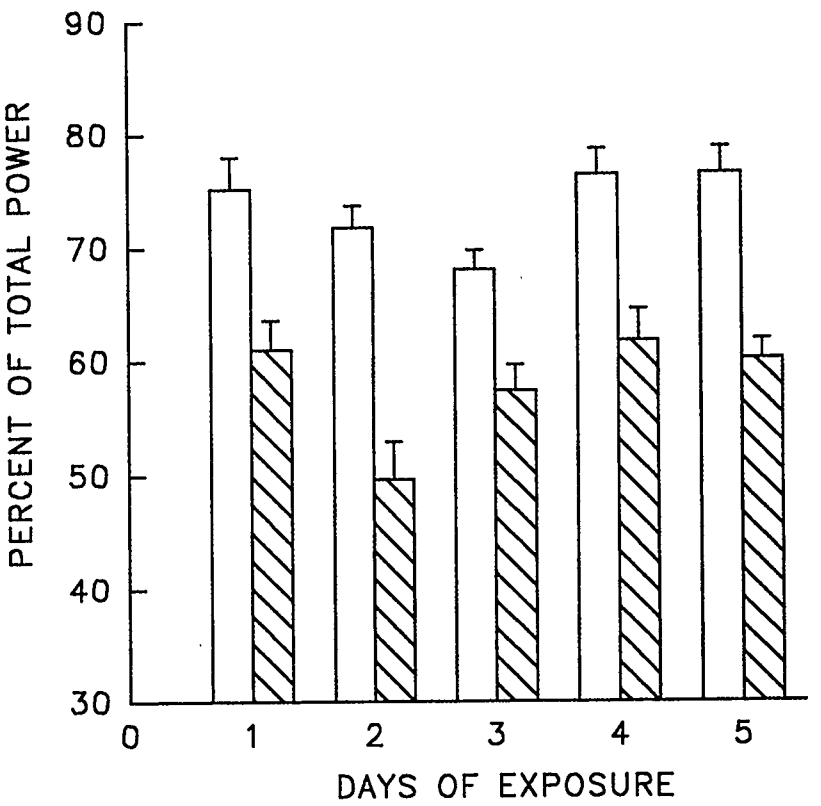

PERCENT OF TOTAL POWER
90
80
70
60
50
40
30
0
1
2
3
4
5
DAYS OF EXPOSURE

Figure 9. Example recordings of hippocampal theta activity recorded from dentate gyrus (DG) and CA1 region of a single animal prior to first exposure to 100 ppm of H_2S (control), following 1 and 3 hours of the first exposure and the next day (day 2) after the first exposure.

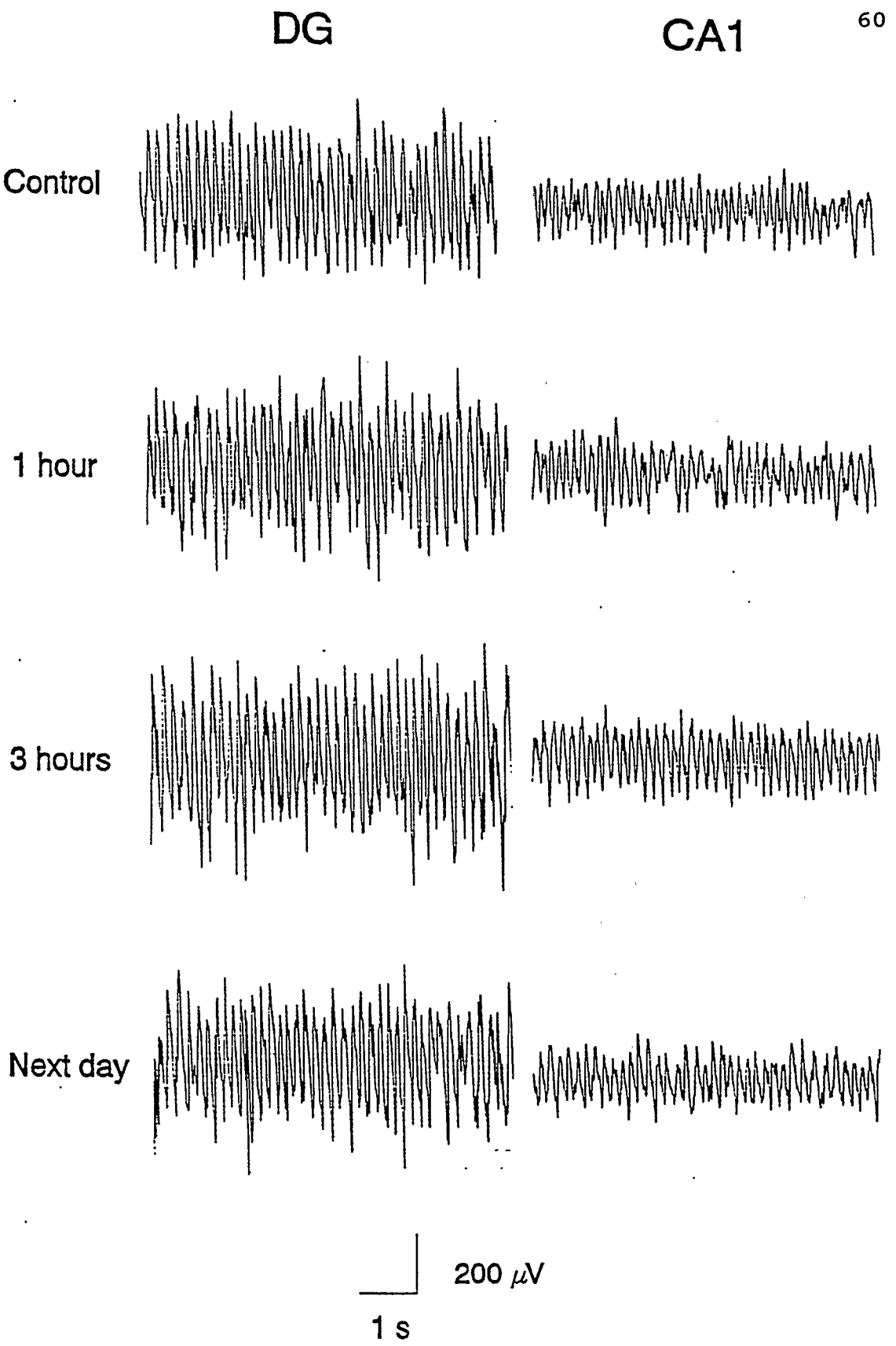

DG
CA1
Control
1 hour
3 hours
Next day
200 μV
1 s

Figure 10. Example recordings of hippocampal EEG activity recorded from dentate gyrus of a rat during walking and immobility prior to and after exposure on day 3 and 5 to 100 ppm H_2S.

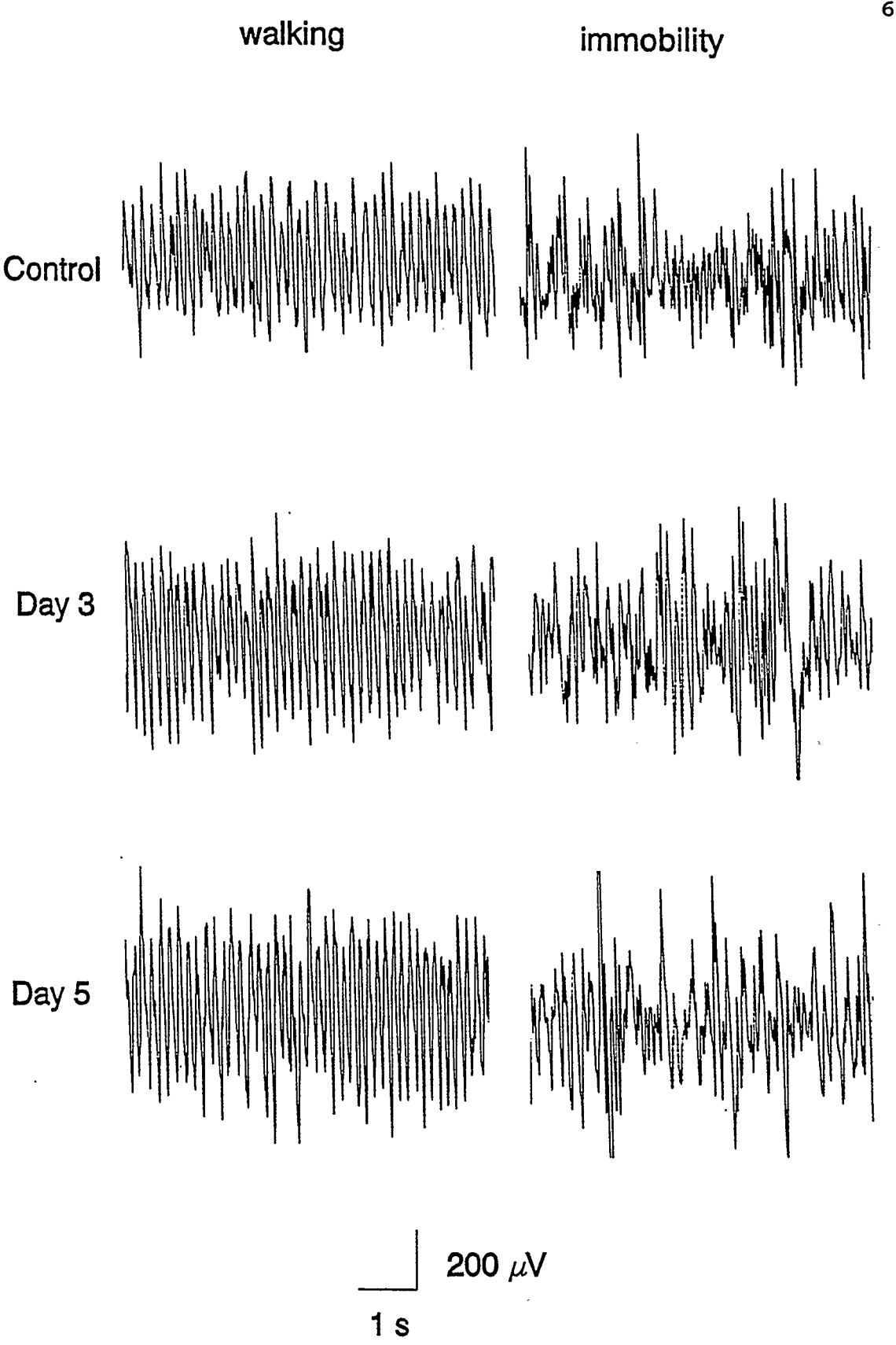
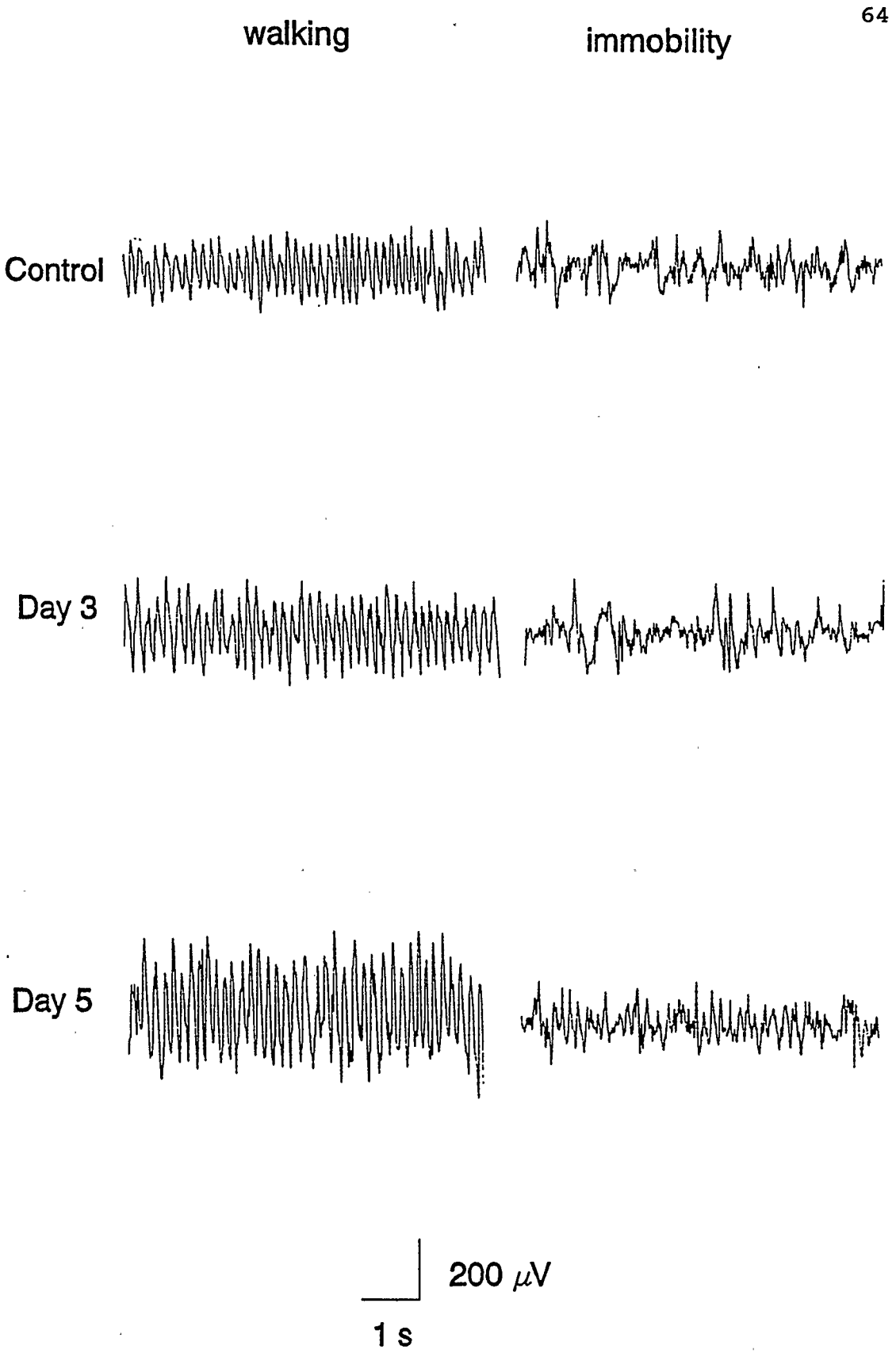

walking
immobility
Control
Day 3
Day 5
200 μV
1 s

Figure 11. Example recordings of hippocampal EEG activity recorded from CA1 region of a rat during walking and immobility prior to and after exposure on day 3 and 5 to 100 ppm H_2S.

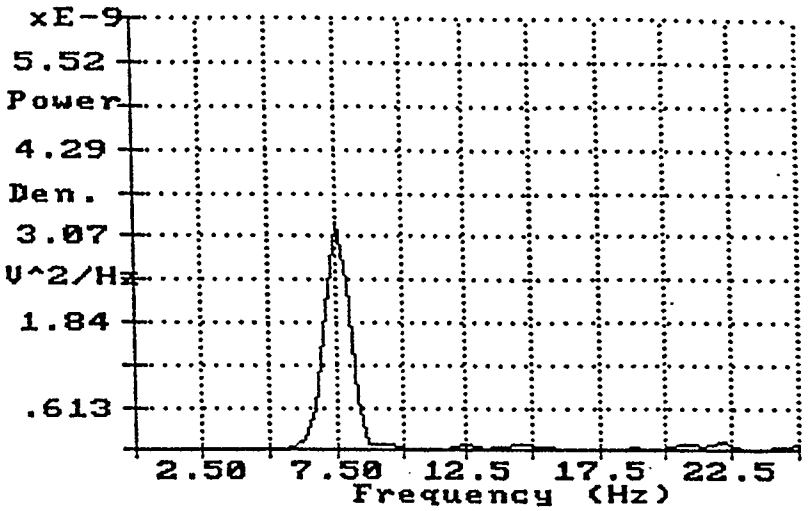

walking
immobility
Control
Day 3
Day 5
200 μV
1 s

present during immobility and automatic behaviour. The amount of time rats spent moving versus immobility was not quantified but it was continuously observed by the experimenter and appeared to be unchanged.

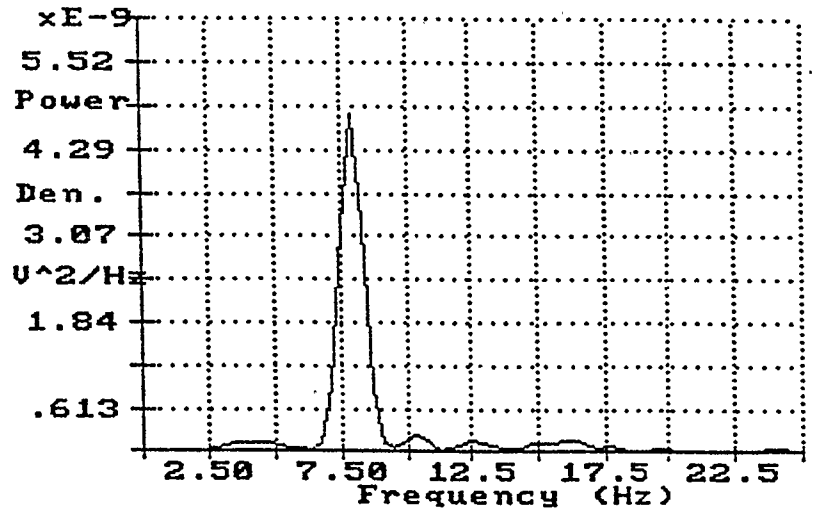
The representative power density spectra of hippocampal theta activity recorded from dentate gyrus of a single animal during the repetitive exposure to 100 ppm of hydrogen sulphide are presented in Figure 12. Repeated exposure to 100 ppm H_2S produced a cumulative increase in power of hippocampal theta activity (Figure 13) in both dentate gyrus and CA1 regions (correlation coefficients (r) were: 0.991 for dentate gyrus and 0.997 for CA1). A comparison of total power of the control activity recorded prior to the first exposure and activity recorded following day 1, 2, 3, 4 or 5 of exposure showed a significance increase on day 3 ($p < 0.05$) as well as on day 4 and 5 ($p < 0.01$). The total power of hippocampal theta activity in dentate gyrus region reached 209.06 ± 8.48% of the control power (power of the activity recorded prior to the first exposure) in dentate gyrus and 197.38 ± 13.07% in CA1 region. The power of this activity recorded just prior to the exposure to H_2S at each day also increased in a cumulative fashion (correlation coefficients were 0.996 and 0.986 for dentate gyrus and CA1 respectively). The two plots of total power of hippocampal theta recorded prior and after the 3 hour exposure to 100 ppm H_2S were almost parallel (Figure 13). The peak frequency of this activity was not significantly altered

Figure 12. Example power spectra of hippocampal theta activity recorded from dentate gyrus of a rat recorded during walking prior to and following exposure to 100 ppm H_2S on day 3 and 5.

CONTROL



DAY 3



DAY 5

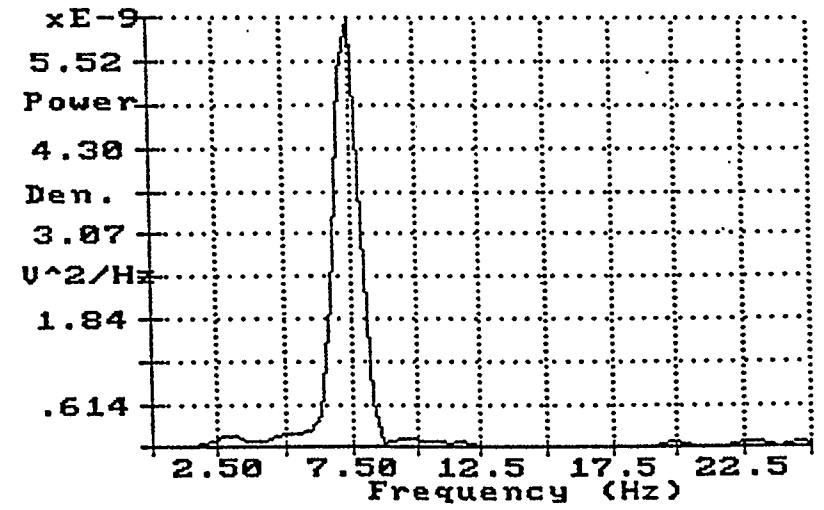
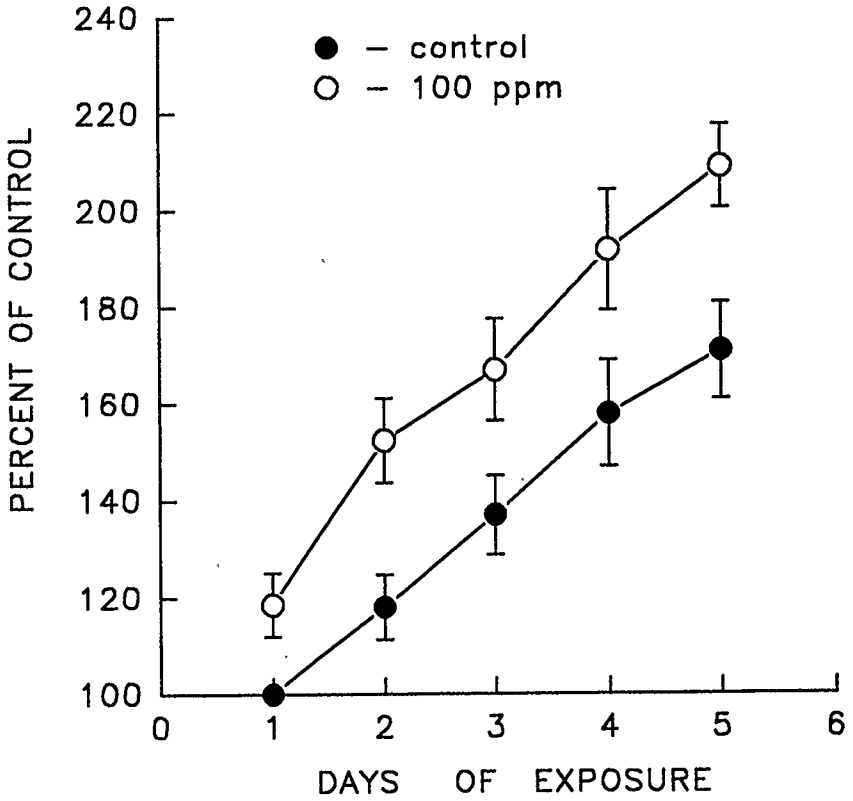


Figure 13. Graphs demonstrating a cumulative effect of the exposure to 100 ppm H_2S on the total power of hippocampal theta activity recorded from dentate gyrus (a) and CA1 (b) regions. Each point represents the mean ± S.E.M. of at least 5 animals. Open circles represent the total power of hippocampal theta activity presented as a percent of the control power (power of the activity recorded prior to exposure - day 1 control) following 3 hours of the exposure to 100 ppm H_2S at five consecutive days. Filled circles demonstrate the power of hippocampal theta recorded just prior to exposure on each day.

a)


● – control
○ – 100 ppm
PERCENT OF CONTROL
240
220
200
180
160
140
120
100
0
1
2
3
4
5
6
DAYS OF EXPOSURE

b)

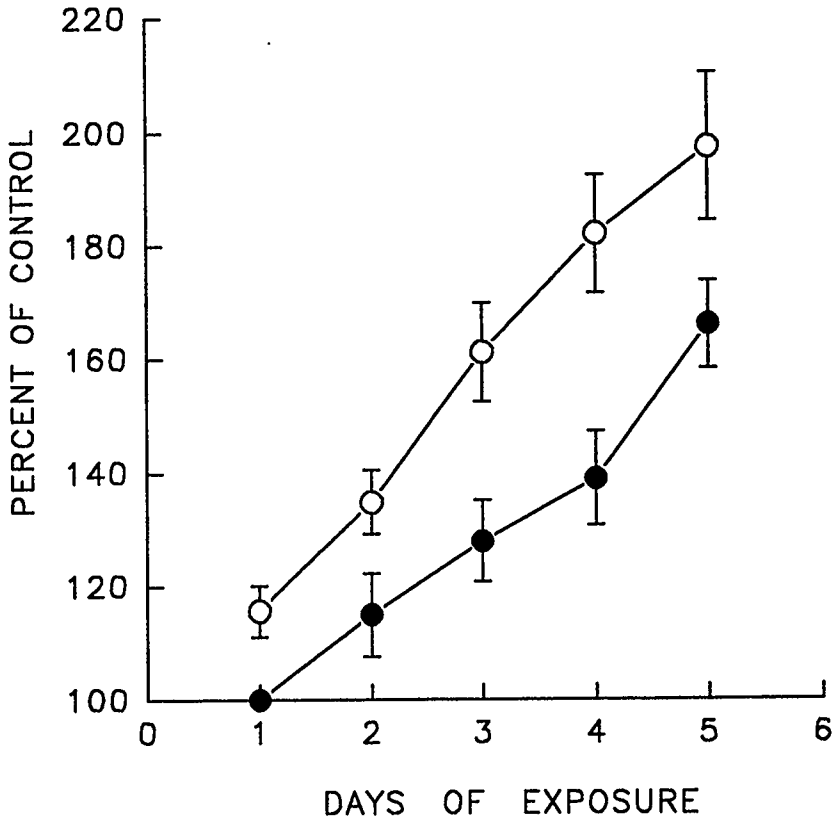
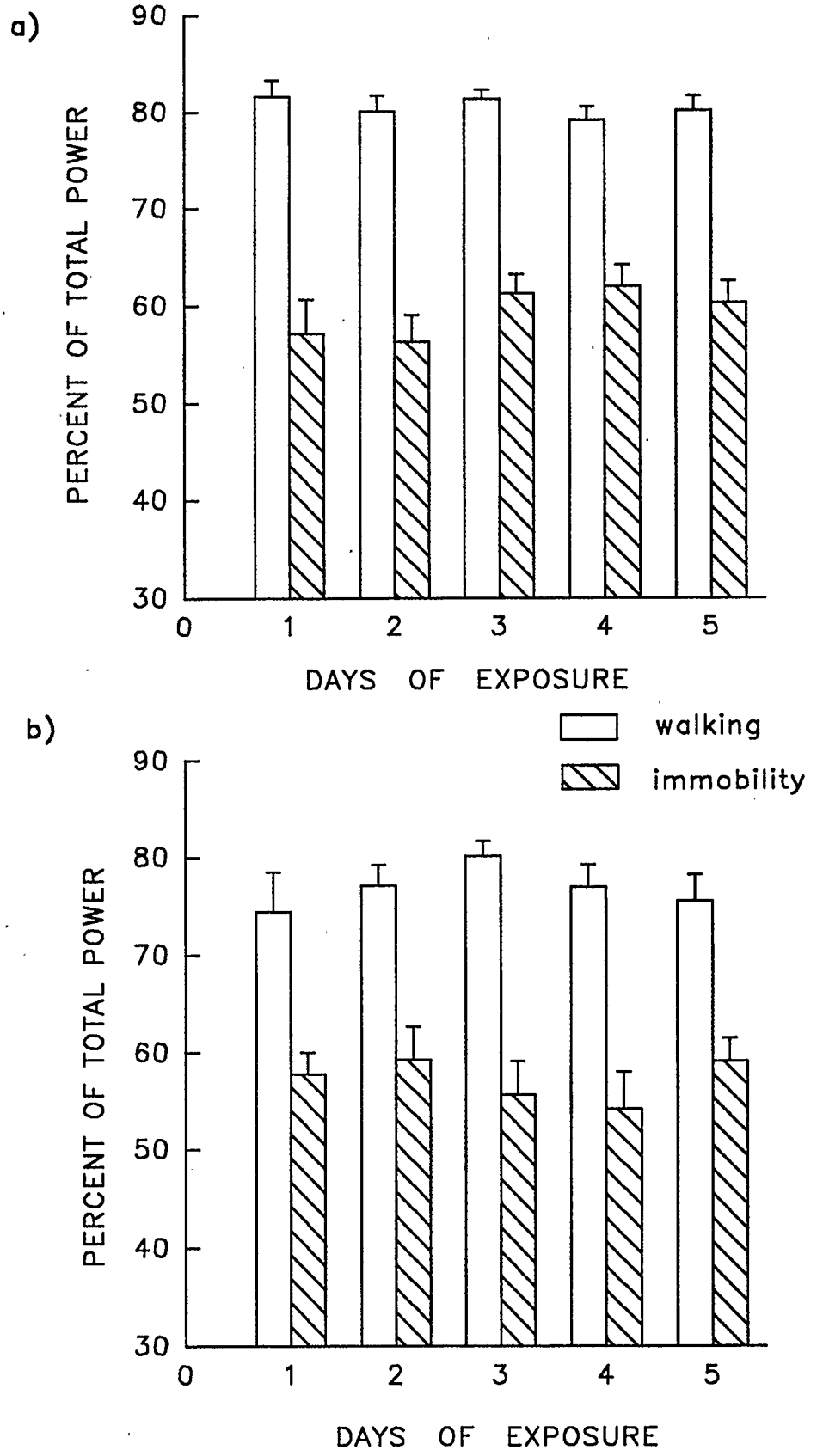

PERCENT OF CONTROL
220
200
180
160
140
120
100
0
1
2
3
4
5
6
DAYS OF EXPOSURE

Figure 14. Histograms of the percentage of total power in the frequency window of 4 - 12 Hz for recordings from dentate gyrus (a) and CA1 (b) regions during exposure to 100 ppm of H_2S. Each bar represents the average ± S.E.M of at least five animals.


a)
PERCENT OF TOTAL POWER
90
80
70
60
50
40
30
0
1
2
3
4
5
DAYS OF EXPOSURE
b)
walking
immobility
PERCENT OF TOTAL POWER
90
80
70
60
50
40
30
0
1
2
3
4
5
DAYS OF EXPOSURE

during the exposure to hydrogen sulphide and was in the range of 7.01 ± 0.79 Hz to 8.13 ± 0.47 Hz for dentate gyrus and from 6.98 ± 0.78 Hz to 7.46 ± 0.88 Hz for CA1. The percentage of total power in the frequency window of 4 - 12 Hz (theta frequency) was also unchanged and was in the range of 79.23 ± 1.4% to 81.42 ± 0.97% for dentate gyrus and 74.47 ± 4.04% to 80.09 ± 1.51% for CA1 (Figure 14). A comparison of the mean noise level of the control activity recorded during walking prior to the first exposure and activity recorded following the last (day 5) day of exposure to 100 ppm H_2S revealed a significant increase of this parameter, however the signal to noise ratio remained unaltered. The administration of 50 mg/kg of atropine prior to the last exposure to H_2S had no significant effect on theta activity present during walking or on sulphide-induced increase in power of this activity.

3.2.3 Recovery Following Repeated Exposure to 100 ppm of H_2S

The increased power of hippocampal theta activity following repeated exposure to 100 ppm of H_2S was found to be reversible, however it required two to three weeks for complete recovery (Figure 15). A second series of exposures (3 hours a day for 5 consecutive days) to 100 ppm H_2S one month after the end of the first trial produced almost identical effects on hippocampal theta as the first trial (Figure 16). The total power of hippocampal theta activity recorded from dentate gyrus during the second series of exposures was

Figure 15. A plot of total power of hippocampal theta activity recorded from CA1 (▽) and dentate gyrus (▼) 1, 2 and 3 weeks after the last exposure showing recovery following repeated exposure to 100 ppm H_2S. Each point represents the mean ± S.E.M of at least five animals.

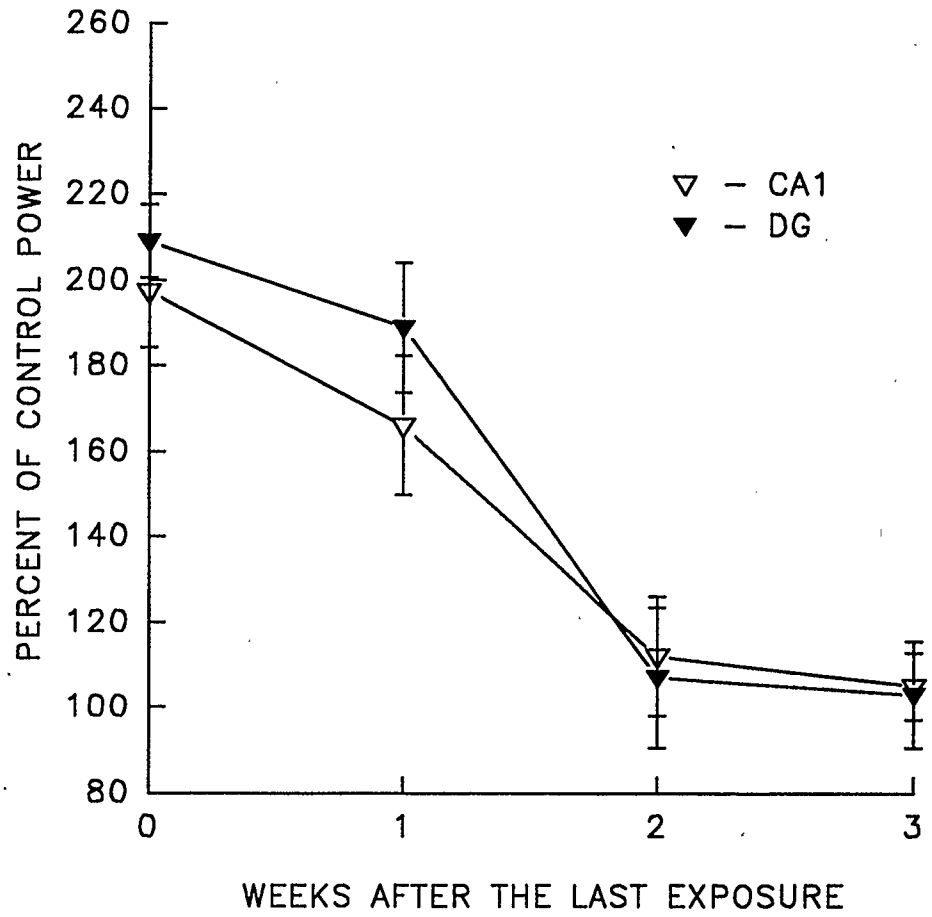
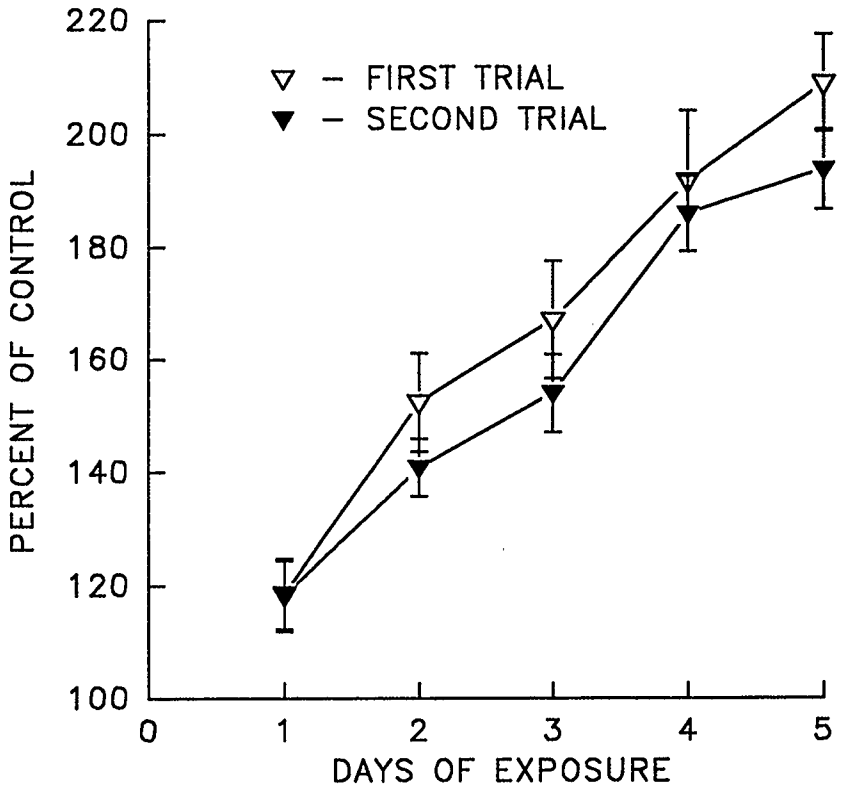

260
240
220
200
180
160
140
120
100
80
PERCENT OF CONTROL POWER
▽ – CA1
▼ – DG
0
1
2
3
WEEKS AFTER THE LAST EXPOSURE

Figure 16. Graphs demonstrating the increase in total power of hippocampal theta activity in dentate gyrus (a) and CA1 (b) regions during the two trials of the exposure to 100 ppm of hydrogen sulphide. The second trial (▼) took place a month after recovery following the first trial (▽). Each point represents average ± S.E.M. for 5 animals.

a)


▽ – FIRST TRIAL
▼ – SECOND TRIAL
PERCENT OF CONTROL
220
200
180
160
140
120
100
0
1
2
3
4
5
DAYS OF EXPOSURE

b)

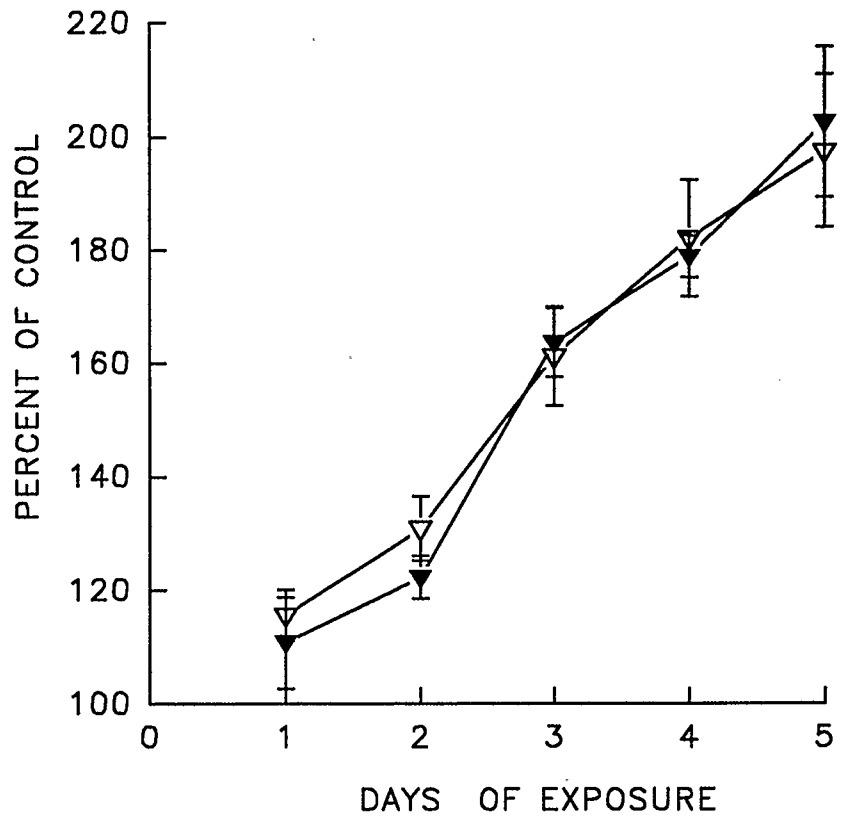
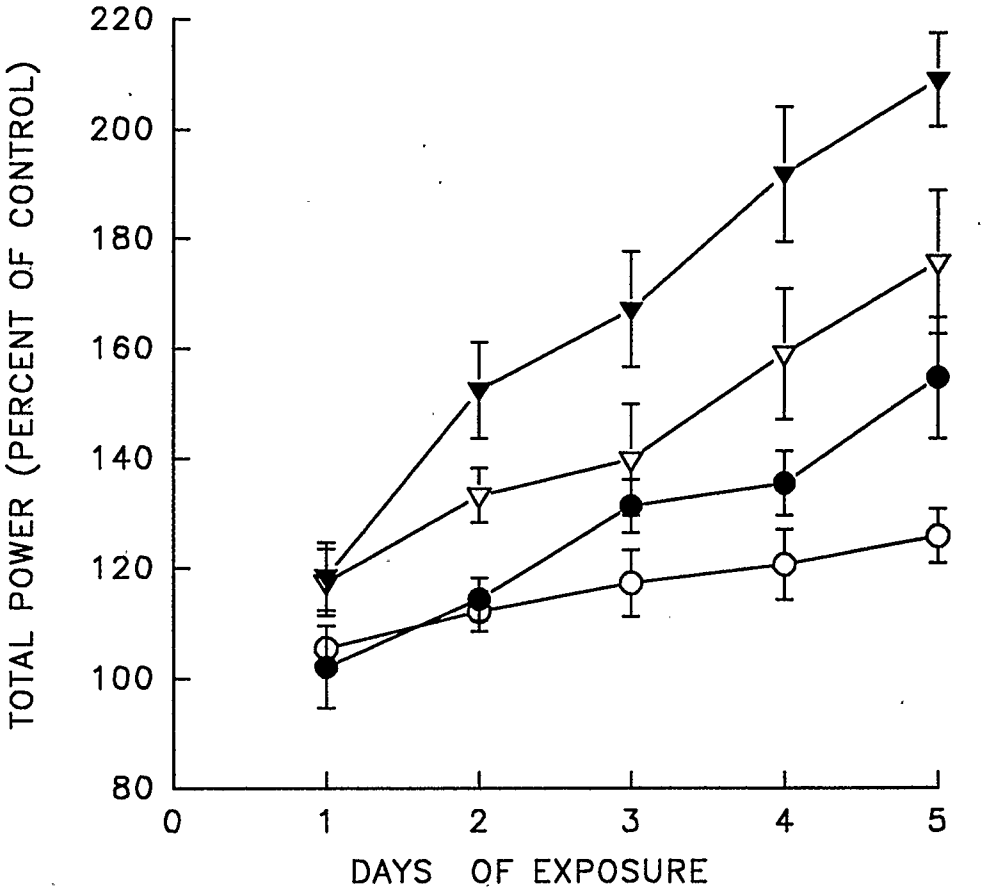
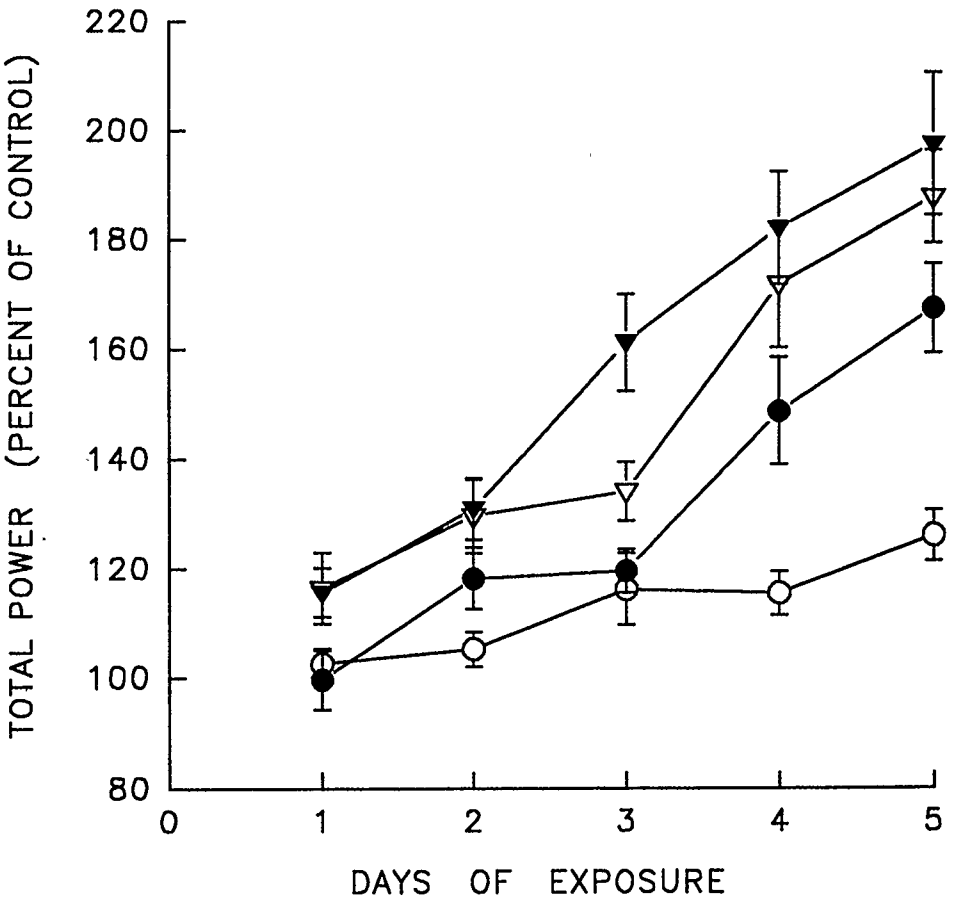

PERCENT OF CONTROL
220
200
180
160
140
120
100
0
1
2
3
4
5
DAYS OF EXPOSURE

Figure 17. A plot of the dose response relationship of hydrogen sulphide concentration and total power of hippocampal theta activity recorded from dentate gyrus during type 1 behaviour. Each point represents the average ± S.E.M. of at least 5 animals.



○ – 25 ppm H_2S
● – 50 ppm H_2S
▽ – 75 ppm H_2S
▼ – 100 ppm H_2S

Figure 18. A plot of the dose response relationship of hydrogen sulphide concentration and total power of hippocampal theta activity recorded from CA1 region during type 1 behaviour. Each point represents the average ± S.E.M. of 5 animals.



○ – 25 ppm H_2S
● – 50 ppm H_2S
▽ – 75 ppm H_2S
▼ – 100 ppm H_2S

elevated to 202.40 ± 13.92% of the control power. In CA1 region it was increased to 193.84 ± 7.09%.

3.3. Dose-response Relationship.

The results of repeated exposures to four different concentrations (100, 75, 50 and 25 ppm) of hydrogen sulphide on hippocampal theta activity recorded from dentate gyrus and CA1 region are presented in Figure 17 and 18 respectively. The lowest concentration, 25 ppm H_2S (○), produced only a slight increase in the power of hippocampal theta. This increase was not statistically significant in the dentate gyrus, whereas in CA1 the power at day 5 of exposure was significantly elevated ($p < 0.05$) and reached 125.92 ± 2.79% of the control power.

Exposure to 50 ppm H_2S (●) resulted in a greater increase in total power of hippocampal theta that reached 167.26 ± 8.12% of the control power in CA1 region and 154.59 ± 11.04% in dentate gyrus. The increase was statistically significant at day 4 and 5 ($p < 0.01$) in CA1 region and at day 5 ($p < 0.01$) in dentate gyrus. Exposure to 75 ppm H_2S (▽) produced further increase in power of hippocampal theta activity. The total power in dentate gyrus region was elevated to 175.67 ± 13.09% of control; significantly increased at day 4 and 5. In the CA1 region, power increased to 187.98 ± 8.49% of control and was statistically significant at day 4 and 5. The results of exposure to 100 ppm H_2S (▼) were described in paragraph 3.2.2.

3.4 Effects of Hydrogen Sulphide Exposure on Hippocampal EEG Compared to Neocortical EEG.

Exposure to 100 ppm H_2S did not produce any significant effects on neocortical EEG (Figure 19), however there was an increase in amplitude of hippocampal theta activity recorded from dentate gyrus of the same animal. The total power of neocortical EEG was not altered (Figure 20a), and neither was the peak frequency (Figure 20b).

3.5 Effects of Hydrogen Sulphide Exposure on Detomidine-Induced Type 2 Hippocampal Theta Activity.

Animals receiving detomidine showed expected behavioral and EEG changes. Rats became hypoactive. Spontaneous walking appeared effortful and was separated by long periods of immobility. All four animals exhibited almost continuous type 2 (immobility theta (Figure 21). The peak frequency of detomidine-induced type 2 theta was 5.3 ± 0.2 Hz. The ensemble 40 s (average) power spectra of a single animal during control walking and immobility behaviour and following detomidine injection are presented in Figure 22.

The example recordings of detomidine-induced type 2 theta activity recorded from dentate gyrus of a single animal during the repeated exposure to 100 ppm H_2S are demonstrated in Figure 23. Repeated exposure to 100 ppm H_2S resulted in a cumulative increase of total power of detomidine-induced type 2 theta activity (Figures 23 and 24). The total power of this

Figure 19. Example recordings of hippocampal and neocortical EEG of the same animal prior to the exposure (control) and at day 3 and 5 of the exposure to 100 ppm H_2S. Notice that the amplitude of hippocampal theta activity increased during the exposure whereas neocortical EEG remained unchanged.

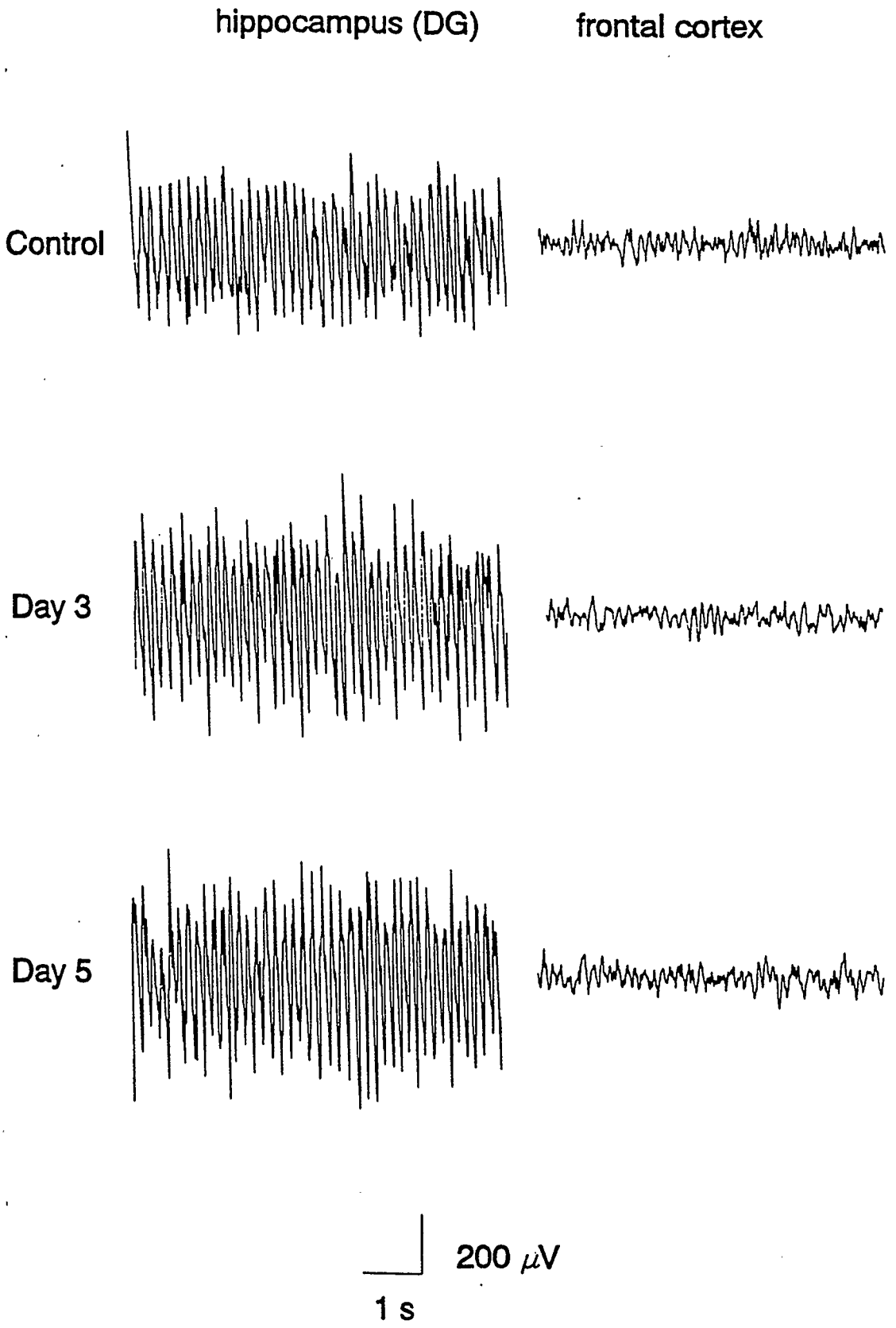
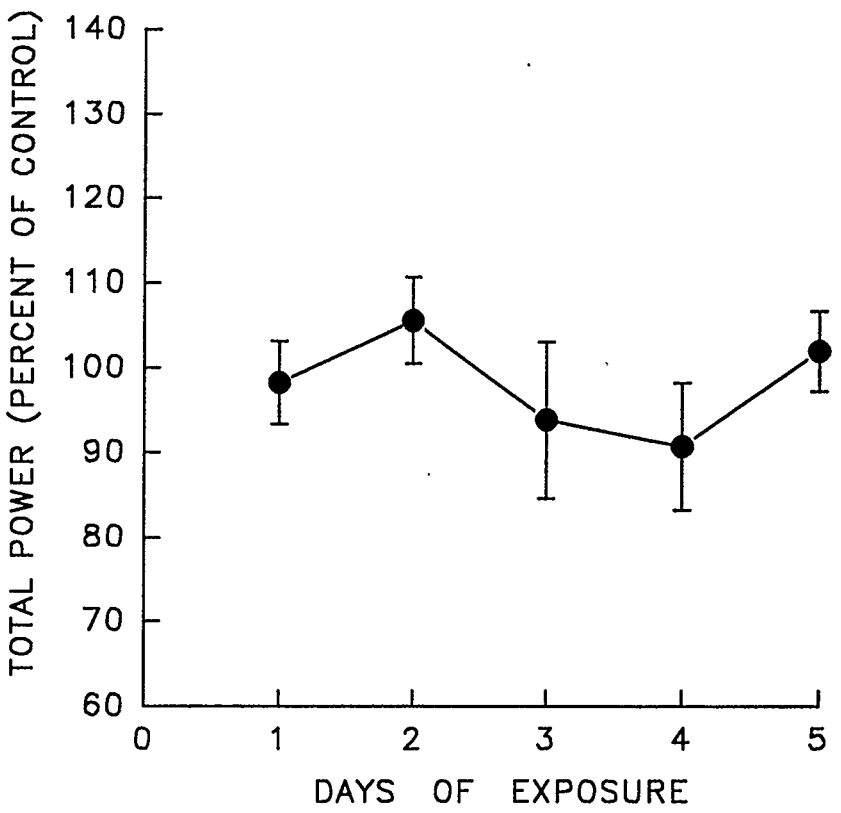

hippocampus (DG)
frontal cortex
Control
Day 3
Day 5
200 μV
1 s

Figure 20. Graphs demonstrating the effect of hydrogen sulphide exposure on the total power (a) and peak frequency (b) of neocortical EEG.

a)


TOTAL POWER (PERCENT OF CONTROL)
140
130
120
110
100
90
80
70
60
0
1
2
3
4
5
DAYS OF EXPOSURE

b)

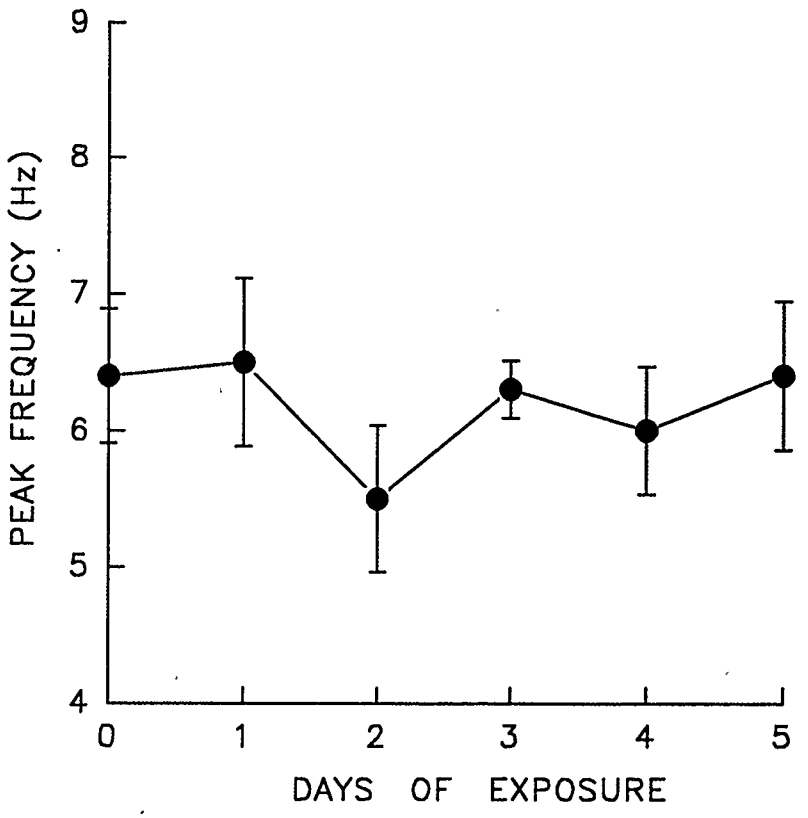

PEAK FREQUENCY (Hz)
9
8
7
6
5
4
0
1
2
3
4
5
DAYS OF EXPOSURE

Figure 21. Example recordings of hippocampal EEG activity recorded from dentate gyrus of a rat during control walking (upper trace) and immobility (middle trace) behaviour and during immobility following detomidine injection (lower trace).

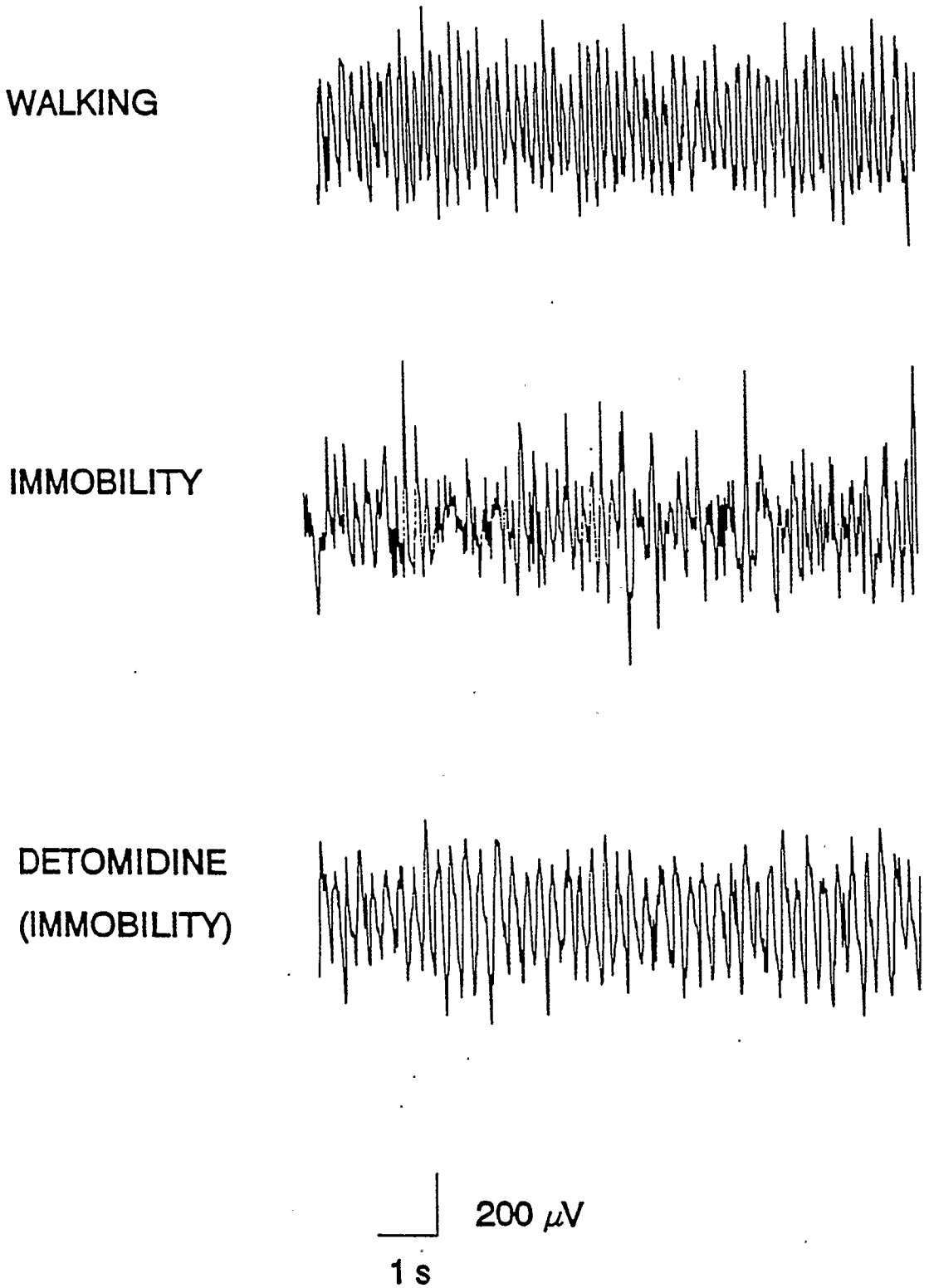
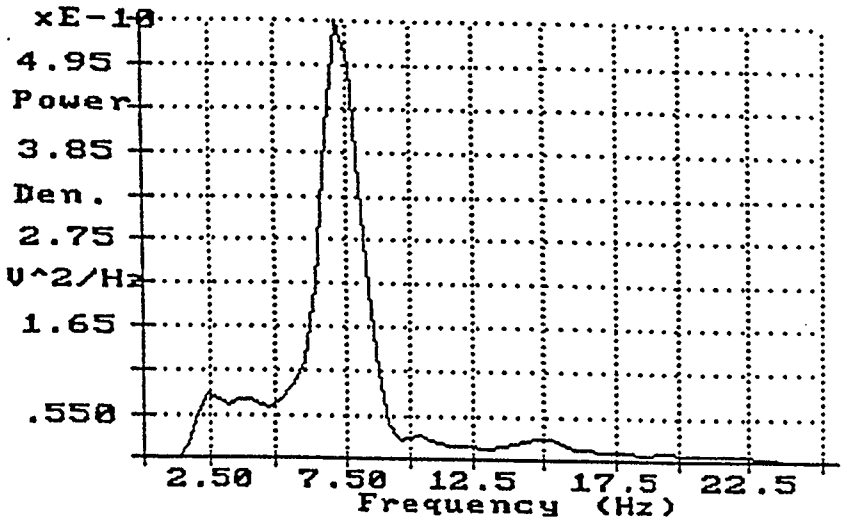
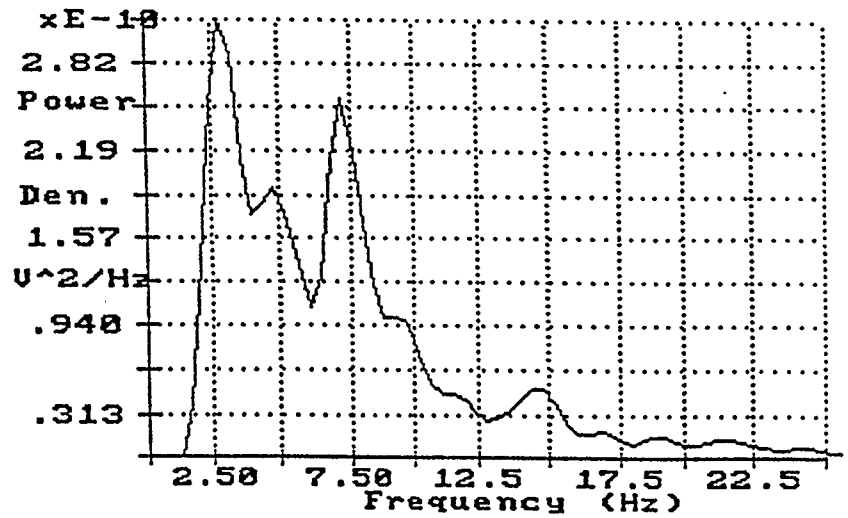

WALKING
IMMOBILITY
DETOMIDINE
(IMMOBILITY)
200 μV
1 s

Figure 22. Example ensemble (average) 40 s power spectra of hippocampal EEG activity recorded from dentate gyrus of a rat during control walking (upper trace) and immobility (middle trace) behaviour and during immobility following detomidine injection (lower trace).

WALKING



IMMOBILITY



DETOMIDINE

(IMMOBILITY)

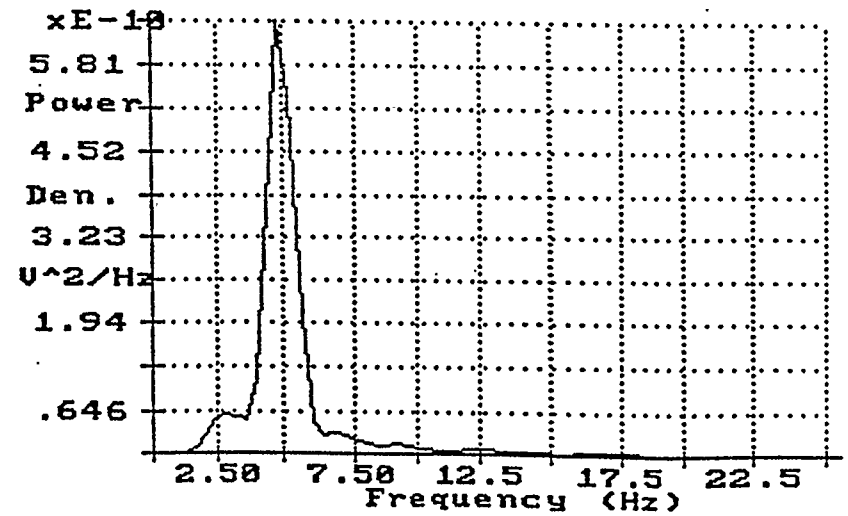


Figure 23. Example recording of detomidine-induced hippocampal type 2 theta activity recorded from dentate gyrus of a rat prior to and following exposure to 100 ppm H_2S on day 1 and 5.

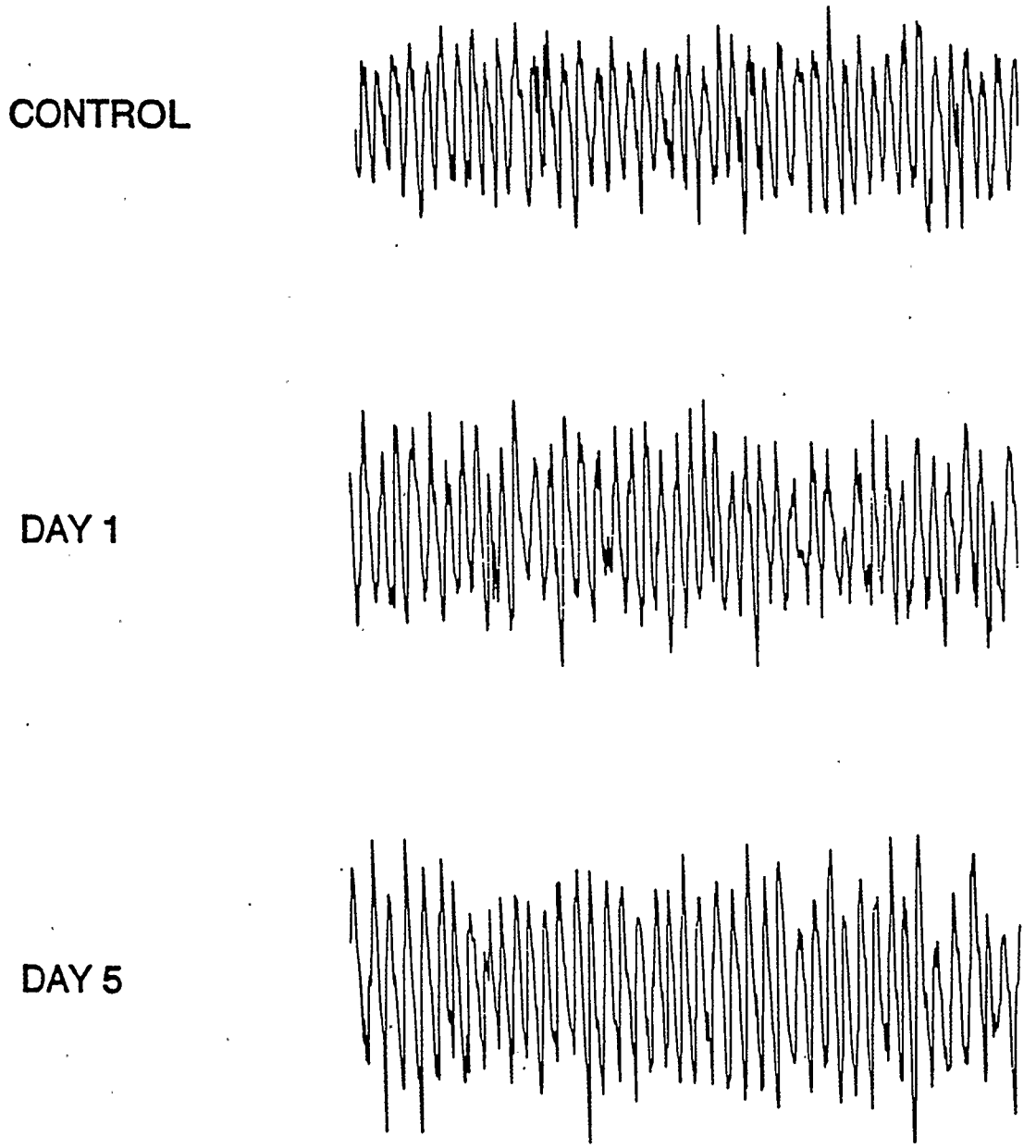
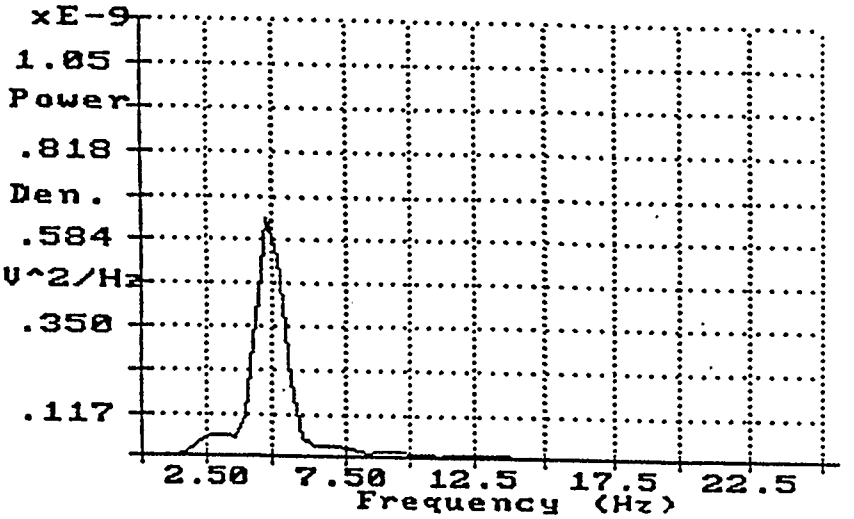
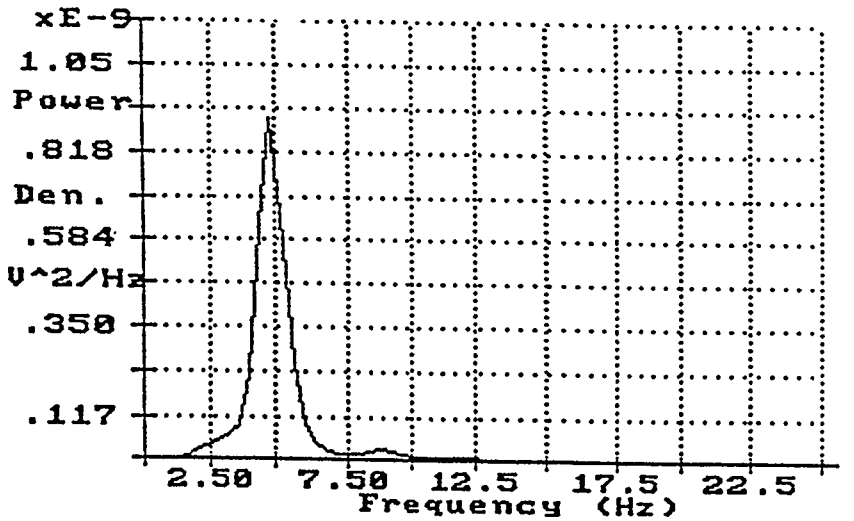

CONTROL
DAY 1
DAY 5
200 μV
1 s

Figure 24. Example ensemble (average) 40 s power spectra of detomidine-induced hippocampal type 2 theta activity recorded from dentate gyrus of a rat prior to and following exposure to 100 ppm H_2S on day 1 and 5.

CONTROL



DAY 1



DAY 5

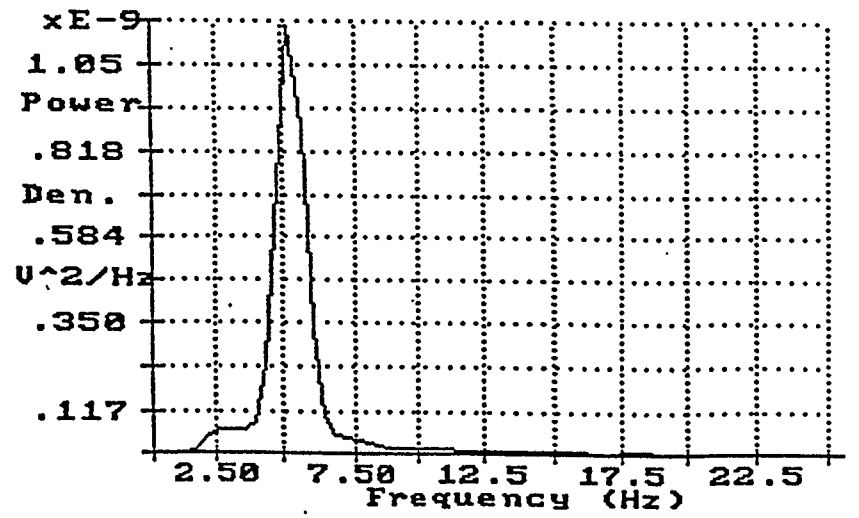
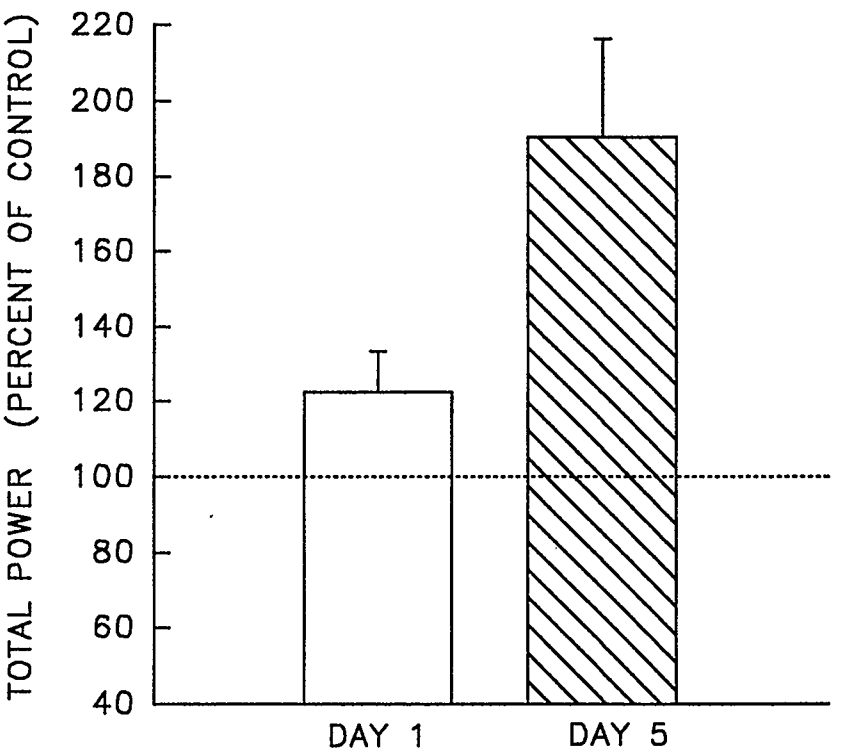


Figure 25. A histogram demonstrating a cumulative effect of the repeated exposure to 100 ppm H_2S on the total power of detomidine-induced type 2 theta activity recorded from dentate gyrus of a rat following exposure on day 1 and 5. Total power on each day is presented as a percent of total power of the control detomidine-induced type 2 theta activity.


TOTAL POWER (PERCENT OF CONTROL)
220
200
180
160
140
120
100
80
60
40
DAY 1
DAY 5

activity reached 122.3 ± 11.06 % of the control power on day first of exposure to 100 ppm H_2S and 190.43 ± 25.43 % on day 5. The peak frequency of detomidine-induced type 2 theta remained unaltered and was in the range of 4.8 ± 0.1 Hz on day 1 and 4.8 ± 0.2 Hz on day 5 of exposure.

3.6 Neurochemical Effects Following Hydrogen Sulphide Exposure.

The effects of exposure to 100 ppm H_2S on monoamine levels in three brain regions, cerebellum, frontal cortex and hippocampus are presented in Table 2 and Figure 26. In the hippocampus, hydrogen sulphide exposure produced a significant ($p < 0.05$) depression of serotonin levels (85.8% of control); however levels of the major metabolite, 5-HIAA, where not significantly altered. The ratio 5-HT/5-HIAA remained unchanged. Two other monoamines, norepinephrine and dopamine were not significantly altered. In the frontal cortex, levels of serotonin and norepinephrine were significantly ($p < 0.05$) increased, and reached 115.9% and 119.5% of control levels respectively. Dopamine and 5-HIAA levels, as well as 5-HT/5-HIAA ratio were not altered. In the cerebellum, exposure to hydrogen sulphide resulted in an increase of 5-HIAA to 118.4% of control levels and norepinephrine to 118.2%. Two other monoamines as well as 5-HT/5-HIAA ratio remained unaltered.

The effects of hydrogen sulphide exposure on amino acid levels are presented in Table 3 and Figure 27. Of the seven

Table 2. Tissue levels of serotonin (5-HT), 5- hydroxyindole--acetic acid (5-HIAA), norepinephrine (NE) and dopamine (DA) in the hippocampus, frontal cortex and cerebellum of control rats, and following exposure to 100 ppm H_2S (3 hours per day for 5 days).

	hippocampus	fr. cortex	cerebellum
5-HT			
control	492.4 ± 22.8	377.2 ± 14.2	61.9 ± 3.8
treated	*422.8 ± 13.1	*437.3 ± 20.9	66.9 ± 3.8
5-HIAA			
control	538.3 ± 14.2	137.9 ± 6.3	33.7 ± 1.4
treated	488.6 ± 23.3	146.7 ± 4.3	*39.9 ± 1.9
NE			
control	275.4 ± 13.9	261.8 ± 14.7	264.5 ± 6.6
treated	260.8 ± 9.8	*312.0 ± 14.4	*312 ± 14.4
DA			
control	21.3 ± 4.2	132.9 ± 23.8	12.9 ± 1.1
treated	24.6 ± 1.9	146.2 ± 27.5	14.5 ± 1.8

Note: Values are expressed as nanograms per gram frozen tissue ± S.E.M.

* - $P < 0.05$

Figure 26. Effects of H_2S exposure on monoamine (serotonin - 5-HT; 5-hydroxyindoleacetic acid - 5-HIAA; norepinephrine - NE; dopamine - DA) levels in hippocampus, frontal cortex and cerebellum. Data are expressed as a percent of control levels.

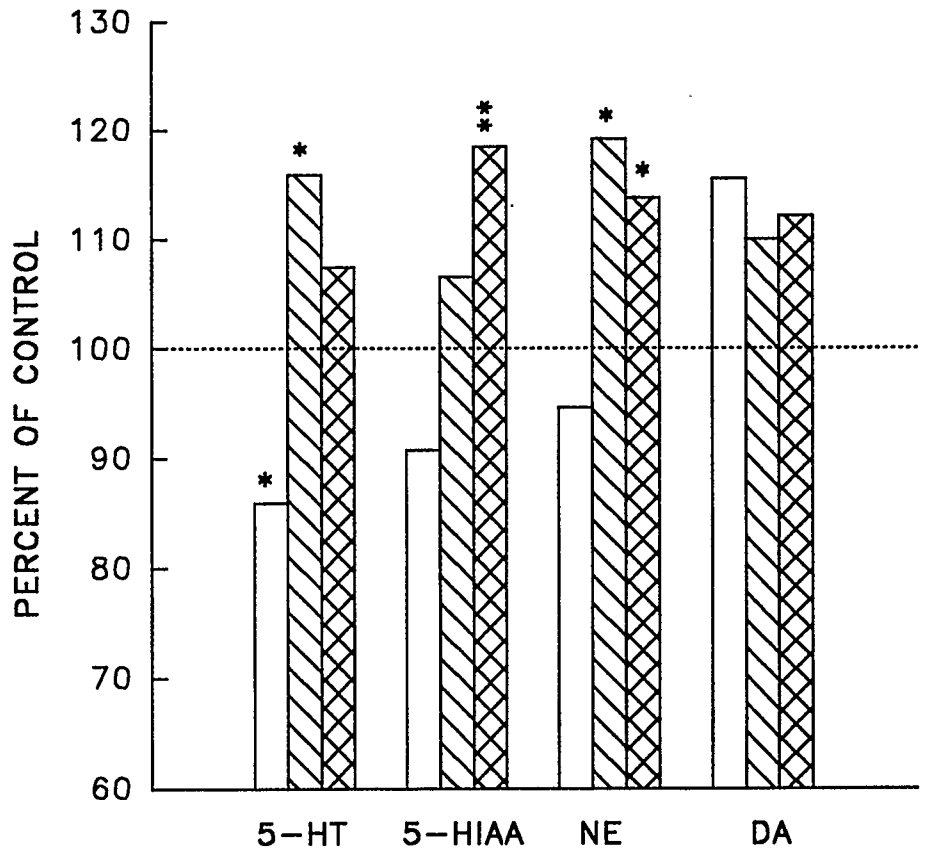
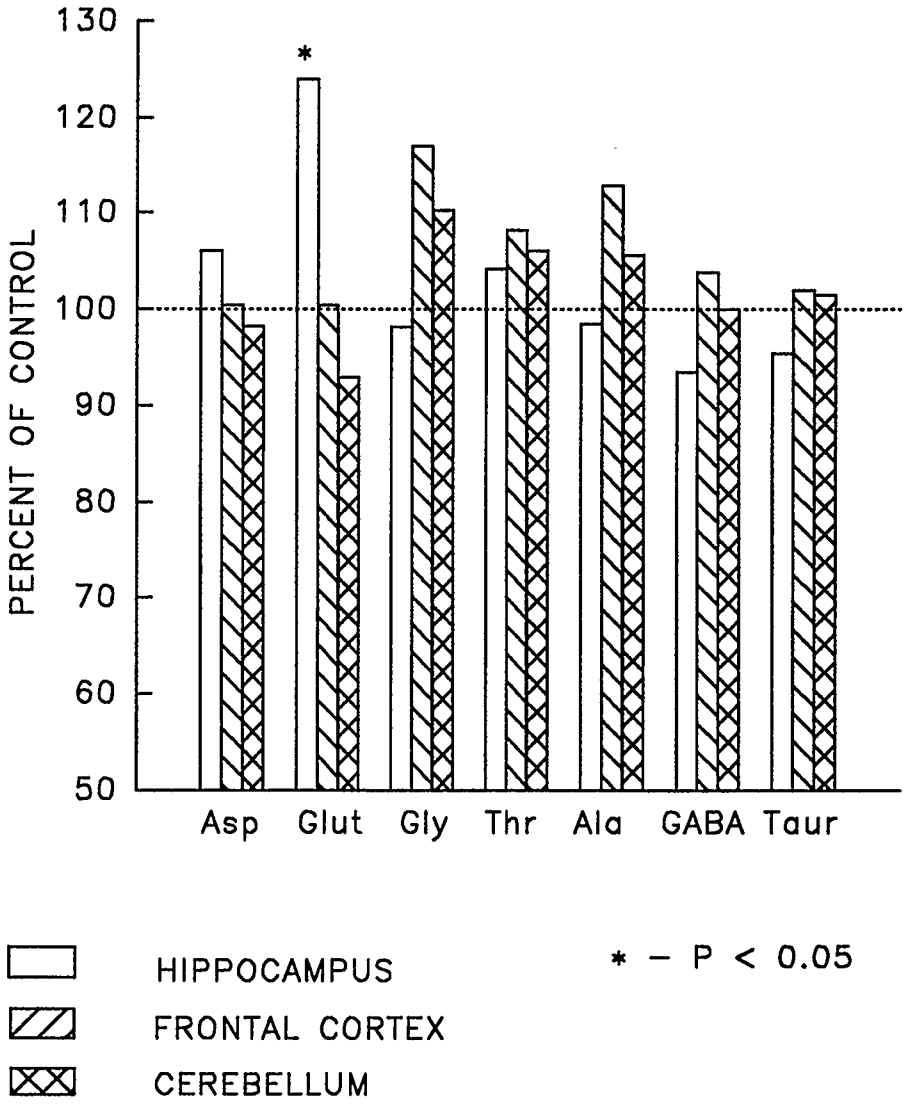

130
120
110
100
90
80
70
60
PERCENT OF CONTROL
5-HT
5-HIAA
NE
DA
HIPPOCAMPUS
FRONTAL CORTEX
CEREBELLUM
* - P < 0.05
** - p < 0.01

Table 3. Tissue levels of aspartate (Asp), glutamate (Glut), glycine (Gly), threonine (Thr), alanine (Ala), GABA and taurine (Taur) in hippocampus, frontal cortex and cerebellum of control rats and following exposure to 100 ppm H_2S (3 hours per day for 5 days).

		hippocampus	fr. cortex	cerebellum
Asp	control	2.04 ± 0.12	2.46 ± 0.17	1.79 ± 0.07
	treated	2.16 ± 0.13	2.47 ± 0.17	1.76 ± 0.09
Glut	control	13.09 ± 0.97	16.21 ± 0.95	14.18 ± 0.77
	treated	*16.24 ± 0.95	16.28 ± 0.74	13.18 ± 1.00
Gly	control	1.74 ± 0.19	1.91 ± 0.16	1.24 ± 0.09
	treated	1.71 ± 0.16	2.23 ± 0.15	1.37 ± 0.09
Thr	control	9.56 ± 0.74	13.83 ± 0.88	12.80 ± 0.81
	treated	9.95 ± 0.68	14.96 ± 1.11	13.57 ± 1.09
Ala	control	0.64 ± 0.04	0.59 ± 0.06	0.75 ± 0.05
	treated	0.63 ± 0.06	0.67 ± 0.06	0.79 ± 0.08
GABA	control	2.12 ± 0.79	1.84 ± 0.15	1.37 ± 0.10
	treated	1.98 ± 0.12	1.91 ± 0.14	1.37 ± 0.09
Taur	control	7.19 ± 0.27	8.00 ± 0.51	6.06 ± 0.21
	treated	6.87 ± 0.22	8.16 ± 0.51	6.15 ± 0.36

Note: Values are expressed as μMol per gram frozen tissue ± S.E.M. * - $P < 0.05$

Figure 27. Effects of H_2S exposure on amino acid (aspartate - Asp; glutamate - Glut; glycine - Gly; threonine - Thr; alanine - Ala; GABA; and taurine - Taur) levels in hippocampus, frontal cortex and cerebellum. Data are expressed as percent of control levels.


130
120
110
100
90
80
70
60
50
PERCENT OF CONTROL
*
Asp
Glut
Gly
Thr
Ala
GABA
Taur
HIPPOCAMPUS
FRONTAL CORTEX
CEREBELLUM
* – P < 0.05

amino acid measured in three brain regions, only glutamate levels in the hippocampus were significantly altered ($p < 0.05$) during exposure to 100 ppm H_2S, increasing to 124.1% of control levels.

4.0 DISCUSSION

4.1 General Considerations

In order to exclude the effects of anoxic conditions that might have occurred in the chamber during exposure to hydrogen sulphide (since H_2S was balanced in nitrogen), control exposures to nitrogen/air mixture were carried out (see section 2.3). The control exposure did not result in any significant changes in hippocampal EEG activity or in behaviour of the animal, thus ruling out effects of possible anoxic conditions on hippocampal theta activity. Hydrogen sulphide has a strong offensive odour, thus the effect of odorants on hippocampal theta activity must be considered in analyzing the results. The study by Vanderwolf (Vanderwolf, 1992), however, demonstrated that olfactory stimulation of sniffing behaviour was not correlated with the production of theta activity. Thus the observed H_2S-induced alteration of hippocampal theta activity was not a result of anoxia or olfactory stimulation.

4.2 Cumulative Effects of Hydrogen Sulphide Exposure on Hippocampal Theta Activity

Perhaps the most significant finding of this study is that repeated exposure to low concentrations of hydrogen sulphide produced a cumulative increase in total power of hippocampal theta activity in freely moving rats. The effects

of chronic hydrogen sulphide intoxication have been controversial. It was proposed that H_2S is a noncumulative poison because of its rapid metabolism and excretion (Beauchamp et al., 1984). This was confirmed by the study that showed the majority of administrated [S^{35}]-sodium sulphide was excreted in the urine as sulphate in the first 6 - 12 hours after dosing (Cutris et al., 1972). However it has also been reported that H_2S toxicity may be cumulative (Savolainen et al., 1980). The study by Savolainen demonstrated that repeated exposure to 100 ppm hydrogen sulphide resulted in increasing inhibition of cerebral cytochrome oxidase activity, depression of RNA and reduced uptake of orotic acid in the RNA fraction (Savolainen et al., 1980). These effects were attributed to the cumulative inhibition of the cytochrome oxidase. It was suggested that slow dissociation of the haem-sulphide complex and the rate of the synthesis of the new haem could be the limiting factors for the recovery following H_2S intoxication (Savolainen et. al., 1980). The half-life of cerebral cytochrome haem in rat brain, however, has been estimated to exceed 24 hours (Schanley et al., 1977), whereas the effects of hydrogen sulphide exposure on hippocampal theta activity persisted for two to three weeks. This would suggest that the increase in total power of hippocampal theta activity is probably due to mechanisms other than inhibition of cytochrome oxidase.

It has also been demonstrated that another enzyme,

monoamine oxidase (MAO), is readily inactivated by sulfhydryl reagents (Yasunobu and Tan, 1985; Weyler and Salach, 1985). High doses (10 - 100 mg/kg) of sodium bisulphide were shown to inhibit monamine oxidase activity in a dose dependent manner (Warenycia et al., 1990). The mechanisms and possible reversibility of sulphide-induced MAO inhibition are unknown. It was postulated, however, that modification of this enzyme does not take place at a direct catalytic site (Weyler et al., 1990). The half-life for the recovery of rat brain MAO after irreversible inhibition was reported to be up to 30 days (Egashira and Yamanaka, 1981; Corte and Tipton, 1980; Arnett et al., 1987). Since the time of recovery of MAO activity correlates with the time required for the recovery of the total power of hippocampal theta activity following exposure to hydrogen sulphide, it is possible that elevated catecholamine and/or serotonin levels resulting from the inhibition of monoamine oxidase may be involved in the sulphide-induced alteration of hippocampal theta activity.

4.3 Neurochemical Effects of Hydrogen Sulphide Exposure

Following the exposure to 100 ppm of H_2S, the levels of serotonin in the hippocampal formation were significantly lower than the control levels, whereas in the frontal cortex they were significantly elevated. Norepinephrine levels were unaltered in hippocampus, but increased significantly in the cerebellum and frontal cortex. These data agree with those

reported by Warenycia (Warenycia et al. 1989). That study demonstrated that administration of a high dose (twice the LD_{50}) of sodium bisulphide (volatile salt of hydrogen sulphide) increased norepinephrine and epinephrine levels in the hippocampus. In the brainstem all three catecholamines and serotonin were increased. Similar effects were shown following chronic exposure to low levels of H_2S during brain development in the rat (Skrajny et al., 1993). Exposure to 75 ppm H_2S resulted in increased levels of serotonin and norepinephrine in cerebellum and frontal cortex. The increased levels of monoamines could be explained by the inhibition of the degradative enzymes, either catechol O-methyl transferase (COMT) or monoamine oxidase (MAO). It was demonstrated previously that high doses (10 - 100 mg/kg); 70 - 667% of the LD_{50}) of sodium bisulphide inhibit monoamine oxidase activity in a dose dependent manner (Warenycia et al., 1989). Although there is no report available on the effects of sulphide treatment on COMT activity, the sensitivity of this enzyme to sulfhydryl reagents (Borchardt, 1977), suggests that it may also be susceptible to sulphide inhibition. The calculated turnover ratio of serotonin (5-HIAA/5HT) in three brain regions studied in this experiment, however, did not change suggesting that a mechanism other than inhibition of a degradation process may be involved. It is possible that H_2S alters more than one biochemical process involved in monoamine turnover, such as synthesis, release or biotransformation.

Among seven amino acids studied in three brain regions, only glutamate levels in the hippocampus were increased following H_2S exposure. No changes in central amino acid levels were observed in the frontal cortex and cerebellum. Similar effects were demonstrated in an earlier study by Kombian (Kombian et al., 1988). It was shown that acute exposure to high dose of sodium bisulphide (twice the LD_{50}) produced an increase of amino acid levels in brainstem, while no significant effects were detected in other brain regions: cerebral cortex, striatum and hippocampus. The increased levels of glutamate in the hippocampal formation observed in the present study could result from a cumulative effect of repeated exposure to hydrogen sulphide since acute treatment with a much higher dose did not have any effect on the levels of glutamate in this brain region. The mechanism responsible for the sulphide-induced increase of glutamate levels will need further investigation. It is well established that there are multiple metabolic compartments or pools of glutamate in the CNS, as well as many potential precursors and metabolic pathways for its biosynthesis (Shank et al., 1983). In the present study the whole tissue levels of glutamate were measured. Since the transmitter pool of glutamate represents only a fraction of the total free glutamate in the CNS, it is impossible to state whether the increased whole tissue levels of this amino acid relate to any alteration in the neurotransmitter pool. However it has also been estimated that

the transmitter pool of glutamate may be as large as 40 to 45% of the total glutamate present in regions of the brain with dense glutamatergic innervation (Fonnum et al., 1981; Young et al., 1974). Abnormally enhanced glutamatergic neurotransmission has been reported to cause excitotoxic cell damage (Greenamyre, 1986), and neuronal death associated with olivopontocerebellar atrophy, Huntington's disease, status epilepticus and hypoxia/ischemia (Greenamyre, 1986). It has also been suggested that chronic overactivity of the hippocampal glutamatergic system may lead to degeneration seen in Alzheimer's disease. Among other important roles, glutamate is involved in long-term potentiation and memory (Headley and Grillner, 1990). The present study may thus provide some support for the involvement of glutamate in alteration in memory following the exposure to hydrogen sulphide.

Whole tissue levels of neurotransmitters measured in this study do not reflect extracellular changes. A microdialysis study of the extracelluar pool of major neurotransmitters involved in the production of hippocampal theta activity measured in different brain regions following the exposure to hydrogen sulphide could have been helpful in understanding the mechanism of action of this toxicant. However, this technique despite increasing popularity is still far from being a routine method and presents several methodological limitations. First, the amount of substances measured only partially reflect their true concentration in the

extracellular space (Nakahara et al., 1991). Second there is tissue damage by the microdialysis probe including inflammatory changes and subsequent gliosis and fibrosis in the area surrounding the probe (Benveniste et al., 1989); in addition tissue diffusion characteristics and local cerebral metabolism as well as blood flow may change (Benveniste and Hüttemeier, 1990). Third, neurotransmitters recovered in the dialysate are sometimes not involved in neurotransmission (Lonnroth et al., 1987).

4.4 Possible Cellular Mechanisms of Hippocampal Theta Activity

The precise cellular mechanisms by which hippocampal theta activity is induced are unknown. Some insight may be gained from studies using the hippocampal slice preparation and a recently proposed computer model of carbachol-driven rhythmic population oscillations in the CA3 region (Traub et al., 1992). In this model 5 Hz oscillations were obtained under the following conditions: (1) excitatory synaptic conductance was within a limited range; (2) there was a blockade of fast and slow IPSPs; (3) the after-hyperpolarization conductance was reduced, but not abolished; (4) the apical dendrites of the pyramidal cells were depolarized. All these actions are consistent with the action of acetylcholine and carbachol on hippocampal neurons (Halliwell, 1990, Ben-Ari et al., 1981). It was also suggested that this activity occurs synchronously among a large

population of pyramidal neurones and is driven by a polysynaptic recurrent circuit involving non-NMDA glutamate receptors (probably at the level of the interneuron), but not $GABA_A$ or $GABA_B$ receptors (MacVicar and Tse, 1989). Heynen and Bilkey (1991), on the other hand, reported that simultaneous blockade of GABA-ergic IPSPs and excitation by glutamate could produce activity similar to that induced by carbachol. Thus, the neural network that generates carbachol-induced rhythmical slow activity appears to involve two major excitatory synapses (cholinergic and glutamatergic) as well as inhibitory GABAergic synapses. In the simplest model that can be proposed for the induction of rhythmical activity in hippocampal slices, the principal cells (pyramidal and granule cells) receive excitatory cholinergic projections and then send excitatory glutamatergic projections to local interneurons which in turn send inhibitory GABAergic connections to the principal cells.

The generation of theta activity in the *whole animal* appears to be more complex. Two types of theta have been differentiated (see section 1.8.1): type 1, atropine-resistant and type 2 atropine-sensitive. In 1983, Buzsaki proposed a model of generation of hippocampal atropine-sensitive theta activity in a whole animal which was based on feed-forward inhibition from the septum and a direct excitation by the entorhinal input (Buzsaki et al., 1983). In this model it was assumed that pyramidal and granule cells receive almost

synchronous dendritic depolarization via perforant path and inhibitory projections from interneurons at their somata. The interplay between the two processes determine whether a given hippocampal cell will be discharged (Buzsaki et al., 1983). It has been suggested that septal neurons may act as a pacemaker for the hippocampal rhythm (Petsche et al., 1962; Stumpf, 1965). However more recent studies (Ott et al., 1977; Rowntree and Bland, 1986) have demonstrated that the hippocampus can generate theta activity in the absence of a rhythmical cholinergic input, however, a nonrhythmical cholinergic input must be present. Thus, it is possible that mechanisms favouring rhythmical activity are present in both the septal nuclei and the hippocampus. Under normal conditions both may contribute to the development of theta activity in the hippocampus.

Type 1 theta activity is thought to be dependent on the serotonergic pathways ascending from the brainstem to the hippocampal formation (Robertson et al., 1991; Vanderwolf, 1988). It was reported these activity is attenuated by treatment with p-chlorophenylalanine or reserpine and restored by serotonergic agonists (Vanderwolf et al., 1986). Serotonergic activation is probably dependent on both 5-HT_1 and 5-HT_2 receptors (Watson et al., 1992). It was also suggested that theta activity during type 1 behaviour is produced by joint action of cholinergic and serotonergic inputs to the hippocampal formation (Vanderwolf, 1988). If

either of these inputs is inactivated, the other input is able to produce theta activity. During immobility and automatic behaviour, both inputs are inactive allowing large amplitude irregular activity to occur (Vanderwolf, 1988).

The cellular mechanism by which serotonin induces theta activity is still unknown. It was suggested that serotonin has a dual effect on dentate gyrus granule cells; suppressing spontaneous activity while enhancing excitability to afferent stimulation (Richter-Levin and Segal, 1990). In CA1 cells, the effect of serotonin is even more complex. First, it activates a Ca-dependent K-current which is inhibitory. Second, it suppresses the slow Ca-dependent K-conductance which produces an increase in neuronal discharge in response to depolarizing input. Third, serotonin produces a more slowly developing and long lasting suppression of an intrinsic voltage-dependent K-conductance, leading to neuronal depolarization (Colino and Halliwell, 1987). It was suggested that the effects of serotonin may in part be mediated via an interaction with glutamate (Beck, 1992). For example, it has been suggested that serotonin enhances glutamatergic potentials in the neocortex (Reynolds et al, 1988). It was also suggested that release of serotonin in the hippocampus could facilitate rhythmical excitatory glutamatergic perforant path input to the hippocampus and dentate gyrus (Vanderwolf, 1988).

4.5 Possible Mechanisms and Site of Action of H_2S on hippocampal Theta Activity.

Because the cellular mechanisms of theta generation are still not clear, and there are a limited number of studies on the electrophysiological effects of hydrogen sulphide, it is difficult to precisely define site and mechanism of action of this toxicant. Exposure to hydrogen sulphide appeared to affect both types of theta activity: type 1, atropine resistant and type 2, atropine sensitive theta in both CA1 and dentate gyrus regions. Both the power of hippocampal theta activity in the frequencies 4 - 12 Hz as well as noise levels (frequencies between 20-25 Hz) were increased, whereas the signal to noise ratio remained unaltered. The percentage of the total power of hippocampal theta activity in the frequency window 4 - 12 Hz remained statistically unchanged during exposure to hydrogen sulphide.

It was reported that blockade of $GABA_A$ mediated fast inhibition in hippocampal slices produced an increase in amplitude of synchronized activity and a reduction in frequency (Schneiderman, 1986). Since no effects on frequency of hippocampal activity were observed in the present study, it may be suggested that hydrogen sulphide acts via mechanisms other than blockade of $GABA_A$ mediated inhibition. In fact, in the study by Baldelli it was demonstrated that sodium bisulphide (volatile salt of hydrogen sulphide) has an inhibitory effect on hippocampal neurons. These effects were

suggested to be due to the opening of a K channel and activation of Na^+/K^+ ATPase (Baldelli et al., 1990). However, these observations were based on very high doses of sulphide (80-200 μM; 107-267% of LD_{50}), whereas in the present study the effects of low doses of hydrogen sulphide were examined.

Based on previous studies (Vanderwolf et al., 1986; Robertson et al., 1992), it could be hypothesised that sulphide-induced increase in amplitude of type 1 hippocampal theta activity may be related to increased serotonergic transmission. This hypothesis, however, presents several limitations. First, it is not supported by the neurochemical data; in fact, serotonin levels in the hippocampal formation were found to be significantly depressed. It could be argued, however, that the whole tissue content of serotonin measured in this study does not necessary reflect the "pool" available for neurotransmission. Second, this hypothesis would not provide an explanation for the sulphide-induced alteration of type 2 theta activity, which is cholinergically mediated.

The study by Sainsbury (Sainsbury and Partlo, 1991) demonstrated that norepinephrine may possess a neuromodulatory role on hippocampal theta activity. In freely moving rats, the $alpha_2$ agonist detomidine produced an almost continuous type 2 (immobility) theta and attenuation of theta during movement. The present study showed that hydrogen sulphide produced an increase in power of detomidine-induced type 2 theta activity. Thus the effects of the $alpha_2$-agonist, detomidine, and of

hydrogen sulphide on theta activity appear to be additive. The mechanism involved in the modulation of detomidine-induced theta activity will require further investigation. It was suggested that detomidine releases type 2 theta activity at the median raphe nucleus (MRN) level by acting on alpha$_2$-receptors on serotonergic neurons which in turn removes the inhibitory effect of serotonin on the cholinergic system (Sainsbury and Partlo, 1993). Norepinephrine levels in the septal nuclei were not examined in the present study, however, increased levels of this neurotransmitter were found in two other brain regions (frontal cortex and cerebellum, but not in the hippocampus). Thus it is possible that increased levels of norepinephrine in the medial septum may add to the effects of detomidine in releasing type 2 theta activity.

It was demonstrated previously in hippocampal slice preparations that blockade of excitatory glutamatergic synaptic transmission (non-NMDA) leads to rhythmic activity at the same frequency but reduced amplitude (MacVicar and Tse, 1986). It can be hypothesised that the increased levels of glutamate in the hippocampus following exposure to hydrogen sulphide may, in part, account for the effects of this toxicant on hippocampal theta activity.

It is well established that cholinergic transmission is necessary for the induction of type 2 theta activity (Kramis et al., 1975). It may also be involved in the generation of type 1 theta activity (Vanderwolf, 1988). Since both types of

theta appear to be affected by the exposure to hydrogen sulphide it is possible that alteration of a neurotransmitter pool of acetylcholine may be involved in H_2S-induced effects on hippocampal theta activity.

It was noted that exposure to 100 ppm hydrogen sulphide produced no significant effects on EEG activity recorded from the frontal cortex. One of the possible explanations for different sensitivity of these two brain regions to hydrogen sulphide exposure could be that the detoxification processes in frontal cortex are more efficient in removing H_2S, possibly through its oxidation to sulphate (Beauchamp et al., 1984; Curtis et al., 1972). Alternatively neurochemical data revealed that the exposure to the same concentration of hydrogen sulphide significantly altered monoamine and amino acid levels in both hippocampus and frontal cortex regions. The pattern of changes, however, was different in these two brain regions. For example glutamate was significantly increased only in hippocampus, whereas in frontal cortex norepinephrine and serotonin levels were elevated. These data suggest that hydrogen sulphide may exert its toxic effects upon these two brain regions via different mechanisms, although selective accumulation of this toxicant by different brain regions may also be involved (Warenycia et al., 1989).

The two major inputs to hippocampal formation arise from septum and entorhinal cortex (Lopes da Silva, 1990) and both of these two brain regions were shown to be involved in the

production of hippocampal theta activity. It was demonstrated that large lesions of the medial septum eliminated theta production (Rawlins et al., 1979; Sainsbury and Bland, 1981) in both the CA1 and dentate gyrus regions and both types of theta were equally affected. It was also suggested that the entorhinal cortex is an integral part of both systems responsible for the production of type 1 and type 2 theta activity (Montoya and Sainsbury, 1984). This observation was based on the study of bilateral lesions of the entorhinal cortex which resulted in a reduction of theta amplitude. In addition the lesions resulted in disruption of the correlation of type 1 theta activity with voluntary movements as well as of type 2 theta with sensory stimulation (Montoya and Sainsbury, 1984). Thus it is possible that inputs coming from the outside of the hippocampal formation, medial septum and/or entorhinal cortex, in particular, may be relevant to the effects on hippocampal theta activity observed following hydrogen sulphide exposure. The involvement of the entorhinal cortex in this process is less likely since the exposure to hydrogen sulphide did not change the correlation between the presence of type 1 theta and voluntary movements. However previous experiments were based on bilateral lesions of entorhinal cortex and the present study may involve only very subtle alteration in neurochemistry and/or electrophysiology of this structure.

4.6 Involvement of Trace Metals

One of the peculiar features of the hippocampus is a high concentration of trace metals, predominantly zinc, confined to the terminal fields of the axons of mossy fibre system (McLardy, 1960); Haug, 1967). Zinc occurs primarily within vesicles and it has been postulated that it may act as an enzyme constituent (Vallee and Galdes, 1984), serve as co-factor (Crawford and Connor, 1975) or intracellular messenger substance (Assaf and Chung, 1984). It has been demonstrated that zinc has profound modulatory effects upon nerve cells. For example, it can modulate the binding affinities of enkephalin (Stengaard-Peterson, et al., 1991), GABA receptors (Westbrook and Mayer, 1987) or alter the activity of ionic pumps and channels (Ebadi and Hama, 1986; Frederickson, 1989). It was also reported that millimolar concentration of Zn^{++} interfered directly with the binding of L-glutamate to NMDA receptors, acting as a potent noncompetitive antagonist of NMDA receptors on cultured hippocampal neurons (Westbrook and Mayer, 1987). Exposure to hydrogen sulphide may cause a precipitation of zinc as the sulphide salt resulting in depletion of the metal from the mossy fibres and thus produce alteration of neuronal function. It has been demonstrated that chronic zinc deficiency alters neuronal function of hippocampal mossy fibres in adult rats (Hesse, 1979). It is well established that activation of NMDA glutamate receptors is required for development of long-term potentiation, a model

for learning and memory (Collingridge, 1985). Blockade of the NMDA receptors reversibly impairs spatial discrimination learning and long-term potentiation in vivo (Morris et al., 1987). Thus it might be expected that a blockade of NMDA receptors with zinc sulphide may result in an increased glutamatergic transmitter pool, and associated with memory dysfunction. It is also possible that increased levels of glutamate could be responsible for the increase in amplitude of theta activity following hydrogen sulphide exposure.

4.7 Hippocampal Theta Activity as an Electrophysiological Parameter for the Study of Neurotoxic Agents

Hippocampal theta activity proved to be a useful parameter for the study of a neurotoxic agent for several reasons. (1) This activity was found to be very sensitive to exposure to hydrogen sulphide. The effects on total power of theta activity were observed at concentrations as low as 50 ppm. This concentration correlates to about 1/8 of the LD_{50} dose reported for rat. In the *in vitro* experiments on hippocampal slices the effects of sulphide treatment could be seen at doses corresponding to the LD_{50} (Reiffenstein, 1989). In humans, 50 ppm of hydrogen sulphide produces eye irritation in 30 minutes (Milby, 1962). Following repeated exposure, neurological symptoms such as mental depression, irritability, poor memory and fatigue can occur (Beauchamp et al., 1984; Arnold et al., 1985). (2) The use of a whole animal

preparation allows for the investigation of the effects of repeated and chronic exposure. It was demonstrated that repeated exposure to hydrogen sulphide results in a cumulative increase of the total power of hippocampal theta activity. (3) This model preserves the major site of entry (lung) for hydrogen sulphide as well as its distribution, biotransformation and elimination. Another advantage of this model is the preservation of the intact blood brain barrier that is a very important factor in studying the effects of inhalation neurotoxicants. It can be proposed that hippocampal theta activity in the freely moving rat may be useful as a valuable parameter to investigate the neurotoxic effects of hydrogen sulphide as well as other toxicants.

4.8 General Conclusions

The hippocampal formation is believed to be involved in emotion (Papez, 1937), memory (Scoville and Milner, 1957; Butters and Cermak, 1975) and spatial-cognitive abilities (O'Keefe and Nadel, 1978). There has also been a considerable amount of speculation about the role of hippocampal theta activity in memory (Landfield 1976; Vertes, 1986). For example, experiments with hippocampal slices showed that stimulation of the Schaffer-commissural projections to CA1 field with short bursts were optimal for producing long-term potentiation when they were applied at frequencies of theta (4-12 Hz) (Larson et al., 1986). Any alteration in the

neurochemistry or electrophysiology of this structure may then affect higher neurological processes in which it is involved. This study demonstrated that exposure to low levels of hydrogen sulphide altered the neurochemistry and electrophysiology of the hippocampal formation. It was also shown that exposure to hydrogen sulphide produces an increase in total power of hippocampal theta activity in a dose dependent manner. Multiple exposures produced a cumulative effect on hippocampal theta that required 2-3 weeks for complete recovery. It was also demonstrated that exposure to 100 ppm of hydrogen sulphide altered monoamine and amino acid levels in hippocampus, frontal cortex and cerebellum. It is suggested that the observed neurochemical and electrophysiological effects may be relevant to neurological symptoms described for humans following hydrogen sulphide intoxication.

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