The Influence of Hypoxia on Avoidance Learning, Intra-Cranial Self-Stimulation and Drinking Behavior

door

Gilbert Henri Camiel CLINCKE

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De invloed van hypoxie op het leren van vermijdingsgedrag, zelfstimulatiegedrag en drinkgedrag

PROEFSCHRIFT

ter verkrijging van de graad van doctor in de geneeskunde aan de
Erasmus Universiteit Rotterdam op gezag van de rector magnificus
Prof. Dr. M.W. van Hof en volgens besluit van het college van dekanen.
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Promotor: Prof. Dr. M.W. van Hof

Overige leden: Prof. Dr. H. Delooz
Prof. Dr. I.S. Russell
Prof. Dr. J.J. van der Werff ten Bosch

''Why is it, that when somebody learns how to study a single nerve cell or a single renal tubule or to isolate a single enzyme every one (rightly) says 'Bravo'; but when attempts are made to isolate functional units of behavior for study many peoply say 'Ah, but you are neglecting all other concurrent behavior and therefore your results are meaningless'.''

P.W. Dews (1958)

Studies on behaviour. IV. Stimulant actions of methamphetamine. J. Pharmac. Exp. Ther. 122: 137-147

Aan Hilde

Bram

Kim

Leen

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Voorwoord

Het voltooien van een proefschrift is onmogelijk zonder de hulp van een aantal mensen. In de eerste plaats wil ik hen bedanken die mede aan de basis liggen van het ontstaan van dit werk. Dr. P.A.J. Janssen heeft door het ter beschikking stellen van zij laboratoria en het verstrekken van de nodige faciliteiten de realisatie van dit werk mogelijk gemaakt. Zijn blijvende belangstelling en aanmoediging worden dan ook erg gewaardeerd. Een belangrijke bijdrage werd geleverd door Dr. A. Wauquier die de leiding heeft over de afdeling neurofarmacologie waar de experimenten werden uitgevoerd. Niet alleen mijn opleiding in de farmacologie dank ik aan hem. Veel van de uitgewerkte ideeën zijn ontstaan tijdens onze gezamelijke vrijdagse "brainstorming". Mijn promotor Prof. Dr. M.W. van Hof ben ik een speciaal woord van dank verschuldigd voor zijn enthousiaste begeleiding en zijn aktief bijsturen van zowel het experimenteel werk als het op schrift stellen van de resultaten.

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Bert.

Introduction

Guyton (1981) in the last edition of his "Textbook of Medical Physiology" extensively describes the physiological determinants of tissue oxygenation but he discusses the subject of hypoxia with a single page (p. 535) of the 1074-page book. If the importance of a physiological condition for health and medical practice is proportional to the space allocated to it in medical textbooks one is bound to conclude that hypoxia is of rather limited interest. However, the large list of different causes of hypoxia indicates that this condition occurs frequently in a great number of pathological conditions.

Hypoxia is not a disease but a condition that refers to the reduced oxygen availability in an organism, tissue or cell. The vital importance of continuous and sufficient oxygen delivery for survival and normal functioning of tissues is perhaps best illustrated by the high consumption rate. On average 300 ml oxygen per minute are needed by a human organism. Reduced availability is therefore a major medical problem for which the interest has grown over the last decades. Consequently, hypoxia has been an important subject in medical research and the literature of the early eighties (Wauquier *et al.*, 1982; Clifford Rose and Amery, 1982).

Oxygen transport mechanisms and causal factors of hypoxia

The causes of hypoxia are related to the different mechanisms involved in the transport of oxygen atoms from the atmosphere to the cell where they are used in the oxidative phosphorylation to form ATP. Therefore, the different forms of hypoxia can be discussed logically by relating them to sequential events in oxygen recruitment by the organism.

Oxygenation of the lung is the first important factor in the oxygen transport to the tissues. The lung is the link between the ambient oxygen and the internal transport system. Hypoxia caused by deficient oxygenation will lead to a reduced PO_2 of the arterial blood and deficient oxygen saturation of hemoglobin (hypoxic hypoxia).

The origins of hypoxic hypoxia can be found in the composition of the inspired air or in diseases of the respiratory system that compromise diffusion to the blood. Decreased oxygen tension of the inspired air is a typical condition that occurs at high altitudes (hypobaric hypoxia). People in general are more exposed to it than one would expect. A mild form of hypobaric hypoxia is inflicted upon each passenger during all commercial aircraft flights (Harding and Mills, 1983). Aircraft cabins are not pressurized to sea level but to an atmospheric pressure equivalent to the pressure at an altitude of approximately 2000 m. Physiologically this means that the alveolar PO_2 is decreased from 102 mm.Hg to 74 mm.Hg.

A large number of pulmonary diseases can cause hypoxia through different mechanisms. A first mechanism is reduction of alveolar ventilation. This can result from paralysis of the respiratory smooth muscles (e.g. drug induced,

myasthenia gravis) or from increased airway resistance and decreased compliance of the lung and chest wall. A second mechanism is decrease of the diffusing capacity of the lung. This mechanism becomes operational when the surface of the respiratory membrane is changed. Either reduction or increased thickness of the membrane will hamper diffusion of oxygen to the blood. A third mechanism is abnormal ventilation-perfusion ratios. Most lung abnormalities as pneumonia, pulmonary edema, lung infections and emphysema induce respiratory insufficiency and hypoxia through a combination of the described mechanisms.

The cardiovascular system controls the second major event in the oxygen supply which is the active transport to the tissues. In the system the heart has a pump function and the hemoglobin in the erythrocytes operates as oxygen carrier in the plasma. Conditions in which the blood flow completely stops lead to hypoxia which is called ischemia. This can occur in all tissues at the same time after cardiac arrest (global complete ischemia) or in specific tissues and regions after vascular occlusion (regional complete ischemia). Reduction of the blood flow can lead to what is called oligemic hypoxia or global incomplete ischemia. Global incomplete ischemia is caused by abnormal low cardiac output and regional incomplete ischemia is associated with reduced flow due to vasospasm and arterial narrowing. One of the most frequent causes of reduced blood supply is atherosclerotic narrowing of arteries. This condition leads to a chronic hypoxic condition of the tissue. A special case related to hypoxia induced by blood flow anomaly is the venous-to-arterial shunt. In this condition there is no alteration in the blood supply but there is a change in the composition of the blood that reaches the tissue. Venous blood with low PO_2 supplies a region where well oxygenated arterial blood is needed.

Hypoxia caused by alterations of the oxygen carrier is called anemic hypoxia. Reduction in the carrier that limits the oxygen content in the system is caused by blood loss (hypovolemia) or by reduced numbers of erythrocytes (anemia). Carbon monoxide intoxication leads to severe global hypoxia in all tissues. In this condition the carbon monoxide binds to the same place on the hemoglobin as oxygen. Since the carbon monoxide is competitive for the binding site at a pressure 230 times lower than oxygen, it occupies all available binding sites at an alveolar pressure of 0.7 mm.Hg (lethal condition). The oxygen binding capacity of the hemoglobin can be permanently decreased by this mechanism in chain smokers. This gives rise to one of the forms of chronic hypoxia.

The last step before oxygen reaches the cell is the passive diffusion from the blood to the cell. The oxygen has to pass the extracellular space between the capillary and the cell membrane. When the extracellular space is enlarged due to edema, oxygen can be prevented to reach the cell. This type of hypoxia almost always leads to cell loss in the edematous tissue. Another oxygen deficit at the cell level can be induced by blocking intracellular enzymes (histotoxic hypoxia). In such condition (e.g. cyanide poisoning) the oxygen supply can be perfectly normal, but the cell cannot use the available oxygen because cytochrome oxidase is blocked.

A special form of hypoxia is fetal hypoxia during gestation and labor. The causes here are not necessarily related to the fetal circulation but rather dependent upon maternal and/or placental functioning. Perinatal hypoxia is an important problem since it is known to induce neurological abnormalities wich can persist into the later lifespan (Prechtl and Stemmer, 1962; Prechtl, 1968; Touwen, 1981).

The multiple causes of hypoxia that were mentioned above more often occur in combination with each other than alone (Siesjö, 1978). This is certainly the case when post-operative hypoxia develops (Amery, 1982). Some good examples of the way in which well controlled health problems can lead to serious hypoxia when they are combined with an additional minor challenge are found in aviation medicine. The level of cabin pressurization of aircraft has been based on physiological properties of the hemoglobin. At a cabin altitude of 2000 m the alveolar PO_2 is decreased from 102 mm.Hg to 74 mm.Hg. Due to the sigmoid shape of the hemoglobin oxygen dissociation curve, the saturation of hemoglobin is still more than 95 % under these circumstances. This is the point where a normal healthy passenger can stand the mild hypoxia without any adverse effects. However, this relatively small decrease in oxygen tension can induce symptoms of hypoxia in people who function normally at sea level but are more susceptible. Amongst those are persons with respiratory diseases as chronic bronchitis, lung emphysema, bronchiectasis and cor pulmonale (Green, 1977). Cardiovascular and neurological problems especially atherosclerosis might quickly induce pronounced cerebral hypoxia. Even heavy smokers can become hypoxic since the carbon monoxide shifts the oxygen dissociation curve in such a way that less oxygen becomes available to the tissues (Perutz, 1978).

The brain: a sensitive and vulnerable tissue

In principle all organs or tissues can become hypoxic but there are large differences in the sensitivity towards hypoxia among tissues. This means that comparable levels of hypoxia can have quite different consequences according to the tissue involved. Heart and brain have attracted most attention of both the clinician and the researcher for obvious reasons. Both organs have an essential role in survival. The extreme vulnerability of the brain and the profound effects of brain damage on the quality of life and behavioral functioning explain the recent interest in cerebral protection and the treatment of brain ischemia/hypoxia (Barnett *et al.*, 1981; Stefanovich, 1981; Krieglstein and Stefanovich, 1982; Janssen and Reneman, 1982; Wauquier *et al.*, 1982; Wiedemann and Hoyer, 1983).

The brain's sensitivity to hypoxia is related to the fact that it has few energy stores and is almost completely dependent upon aerobic glycolysis for its energy production. Approximately 20 % of the total amount of oxygen needed by the organism is consumed by the brain. Therefore constant delivery of oxygen and substrate is essential for energy production. This energy is needed for adequate cellular function as well as for the preservation of neuronal

integrity. ATP in the brain is produced by oxidative phosphorylation so that 1 M glucose is transformed into 36 M ATP. The brain uses this energy for several purposes. ATP is needed for electrical conductivity and synaptic activity of cells, for active ion transport across the membranes and for synthesis of neurotransmitters. ATP dependent ion exchange pumps keep the balance between intracellular and extracellular ion gradients and remove metabolic waste products. Finally resynthesis of membrane constituents and intracellular transport require energy (Kalimo *et al.*, 1983).

If oxygen and/or substrate supply are deficient during hypoxia/ ischemia the brain cells will lose first their ability to transmit electrical impulses. In clinical neurophysiological investigations, the affected electrical function will be manifested in the EEG traces. The EEG frequency will slow down in the mild cases and flattening and electrocortical silence will become apparent in more pronounced cases. Electrocortical silence occurs in man (Trojaborg and Boysen, 1973; Astrup *et al.* 1977; Wauquier and Declerck, 1982) and in animals (Branston *et al.*, 1974) when the cerebral blood flow (CBF) falls below a critical level of 15 ml/100 gram/min. In such condition the neurons do not lose their integrity and recovery will occur after normalization of the supply.
Further decrease of the CBF however will lead to irreversible cell damage. With a CBF under 10 ml/100 gram/min, the cells become depleted of ATP and active ion transport which is ATP dependent will stop. At that moment K^+ will leak from the cell and Na^+, Cl^- and Ca^{2+} will enter. The membranes depolarize and water enters the cells due to the changed osmolarity.
Edema formation is most pronounced during recirculation after complete circulatory arrest. The volume of the brain will increase and give rise to an elevated intracranial pressure which in return can compromise brain perfusion (Hossman, 1977). Membrane depolarization and ion leakage is a general phenomenon known as asphyxial or anoxic spreading depression which is associated with hypoxia/ischemia. These processes occur extremely fast in brain cells as compaired with other tissue cells (Astrup, 1983). It is now generally accepted that no irreversible damage occurs as long as the cell membranes do not depolarize. Hossmann (1982) calls the time between the onset of the hypoxic/ischemic event and the occurrence of depolarization of cortical steady potential "safe" revival time. This refers to the period during which it is possible to restore normal physiological and metabolic functions of the brain, provided that adequate measures are taken to eliminate the causes of hypoxia. Recent evidence suggests that revival of CNS neurons is possible up to 20 minutes following cardiac arrest (White *et al.*, 1983; Edmonds *et al.*, 1984). However, post-ischemic secondary events may compromise the survival chances of the brain. Many factors can be involved in the development of brain injury in the post-ischemic period. Some of the most important are: increased intracranial pressure due to edema, post-ischemic hypotension, intravascular disseminated coagulopathy, respiratory insufficiency of neurogenic origin and post-ischemic hypermetabolism.

Treatment of brain hypoxia/ischemia

The recognition of the sensitivity of the brain and the first attempts to protect it are found very early in the history of medicine. Frost (1981) in her article on brain preservation, mentions that already in the second century A.D. Areteus a Greek physician suggested the use of hypothermia. The protective effect of hypothermia is based on general inhibition of metabolic activity. Even a relatively small reduction of the temperature to 30°C causes a decrease of 50 % in oxygen consumption (Davis *et al.*, 1977). According to Pronk (1982), deep cooling to 15°C gives such a profound metabolic depression that extracorporeal circulation during surgery can be stopped without risk of brain damage. Hypothermia in different degrees is therefore used as a prophylactic measure in open heart surgery (Dillard *et al.*, 1971) and neurosurgery (Silverberg *et al.*, 1981). However, the technique has some limitations and brain damage has been reported even after deep hypothermia (Björk and Hultquist, 1960). Safe limits are dependent upon both the duration and the level of cooling. Approximately 30 minutes protection can be expected with cooling to 25°C (Lam *et al.*,1950; Pontius *et al.*, 1954) and this time is doubled when the temperature is further decreased to 8°C or lower (White *et al.*,1973). Extending the period beyond one hour requires additional interventions such as hemodilution (Copeland *et al.*, 1974; Laver *et al.*, 1975) and rinsing with Tris-saline (Bacalzo and Wolfson, 1971). Practical reasons have restricted the use of hypothermia to surgical interventions. Therefore alternative means of protecting the brain have been explored.

An obvious step was to look for pharmacological substances that could have beneficial effects (Michenfelder and Milde, 1976; Safar, 1978; Ping and Jenkins, 1978). Among the anesthetics, barbiturates were the first drugs that were suggested to ameliorate the outcome of hypoxia/ ischemia (Goldstein *et al.*, 1966; Michenfelder and Theye, 1973; Smith, 1977). Protective effects were found in simple models of anoxia (Arnfred and Secher, 1962) and hypoxic hypoxia (Wauquier *et al.*, 1981).

The possibility to reduce infarct size after middle cerebral artery occlusion with barbiturates given up to 2 hours after the onset of ischemia is well documented (Smith *et al.*, 1974; Hoff *et al.*, 1975; Mosely *et al.*, 1975; Corkill *et al.*, 1976; Levy and Brierley, 1979). As a consequence barbiturates have been used in humans to protect the brain against hypoxia/ ischemia or to ameliorate the outcome in neurosurgery and after head injury (Marshall *et al.*, 1979; Hoff and Marshall, 1979; Miller, 1979; Belopawlovic and Buchtal, 1980; Safar, 1980). The initial positive results with barbiturates given after global ischemia in primates (Bleyaert *et al.*, 1978) could not be confirmed in subsequent experimental work (Steen *et al.*, 1979; Gisvold *et al.*, 1981; Todd *et al.*, 1982) and in a clinical trial (Abramson *et al.*, 1983). The major reason for the negative results and the growing skepticism amongst clinicians (Rockoff *et al.*, 1979; Michenfelder, 1982; Steer, 1982) is the severe cardiorespiratory depression associated with barbiturate therapy. Post-hypoxic hypoperfusion can be increased by a suppression of the cadiovascular system. Due to barbiturate therapy, this condition can occur and create a second hypoxic episode which

in turn can affect negatively nerve tissue. The problems with barbiturates have triggered the search for other compounds that lack cardiovascular side effects. Etomidate for instance, a non-barbiturate hypnotic, has been described as a potent drug against different forms of hypoxia (Wauquier *et al.*, 1980, 1981). Recently it was shown that lidoflazine, an adenosine-sparing agent (Van Belle, 1970), prevented the hypoperfusion occurring after cardiopulmonary arrest in dogs (Winegar *et al.*, 1983).

The role of calcium in the pathophysiology of hypoxia/ischemia

Calcium seems to play a central role in irriversible cell damage observed in different tissues after severe hypoxia (Shay, 1973; Farber, 1981; Hass, 1981). Siesjö (1981) has made a synthesis of the pivotal role of calcium in the origin of cell damage in the brain. During hypoxia/ischemia energy failure leads to ion schifts across the cell membrane. K^+ leaves and Na^+ and Ca^{2+} enter the cell. Ca^{2+} activity in the cytosol increases because Ca^{2+}-sequestering mechanisms are blocked at that moment. Phospholipases are activated due to the Ca^{2+} accumulation and result in an accumulation of free fatty accids (FFA). The latter and the uptake of K^+ and Cl^- into glial cells lead to edema and swelling of the astrocytes. This swelling and the oxygen consumption by the glial cells further prevents the supply to the neurons. Once that condition is reached the resupply of oxygen will lead to oxidation of arachidonic acid and formation of prostaglandin-like substances and leucotrienes. As a result thromboxanes are formed which can hamper microcirculation and free radicals can induce additional vascular damage. The increasing knowledge of the pathophysiology of hypoxia/ischemia and the evidence that conditions in the post-hypoxic period can compromise subsequent outcome have raised the interest for other drugs that can influence these conditions such as Ca^{2+}-entry blockers and anticonvulsants (for review see Hossmann, 1982; Meldrum *et al.*, 1982; Aldrete *et al.*, 1979, 1981). It is clear that in such a sequence of events the prevention of Ca^{2+} overload can be a basic mechanism to prevent cellular damage. Therefore Ca^{2+} entry or overload blockers are now widely tested for their potential protective effect on mainly heart and brain against hypoxia/ischemia (Wauquier *et al.*, 1982; Godfraind *et al.*,1984). The possibility to prevent damage in the cerebral cortex after severe hypoxic-ischemic challenge has recently been demonstrated with flunarizine (Van Reempts *et al.*, 1983).

Protection versus resuscitation

The possibility clearly exists to increase the "safe" revival time of the brain and to ameliorate the outcome after hypoxia by means of pharmacological substances. From a clinical point of view it is necessary to make a distinction between the use of a drug as a prophylactic measure to protect the brain and its use during resuscitation after a hypoxic/ ischemic insult. To protect the

brain, a drug is given before the onset or during the hypoxia. Here the aim is to prevent the final damage. When a drug is given during resuscitation the aim is clearly different. The treatment is then aimed at resolving the dangerous conditions that might induce damage secondary to the original insult. Resolving edema, preventing hypoperfusion and increased intracranial pressure, altering blood viscosity, free radical scavenging, etc. can all contribute to a better outcome during the post-hypoxic period. Although some of the characteristics might be useful in both protection and resuscitation it is obvious that probably no drug will ever combine a favourable effect on all of these factors. Therefore it is necessary to specify protective and resuscitative effects in the pharmacological profile of drugs.

Clinically, protective capacities of drugs can be used in conditions where hypoxia can be foreseen or an enhanced risk is expected. This is the case in neurosurgery and cardiac surgery with cardiopulmonary bypass. Although in these surgical procedures hypothermia and hyperventilation are used as protective measures, additional protection seems to be required. In the literature a considerable incidence of neurological and functional problems are reported after these types of surgery (Brierly, 1967; Aguilar *et al.*, 1971; Stockard *et al.*,1973; Kolka and Hilberman, 1980; Malone *et al.* 1981; Hempelmann *et al.*, 1982, Lancet, 1982). Cerebral hypoxia is associated with the pathogenesis of migraine (Clifford Rose and Amery, 1982). This is another typical example where protective effects of drugs are desired. Recently it has been shown that an antimigraine drug can protect against cerebral hypoxia in different animal models (Wauquier *et al.*, 1984). Protection is further useful in conditions of chronic hypoxia due to pulmonary insufficiency and/or atheriosclerosis.

In stroke, cardiac arrest, head injury and all conditions in which cardiopulmonary resuscitation is needed drugs might be used to ameliorate the subsequent outcome. The treatment target of the drugs can be different here according to secondary pathophysiology of the insult.

Animal models to find new drugs

To find new pharmacological agents that have beneficial effects in hypoxic/ischemic and to determine the pharmacological profile of these drugs animal models have to be used. A large number of such models exist (for review see Stefanovich,1979; Wauquier *et al.*,1981). The method of hypoxia induction applied in these models relates them to the different forms of hypoxia described earlier. For instance, high pressure neck tourniquet, cardiac arrest and decapitation cause circulatory arrest in the brain and might therefore be considered models of global ischemic hypoxia. Hypovolemia and systemic hypotension can be used to study oligemic hypoxia. Occlusion of the medial cerebral artery is a model of regional or focal ischemic hypoxia. Hypoxic hypoxia is induced by altering the oxygen content in the inspired air. This is realised by lowering the atmospheric pressure (hypobaric hypoxia) or by changing the composition of the inspired gas mixture (normobaric hypoxia).

Another important distiction to be made is the level of measurement which is used in a model. Let us take a hypothetical cardiac arrest (CA) experiment in the dog as an example to illustrate this issue. In the model CA is induced by electrical stimulation and CPR is given 10 minutes after the onset of the arrest. Within that same model different parameters can be measured. Biochemical parameters such as FFA accumulation, levels of catecholamines and substrates (glucose, glycogen, pyruvate, lactate, ADP, ATP, AMP and creatine-phosphate) etc. can be measured at different time intervals during and after CA provided the dog is sacrificed and the brain is removed. The same holds when the experimenter wants to evaluate structural changes in the brain tissue by histological examination. Physiological parameters might be focused in the same model. Cardiac output, blood gases, cortical and subcortical blood flow, intracranial pressure, etc. can then be measured. This type of measurements would require major surgical interventions so that the animals have to be sacrificed in most cases. Also the effect on different functions might be studied in such experiment. Neurophysiological measurements of brain function such as evoked potentials and EEG could be made in acute preparations where the animals do not survive. It is also possible to study the effect of CA on behavioral functions and their recuperation with this model but it cannot be done in combination with measurements of other parameters mentioned above.

The pharmacological profile of a drug can be made when the different parameters are evaluated in independent experiments. In the hypothetical CA model both protective and resuscitative characteristics can be studied depending on the point in time when the drug was given (before, during or after CA). This is not always the case in other models. For pharmacological screening of antihypoxic properties, animals are often exposed to extreme hypoxic conditions and prolongation of survival is scored. In this type of experiment only protection can be evaluated. In addition no information can be obtained on subsequent functioning since none of the controls survives and consequently a reference point for drug evaluation is lacking.

Aims of the study

The first aim of the present study was to develop some models in which it was possible to quantify the effects of hypoxia on behavioral functions. Survival is still the primary target in emergency cases involving hypoxic/ischemic insults, but due to improvements in CPR most patients do survive such episodes. Obviously restoring the quality of life is then the major concern in medical practice. Neurological problems and functional deficits in the post-hypoxic period create discomfort to patients that survive acute hypoxic/ischemic insults. Memory problems due to hypoxic/ischemic brain injury after cardiac or respiratory arrest occur frequently (Volpe and Hirst, 1983). Conditions associated with aging, such as atherosclerosis and cardiovascular insults are also known to induce different types and degrees of memory impairment (Huppert, 1982).

Therefore it is of interest to know whether pharmacological treatment can have beneficial effects on disturbed behavioral functions and their recuperation after hypoxia. Consequently a second goal of this study was to construct the models in such a way that they could be used in pharmacological research.

Models designed to evaluate functional behavior in pharmacological research have to meet certain requirements. Since the aim was to study the effects on functional behavior it was imperative to choose forms and levels of hypoxia which resulted in maximal survival of the animals. Large experimental mortality with survival of strong animals would bias the result towards a positve outcome and jeopardize the validity of any generalization to a normal population. However, the survival rate is not only dependent upon the type and duration of hypoxia. Identical levels of hypoxia result in different survival rates according to the species used. A good illustration of this are the survival times of different species when exposed to sudden severe hypobaric hypoxia. When brought within two minutes to a simulated height of 35,000 Ft in a decompression chamber mice die within 4 minutes. Wistar rats survive between 6 and 45 minutes in this condition. Larger animals as guinea-pigs and rabbits adapt quite well to the same condition. They become cyanotic, lose their righting reflex but regain their postural reflexes after some minutes and can be kept for long periods (more than one hour) in the hypoxic environment. To secure maximal survival rate, the induced hypoxia in this study was limited to tolerable levels of hypobaric and normobaric hypoxia according to the species used.

Good models for pharmacological research, which often require testing of large numbers of compounds at different doses, need to be relatively simple and economical. This means that in ideal circumstances the animals can be tested several times with different drugs and at different doses. With respect to protective effects of drugs against hypoxia it is also important that a model allows evaluation of chronic treatment with drugs. This requirement is needed to obtain information on possible protective effects against chronic hypoxia.

Stability of the induced effects is one of the most important elements before a model can be used in pharmacological research. It is a necessary condition to evaluate antagonism of the induced phenomenon by drugs. Hypoxia as a method to affect behavioral functions might cause some problems in this respect. Adaptation to hypoxia is not only a phenomenon related to high altitude physiology. Behavioral adaptation has been observed in experimental models already after the second exposure to hypoxia (Dogterom, 1983). These findings suggest that the sensitivity towards hypoxia is altered in some way. It is obvious that if a drug is given before the second exposure one could erroneously attribute the improved behavior to a protective drug effect. To avoid this problem the models presented in this study all involve multiple exposure to the same form and level of hypoxia over extended periods of time using the same animals. This made it possible to examine whether or not a steady-state effect could be reached.

A last major requirement is related to the interpretation of drug effects when using a particular model. Van Hof (1982) has pointed out by using the experiments on retrograde amnesia (Flohr, 1979) for example, that it is often

difficult to decide whether observed protection with drugs should be attributed to direct effects on the nervous system or to effects on cardiovascular functioning. Although it is difficult to examine these two factors independently during *in vivo* behavioral experiments, some precautions can be built into the models. Careful selection of behaviors which are heavily dependent upon neuronal integrative processes in the CNS, and the choice of sufficiently deep levels of hypoxia can be helpful in this respect. The exact mechanism of action of drugs and the proof of direct neuronal effects can only be obtained in independent experiments. This has recently been demonstrated by using the *in vitro* hippocampal slice (Wauquier *et al.*, 1984). It was shown that flunarizine improved the electrophysiological recovery of the slices from hypoxia. The positive effects were observed in the absence of any circulation so that beyond any doubt the compound exerted its effects directly on the neurons.

This study will attempt to answer following questions: 1. What is the effect of hypoxia on a number of different behaviors, provided suitable induction methods are used to guarantee maximal survival of the species used? 2. Do the different models sufficiently meet the requirements to be used in pharmacological research? 3. Is it possible to obtain protection against the behavioral deficits with pharmacological substances?

Three different types of behaviors were selected for this study. In chapter 1, the effect of hypoxia on the acquisition of two-way avoidance learning in the guinea-pig are described. Chapter 2 deals with pharmacological protection with Ca^{2+} entry blockers against the hypoxia induced effects on avoidance learning. In chapters 3 to 5, experiments are presented in which intra-cranial self-stimulation was used as behavioral parameter. In chapter 3, the effects of hypoxia on subsequent self-stimulation behavior are described. Chapter 4 deals with selfstimulation behavior during hypoxia. In chapter 5, a method is described in which self-stimulation behavior can be used to investigate the effects of hypoxia on stimulus discrimination. Chapters 6 to 8 contain experiments on drinking behavior in the rat. In chapter 6, adaptation of drinking behavior to restricted drinking times as well as the influence of repeated water injections on the intake are discussed. The influence of hypoxia on subsequent drinking behavior is the subject of chapter 7. Finally, chapter 8 contains a published article on the protective effect of the Ca^{2+} entry blockers flunarizine and verapamil on water intake attenuated by hypoxia. In the discussion the value of the different models will be evaluated with reference to their utility for pharmacological research purposes in the future.

Chapter 1
The Effects of Hypoxia on the Acquisition of a Two-Way Avoidance Task in the Guinea-Pig

1.1. Introduction

Hypoxia has been used with variable success to induce amnesia in learning and memory experiments. In spite of the often conflicting results it was possible to infer some general properties of hypoxia as an amnesic agent. First, hypoxia seemed to be less effective when it was used to affect strong memory traces which were established after multitrial training. Hypoxia never completely destroyed memory traces (Van Harreveld, 1965; Nielson *et al.*, 1963; Van Harreveld *et al.*, 1981; Dogterom, 1983). Secondly, it proved to be very difficult to block the acquisition of a learning task (Bryant and Thompson, 1958; Baldwin and Soltysik, 1965; Giurgea *et al.*, 1971). Thirdly, the amnesic effects tended to disappear after repeated exposures (Ledwith, 1967; Flohr, 1979).

Flohr (1979) has pointed out that the large difference in induction methods and depth of hypoxia might explain to some extent the conflicting results. However, the theoretical framework in which the experiments were designed was probably a far more important origin of the variable results.
Most investigators tried to find evidence supporting either the consolidation or retrieval hypothesis with regard to the phenomenon of retrograde amnesia. By doing so they assumed a two-process model of memory formation (McGaugh, 1966; Baronades, 1970). In this model a short-term memory (STM) holding mechanism with a fast decay is transcribed in a sequential and/or parallel way into a long-term memory mechanism (LTM). The latter trace is then responsible for the holding of the information over a prolonged time period (weeks, months, years). Working with such a model the investigators tried to find out whether amnesia originated from a deficient consolidation (transcription into LTM fails) or from a blockade of retrieval systems (LTM trace is present but cannot be recalled).
As a result memory was tested after a rather long-time interval following training when consolidation was completed. This means that the early stages of memory formation were seldom studied. In addition the two-phase model proved to be invalid since at least three stages of memory formation could be experimentally defined (Gibbs and Ng, 1976, 1977). It is evident that experiments designed within a partly deficient model can generate contradictory results especially when different experimental techniques are used as well.

1.2. Experimental evidence for a multistage memory formation

Frieder and Allweis (1978) used a brief but intense hypoxia to provide strong evidence for a three-stage memory formation hypothesis. In their experiments they trained rats in an active one-way avoidance task until the animals avoided shock at a criterion level (5 avoidances in 5 consecutive trials). At different time intervals after the criterion score was reached the animals were exposed to hypoxia (pure N_2 or 2 % O_2 in N_2) for 20 to 40 seconds. The exposure time was dependent on the speed with which the animals lost consciousness. Retention tests were carried out from 5 minutes to 20 hours after training. Their results suggested that there exists a period of 15 minutes after training during which a memory consolidating process is vulnerable to hypoxia. However, the induced amnesia was transient. The onset occurred 15 to 20 minutes after training and complete recovery was seen 3 to 4 hours later. This form of transient amnesia was most marked when the hypoxia immediately followed the training session. These results suggested the existence of a medium-term memory trace that bridges the gap between the decay of the STM and the onset of the LTM. The induced amnesia seemed to be very specific for the MTM in the sense that only the processes responsible for transcription from STM to MTM were blocked. No interference with transcription from STM to LTM or with retrieval mechanisms was found. In addition it was demonstrated that the hypoxia did not affect memory recall from MTM and was concluded that: "... hypoxia prevents the formation of the hypoxia-resistant memory-holding mechanism that is normally established by about 20 min after training and which persists at least until LTM becomes effective, about 200 min after training.". In more recent experiments (Frieder and Allweis, 1982 a,b) it was shown that the hypoxia-induced amnesia could be antagonized by post-hypoxic hyperoxia. The antagonism was only possible when the interval between hypoxia and hyperoxia was smaller than 90 seconds. Based on these results the assumption was made that a "very short term memory trace" (VSTM) exists during a short period after training. The hypothesis now is that MTM development is initiated from VSTM. Since VSTM is short lasting and is masked during retrieval by STM we will further discuss hypoxic effects with reference to the three retrievable traces STM, MTM and LTM.

These experiments clearly indicated that short but intense hypoxia given immediately after training generates a specific effect. It blocks the processes for transcription from VSTM or STM to MTM without interfering with transcription from STM to LTM and retrieval mechanisms. It has to be kept in mind that the amnesic effects can only be observed when the retention tests are executed during the period in which MTM is the principal holding mechanism from which retrieval must take place.

The Frieder and Allweis (1978) data are restricted to the experimental situation where acquisition was completed in a single training session and retention was tested after different training intervals. The same holds for

recently published results (Allweis *et al.*, 1984) on retention of a passive avoidance task in the chick. In this model, hypoxia induced similar memory deficits as in the rat, but again the animals had completed the training before retention tests were held. However, the specificity of the described effects allows to make predictions about the effects of hypoxia on acquisition curves of learning tasks with higher complexity.
In the next section we will describe how a multistage model can be used to predict the influence of hypoxia on acquisition of conditioned two-way shuttle behavior to acoustic stimuli.

1.3. A multistage model of memory formation: predictions about post-training hypoxia on the acquisition of two-way shuttle behavior

Conditioned shuttle behavior responses to acoustic stimuli in two-way avoidance paradigms are fairly difficult for animals to learn. Multitrial sessions spread over several training days are needed before a reasonable level of performance is reached. Each training session following the initial one can partly be considered a retention test. Thus the acquisition curves will contain information about the different stages of memory formation. The intersession intervals will determine to a large extent from which memory trace retrieval will take place during a particular session. The different phases of memory formation as well as their relation with retrieval and performance are depicted in Figure 1.1. This general scheme is a modification of the one presented by Matthies (1979). It is clear that valid conclusions about the memory traces can only be made when retrieval processes and motor systems have a normal function during the retention performance.

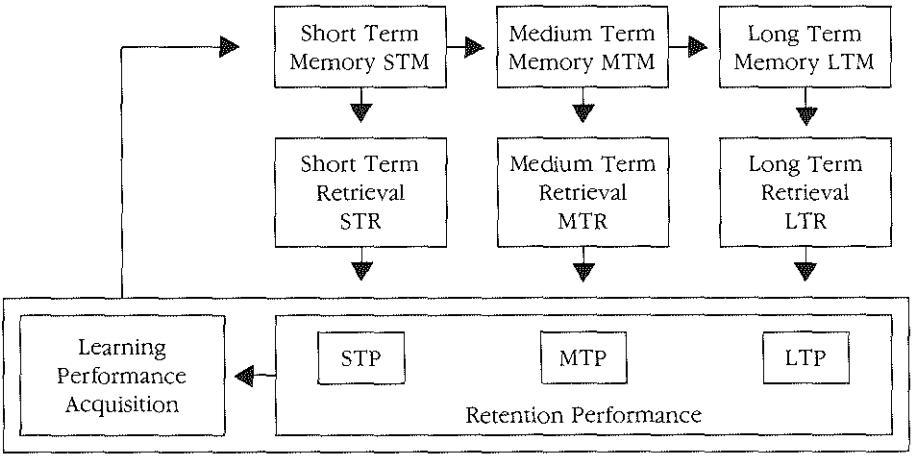

Figure 1.1. Relations between performance, memory traces and retrieval mechanisms in learning and memory.

The STM is built up immediately during acquisition performance and lasts only seconds or minutes. The MTM develops shortly after training sessions but lasts for minutes or hours before it decays. The LTM is the last stage in the sequence and it arises with some delay, but lasts for days and even months or years. In experiments where acquisition cannot be completed in a single session (one or multitrial) it is clear that the performance on subsequent sessions will be determined by a combination of retrieval from the different traces (STM + MTM + LTM). When two sessions are held with an intersession interval of 30 to 150 minutes, retrieval from MTM should be the mechanism responsible for a gain in performances seen during the second session. The gain seen after intersession intervals of 24 hours should largely depend on retrieval from LTM. As a consequence any learning experiment based on a paradigm where two daily sessions are held with intersession intervals between 30 to 150 minutes, is expected to generate parallel acquisition curves over the training days. The performance in the second session should always be superior to that in the first one. Given the intersession interval the observed gain can then be attributed to retrieval from the MTM trace which, at that point, is the prime holding mechanism from newly acquired information during the first daily session.

In the assumption that the Frieder and Allweis results can be reproduced in a multi-session learning experiment, a number of predictions can be made about the effects of post-session hypoxia on the acquisition of a two-way avoidance task. First, acute post-training hypoxia will not prevent the acquisition of the learning task. Secondly, it should be possible to antagonize the gain in performance between the two sessions by induced hypoxia since the gain is dependent on MTM formation. In the next section we will describe two experiments in which different intersession intervals between daily sessions are used, to evaluate the above predictions.

1.4. Experiment 1 : Intersession interval 2 hours

1.4.1. Materials and methods

1.4.1.1. Animals

Twenty male guinea-pigs from the Janssen breeding facilities weighing 250 ($\pm$ 10) g were used. Guinea-pigs were preferred over rats because acquisition of conditioned shuttle behavior in a two-way avoidance task occurs faster and more efficiently in this species. The animals were individually housed three days before the first training day and kept under standard laboratory conditions.

1.4.1.2. Avoidance apparatus

The shock avoidance apparatus consisted of a perspex box (45 x 15 x 29 cm high) divided into two compartments by a 5 cm high hurdle. This box was enclosed in a larger sound attenuating cage. The bottom of each compartment was a grid floor which could be electrified. Placing an animal on either floor

closed a relay so that its position could be detected. Automatic position detection was carried out by a Rockwell AIM65 micro-computer. Training sessions and data registration were automatically controlled by the same computer. The complete set-up is shown in Illustration 1.1.

1.4.1.3. Training-treatment procedure

Groups of ten animals were assigned at random to two different training-treatment conditions. For both groups, training was given on five consecutive days. Daily training consisted of two sessions held with an intersession interval of 120 minutes. All animals received daily a s.c. injection of saline at a volume of 1 ml/100 g body weight.

Illustration 1.1.
Two-way avoidance shuttle box and automatic control unit (Rockwell AIM65 micro-computer). During experimental sessions the transparent front wall seen on the picture was shielded.

The injections were given one hour before the first daily session. The animals of one group were exposed to acute hypoxia (group H) immediately after each of the two daily sessions. The animals of the other group were not exposed to hypoxia (group NH) but were transferred to their home cages immediately after each session.

Each training session consisted of 20 trials each lasting 30 seconds (total duration 10 minutes). The animals were placed in the left compartment of the avoidance apparatus and the session was started after a 20-second delay. At the start of the first trial in the session a tone (500 Hz) was presented for 10 seconds as a conditioned stimulus (CS) to warn the animal that shock would be delivered through the compartment grid where it was positioned. As long as the CS was on, none of the compartment grids were electrified. At the end of the CS the compartment where the animal was positioned at the onset of the trial was electrified.
The shock was left on for the remaining time of the trial. Avoiding a shock by jumping to the opposite grid during the CS was scored as a correct response. Upon each avoidance response the CS was turned off and the grid just left was electrified for the remaining time of the trial. If the animal stayed in the same position exceeding the CS duration, it received a shock (1.5 mA RMS) through the paws as unconditioned stimulus (UCS) and could escape to the opposite grid. The computer system automatically registered the number of avoidances (correct responses) and calculated the mean escape time for the trials where the UCS was received. At the end of each session the animals were put back in their home cage until the next session was started.

This experimental procedure was a variation on Duncan's (1949) procedure which has been mainly used for shuttle avoidance conditioning in the rat (Theios and Dunaway, 1964; Theios *et al.*, 1966).

1.4.1.4. Hypoxia

The apparatus to induce hypoxia was a perspex cylinder (Ø 30 cm, height 15 cm). The cylinder was continuously flushed with pure nitrogen.
The composition of the air in the cylinder was permanently monitored and analysed by a Sybron/Taylor Servomex O_2 analyzer OA570. The complete equipment is shown in illustration 1.2.

The animals from group H were exposed to hypoxia immediately after each session. They were put for 1 minute in a pure (100 %) nitrogen environment. The nitrogen concentration decreased to 99.7 ($\pm$ 0.2) % when the animals were put in the apparatus but it returned to 100 % within three seconds. Upon removal recuperation was assisted by slight rhythmic compression of the chest to overcome the pronounced bradycardia and respiratory failure. The gasping reflex occurred within 15 seconds. From that point on chest compression was discontinued. This was sufficient to induce spontaneous breathing in all cases.

1.4.1.5. Data presentation and statistical analysis

Acquisition curves (mean number of avoidances) were separately plotted for the first and second daily sessions in each of the training-treatment combinations.

Group differences were evaluated using analysis of variance having repeated measures of two independent factors (training days and sessions). Comparisons and analysis of major interactions between factors were based on the general analysis with techniques described in Keppel (1973).

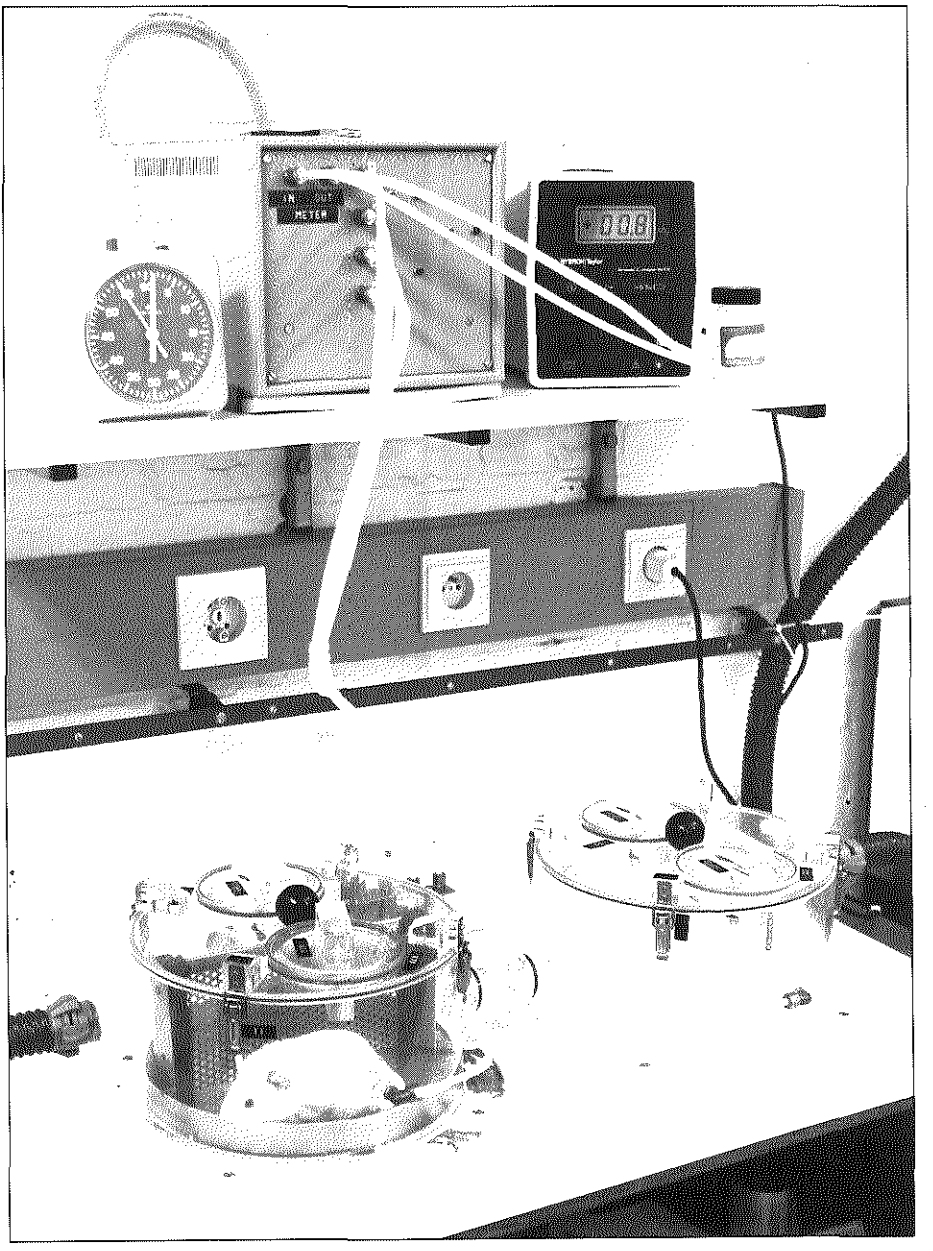

Illustration 1.2. Equipment used to induce hypoxia and to control gas mixtures inside the container.

1.4.2. Results

1.4.2.1. Avoidance responses

Figure 1.2. shows the acquisition curves for the two training-treatment conditions. Although the values obtained for the two daily sessions should be separated on the time axis, they were put above the same time point (training day) to obtain a better picture of the differences between the sessions run on the same day.

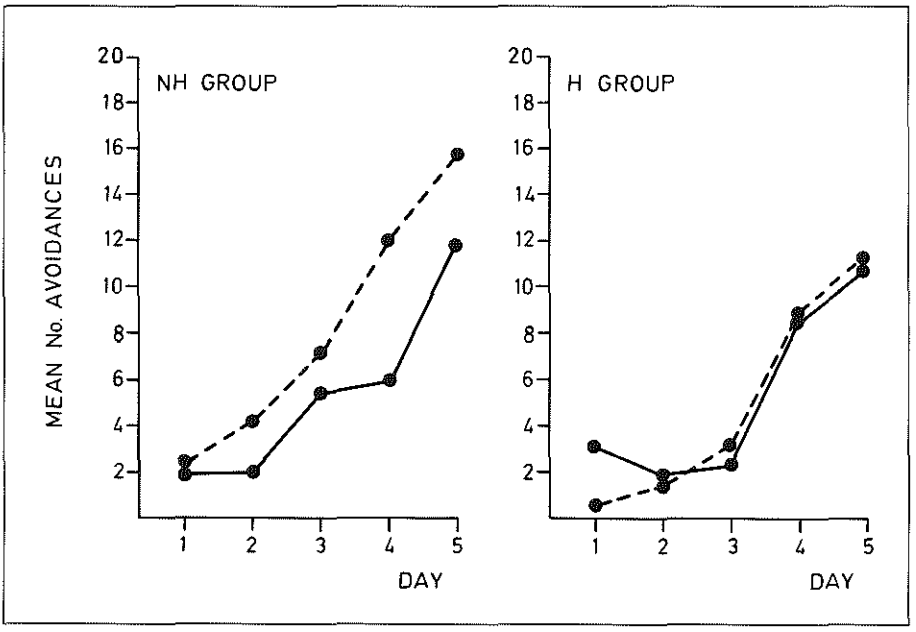

Figure 1.2. Mean number of avoidance responses during two daily sessions for different training-treatment condition (NH = no hypoxia group, n = 10; H = post-session hypoxia group, n = 10). The interval between daily sessions was 120 minutes.
Acquisition curves based on session 1 ●—● and session 2 ●- - -●

For comparisons between the hypoxic and non-hypoxic group the data were analyzed in an A x (B x C x S) mixed repeated measures design with as independent factors: post-session treatment (A), training days (B) and daily sessions (C). The statistical analysis is summarized in Table 1.1.

Table 1.1.
Analysis of variance on the number of avoidances in groups NH and H. A x (B x C x S) repeated measures design with independent factors: A = post-session treatment, B = training days and C = daily sessions.

Source	SS	df	MS	F
A	149.44	1	149.44	1.27
S/A	2112.81	18	117.38	
B	3227.13	4	806.78	32.02***
A x B	86.13	4	21.53	< 1
B x S/A	1814.74	72	25.20	
C	88.44	1	88.44	10.31**
A x C	126.41	1	126.41	14.73**
C x S/A	154.45	18	8.58	
B x C	99.33	4	24.83	3.04*
A x B x C	32.17	4	8.04	< 1
B x C x S/A	587.70	72	8.16	

* $p < .05$; ** $p < .01$; *** $p < .001$.

In both groups, performance significantly improved over the training days [B: $F(4/72) = 32.02$, $p < .01$] and no significant difference in acquisition efficiency was found due to post-session hypoxia [A: $F(1/18) = 1.27$, n.s. and A x B: $F(4/72) = < 1$, n.s.].

A significant difference was found between the consecutive daily sessions [C: $F(1/18) = 10.31$, $p < .01$] but the effect was dependent upon the post-session treatment [A x C: $F(1/18) = 14.73$, $p < .01$]. An analysis of this interaction (Table 1.2.) revealed that the performance during the second session was dependent upon the post-session treatment given [A at C_2 level: $F(1/18) = 4.54$, $p < .05$] after the first daily session as can easily be seen on Figure 1.2. Hypoxia treatment, however, did not affect the performance on the first daily session.

Table 1.2.
Differential effects of post-session treatment (A) at the two daily sessions: C_1 = first session, C_2 = second session.

Source	SS	df	MS	F
A at C_1	0.49	1	0.49	1
A at C_2	275.56	1	275.56	4.54*
S/A at C_1	1173.86	18	65.21	
S/A at C_2	1093.40	18	60.74	

* $p < .05$.

Analysis of the significant session effect (Table 1.3.) indicated that there was only a significant higher performance in the second session when no hypoxia was given [C at A_1: $F(1/9) = 24.56$, $p < .001$]. Post-session hypoxia reduced the performance in the second session to the level reached during the first session [C at A_2: $F(1/9) = < 1$, n.s.].

Table 1.3.
Differences between the two daily sessions under the two treatment conditions: A_1 = no hypoxia, A_2 = post-session hypoxia.

Source	SS	df	MS	F
C at A_1	213.16	1	213.16	24.56***
C at A_2	1.69	1	1.69	< 1
C x S at A_1	78.14	9	8.68	
C x S at A_2	75.61	9	8.40	

*** $p < .001$.

1.4.2.2. Escape times in hypoxia-treated animals

Since motor and sensory impairment can influence performance it was important to know whether our results with hypoxia-treated animals were related to such effects. Some information on this matter could be obtained by analyzing the escape times recorded when animals failed to avoid the shock. Increased escape times after hypoxia would indicate that the motor or sensory systems of the treated animals were affected. The latter could be a factor influencing performance which is not related to memory holding mechanisms.

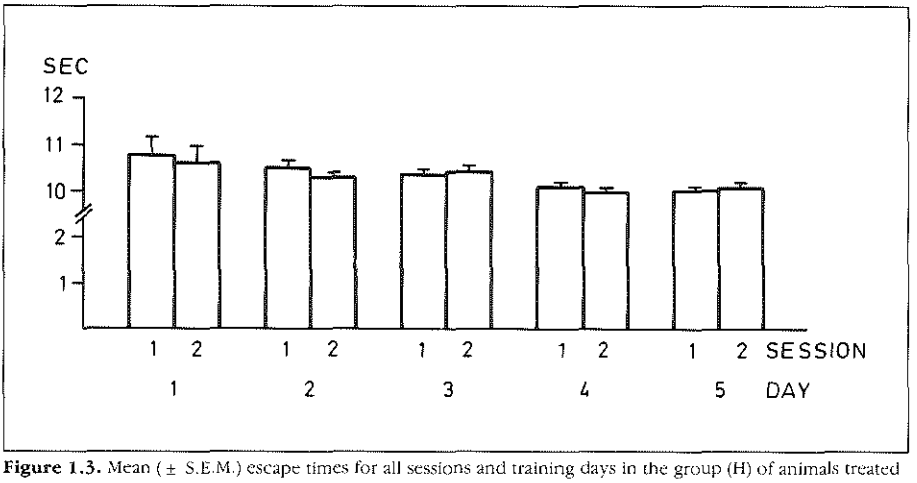

Figure 1.3. Mean (± S.E.M.) escape times for all sessions and training days in the group (H) of animals treated with post-session hypoxia.

Figure 1.3. shows the mean escape times for the hypoxia group (H) during all sessions and training days. As can be seen there was a general tendency for a decrease in escape times over the training days. There was no apparent effect of the hypoxia after session 1 on the escape times during session 2 on the same day. If anything can be seen it is rather a decrease of the escape times during the second session. The data were analyzed in an (A x B x S) repeated measure design with as independent factors training days (A) and daily sessions (B). Table 1.4. shows the results of the analysis for the H group. A significant decrease [A: $F(4/36) = 5.38$, $p < .01$] was observed over the training days, but the escape times on consecutive daily sessions were not significantly different [B: $F(1/9) = < 1$, n.s.] from each other.

Table 1.4.
Analysis of variance on the escape times in the hypoxia group. (A x B x S) repeated measures design with independent factors: A = training days and B = daily sessions.

Source	SS	df	MS	F
A	6.22	4	1.56	5.38**
S	1.26	9	.14	
A x S	10.44	36	.29	
B	.16	1	.16	< 1
B x S	3.89	9	.43	
A x B	.33	4	.08	< 1
A x B x S	9.58	36	.27	

** $p < .01$.

1.4.3. Conclusions

The results from this experiment showed that guinea-pigs quickly acquire conditioned shuttle behavior in a two-way avoidance paradigm if training is spread over two daily sessions. In the NH group the performance during the second session was systematically higher than during the first session on the same day. As a consequence acquisition curves based on performance in the second session lie above those based on performance in the first session. This is in line with the theoretical concept that in such a learning procedure, performance in the first daily session is mainly dependent on retrieval from STM and LTM. Performance in the second session depends on retrieval from STM and LTM but also on retrieval from MTM. MTM is the holding mechanism for the freshly acquired information from the first session.

According to Frieder and Allweis (1978) short but intense hypoxia prevents MTM formation without interfering with either retrieval or with transcription from STM to LTM. The results obtained with post-session hypoxia in our experiment confirm this assumption. Given the intersession interval used (120 min) the increase in performance between session one and two can be

attributed to retrieval from MTM. This increase was disrupted by post-session hypoxia without disturbing normal acquisition and formation of LTM.
The acquisition curves based on the performance in the first session were not different in hypoxia-treated animals from those in non-treated animals.
This indicated that the transcription from STM to LTM was not attenuated.
It was also evident that during the second session retrieval from LTM and STM was still possible. If the latter were not the case, it would not have been possible to obtain any acquisition curve from the performance during the second daily session in hypoxia-treated animals.

As predicted the only effect of post-session hypoxia on learning in the two-way avoidance task was a reduction of the performance in the second session towards the level of the first session. Since retrieval from the MTM holding mechanism is believed to be responsible for the better performance during the second session and retrieval mechanisms were apparently intact, it follows that under the conditions employed, intense hypoxia prevented the development of MTM holding mechanisms.

An alternative interpretation could be that the results are due to anterograde amnesia effects on the learning in the second session. Although this is certainly plausible, we think that such effects would also result in impaired acquisition. However, this was not seen in our experiment.

In contrast to the effect of hypoxia on other functional behaviors, no adaptation effects were observed (Weinstein and Annau, 1969; Dogterom, 1983; and chapter 3-7 in this study). The induced hypoxia seemed to reliably block MTM formation on all training days. As a consequence no interaction between training days and hypoxia treatment was observed in the statistical analysis. This is a very interesting aspect since it means that this form of hypoxia in the paradigm presented has a stable effect that can be quantified.

1.5. Experiment 2: Intersession interval 1 hour

The results of the first experiment were consistent with the theoretical expectations based on the results obtained with rats in a different paradigm. To further investigate the temporal characteristics of MTM in the guinea-pig during multi-session two-way avoidance learning a second experiment was carried out. In this experiment the intersession interval was reduced to one hour. This was still within the limits during which amnesia was observed after post-training hypoxia in the rat.

1.5.1. Materials and methods

As in the first experiment (see 1.4.) twenty male guinea-pigs were used and assigned at random to two different training-treatment conditions. Training and treatment were identical to the first experiment except for the intersession interval between the two daily sessions. This was reduced to 60 minutes.

The same variables were measured as in the first experiment and in addition the latency to regain the righting reflex after hypoxia was measured in the hypoxia group (H). As before, statistical analyses were based on analysis of variance having repeated measures.

1.5.2. Results

1.5.2.1. Avoidance responses

The acquisition curves (groups NH and H) can be seen in Figure 1.4. and the statistical analysis on the number of avoidances is summarized in Table 1.5.

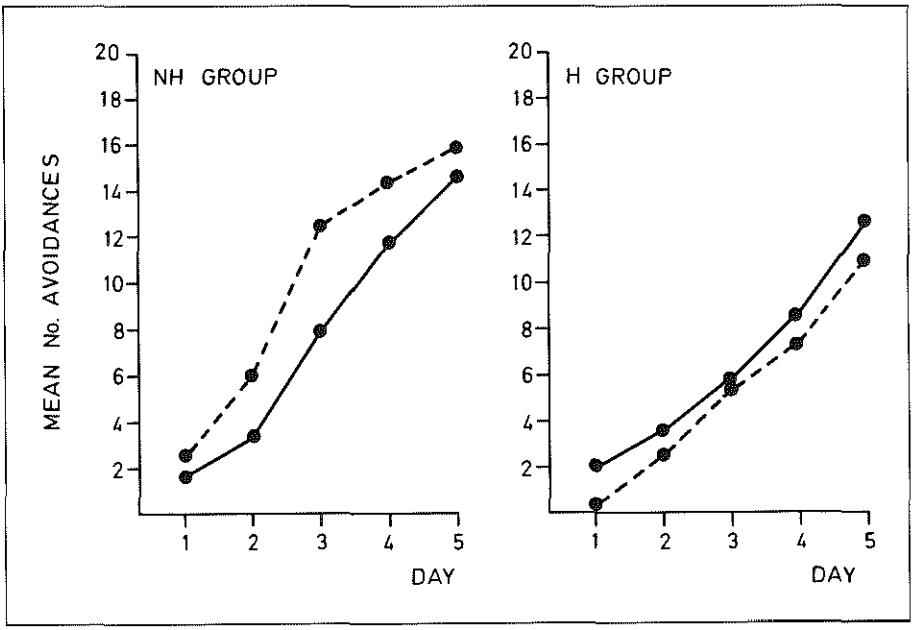

Figure 1.4 Mean number of avoidance responses during two daily sessions for different training-treatment conditions (NH = no hypoxia group, n = 10; H = post-session hypoxia group, n = 10). The interval between daily sessions was 60 minutes.
Acquisition curves based on session 1 ●——● and session 2 ●- - -●

Table 1.5.
Analysis of variance on the number of avoidances in groups NH and H. A x (B x C x S) repeated measures design with independent factors: A = post-session treatment; B = training days and C = daily sessions.

Source	SS	df	MS	F
A	518.42	1	518.42	5.46*
S/A	1709.36	18	94.96	
B	3721.58	4	930.40	46.29***
A x B	136.68	4	34.17	1.70
B x S/A	1446.94	72	20.10	
C	18.00	1	18.00	5.42*
A x C	154.88	1	154.88	46.65***
C x S/A	59.72	18	3.32	
B x C	41.90	4	10.48	2.06*
A x B x C	7.92	4	1.98	< 1
B x C x S/A	366.58	72	5.09	

* $p < .05$; *** $p < .001$.

An improved performance over the training days was observed for both groups [B: $F(4/72) = 46.29$, $p < .001$], indicating that the acquisition process of the task was not affected by the hypoxia treatment [A x B: $F(4/72) = 1.70$, n.s.]. However, the total number of avoidances over all sessions and training days was significantly reduced by post-training hypoxia [A: $F(1/18) = 5.46$, $p < .05$]. The highly significant interaction between the treatment conditions and the daily sessions [A x C: $F(1/18) = 46.65$, $p < .001$] suggests a specific action of the hypoxia on one of the two daily sessions. Therefore this interaction was further analyzed in detail and a summary of this analysis is presented in Table 1.6.

Table 1.6.
Differential effects of post-session treatment (A) at the two daily sessions: C_1 = first, C_2 = second.

Source	SS	df	MS	F
A at C_1	53.29	1	53.29	1.18
A at C_2	620.01	1	620.01	11.67**
S/A ar C_1	812.90	18	45.16	
S/A at C_2	956.18	18	53.12	

** $p < .01$.

As can be seen the main effect of post-session hypoxia is only seen during the second session of the day [A at C_2: $F(1/18) = 11.67$, $p < .01$] where MTM is the holding mechanism for newly acquired information. Hypoxia does not affect the performance on the first daily session where LTM is the principal source for retrieval [A at C_1: $F(1/18) = 1.18$, n.s.].

A general significant difference between the performance on the two daily sessions was observed [C: $F(1/18) = 5.42$, $p < .05$]. In the non-hypoxia group (NH) performance in the second session was systematically higher as compared with the first one, whereas in the hypoxia-treated group (H) the reverse was observed. An analysis (Table 1.7.) was done to check whether these effects were significant in both treatment conditions since the significant main effect (factor C) could be partially masked by the significant interaction between treatment and sessions (A x C).

Table 1.7.
Differences between the two daily sessions under the two treatment conditions: A_1 = no hypoxia, A_2 = post-session hypoxia.

Source	SS	df	MS	F
C at A_1	139.24	1	139.24	39.02***
C at A_2	33.64	1	33.64	10.99**
C x S at A_1	32.16	9	3.57	
C x S at A_2	27.56	9	3.06	

** $p < .01$; *** $p < .001$.

The analysis confirmed that the performance in the second session was significantly higher when the animals did not receive hypoxia [C at A_1: $F(1/9) = 39.02$, $p < .001$], but it was significantly reduced when post-session hypoxia was applied [C at A_2: $F(1/9) = 10.99$, $p < .01$].

1.5.2.2. Escape times in hypoxia-treated animals

Figure 1.5. shows the mean escape times for the hypoxia group (H) during all sessions and training days. Again there was a general tendency for a decrease in escape times over the training days. Escape times during session 2 were not higher than escape times during session 1 on the same day.

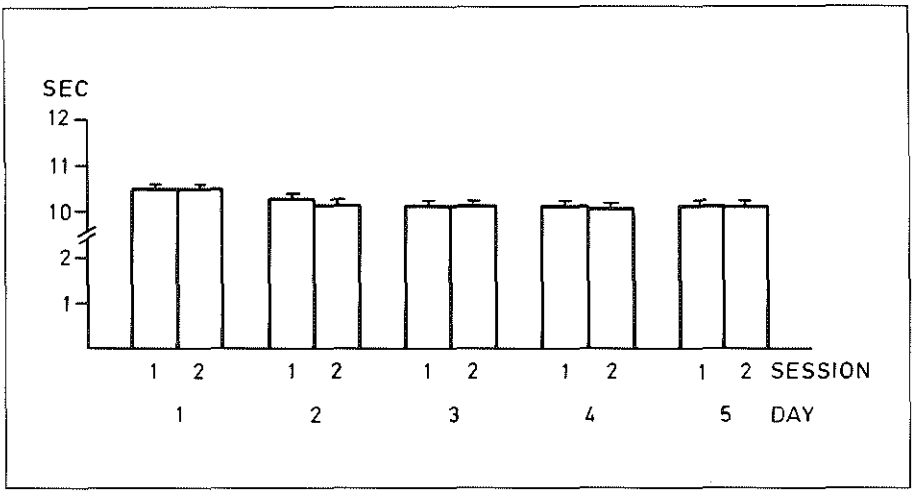

Figure 1.5. Mean (± S.E.M.) escape times for all sessions and training days in the group (H) of animals treated with post-session hypoxia.

Table 1.8. summarizes the analysis of variance on the escape times from the hypoxia group. Again a significant decrease [A: $F(4/36) = 15.67$, $p < .001$] was observed over the five training days. However, there was no significant difference [B: $F(1/9) = 1.25$, n.s.] between the escape times on subsequent daily sessions.

1.5.2.3. Latency to regain righting reflex after hypoxia

The latency to regain the righting reflex was measured after each exposure to hypoxia. Figure 1.6. depicts the results for the five training days. The figure suggests that the latency was increased after the second exposure especially on training day one and two.

Table 1.8.

Analysis of variance on the escape times in group H. (A x B x S) repeated measures design with independent factors: A = training days and B = daily sessions.

Source	SS	df	MS	F
A	1.88	4	.47	15.67 ***
S	.91	9	.10	
A x S	1.24	36	.03	
B	.05	1	.05	1.25
B x S	.35	9	.04	
A x B	.06	4	.02	< 1
A x B x S	1.30	36	.04	

*** $p < .001$.

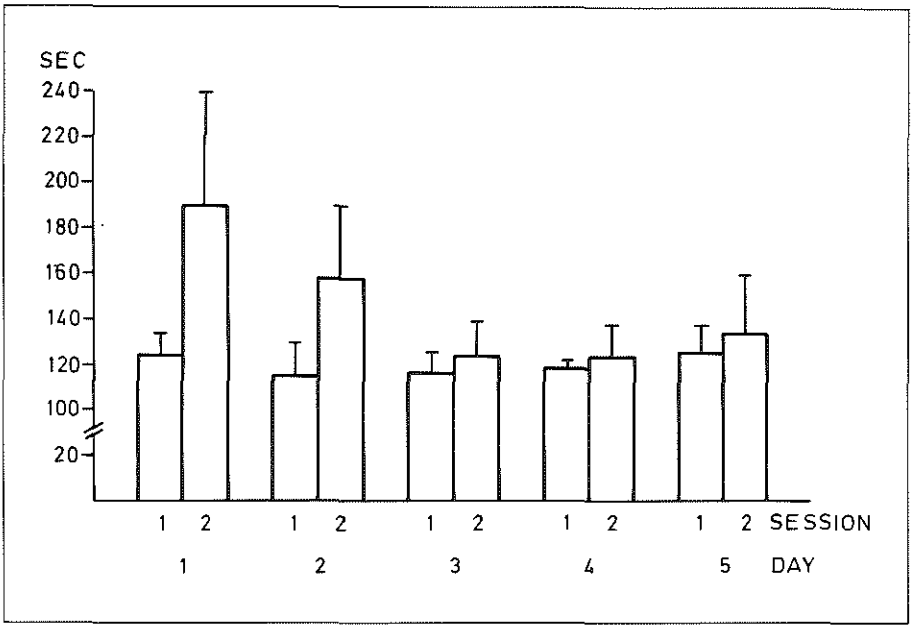

Figure 1.6. Mean (± S.E.M.) latency for the righting reflex to occur after post-session hypoxia over the five training days of group H.

However, statistical analysis (Table 1.9.) showed that there was no significant difference between latencies observed after the two consecutive exposures [B: $F(1/9) = 1.89$, n.s.]. In addition no significant differences were found between recuperation times on the different training days [A: $F(4/36) = 1$, n.s.].
The elevated mean latencies after the second exposure on training day one and two are due to the fact that some animals had very long recuperation times after the second exposure. However, different animals were responsible for the similar effect on both days. The latter is clearly illustrated by the fact that the correlation between the latencies observed on training day one and two was very low and not significant ($r = .22$; t-test linear regression $t = .94$, n.s.).
In addition half of the animals show increased latencies after the second daily exposure whereas the other half had decreased times. The combination of these factors explains why the observed differences were not significant.
Most animals had recuperated within two minutes after removal from the nitrogen environment.

1.5.3. Conclusions

Reducing the intersession interval between daily sessions to 60 minutes did not change the acquisition of the task in the NH group. The performance during the second session was again systematically higher than during the first session on the same day.

Table 1.9.
Analysis of variance on the latency for righting reflex in group H. (A x B x S) repeated measures design with independent factors: A = training days and B = daily sessions.

Source	SS	df	MS	F
A	19051.74	4	4762.94	< 1
S	79225.89	9	8802.88	
A x S	172190.86	36	4783.08	
B	17397.61	1	17397.61	1.89
B x S	82717.09	9	9190.79	
A x B	14588.94	4	3647.24	< 1
A x B x S	138784.86	36	3855.14	

Although acquisition was not blocked, it was clear that post-session hypoxia had more pronounced effects on the performance in the second session with the shorter intersession interval. Instead of reducing the performance to the level of the first session, a significant decrease below that level was observed. This indicated that apart from the effect on MTM either STM or LTM was affected or that a greater anterograde effect was induced. Most probably retrieval from one of these traces was blocked. Given the fact that a substantial performance was still seen during the second session it is very likely that the effect points towards a suppression of STM retrieval. If LTM retrieval would have been blocked, one would not have obtained an acquisition curve from the performance during the second session. Blockade of the development of STM can be ruled out since it would have led to absence of any acquisition. An alternative interpretation can be that the decrease in performance reflected a hang-over related to motor or sensory inhibition due to insufficient recovery. This is very unlikely since no enhancement of the escape times during the second session could be found. The escape times even significantly decreased over the training days.

The latency for the righting reflex to occur seemed to have limited value as a parameter to assess recuperation after hypoxia. The intra-individual variability was very high and no systematic trends were observed. Enhanced recuperation will be difficult to prove with this parameter. However, it can have some value as indicator for negative drug effects. Suppressive effect on the cardiopulmonary function might well compromise recovery and can considerably prolong the time before postural reflexes are regained.

1.6. Discussion

The evidence from both human and animal experimental data suggest the existence of at least three stages of memory formation. However, none of the proposed variants has met with universal acceptance yet (Booth, 1970; Halstead and Rucker, 1970; Matthies, 1974; Squire, 1975; Gibbs and Ng, 1976). This is related to the fact that temporal parameters which can be quite different depending on the species and task used, determine to a large extent the choice of terminology. So far, we have interpreted and discussed our results by using the model and terminology of Frieder and Allweis (1978) but a dual trace or single trace dual-process model could be used as well. An alternative interpretation in such a model could be that hypoxia displaced the decay of STM and the formation of LTM. The transient retention deficit would then be equivalent to a Kamin (1963) effect which is due to insufficient overlap of STM and LTM. We favor a three phase model as working hypothesis but additional experiments are needed to describe the time course of formation and decay of memory traces in the guinea-pig. Although these theoretical issues are important our main objective was to induce stable and reproducible effects on learning and memory.

As we have pointed out in the introduction, stability of the hypoxia-induced effects on behavior is a major requirement to study the antagonism of these effects by pharmacological treatment. The effects of post-session hypoxia on conditioned two-way shuttle behavior described in this chapter seem to meet this requirement perfectly. In both experiments hypoxia reliably induced a decrease in performance during the second daily session. In addition, no adaptation to hypoxia was observed after multiple exposures in the course of the experimental week. The latter characteristic is one of the major assets of this behavioral paradigm. Apparently, sustained training and multiple exposures were not required to arrive at a steady-state effect. For drug treatment studies this implies that treatment can be started from the very first training day on. Consequently, the multisession, multi-exposure paradigm used in the two-way avoidance task presented here allows to study the effect of chronic drug treatment on hypoxia-induced memory deficits.

Another advantage of this type of experiments is the specificity of the induced effects. Certainly if one considers the two-hour interval study, it is probable that hypoxia only affects a single memory trace, i.e. MTM. Moreover, the effects seem to be independent of general behavioral suppression or other secondary effects produced by hypoxia. This condition is favourable to arrive at straightforward interpretations of drug effects. The fact that the normal acquisition of the learning task is not blocked by hypoxia is an additional advantage for application of the paradigm in drug studies. Under these circumstances it becomes possible to study the protective potential of a drug against hypoxia along with its proper effects on the acquisition of a complex learning task in a single experiment. It is conceivable that certain drugs or high doses of drugs which have anti-hypoxic effects in simple survival models attenuate acquisition by causing different degrees of neurological disturbances.

As a consequence performance during the sessions can be affected and reduced learning caused by the side-effects of the drug will be observed. Thus the paradigm will allow to find out if a drug can be given in a dose that protects against hypoxia without interfering with normal functioning and learning capabilities through secondary drug effects.

The type of hypoxia employed in the learning experiments is an additional favourable element for the evaluation of possible working mechanisms of the drugs. In many other experiments, hypoxia was induced by exposing the animals to an environment where the oxygen saturation of the inspired air was partly reduced (normobaric or hypobaric). This means that during the exposure, although limited, oxygen is still available to vital tissues.
The efficiency with which the oxygen is delivered to these tissues is dependent upon an intact cardiovascular transport system. Hence, it is possible to attribute protective drug effects to intact or even improved cardiovascular functioning under hypoxic conditions (Van Hof, 1982). A similar interpretation can be excluded when the animals are exposed to pure nitrogen. Since no oxygen is available in the inspired air and oxygen tension in the tissues decreases rapidly to very low levels, transport mechanisms during hypoxia cannot account for a post-hypoxic improvement. This means that in the present paradigm any protection can be attributed with a greater certainty to direct protection of the brain. However, the possibility still exists that protective effects originate from improved cardiovascular functioning during the intersession interval. The ECG recovery after hypoxia might be used then in a number of independent experiments to investigate cardiovascular effects of the drug treatment.

Apart from the possibility to study protective drug effects, the paradigm allows to study resuscitative potentials of drugs. Experiments can be designed where the drug is given immediately after the first daily exposure to hypoxia. This will then allow to find out if the compound can restore the drop out of MTM observed in the second session. This aspect is important for a large number of clinical indications where drugs can only be given after the insult as, for example in cardiac arrest and stroke.
That resuscitative measures can be effective has already been demonstrated for hyperoxia treatment shortly after the induced hypoxia (Frieder and Allweis, 1982 a).

With regard to the two intersession intervals used, it is important to stress that the two-hour interval might be the best choice for drug studies. It was clear from the experiments that the results obtained with this interval fitted perfectly with the theoretical assumption and the evidence from the Frieder and Allweis experiments. Although the results with the one-hour interval are not in contradiction with the theory, they point towards the existence of a more complicated effect. The origin of this is not clear, although it is very likely that a retrieval block is involved. One has to assume that with the shortest intersession interval, different putative mechanisms are either still affected or not recovered after the hypoxia. Several neurophysiological mechanisms are involved in the formation of memory traces. It has been shown, for instance, that normal norepinephrine (NE) levels are essential for the formation of MTM. Intracisternal injection of diethyldithiocarbamate (DDC), a NE synthesis

inhibitor, caused amnesia with an identical time course as the amnesia induced by hypoxia (Frieder and Allweis, 1982 b). The amnesic effect of drugs like ouabain, ethacrynic acid and KCl (2.5 to 5 mM) suggest the involvement of Na^+/K^+ ATPase in the formation of MTM (Gibbs and Ng, 1976, 1977). Besides the different neurophysiological mechanisms involved in the formation of both traces, retrieval failure can still be based on other mechanisms than NE levels and sodium pump activity. It is conceivable that a drug prevents the hypoxia-induced blockade of the MTM trace by activating and/or stabilizing the sodium pump activity. Such drug would then restore the behavioral effects observed in an experimental paradigm with the two-hour intersession interval. However, it is not certain that the same drug will be effective against the behavioral effects seen in an experiment where the one-hour interval is used.

Given the specificity and stability of the effects seen in this experimental paradigm we may conclude that avoidance learning will be a suitable method to study pharmacological protection against hypoxia-induced functional deficits. As a model it has direct clinical relevance related to cognitive impairments after hypoxic insults in humans. Cardiac and respiratory arrest as well as hypoxic insults during coronary bypass operations are known to induce amnesic events in patients (Volpe and Hirst, 1983; Huppert, 1983; Meltzer, 1983). Drugs that are found active in the model might therefore be indicated for use as a prophylactic measure in interventions that are known to present the risk of being accompanied with hypoxic episodes.

Chapter 2
Pharmacological Protection Against Hypoxia-Induced Effects on Avoidance Learning

2.1. Introduction

The possibility to antagonize selective hypoxia-induced effects on medium-term memory (MTM) was clearly demonstrated in the rat by application of hyperoxia treatment (Frieder and Allweis, 1982a). We tried to accomplish the same result by pharmacological treatment. The two-way avoidance paradigm described in the first chapter was used in the experiments which will be presented here. The aim was to evaluate the utility of the new paradigm for pharmacological research purposes. At the same time information could be obtained on the effect of chronic drug treatment against hypoxia-induced memory deficits.

To demonstrate the possibilities with the paradigm a choice had to be made between different categories of drugs that have antihypoxic properties. In simple models where survival is used as a criterion for protection many drugs that belong to different classes are active. Barbiturates and other hypnotics (Wauquier *et al.*, 1981), some benzodiazepines (Nakanishi *et al.*, 1973; Nugent *et al.*, 1982), anticonvulsants (Caillard *et al.*, 1976), calcium entry blockers (Wauquier, 1984) and antimigraine drugs (Wauquier *et al.*, 1984) were able to prolong survival in extreme hypoxic conditions.

Prolongation of survival however, is not necessarily a good predictor for protective effects against functional deficits in the field of learning and memory. To optimize the chance that protection could be found it was decided to concentrate on a group of drugs for which both protective effects against learning and memory deficits and protection against mortality due to nitrogen exposure were described. The choice of drugs was limited in the first place by the specific characteristics of the behavioral paradigm to be used. Barbiturates and hypnotics were excluded because carry-over effects on behavior could be expected. In the survival models, optimal results with these compounds were obtained when the animals were asleep during exposure to hypoxia. Evidently, avoidance learning cannot be accomplished during sleep.

To study the pharmacological protection against hypoxic effects on learning and memory two types of experiments were described in the literature. In the first type, conditioning (one-way avoidance) was studied during hypobaric hypoxia (Hurwitz *et al.*, 1971; Boismare *et al.*, 1977). Central dopaminergic stimulation by low doses of peribedil, amantadine, bromocryptine and apomorphine improved learning during hypobaric hypoxia (Saligaut *et al.*, 1981 a,b). Protection was also observed after combined treatment with L-dopa and benserazide (Boismare *et al.*, 1975). We did not choose dopaminergic agonists as test compounds in our experiments for several reasons. First, these compounds are also known to alter behavior in non-hypoxic conditions. Secondly, we could not find any protection against exposure to pure nitrogen with these compounds. Finally, improving avoidance

performance during hypoxia is probably quite different from antagonizing amnesia due to hypoxia since different mechanisms might be involved.

In the second type of studies retrograde amnesia was induced by exposure to hypoxia after initial one-trial passive avoidance training. Protective effects of piracetam were shown in this paradigm (Sara and Lefevre, 1972; Gouret and Raynaud, 1976) and in operant conditioning with a visual stimulus as CS (Giurgea *et al.*, 1971). In all these experiments a gas mixture with 3-4 % oxygen content was used to induce hypoxia.

However, protection against pure nitrogen exposure in the rat could not be obtained with piracetam (Wauquier *et al.*, 1982). The same holds for other compounds including vincamine and hydergine which were active against hypoxia-induced amnesia in the one-trial passive avoidance paradigm (Gouret and Raynaud, 1976). A general observation in these experiments was that the drugs were only active in a narrow dose range.

Hoffmeister *et al.* (1982) reported that the dihydropyridine derivative nimodipine had potent antiamnesic effects in a very broad dose range. As in the other experiments mentioned a passive avoidance task was used and the animals were exposed after initial training to a gas mixture containing 3.8 % oxygen. The ED_{50} for p.o. administration was 0.1 mg/kg and protection was seen after pretreatment intervals between 30 minutes and 24 hours. Nimodipine also protected against mortality following a 1 minute exposure to 100 % nitrogen (Wauquier *et al.*, 1984). Nimodipine belongs to a heterogenous class of drugs called "calcium entry blockers" according to their effects on calcium-mediated processes in vascular and/or cardiac tissues. Two other compounds that have been classified in this group, flunarizine a piperazine derivative and nifedipine a structural analogue of nimodipine protect against pure nitrogen exposure. Interestingly antiamnesic effects in a passive avoidance paradigm were also described for flunarizine (Wauquier *et al.*, 1982).

Based on these findings reported in the literature and the important role of calcium in the cascade that leads to cell death after severe hypoxic insults we decided to study a number of these "calcium entry blockers". Flunarizine and nimodipine were chosen because they both are known to protect against retrograde amnesia and mortality after nitrogen exposure. The later is important because exposure to pure nitrogen is used as challenge in our experiments. Nifedipine and verapamil have their main effects on the slow calcium channels (voltage-activated) in the coronary vasculature and cardiac tissue. Nifedipine has weak activity and verapamil is not active against nitrogen exposure.

This indicates that these drugs do not enter the brain or that they lack central activity. They were included in the study to determine if drugs with primarily cardiovascular activity protect against the centrally mediated hypoxic effects on the two-way avoidance learning.

2.2. Materials and methods

2.2.1. Animals and experimental procedure

Seven groups of ten male guinea-pigs were given two-way avoidance training on five consecutive days. Daily training consisted of two sessions which were held with an intersession interval of 120 minutes. The training procedure is described in detail in the first chapter. Immediately after each of the daily sessions, the animals were exposed for 1 minute to a 100% nitrogen environment.

On all training days each group (N = 10) was pretreated with a dose of either flunarizine (0.16 or 0.63 mg/kg s.c.), nimodipine (0.16 or 0.63 mg/kg p.o.), nifedipine (0.16 or 0.63 mg/kg p.o.) or verapamil (0.63 or 2.5 mg/kg s.c.) one hour before the first daily session. One group received 0.63 mg/kg flunarizine s.c. four hours before the first daily session. All drugs were freshly prepared and given as a solution (flunarizine and verapamil) or as a suspension (nifedipine and nimodipine) in water. Data recording was the same as for the experiments on saline-treated animals which were presented in Chapter 1.

2.2.2. Statistical analysis

The effectiveness of drug treatment can be evaluated by different types of statistical analysis. In a first type of analysis we can evaluate within each individual treatment group whether or not a specific dose of a drug was capable of antagonizing the hypoxic effect. To do so the data are analyzed in a repeated measures design (A x B x S) with as independent factors training days (A) and daily sessions (B). When protection occurs a significant main effect of factor B will be found. In the same analysis a significant main effect of factor A will indicate significant acquisition. If the drug in combination with hypoxia blocks learning no significant effect will be found.

In the second type of analysis the results of a drug-treated group can be analysed together with the results of the control H group of Chapter 1. Then an A x (B x C x S) mixed repeated measures design with as independent factors pre-treatment (A), training days (B) and daily sessions (C) can be used. In such a design the interaction between pre-treatment and daily sessions (A x C) will become significant when protection occurs. To evaluate whether this general significant interaction is due to an effect on the second session in the drug-treated group an analysis of the simple main effects in the interaction needs to be done (for example see Chapter 1, Table 1.3.). In such analysis the mean squares and the error terms of the F-ratio to evaluate the differences between daily sessions in both groups are the same as in a separate analysis of the first type on both groups. Hence, in both types of analysis the same variance components are used to decide on the significance of drug protection. In this respect both techniques are completely equivalent. The advantage of the second type of analysis is the possibility to estimate the relative learning efficiency in both pre-treatment conditions. However, using the same control group to compare it with the nine groups of drug pretreated animals can lead

to wrong conclusions about observed differences. The chance of finding a significant difference increases as a function of the number of comparisons made with a single control group. A better way to evaluate the relative efficiency of learning in drug pre-treated groups is to create a large control group for which confidence intervals can be calculated. Then one can estimate if the data for drug pre-treated groups fall within normal control limits. This was not possible with the present material.

Since the main objective of the present experiment was to evaluate drug protection and both types of analysis are equivalent for this purpose we decide to use the first type (A x B x S) design.

Analysis of variance on the number of avoidances, escape times and latency of righting were done for each group. The repeated measures design (A x B x S) had as independent factors training days (A) and daily sessions (B). All details on the analysis that were performed can be found in Addendum 1 (flunarizine: Tables 1 to 9; nimodipine: Tables 10 to 15; nifedipine: Tables 16 to 21 and verapamil: Tables 22 to 27).

2.3. Results

2.3.1. Flunarizine

Figure 2.1. shows the acquisition curves of the groups pretreated with flunarizine one hour before the first session. The number of avoidances significantly increased in both the 0.16 mg/kg [$F(4/36) = 11.16$, $p < .001$] and 0.63 mg/kg group [$F(4/36) = 8.58$, $p < .001$]. At the dose of 0.16 mg/kg flunarizine protected against hypoxia since the performance during the second session was significantly higher than during the first session [$F(1/9) = 5.5$, $p < .05$]. At the dose of 0.63 mg/kg this main effect was not observed. However, the significant interaction [$F(4/36) = 3.18$, $p < .05$] between training days and sessions suggested protection from day 2 to 4 as can be seen in Figure 2.1. (right part).

Protection was not seen on the last training day. This was mainly due to the fact that 6 out of 10 animals reached a performance of 80 % or more avoidance responses on day 5. The decrease during the second session was therefore probably related to a ceiling effect in the first session.

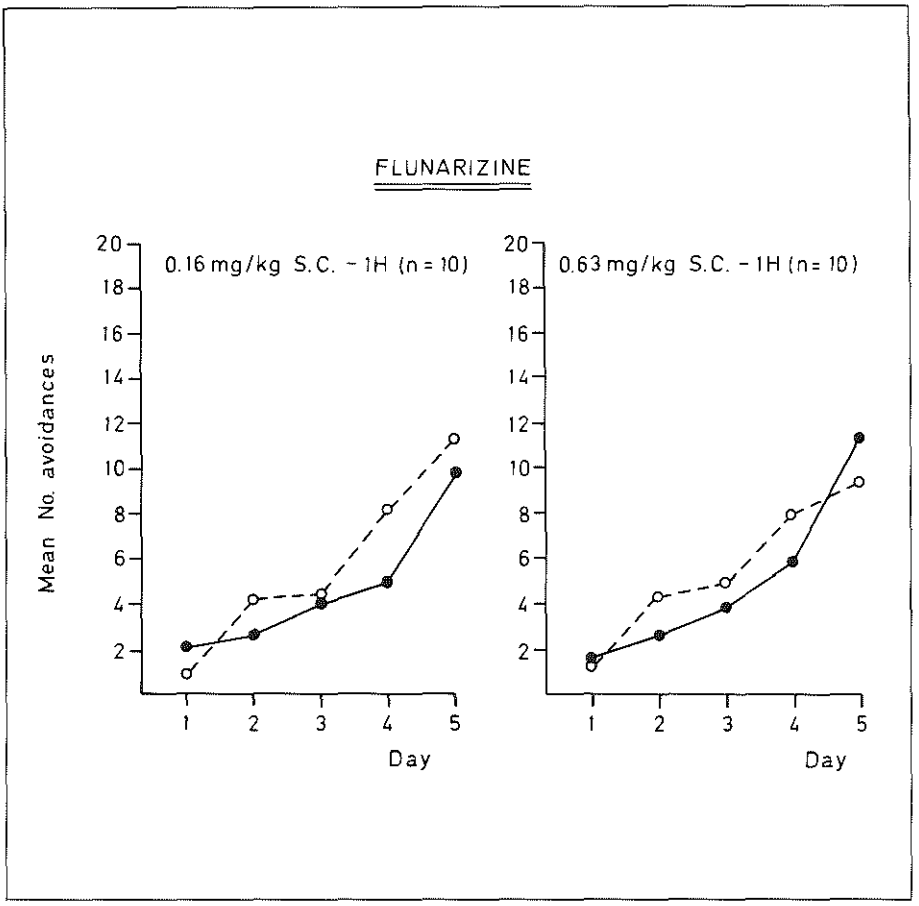

Figure 2.1. Mean number of avoidances during daily sessions on five consecutive training days in two groups of animals pretreated with different doses of flunarizine one hour before the first daily session. Acquisition curves based on session 1 (full line) and session 2 (broken line).

No significant main effects were found on the mean escape times. Escape times did not change over the five training days and no systematic differences occurred between the daily sessions as can be seen in Figure 2.2.

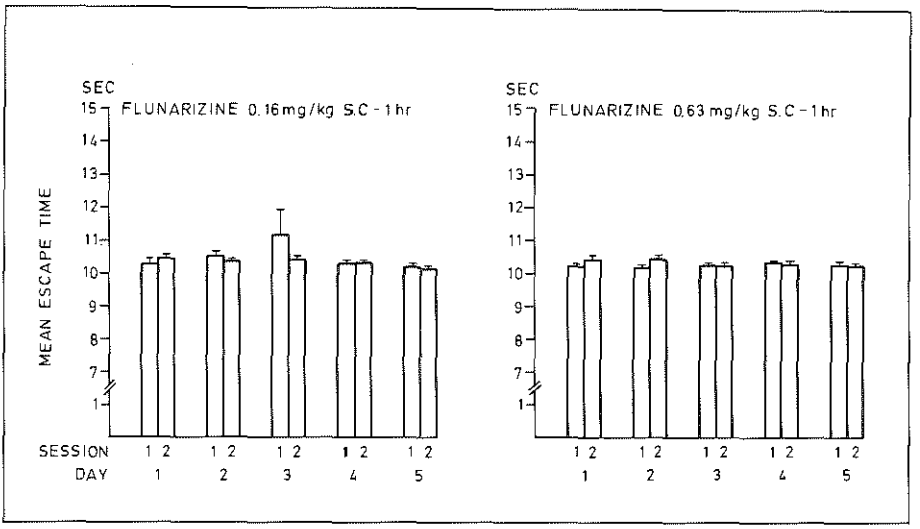

Figure 2.2. Mean (± S.D.) escape times during daily sessions in two groups of animals pretreated with different doses of flunarizine one hour before the first daily session.

The mean latencies of righting are depicted in Figure 2.3. For the group treated with the lowest dose no significant main effects were found. The significant interaction [$F(4/36) = 3.15$, $p < .05$] between training days and sessions was caused by the sharp decrease between session 1 and 2 on the first day. For the group treated with the highest dose a significant decrease over the training days [$F(4/36) = 7.58$, $p < .001$] and between daily sessions [$F(1/9) = 15.38$, $p < .01$] was found.

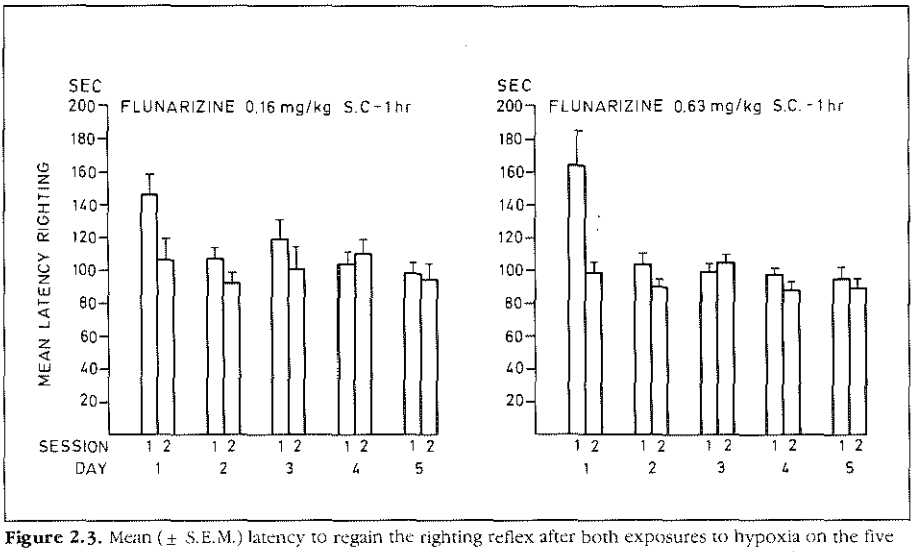

Figure 2.3. Mean (± S.E.M.) latency to regain the righting reflex after both exposures to hypoxia on the five training days in two groups of animals pretreated with different doses of flunarizine one hour before the first daily session.

To investigate whether the partial protection with 0.63 mg/kg was a real protective effect that was attenuated by a ceiling phenomenon, a group of animals was treated with the same dose four hours before the first daily session. As can be seen in Figure 2.4. significant acquisition [$F(4/36) = 11.8$, $p < .001$] of the task together with a significant protection [$F(1/9) = 9.66$, $p < .01$] against hypoxia was observed.

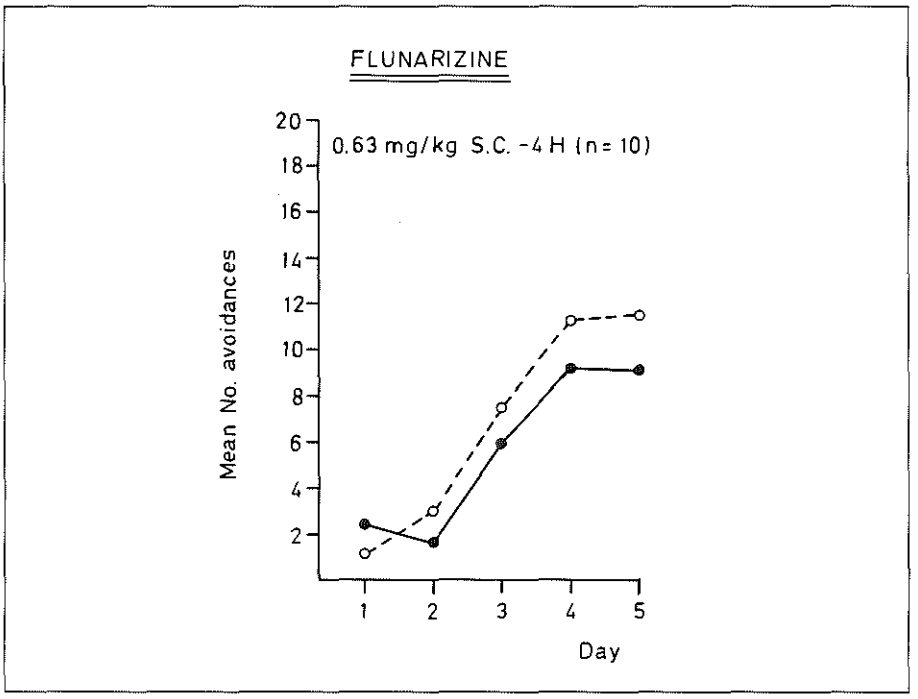

Figure 2.4. Mean number of avoidances during daily sessions on five consecutive training days in a group pretreated with flunarizine four hours before the first daily session. Acquisition curves based on session 1 (full line) and session 2 (broken line).

No significant trends were observed on the mean escape times and the mean latency of righting (Figure 2.5.).

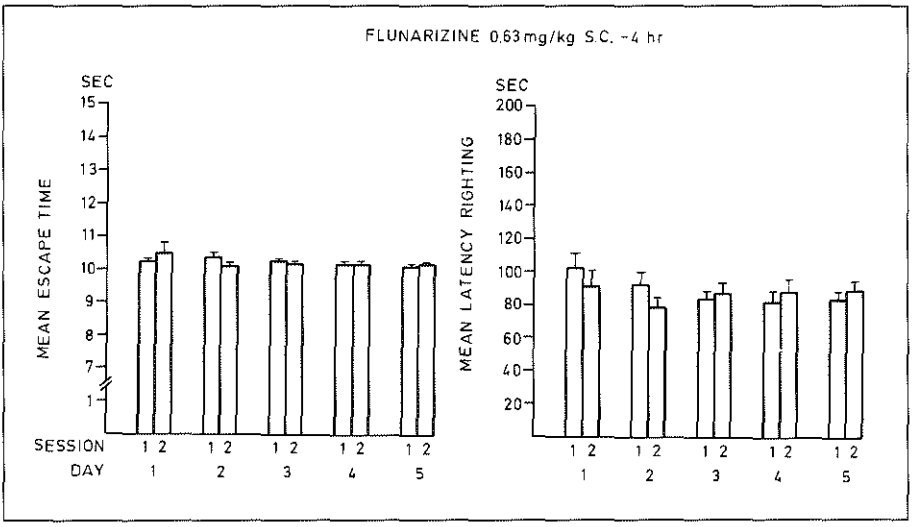

Figure 2.5. Mean escape times (left part) and mean latency of righting reflex (right part) in a group of animals pretreated with flunarizine four hours before the first daily session.

2.3.2. Nimodipine

In nimodipine pretreated groups the number of avoidances significantly increased over the training days with the lowest [$F(4/36) = 8.91$, $p < .001$] and highest dose [$F(4/28) = 5.95$, $p < .01$] tested. As can be seen in Figure 2.6. the group pretreated with 0.16 mg/kg made significantly fewer correct responses during the second session [$F(1/9) = 5.36$, $p < .05$]. No difference between the session was observed in the group pretreated with 0.63 mg/kg. These results indicate that nimodipine had no protective effects against hypoxia at the doses that were given. In the group treated with the highest dose, two animals could not be reanimated after exposure to hypoxia.

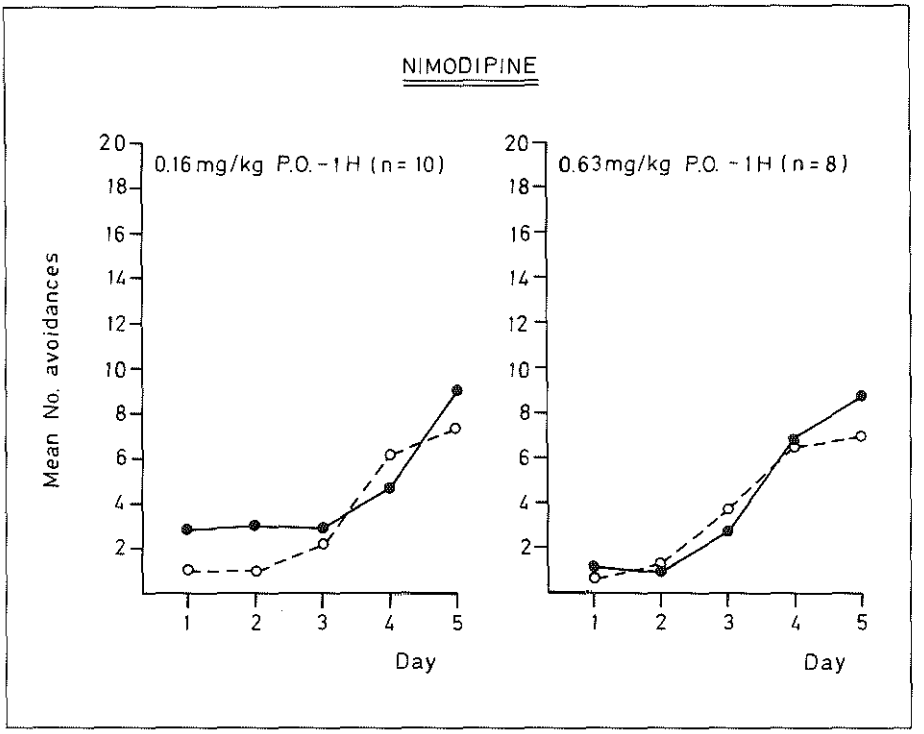

Figure 2.6. Mean number of avoidances during daily sessions on five consecutive training days in two groups of animals pretreated with different doses of nimodipine one hour before the first daily session. Acquisition curves based on session 1 (full line) and session 2 (broken line).

In the group treated with 0.16 mg/kg there was a slight but significant [$F(4/36) = 3.32$, $p < .05$] decrease of escape times in the course of the training period. This effect was not seen with the higher dose (Figure 2.7.).

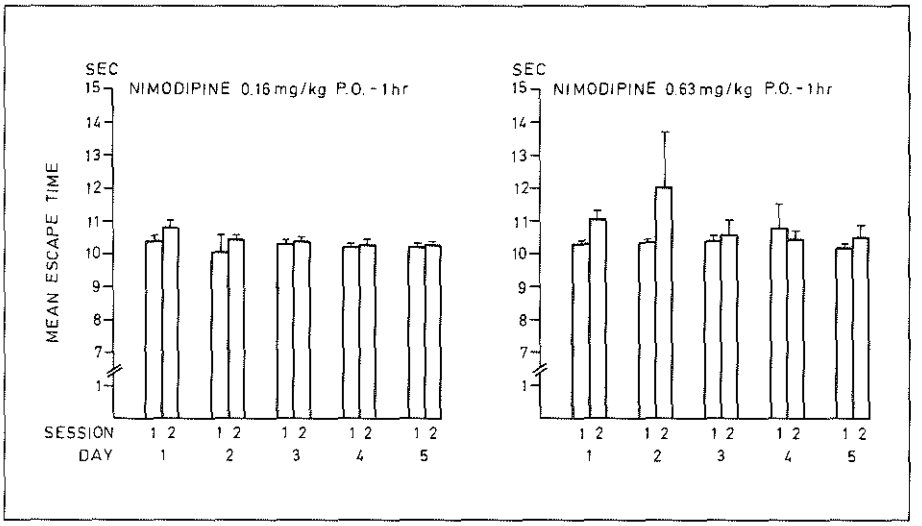

Figure 2.7. Mean (± S.D.) escape times during daily sessions in two groups of animals pretreated with different doses of nimodipine one hour before the first daily session.

The latency of righting significantly [$F(4/36) = 4.04$, $p < .01$] decreased in the course of the training period of the group treated with the lowest dose. Although Figure 2.8. (left part) suggests that the latency is consistently lower after the second exposure, the analysis did not reveal a significant trend. After the highest dose no significant main effects were observed.

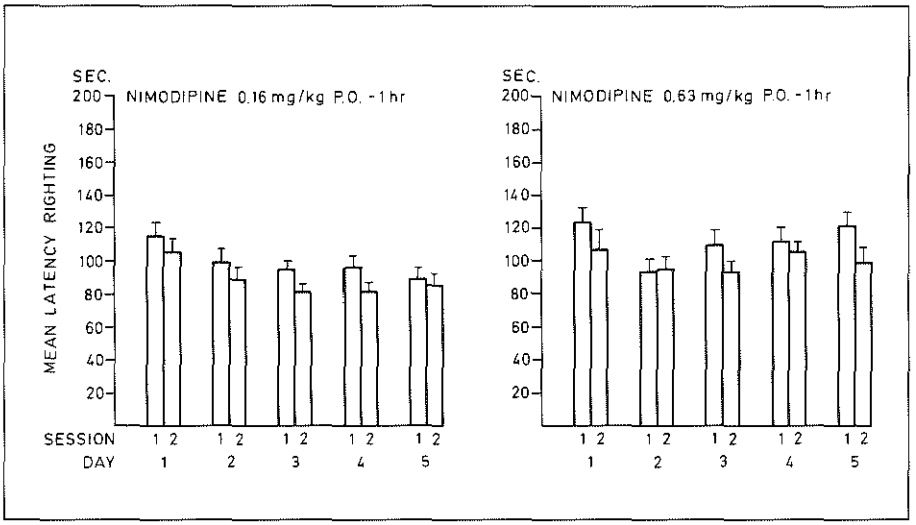

Figure 2.8. Mean (± S.E.M.) latency to regain the righting reflex after both exposures to hypoxia on the five training days in two groups of animals pretreated with different doses of nimodipine one hour before the first daily session.

2.3.3. Nifedipine

Significant acquisition of the task was seen in the groups treated with 0.16 mg/kg [$F(4/9) = 6.57$, $p < .001$] and 0.63 mg/kg [$F(4/9) = 12.3$, $p < .001$] nifedipine. However, Figure 2.9. suggests that the compound had no protective effect. This was confirmed by the statistical analysis since no significant differences were found between daily sessions.

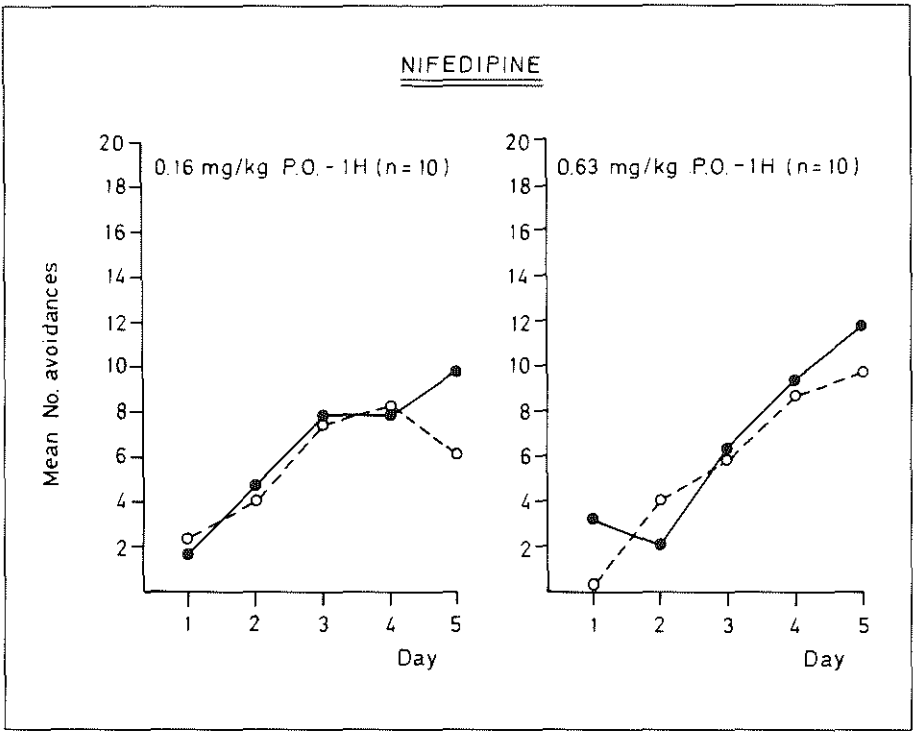

Figure 2.9. Mean number of avoidances during daily sessions on five consecutive training days in two groups of animals pretreated with different doses of nifedipine one hour before the first daily session. Acquisition curves based on session 1 (full line) and session 2 (broken line).

The mean escape times slightly changed during the experimental period as can be seen in Figure 2.10. However, the analysis indicated a small but significant decrease over the training days in the group treated with 0.16 mg/kg [$F(4/36) = 3.17$, $p < .05$] and 0.63 mg/kg [$F(4/36) = 3.13$, $p < .05$] nifedipine.

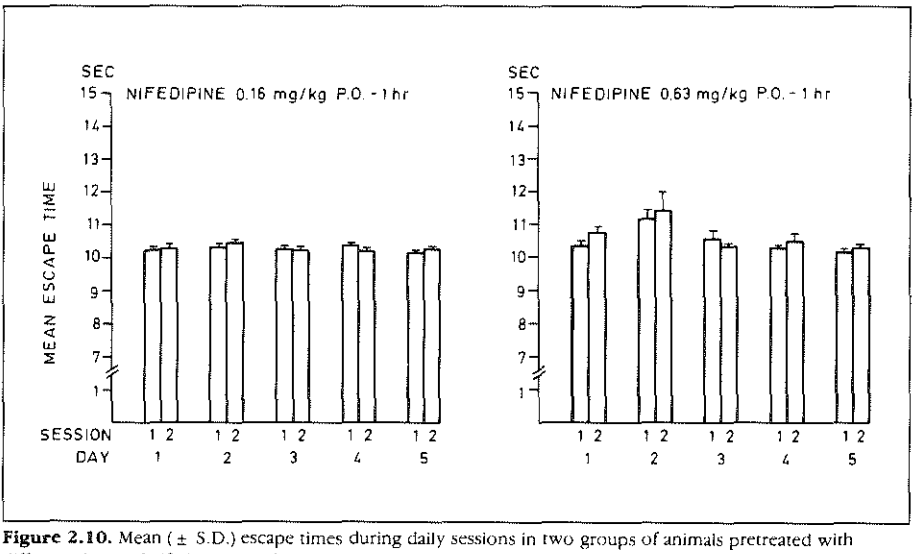

Figure 2.10. Mean (± S.D.) escape times during daily sessions in two groups of animals pretreated with different doses of nifedipine one hour before the first daily session.

Figure 2.11. suggests that the latency to regain the righting reflex was systematically lower after the exposure following the second daily session in the group treated with 0.16 mg/kg. This was confirmed by the statistical analysis [$F(1/9) = 7.66$, $p < .05$] but no general decrease in the latencies was found in the course of the experimental week. This effect occurred in the group treated with the highest dose [$F(4/36) = 5.47$, $p < .01$] but here no significant differences between the daily sessions were found.

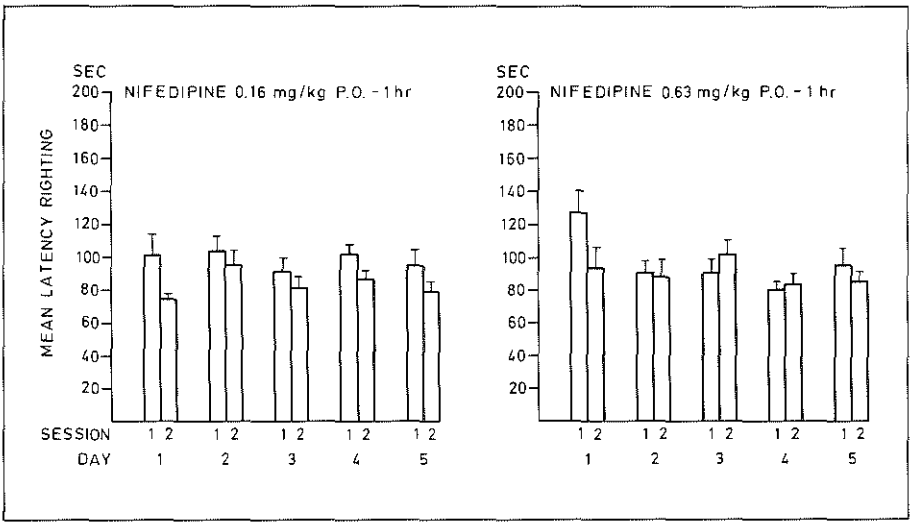

Figure 2.11. Mean (± S.E.M.) latency to regain the righting reflex after both exposures to hypoxia on the five training days in two groups of animals pretreated with different doses of nifedipine one hour before the first daily session.

2.3.4. Verapamil

As can be seen in Figure 2.12. acquisition was minimal after treatment with both doses of verapamil. Consequently no significant gain was revealed by the statistical analysis on the number of avoidances. In addition no protective effects were seen. One animal treated with 2.5 mg/kg died after exposure to nitrogen.

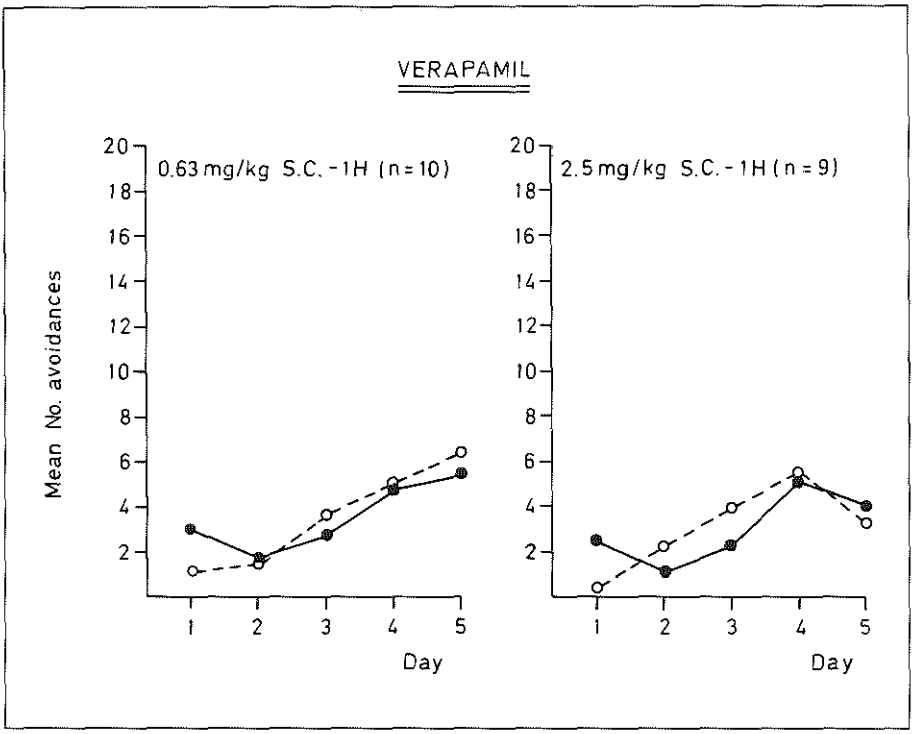

Figure 2.12. Mean number of avoidances during daily sessions on five consecutive training days in two groups of animals pretreated with different doses of verapamil one hour before the first daily session. Acquisition curves based on session 1 (full line) and session 2 (broken line).

Figure 2.13. indicates that the mean escape times were increased during the second daily session. This effect was significant in the group treated with the lowest dose [$F(1/9) = 9.45$, $p < .05$] and did just not reach significance in the other group.

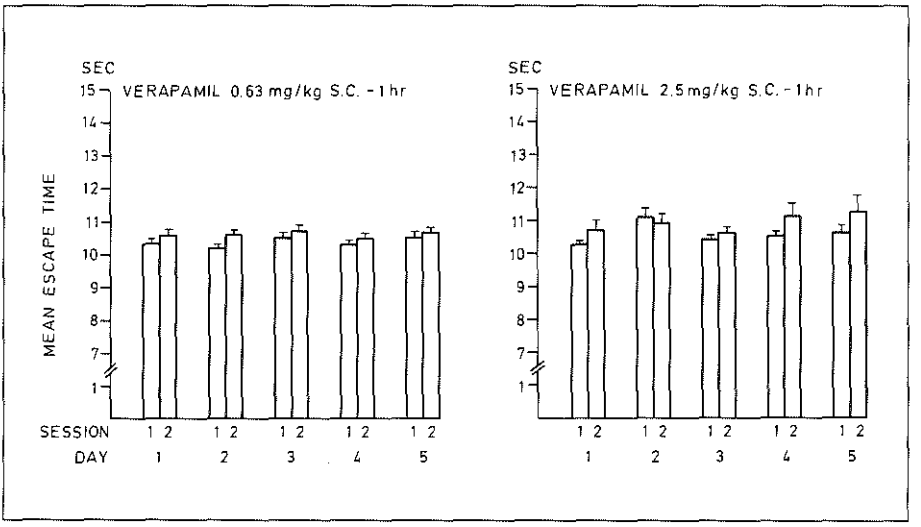

Figure 2.13. Mean (± S.D.) escape times during daily sessions in two groups of animals pretreated with different doses of verapamil one hour before the first daily session.

The latency to regain righting decreased in the course of the training period in the group treated with 0.63 mg/kg verapamil [$F(4/36) = 3.44$, $p < .05$]. No significant main effects were found in the other group.

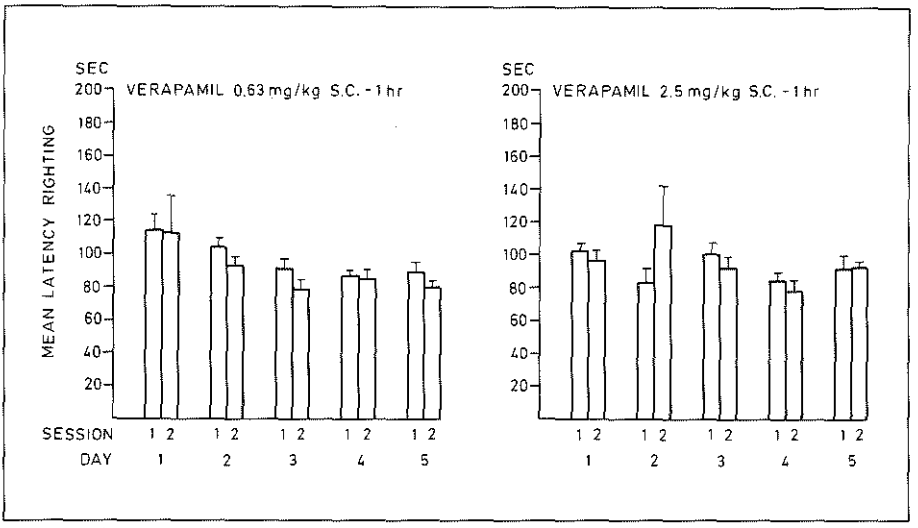

Figure 2.14. Mean (± S.E.M.) latency to regain the righting reflex after both exposures to hypoxia on the five training days in two groups of animals pretreated with different doses of verapamil one hour before the first daily session.

2.4. Discussion

The results obtained with flunarizine demonstrate that the selective hypoxic effects on MTM can be antagonized by pharmacological treatment. In a more general sense this means that the two-way avoidance paradigm is a suitable tool for pharmacological research purposes. An important aspect in this respect is the fact that the efficacy of chronic drug treatment can be evaluated. With flunarizine no signs of tolerance were seen, but the paradigm should reveal tolerance if it occurs. However, the ceiling effect seen on the last training day in one of the groups treated with flunarizine hampered a simple interpretation of the results. This problem can perhaps be avoided in the future by a change in the experimental paradigm. Progressive increments of the number of trials in each session on the consecutive training days will probably prevent ceiling effects in the results.

The experiments with verapamil indicated that pharmacological treatment can also worsen the hypoxic effects on learning and memory. In both groups treated with verapamil the acquisition of the task was almost completely blocked. This effect was probably not caused by direct interference on the CNS level unless the hypoxia had caused damage to the blood-brain barrier.
The good behavioral state of the animals after drug administration did not point towards the existence of such damage. After most hypoxic/ischemic insults secondary effects occur after reoxygenation (Siesjo, 1981). These include a period of hypoperfusion of the brain in combination with hypermetabolism.

The oscillations in brain tissue PO_2 after short lasting inhalation of low oxygen concentrations indicate that post-hypoxic hypoperfusion is possible when brief hypoxia without blood stasis is the precipitant factor (Metzger *et al.*, 1971). The origin of the negative effects with verapamil might be found there. Pretreatment with verapamil can have aggravated this hypoperfusion since the drug has negative-inotropic effects and is known to induce vasodilation (Fleckenstein, 1981). The combination of these effects can lead to a strong secondary hypoxic insult which is normally not seen in control animals originally exposed to the same type of hypoxia. Prolonged secondary hypoxia could therefore be responsible for a failure in transcription to LTM in animals treated with verapamil. Such effect ultimately would result in the absence of acquisition.

The paradigm seems to be a very sensitive method for determination of protective drug effects. The active doses of flunarizine in our experiments were a hundred times lower than those required to protect against mortality in the nitrogen test in the rat (Wauquier, 1984). It was therefore surprising that no protective effects were found with nimodipine at doses which protected against retrograde amnesia in the passive-avoidance paradigm described by Hoffmeister *et al.* (1982). This discrepancy can be related to the differences between the paradigms and to the memory traces that are involved in the amnesia. In the single trial passive-avoidance test the animals are exposed after the trial to a gas mixture with a reduced oxygen percentage (3.8 %) for a period up to 15 minutes. The retention test is given 24 hours later and the observed amnesia reflects an effect on LTM formation. This effect can be caused during the hypoxia but also partly during a secondary hypoperfusion phase. Nimodipine has predominant cerebral vasodilating effects (Kazda *et al.*, 1982; Towart *et al.*, 1982), and could therefore have increased oxygen delivery to the brain both during and after hypoxia. Its beneficial effect on the outcome of complete global brain ischemia in monkeys (Gisvold, 1984) can also be attributed to vascular effects. In our two-way avoidance paradigm the animals were exposed to pure nitrogen and MTM formation was blocked. No effects on LTM were observed. Protection in this model requires direct effects on the CNS and cardiovascular effects were not expected to be beneficial in this paradigm (see Discussion Chapter 1). The present results confirm this since both nimodipine and nifidipine did not protect against the induced amnesia. In addition, direct neuronal effects of flunarizine have been shown in the hippocampal slice (Wauquier *et al.*, 1984). In the present model flunarizine was also the only compound of the drugs tested that protected against hypoxia induced amnesia.

The effects on the escape times were minor. If they occurred at all, an improvement was seen in the course of the training period. Similar results were seen in control animals receiving post-training hypoxia in the experiments discussed in chapter 1. The only exception was found in the group treated with the lowest dose of verapamil in which the escape times were slightly increased during the second session. The latency to regain the righting reflex appeared to be a rather unstable parameter. No real systematic effects were observed and a decrease in latency during the training period was certainly not related to protective drug effects against the amnesia.

These results further strenghten the hypothesis that the hypoxic effects and the protection against them in this paradigm were primarily centrally mediated. Therefore the two-way avoidance paradigm appears to be a good method to study central protective effects of drugs in a behavioral model.

Addendum 1

Table 1

ANOVA on number of avoidances.
Pretreatment: flunarizine 0.16 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	959.66	4	239.92	11.16 ***
S	1415.61	9	157.29	
A x S	773.94	36	21.50	
B (ses.)	28.09	1	28.09	5.50 *
B x S	46.01	9	5.11	
A x B	50.46	4	12.62	1.99
A x B x S	227.94	36	6.33	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 2

ANOVA on mean escape time.
Pretreatment: flunarizine 0.16 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	4.17	4	1.04	1.43
S	8.88	9	0.99	
A x S	26.23	36	0.73	
B (ses.)	0.62	1	0.62	< 1
B x S	6.28	9	0.70	
A x B	2.44	4	0.61	1
A x B x S	21.98	36	0.61	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 3

ANOVA on latency righting.
Pretreatment: flunarizine 0.16 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	11190.16	4	2797.54	2.41
S	12923.16	9	1435.91	
A x S	41811.04	36	1161.42	
B (ses.)	4515.84	1	4515.84	2.79
B x S	14547.36	9	1616.37	
A x B	6325.76	4	1581.44	3.15 *
A x B x S	18063.04	36	501.75	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 4

ANOVA on number of avoidances.
Pretreatment: flunarizine 0.63 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	938.24	4	234.56	8.58 ***
S	1772.84	9	196.98	
A x S	984.16	36	27.34	
B (ses.)	6.76	1	6.76	2.74
B x S	22.24	9	2.47	
A x B	53.24	4	13.31	3.18 *
A x B x S	150.76	36	4.19	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 5

ANOVA on mean escape time.
Pretreatment: flunarizine 0.63 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	0.12	4	0.03	< 1
S	2.52	9	0.28	
A x S	3.02	36	0.08	
B (ses.)	0.15	1	0.15	3.77
B x S	0.36	9	0.04	
A x B	0.43	4	0.11	4.53 **
A x B x S	0.84	36	0.02	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 6

ANOVA on latency righting.
Pretreatment: flunarizine 0.63 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	21249.34	4	5312.34	7.58 ***
S	15565.44	9	1729.49	
A x S	25236.86	36	701.02	
B (ses.)	8028.16	1	8028.16	15.38 **
B x S	4699.24	9	522.14	
A x B	15438.14	4	3859.54	6.80 ***
A x B x S	20436.46	36	567.68	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 7
ANOVA on number of avoidances.
Pretreatment: flunarizine 0.63 mg/kg s.c. -4 h.

Source	SS	df	MS	F
A (days)	1361.26	4	340.32	11.80 ***
S	1489.41	9	165.49	
A x S	1038.54	36	28.85	
B (ses.)	37.21	1	37.21	9.66 *
B x S	34.69	9	3.85	
A x B	43.34	4	10.84	1.79
A x B x S	217.26	36	6.04	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 8
ANOVA on mean escape time.
Pretreatment: flunarizine 0.63 mg/kg s.c. -4 h.

Source	SS	df	MS	F
A (days)	0.51	4	0.13	< 1
S	1.73	9	0.19	
A x S	5.57	36	0.15	
B (ses.)	0.01	1	0.01	< 1
B x S	0.99	9	0.11	
A x B	0.66	4	0.17	1.89
A x B x S	3.31	36	0.09	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 9
ANOVA on latency righting.
Pretreatment: flunarizine 0.63 mg/kg s.c. -4 h.

Source	SS	df	MS	F
A (days)	1950.46	4	487.62	1.23
S	16623.01	9	1847.00	
A x S	14233.74	36	395.38	
B (ses.)	114.49	1	114.49	< 1
B x S	3516.61	9	390.73	
A x B	1761.26	4	440.32	1.34
A x B x S	11836.14	36	328.78	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 10

ANOVA on number of avoidances.
Pretreatment: nimodipine 0.16 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	589.74	4	147.44	8.91 ***
S	1062.29	9	118.03	
A x S	595.46	36	16.54	
B (ses.)	24.01	1	24.01	5.36 *
B x S	40.29	9	4.48	
A x B	40.74	4	10.19	< 1
A x B x S	374.46	36	10.40	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 11

ANOVA on mean escape time.
Pretreatment: nimodipine 0.16 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	4.52	4	1.13	3.32 *
S	9.49	9	1.05	
A x S	12.29	36	0.34	
B (ses.)	0.00	1	0.00	< 1
B x S	5.09	9	0.57	
A x B	2.82	4	0.70	1.56
A x B x S	16.13	36	0.45	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 12

ANOVA on latency righting.
Pretreatment: nimodipine 0.16 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	7938.40	4	1984.60	4.04 **
S	12697.80	9	1410.87	
A x S	17670.80	36	490.86	
B (ses.)	2766.76	1	2766.76	3.84
B x S	4140.84	9	460.09	
A x B	389.04	4	97.26	< 1
A x B x S	13169.36	36	365.82	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 13

ANOVA on number of avoidances.
Pretreatment: nimodipine 0.63 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	647.08	4	161.77	5.95 *
S	1267.09	7	181.01	
A x S	760.73	28	27.17	
B (ses.)	1.51	1	1.51	< 1
B x S	23.39	7	3.34	
A x B	17.43	4	4.36	< 1
A x B x S	199.18	28	7.11	

*** p < .001; ** p < .01; * p < .05.

Table 14

ANOVA on mean escape time.
Pretreatment: nimodipine 0.63 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	6.91	4	1.73	< 1
S	82.42	7	11.77	
A x S	50.53	28	1.80	
B (ses.)	5.36	1	5.36	2.29
B x S	16.40	7	2.34	
A x B	8.86	4	2.22	< 1
A x B x S	74.21	28	2.65	

*** p < .001; ** p < .01; * p < .05.

Table 15

ANOVA on latency righting.
Pretreatment: nimodipine 0.63 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	4137.93	4	1034.48	1.70
S	9782.69	7	1397.53	
A x S	17000.88	28	607.17	
B (ses.)	2964.61	1	2964.61	5.14
B x S	4037.89	7	576.84	
A x B	1343.58	4	335.89	< 1
A x B x S	9848.43	28	351.73	

*** p < .001; ** p < .01; * p < .05.

Table 16

ANOVA on number of avoidances.
Pretreatment: nifedipine 0.16 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	569.86	4	142.47	6.57 ***
S	1452.01	9	161.33	
A x S	782.14	36	21.73	
B (ses.)	13.69	1	13.69	< 1
B x S	191.21	9	21.25	
A x B	59.26	4	14.82	2.06
A x B x S	258.34	36	7.18	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 17

ANOVA on mean escape time.
Pretreatment: nimodipine 0.16 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	0.47	4	0.12	3.17 *
S	1.59	9	0.18	
A x S	1.33	36	0.04	
B (ses.)	0.01	1	0.01	< 1
B x S	0.41	9	0.05	
A x B	0.27	4	0.07	2.36
A x B x S	1.02	36	0.03	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 18

ANOVA on latency righting.
Pretreatment: nimodipine 0.16 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	2762.66	4	690.67	1.36
S	11284.01	9	1253.78	
A x S	18317.14	36	508.81	
B (ses.)	5882.89	1	5882.89	7.66 *
B x S	6914.41	9	768.27	
A x B	998.86	4	249.72	< 1
A x B x S	19571.34	36	543.65	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 19

ANOVA on number of avoidances.
Pretreatment: nifedipine 0.63 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	1147.26	4	286.82	12.30 ***
S	1745.41	9	193.93	
A x S	839.34	36	23.32	
B (ses.)	16.81	1	16.81	1.79
B x S	84.29	9	9.37	
A x B	70.54	4	17.64	2.07
A x B x S	306.86	36	8.52	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 20

ANOVA on mean escape time.
Pretreatment: nimodipine 0.63 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	14.81	4	3.70	5.47 **
S	15.80	9	1.76	
A x S	24.34	36	0.68	
B (ses.)	0.52	1	0.52	1.19
B x S	3.93	9	0.44	
A x B	0.97	4	0.24	< 1
A x B x S	13.26	36	0.37	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 21

ANOVA on latency righting.
Pretreatment: nimodipine 0.63 mg/kg p.o. -1 h.

Source	SS	df	MS	F
A (days)	9415.04	4	2353.76	3.13 *
S	17036.44	9	1892.94	
A x S	27041.96	36	751.17	
B (ses.)	1049.76	1	1049.76	1.70
B x S	5555.84	9	617.32	
A x B	5824.24	4	1456.06	2.10
A x B x S	25003.16	36	694.53	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 22

ANOVA on number of avoidances.
Pretreatment: verapamil 0.63 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	270.20	4	67.55	2.47
S	1516.20	9	168.47	
A x S	984.60	36	27.35	
B (ses.)	0	1	0	< 1
B x S	36.80	9	4.09	
A x B	26.40	4	6.60	1.84
A x B x S	128.80	36	3.58	

*** p < .001; ** p < .01; * p < .05.

Table 23

ANOVA on mean escape time.
Pretreatment: verapamil 0.63 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	0.75	4	0.19	< 1
S	12.28	9	1.36	
A x S	8.55	36	0.24	
B (ses.)	1.04	1	1.04	9.45 *
B x S	0.97	9	0.11	
A x B	0.03	4	0.01	< 1
A x B x S	3.22	36	0.09	

*** p < .001; ** p < .01; * p < .05.

Table 24

ANOVA on latency righting.
Pretreatment: verapamil 0.63 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	13112.70	4	3278.18	3.44 *
S	16387.25	9	1820.81	
A x S	34334.30	36	953.73	
B (ses.)	1376.41	1	1376.41	2.22
B x S	5578.69	9	619.85	
A x B	476.34	4	119.09	< 1
A x B x S	16725.06	36	464.59	

*** p < .001; ** p < .01; * p < .05.

Table 25

ANOVA on number of avoidances.
Pretreatment: verapamil 2.5 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	170.04	4	42.51	2.00
S	1390.49	8	173.81	
A x S	680.96	32	21.28	
B (ses.)	0.28	1	0.28	< 1
B x S	15.82	8	1.98	
A x B	38.67	4	9.67	2.14
A x B x S	144.73	32	4.52	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 26

ANOVA on mean escape time.
Pretreatment: verapamil 2.5 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	3.72	4	0.93	1.90
S	25.69	8	3.21	
A x S	15.63	32	0.49	
B (ses.)	2.47	1	2.47	5.26
B x S	3.77	8	0.47	
A x B	2.28	4	0.57	1.16
A x B x S	15.56	32	0.49	

*** $p < .001$; ** $p < .01$; * $p < .05$.

Table 27

ANOVA on latency righting.
Pretreatment: verapamil 2.5 mg/kg s.c. -1 h.

Source	SS	df	MS	F
A (days)	4380.89	4	1095.22	1.19
S	15978.00	8	1997.25	
A x S	29545.11	32	923.28	
B (ses.)	205.51	1	205.51	0.34
B x S	4844.89	8	605.61	
A x B	5758.04	4	1439.51	2.41
A x B x S	19077.56	32	596.17	

** $p < .001$; ** $p < .01$; * $p < .05$.

Chapter 3
Intracranial Self-Stimulation Behavior after Hypoxia

3.1. Introduction

The discovery of the brain self-stimulation phenomenon by Olds and Milner (1954) has generated a great amount of experimental work (survey in Wauquier and Rolls, 1976). Most of the research was directed at unraveling the neurophysiological bases for reward and motivation. Mapping of the brain sites where self-stimulation could be induced, indicated that noradrenergic and dopaminergic systems were involved. As a consequence many studies were directed towards the neurochemical bases of brain self-stimulation. A wide variety of pharmacological studies with centrally active drugs were undertaken to define the neurochemical bases of reward (review see Wauquier, 1979).

Probably due to the specific orientation which the field has taken, only a few studies investigated the influence of hypoxia on brain self-stimulation behavior (Annau and Weinstein, 1967 & 1968; Weinstein and Annau, 1968 & 1969; Koob *et al.*, 1970). In these experiments self-stimulation in the rat was studied during relatively mild normobaric hypoxia (8-11 % oxygen). It was found that short hypoxic periods (16 minutes) lead to a decrease of the rate of self-stimulation. After repeated exposure to hypoxia, adaptation was observed. Despite the adaptation, the animals never reached the prehypoxic level of lever pressing. The number of lever presses increased during the hypoxic sessions. No changes were observed in arterial, pH, PCO_2, PO_2, O_2 saturation and hematocrit during behavioral adaptation. Therefore these improvements were not related to respiratory or acid-base alterations. In contrast to what was seen during short exposure, prolonged exposure (60 hours) to 10 % oxygen produced an increase of self-stimulation and an almost complete suppression of eating and drinking behavior. The authors (Koob *et al.*, 1970) discussed their results in relation to adaptation to high-altitudes. However, reduced barometric pressure is an essential element of high-altitude exposure which was lacking in their experimental model.

It is clear from the results of the studies mentioned above that hypoxia affects self-stimulation performance. Unfortunately this line of research has never been fully developed. No studies could be found in the literature in which post-hypoxic self-stimulation was assessed. To the best of our knowledge no attempts have been made to antagonize hypoxia-induced effects on self-stimulation by pharmacological treatment. Intracranial self-stimulation is a reliable behavioral phenomenon which can easily be quantified. Therefore it seemed a potentially useful method for research on neuropharmacological brain protection against hypoxia. In the present chapter we will investigate the post-hypoxic (normobaric) effects on brain self-stimulation.

3.2. Materials and methods

3.2.1. Animals and housing conditions

Adult male Wistar rats from the Janssen breeding colony, with a body weight of 250 ± 10 g were used. They were kept in individual cages under standard laboratory conditions (12 h light - 12 h dark) and had free access to food and water. During experimental sessions in the self-stimulation cage no food or water was available.

3.2.2. Surgery

All animals were anesthetized with 0.5 ml Thalamonal® (droperidol 2.5 mg and fentanyl citrate 0.0785 mg per ml) injected s.c. and their head was positioned in a David Kopf stereotaxic apparatus.
Twisted bipolar electrodes (0.254 mm ∅ nichrome wires), insulated with ISONEL 31/BA 689 (Schenectady de France, Béthune) except at the tip, were implanted in the lateral hypothalamic region of the brain according to the coordinates (anterior 4.6 mm; lateral 1.5 mm, ventral 3 mm) from the stereotaxic atlas of De Groot (1963). Arbitrarily, these intracranial electrodes were referred to as "1 and 2". A jeweller's screw was placed on the frontal bone and served as an indifferent electrode. Two other screws were placed just anterior to the lambda and served as anchor points for the dental cement that fixed the electrode to the skull of the rat. The animals were allowed one week of recovery before the shaping procedure began.

3.2.3. Self-stimulation cage and equipment

The experimental cage (20 x 25 x 33 cm) was a PVC (polyvinylchloride) box with a 6 x 3 cm stainless steel lever mounted on the back wall. The lever was placed 6 cm above a stainless steel grid floor. The front wall of the cage was transparent so that direct observation of the animal was possible. A mercury contact swivel was mounted on top of the cage and served to guide the electrode leads to the stimulator (JSI, Janssen Scientific Instruments). In illustration 3.1. a rat can be seen in the set-up during self-stimulation.

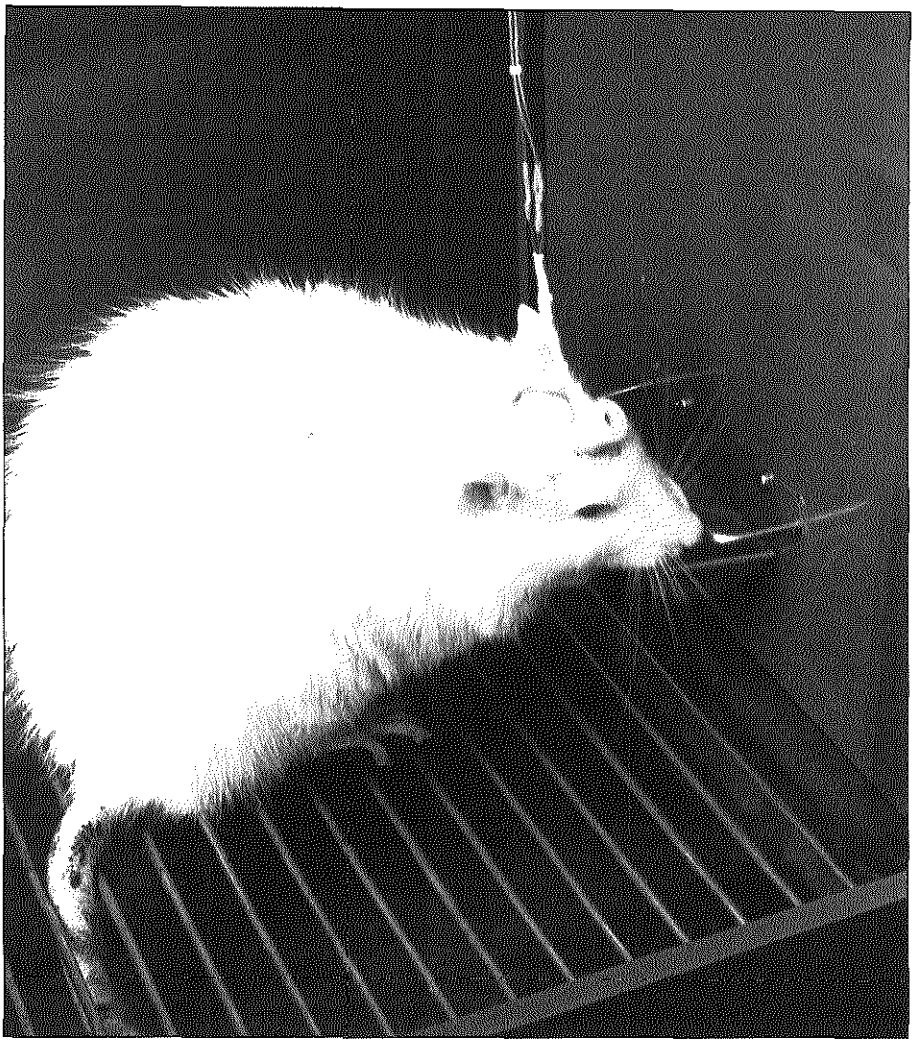

Illustration 3.1. Electrode-implanted Wistar rat during self-stimulation.

Pressing of the lever resulted in brain stimulation. The stimulator generated trains of biphasic rectangular pulses of which intensity, frequency, pulse width and train duration could be adjusted. Brain stimulation was given on fixed ratio schedules 1:1 or 2:1. Thus either each single lever press (1:1) or every second lever press (2:1) was rewarded with a stimulus. Pressing the lever during a stimulation train, however, was not rewarded with a new stimulus.
The number of responses per minute was printed by an Epson printer.

3.2.4. Shaping procedure and choice of the stimulation electrode

For all self-stimulation experiments monopolar stimulation was used. During monopolar stimulation the current was applied between one of the electrodes implanted in the brain and the "indifferent" electrode positioned on the frontal bone.

The shaping procedure was started one week after surgery. During the second week the rats were put in the experimental cage for 30 minutes per day. A session was started by giving an electrical stimulus. After that, the animals could press the lever and received stimuli on a 1:1 reinforcement schedule. In Figure 3.1. the parameters of the stimulus are indicated. Each pulse was alternately positive and negative. Pulse series of 500 msec were given (pulse frequency 80 Hz, pulse intensity 200 μA, pulse duration 4 msec). On alternate days monopolar stimulation was given through electrode "1 or 2".

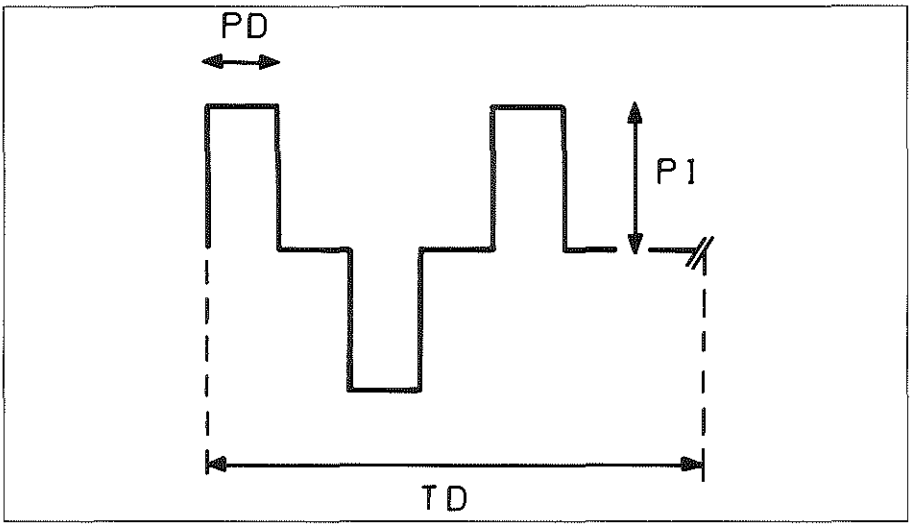

Figure 3.1. Biphasic rectangular pulses: PD: pulse duration = 4 msec; PI: pulse intensity = 200 μA, TD: train duration = 500 msec.

Since the animals entered a new environment during the first days of this shaping week, they showed typical exploratory behavior in the cage. A rat either reacted positively or negatively on the priming stimulus (delivered by the investigator to signal the start of a session). A positive reaction consisted of approach behavior which was characterized by sniffing and intense exploration of the surroundings. During this exploration the rats pressed the lever and discovered that it elicited brain-stimulation. This event then reinforced the lever-pressing behavior. The number of lever pressings then quickly increased during a session. A negative reaction consisted of escape and jumping after stimulation. If such reaction was seen on both electrodes the animal was not used in the experiments.

On alternate days monopolar stimulation was given through either electrode 1 or 2. At the end of the week the best stimulation electrode was chosen for further experiments. In all cases this was the electrode which elicited the greatest behavioral response with minimal side-effects (escape, convulsions, etc.).

3.2.5. Training procedure

After shaping the rats were given daily self-stimulation sessions of twenty-five minutes for two weeks. Training was given on a fixed ratio reinforcement schedule of 2:1. The start of each training session was signaled by a priming stimulus. The stimulus parameters were the same as during the shaping procedure, except for the pulse frequency, which was lowered to 60 Hz. At the end of these two weeks the rats had reached a stable performance level.

3.2.6. Experimental procedure

Nine rats, trained as previously described, were used to investigate the effects of normobaric hypoxia on subsequent self-stimulation behavior. For three weeks the animals were given daily 25-minute self-stimulation sessions (fixed ratio schedule 2:1). The stimulus parameters used were the same as those used during the previous two weeks.

In each of the three weeks, the self-stimulation sessions were preceded by the exposure to a 15-min normobaric hypoxia on Tuesday and Thursday. Normobaric hypoxia was induced by exposing the animals for 15 minutes to an O_2/N_2 gas mixture containing 4.3 $\pm$ 0.1 % oxygen. The rat was placed in a perspex cylinder (∅ 30 cm, height 15 cm), which was continuously flushed with the gas mixture. The composition of the air in the cylinder was permanently monitored and analysed by a Sybron/Taylor Servomex O_2 analyzer OA570. Hypoxia was not given on Monday, Wednesday and Friday (control sessions). At the end of the exposure to hypoxia, the animals were immediately transferred to the self-stimulation cage and self-stimulation was started with a priming stimulus. The time lag between the end of the hypoxia and the priming stimulus was approximately one minute. During the weekends the animals were left in their home cage. For each animal the number of responses (lever pressings) per minute was registered and the mean number of responses per minute was calculated for the whole 25-minute session.

3.2.7. Histological localization of electrodes

A complete overview of the experiment including shaping, training and experimental procedure is given in Figure 3.2.

At the end of the three-week experimental period the animals were sacrificed for histological localization of the electrodes. The rats were anesthetized with ether and injected intravenously with heparin (0.15 ml; 5000 u/ml). The torax was opened and the brain was fixed by intracardiac perfusion with Karnovsky's fixative (2 % formaldehyde and 2.5 % glutaraldehyde in phosphate buffer 0.1 ml/l, pH 7.4) during five minutes at ambient temperature. After decapitation the brain was left overnight in the skull and immersed in the same fixative. Subsequently the brain was removed and a transverse slice which contained the tract of the electrode was made. From this slice 100 μm vibratome sections were cut until a section through the tip of the electrode was obtained. This section was subsequently stained with azur-eosin (pH 4.5) (see Illustration 3.2.).

All electrodes were positioned in the lateral hypothalamic region of the brain.

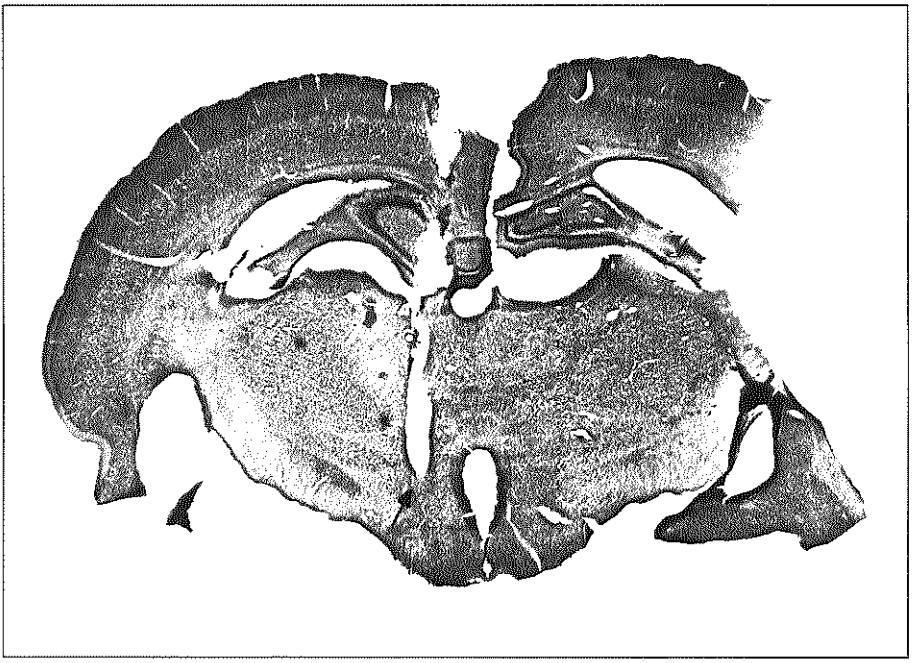

Illustration 3.2. Azur-eosin stained (pH 4.5) 100 μm vibratome section of a rat brain through the tip of the electrode (arrow). Electrode is positioned in the dorsal part of the lateral hypothalamic region beneath the zona incerta.

Weeks	1	2	3 4	5 6 7
	Surgery recovery	Shaping	Training procedure	Experimental Procedure
Stimulation	No	Fr 1:1 TD 500 msec PF 80 Hz PI 200 μA PD 4 msec	Fr 2:1 TD 500 msec PF 60 Hz PI 200 μA PD 4 msec	Fr 2:1 TD 500 msec PF 60 Hz PI 200 μA PD 4 msec
Electrode	—	1-2-1-2 on subsequent days Friday choice 1 or 2	Fixed choice 1 or 2	Fixed choice 1 or 2
Hypoxia	No	No	No	15 min in 4.3 % O_2 before self-stimulation on each Tuesday and Thursday
Session		Daily 30 min	Daily 25 min	Daily 25 min

Figure 3.2. Overview of the experimental design indicating duration of each phase, stimulation characteristics, electrode used, exposure to hypoxia and self-stimulation session duration.
FR: fixed ratio; TD: train duration; PF: pulse frequency; PI: pulse intensity; PD: pulse duration.

3.3. Results

Figure 3.3. depicts the mean (± S.E.M.) number of responses per minute obtained during the 25-minute self-stimulation sessions on control and hypoxia days in the course of the three experimental weeks. General analysis of variance (repeated measures) on all control and hypoxia data revealed no significant general differences between the respective nine control days [F(8/64) = 1.01, n.s.] or between the six hypoxia days [F(5/40) = 2.06, n.s.]. Consequently no systematic trends were found over the whole experimental period for both treatment conditions.

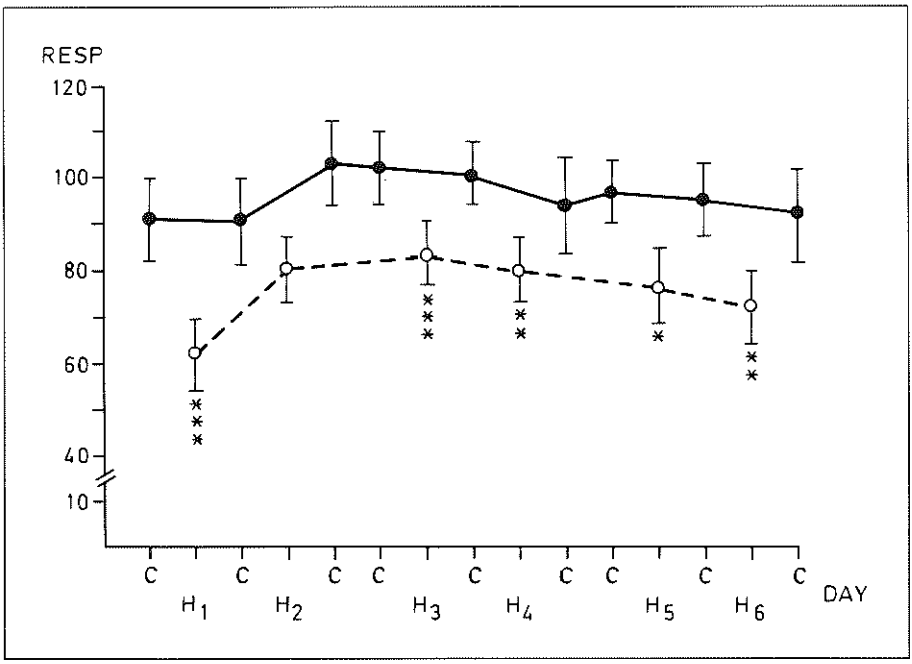

Figure 3.3. Mean (± S.E.M.) response rate per minute (n = 9) during the 25-minute session for consecutive control (C) and hypoxia (H) days in a three-week experiment. Significance of differences calculated against the preceding control day.
* $p < .05$, ** $p < .01$, *** $p < .001$, ●—● control, ●----● post-hypoxia.

Comparisons (one-way analysis of variance with repeated measures) were made between each hypoxia day and the preceding control day. With the exception of the second exposure [F(1/8) = 2.37, n.s.] the mean number of responses had significantly decreased after hypoxia.

This suggests that a considerable adaptation had occurred during the second exposure. The adaptation further seemed to have reached a maximum level, since no systematic change was found in the general analysis of all hypoxia data. This was confirmed by the comparisons between each pair of consecutive hypoxia days (Table 3.1.). A significant increase in mean response rate was found between the first two hypoxia days. From the second exposure on, no further increases were seen and the differences between consecutive hypoxia days were not significant. This indicates that the adaptation had reached a plateau after the second exposure.

Table 3.1.
Mean response rate per minute (over 25 minutes) after the six hypoxia exposures and comparisons between pairs of consecutive hypoxia days.

Hypoxia day	Mean resp./min (± S.E.M.)	Comparison between H days df (1/8)
1	62.51 (± 8.02)	
2	80.83 (± 6.94)	(1-2) F = 11.79 p < .01
3	83.78 (± 7.20)	(2-3) F = 1.16 n.s.
4	80.28 (± 7.11)	(3-4) F = < 1 n.s.
5	76.04 (± 8.13)	(4-5) F = < 1 n.s.
6	72.08 (± 8.09)	(5-6) F = 1.76 n.s.

So far the mean response rate had been determined over the whole 25-minute period. In Figure 3.4. the average response rate was plotted for each minute of the 25-minute session separately. This was done for the first control day and the first three hypoxia days. As can be seen, the response rate was highly reduced in the first minutes of the session and gradually increased to the normal level. It is also evident that the response rate was normalized more rapidly after the second and third exposure. This suggests that the relatively low level of lever pressing after hypoxia shown in Figure 3.3. was due to reduced response rates at the beginning of the session.

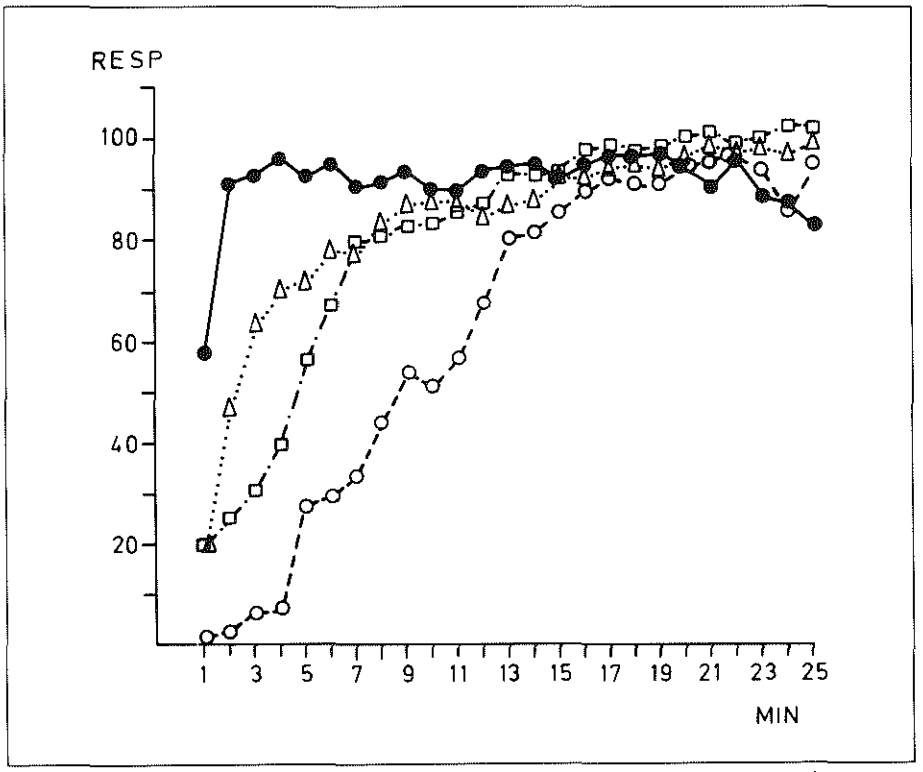

Figure 3.4. Mean response rate (n = 9) during each minute of the self-stimulation session during the first control day and the first three hypoxia days.
●——● control, ●- - -● first hypoxia, □—□ second hypoxia, △- - -△ third hypoxia.

To evaluate this in more detail, mean response rates were calculated for successive 5-minute periods of each session on hypoxia and control days.

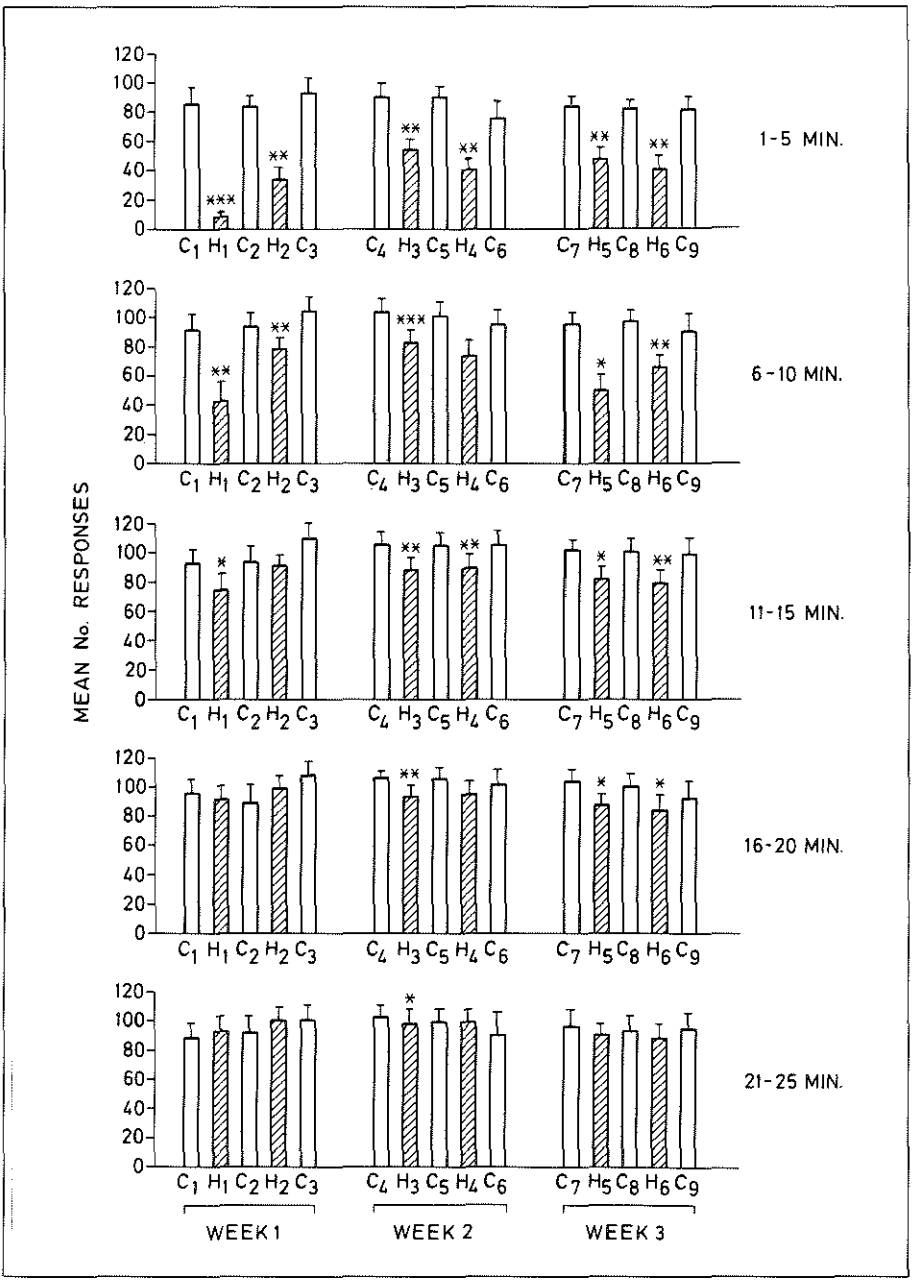

Figure 3.5.
Mean (± S.E.M.) response rate during successive 5-minute periods of the session on control (C, open bars) and hypoxia days (H, hatched bars). Significance of differences between hypoxia and the preceding control day indicated above bars.
* $p < .05$, ** $p < .01$, *** $p < .001$.

As can be seen in Figure 3.5. the mean response rate during the consecutive 5-minute periods of a session remained stable on control days. On all hypoxia days the mean response rate was significantly lower during the first 5-minute period as compared with the response rate during that same period of the session on the preceding control day. Significant differences were observed in the second (6-10 min) and the third (11-15 min) part of the sessions, but it was clear that the effects were less pronounced and became inconsistent. Significant differences during the last parts of the session were only seen on the third hypoxia day. Figure 3.5. also demonstrates that the increase in mean response rate over the whole session between the first two hypoxia days is due to an increase of the response rate during the early parts of the session.

3.4. Conclusions

Brain self-stimulation immediately after a period of normobaric hypoxia was clearly attenuated. However, the observed effects were transient and occurred mainly during the first minutes. Response rates per minute were highly reduced during the beginning of self-stimulation, but then gradually returned to normal levels. This means that the reduction of the mean response rate over the whole session was not due to a prolonged suppression of responding.

The five-minute period analysis showed that adaptation takes place after the first exposure. The effect was most pronounced during the first five minutes of self-stimulation. In addition the adaptation did not increase systematically after the second exposure. This indicates that a maximal adaptation level was already reached after the second exposure. The five-minute period analysis also proved to be a more suitable method for the interpretation of the effects than an analysis based on general response rates calculated over the whole 25-minute sessions. Using the five-minute period analysis it became clear that the response rate was only significantly reduced during the first five minutes of the self-stimulation on all hypoxia days. The effect was not abolished by the observed adaptation during the second and following exposures. This means that normobaric hypoxia had stable and reproducible effects on brain self-stimulation as far as the first five post-hypoxia minutes are concerned.

In general the results suggest that the effects of hypoxia on self-stimulation behavior were short-lasting and not very pronounced. Direct brain stimulation in the lateral hypothalamic area of the rat brain also activates brain stem and thalamic neurones. Arousal and locomotor activity are induced through this neuronal system (Rolls, 1972). Therefore the priming stimulus given to signal the onset of the session probably acted as an antagonist for the suppression induced by hypoxia.

No direct evidence was available from this experiment to identify the nature of the adaptation seen after multiple exposures to hypoxia. However, the fact that it already occurs at a plateau level after the second exposure suggests that it was probably not mediated by slow physiological adaptive mechanisms. A possible explanation might be that the animals had a slightly altered perception of the stimulus complex in the experimental cage after exposure to hypoxia.

Entering the cage after the first exposure is then equivalent to entering a partly new environment and, consequently, habituation might play a role after subsequent exposures. Indeed, the main difference between the first and subsequent hypoxia days was the fact that the delay between the priming stimulus and the onset of lever pressing seen after the first exposure had disappeared from the second exposure on. An empirical test for this hypothesis would be to expose animals first to hypoxia which is not followed by self-stimulation. Then the novelty effect should be seen on self-stimulation after the second exposure if the hypothesis is correct.

Although the effects of hypoxia on subsequent self-stimulation behavior become fairly stable after the second exposure, one might question the utility of the present paradigm for pharmacological research purposes. The hypoxic effects were very short lasting and the spontaneous recovery was rapid, owing to the intrinsic antagonistic properties of the type of reinforcer. Therefore the chance of positively influencing this process through drug treatment is very small. As it is, the paradigm seems to be more suitable to study enhancement of hypoxic effects after drug treatment. Apart from these basic objections the method is not very economical since a long exposure time (15 minutes) is needed to induce minor effects. In addition, possible positive drug effects can be confounded with direct effects of the drug on the operant behavior. This means that each dose of a drug needs to be examined for facilitation of lever pressing in a session that was not preceded by exposure to hypoxia. In conclusion, this paradigm is not recommended to study protective drug effects against hypoxia-induced behavioral deficits.

Chapter 4
Intracranial Self-Stimulation Behavior during Hypoxia

4.1. Introduction

The main focus so far has been on the post-hypoxic period and the quality of the behavioral functions during that period. Of equal importance are the conditions in which preservation of behavioral functions during hypoxia is essential for survival or the maintenance of the quality of daily life. People suffering from vascular diseases, with migraine, atherosclerosis etc., live more or less in a chronic hypoxic condition which can cause a discomfort to themselves and others. People who perform under extreme conditions in a hazardous environment, like mountaineers, experience life-threatening risk when they suffer from the early symptoms of hypobaric hypoxia. Deterioration of psychomotor performance can already occur at an altitude of 2500 m (Denison *et al.*, 1966). Far more dangerous are symptoms like euphoria, lack of judgement, loss of short term memory and mental incoordination which are seen in the early stages of hypoxia. Although problems with cabin pressurization in aircraft are not so frequent, relatively small deviations can induce similar symptoms in crew members and passengers especially in those who are more susceptible due to chronic diseases. In chronic hypoxic conditions and some of the other examples given above drugs might be given as a prophylactic measure to preserve normal functioning.

Models which measure animals' performance under reduced oxygen supply might therefore be very useful in defining the anti-hypoxic effect of drugs. Hypobaric hypoxia was chosen as a method to induce hypoxia because it is equivalent to the conditions encountered in mountaineering and aviation medicine. In addition, avoidance learning (pole jumping) has already been studied during hypobaric hypoxia (Boismare *et al.*, 1980, 1981; Saligaut *et al.*, 1981a, 1982). The latter research determined the effects of hypoxia on behavior which was not yet established and had to be learned under extreme conditions. We decided to use a well-established routine behavior. Intracranial self-stimulation was chosen because it can be attenuated during normobaric hypoxia (Annau and Weinstein, 1967, 1968; Weinstein and Annua, 1968, 1969; Koob *et al.*, 1970).

4.2. Self-stimulation behavior at different levels of atmospheric pressure

4.2.1. Materials and methods

4.2.1.1. Animals and housing conditions

Six male Wistar rats (250 ± 10 g) stereotaxically implanted with twisted bipolar electrodes in the lateral hypothalamic region of the brain (see Chapter 3) were used. They were kept in individual cages under standard laboratory conditions (12 h light - 12 h dark) and had *ad libitum* access to food and water except for the self-stimulation sessions.

4.2.1.2. Self-stimulation cage and equipment

The self-stimulation cage was a transparent PVC cylinder with a 6 x 3 cm stainless steel lever mounted on a built-in wall which protected the leads against damage of the rat. The lever was placed 1 cm above the stainless steel grid floor in the cylinder. This was done to allow the animal to press the lever with a minimal physical effort.

A contact swivel was mounted on a stainless steel bar close to the top of the cylinder. The leads for self-stimulation were guided along the bar through the wall to a stimulator (JSI, Janssen Scientific Instruments). The roof of the cylinder was removable and gave access to the cylinder.

The atmospheric pressure inside the cylinder could be lowered by evacuation with an electrical pump. At a desired pressure a vacuum-valve controlled the reflow into the cylinder to keep the pressure stable.
The pressure in the cylinder was continuously monitored by a digital barometer. Stimulus delivery and recording of the number of lever pressings was controlled by a microprocessor (JSI). The complete set-up is shown in Illustration 4.1.

4.2.1.3. Training and experimental procedure

The shaping procedure and choice of the stimulation electrode were identical to those described in Chapter 3. Subsequently the animals were given the opportunity to adapt to the new cage. They were trained during one week in a self-stimulation procedure which was also used as experimental procedure.

Each animal was put daily in the self-stimulation cage for 34 minutes.
The self-stimulation period was divided in three consecutive sessions of 10 minutes in which the animals could obtain brain stimulation by pressing the lever. Stimulation was given on a fixed ratio schedule 1:1. The stimulus had the following characteristics: pulse train duration 500 msec, pulse duration 4 msec, pulse intensity 200 μA and pulse frequency 60 Hz. The 10-minute periods when the stimulus was available were separated by 2 minutes of time out. During these two minutes no stimulus was available.

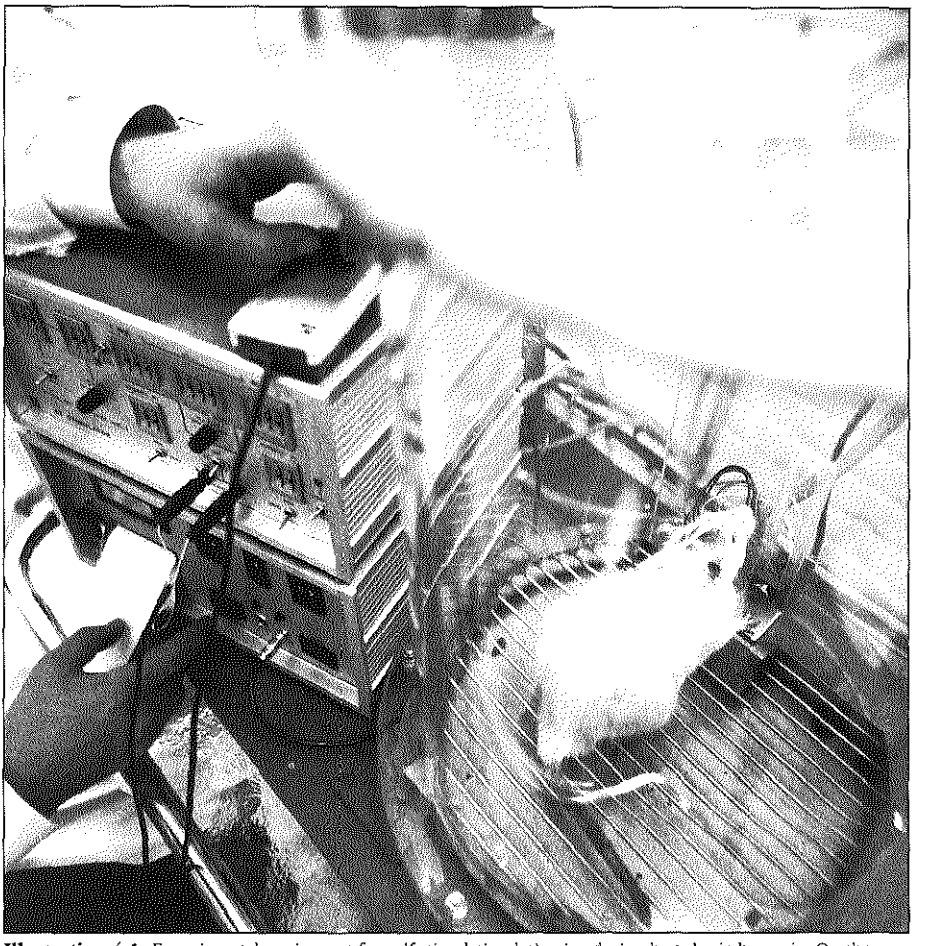

Illustration 4.1. Experimental equipment for self-stimulation behavior during hypobaric hypoxia. On the left, microprocessor for data registration with ST stimulator on top. On the right, rat in the self-stimulation cage.

The experimental procedure was identical to the training procedure with the exception that the second session of each period was held under hypobaric conditions. The atmospheric pressure in the cage was lowered during the two minutes time out between session 1 and 2 and recompression was accomplished during the two minutes between session 2 and 3. Training and experimental procedure are summarized in Table 4.1.

Table 4.1.
Atmospheric pressure during training and experimental procedure.

Daily ICS	Duration	Stimulation	Training pressure	Experimental pressure
Session 1 (S1)	10 min	ON	760 mm.Hg	760 mm.Hg
Time out	2 min	OFF	pump on	decompression
Session 2 (S2)	10 min	ON	760 mm.Hg	600-200 mm.Hg
Time out	2 min	OFF	pump off	recompression
Session 3 (S3)	10 min	ON	760 mm.Hg	760 mm.Hg

The animals were exposed to the experimental procedure for two consecutive weeks. In the first week, the atmospheric pressure during session 2 was decreased to 600 mm.Hg on Monday and then lowered with steps of 100 mm.Hg on subsequent days until 200 mm.Hg on Friday. In the second week the atmospheric pressure was decreased to 400 mm.Hg on Monday and then lowered with steps of 50 mm.Hg on subsequent days until 200 mm.Hg on Friday.

To measure performance under hypobaric hypoxia, the total number of responses during session 2 (hypobaric hypoxia) were expressed as a percentage of the total number of lever pressings in session 1. This was done for each animal on each of the experimental days. A similar calculation was made for the total number of lever pressings in session 3. Thus the daily performance of each animal during hypoxia and in the post-hypoxic period was compared with its own control performance in normal atmospheric pressure.

Statistical evaluation of the data was omitted in this pilot experiment since 3 out of 6 animals died during the course of the two experimental weeks.

4.2.2. Results

In the present experiment experimental mortality was high. Two animals died following the first exposure to 200 mm.Hg for ten minutes during the last day of the first experimental week. One animal died after the exposure to 250 mm.Hg during the second week. This high mortality seems to be related to the temporal relation between behavioral performance and exposure to severe hypobaric hypoxia. Implanted rats normally tolerate exposure to 200 mm.Hg very well when they do not perform just before and during hypoxia (see Chapter 5). The animals in the present experiment were more susceptible. This could be due to an increased oxygen demand related to the physical effort before and during exposure to hypobaric hypoxia.

In Figure 4.1. the results of the two experimental weeks are summarized. When all sessions were held in a normal atmospheric pressure (760 mm.Hg as in training procedure), the mean performance during S2 and S3 was slightly higher than during S1. As can be seen the performance during the exposure to hypobaric hypoxia decreased. Substantial and systematic effects were observed at 300 mm.Hg. In this condition the performance dropped to an average of 51.47 % in the six animals during the first week. However, large differences in

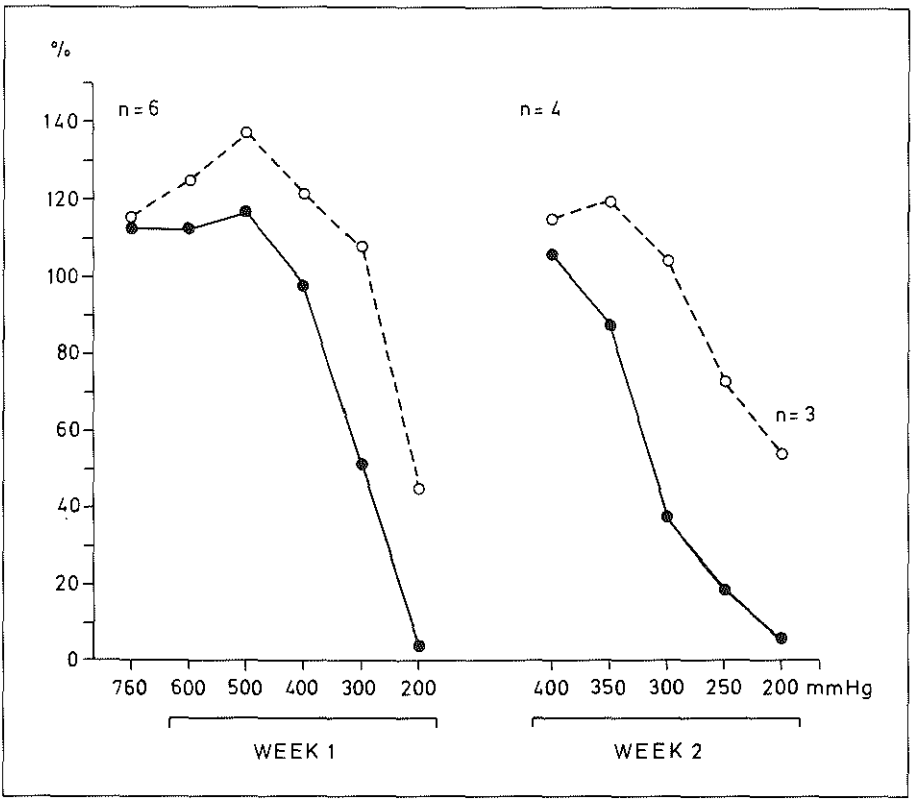

Figure 4.1. Mean % of lever pressings (S1 = 100 %) under different atmospheric pressure (S2 full lines) and during the post-hypoxic session (S3 broken lines) in the two experimental weeks.

sensitivity towards hypoxia were seen. The performance ranged between 96.48 % in the least affected animal and 11.36 % in the most affected animal. At 200 mm.Hg ICS was almost completely blocked. The performance dropped to an average of 4.15 % of the control session. At that atmospheric pressure all animals made some responses by chance. Since the lever was placed only 1 cm above the grid floor, the animals occasionally touched the lever while they were moving around the cage. Although self-stimulation was always initiated, sustained intentional lever pressing was not observed. Two animals did not survive the exposure.

The performance during S3 immediately after the recompression was normal except after the exposure to 200 mm.Hg. Again there was a large variability since two animals did not perform at all and two almost reached S1 performance (98.26 and 81.63 % respectively).

The results obtained during the second week clearly confirmed this picture. Again performance at 300 mm.Hg was seriously depressed (38.64 %) and completely blocked at 200 mm.Hg (6.29 %). At 250 mm.Hg the performance (18.8 %) was between that observed at 300 and 200 mm.Hg, but the general behavioral pattern resembled more that of the animals at 200 mm.Hg.

One animal did not survive the 250 mm.Hg. Performances during S3 were slightly suppressed after hypoxia at 250 mm.Hg and lower. The differential sensitivity of the three rats which completed the experiment is illustrated in Figure 4.2.

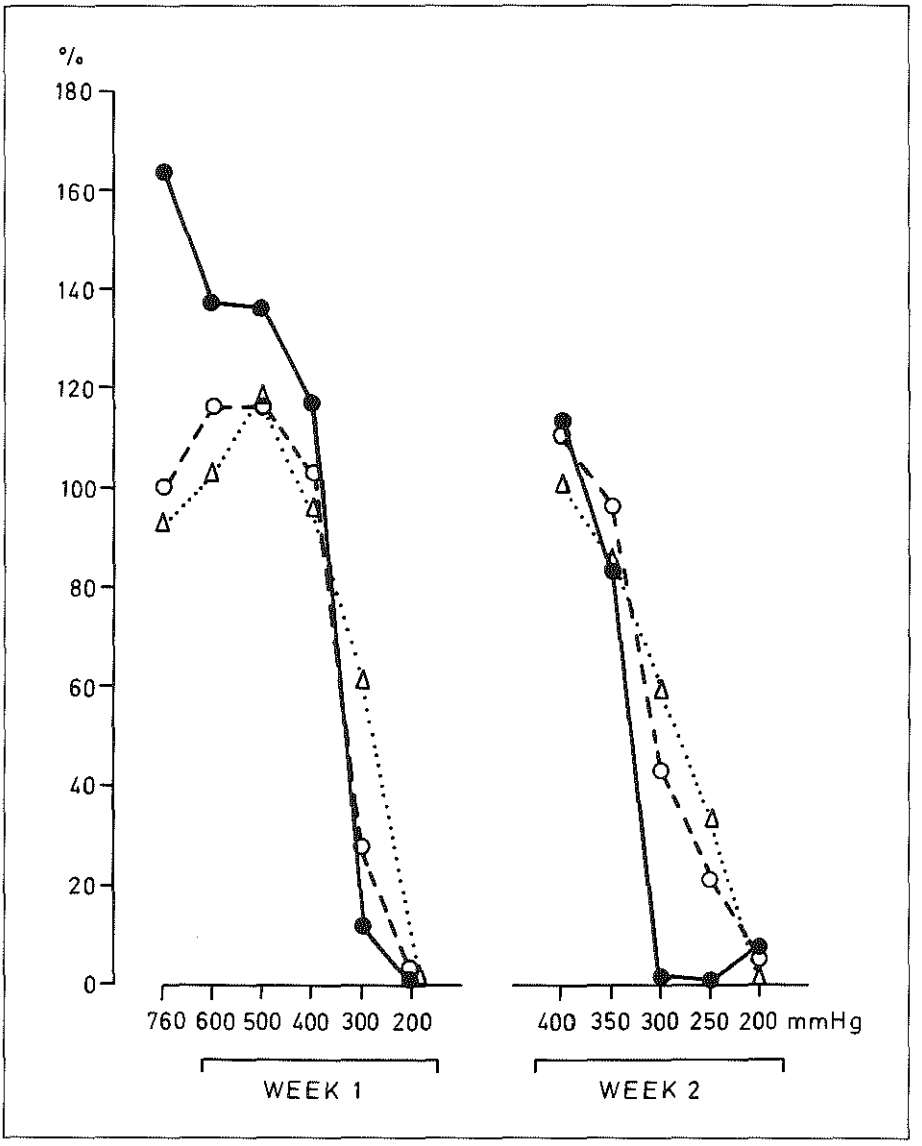

Figure 4.2. % of lever pressings (S1 = 100 %) under different atmospheric pressure. Rat 7674 (full line); rat 7676 (broken line); rat 7679 (dotted line).

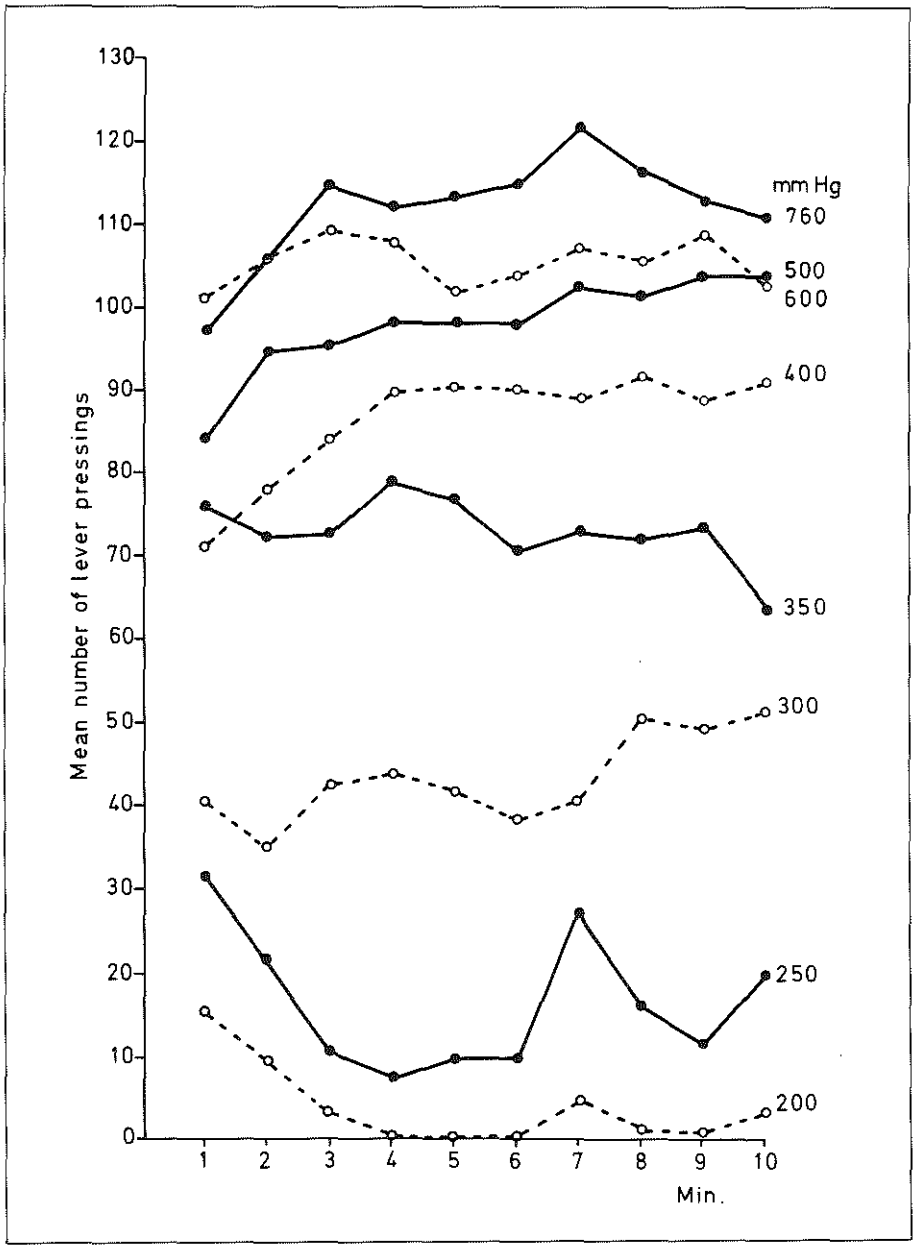

Figure 4.3. Mean response rate during each minute of S2 at different atmospheric pressure.

To investigate whether the decrease in lever pressing under hypobaric conditions was due to a prolonged suppression of responding or to a gradual decrease of performance over time, the mean response rates were calculated for each minute of S2 at different atmospheric pressure. As can be seen in Figure 4.3. lowering the atmospheric pressure caused a general decrease in the mean response rate over the whole session. This means that the initial response rate decreased in proportion to the depth of hypoxia. It was maintained or even slightly increased in the course of the exposure time up to 300 mm.Hg. Beneath that pressure the animals were unable to sustain performance at the initial response rate. At 250 mm.Hg the response rate decreased gradually during the first four minutes. Although it then increased slightly it never again reached the initial value. At 200 mm.Hg the animals started to press the lever at a very low rate but within three minutes intentional lever pressing completely vanished.

The stable mean response rate per minute associated with each level of atmospheric pressure is not an artefact of averaging individual performances. Stable response rates are also seen when the performance of individual animals is plotted separately (see Figure 4.4.).

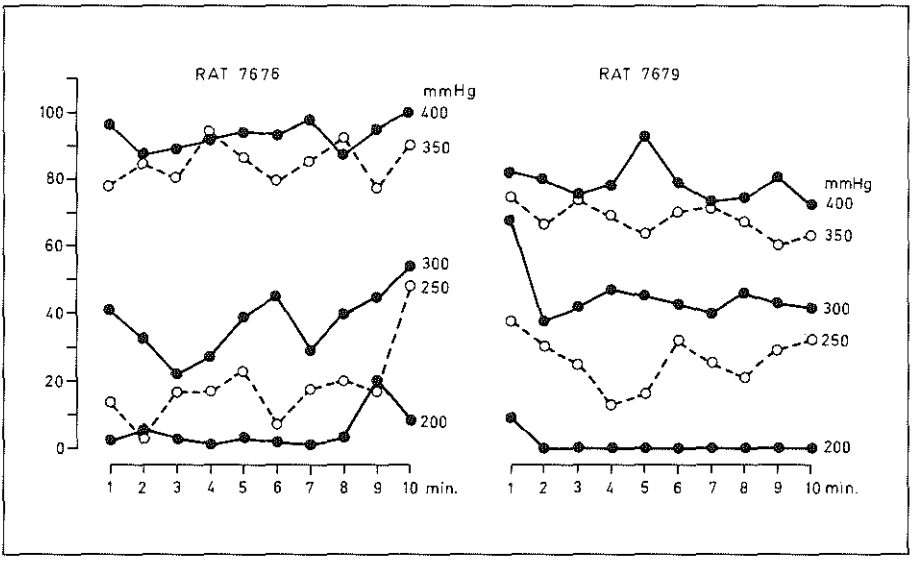

Figure 4.4. Response rate per minute during S2 at different atmospheric pressure in two rats. The data are from the second experimental week.

4.2.3. Conclusions

The results obtained in this pilot experiment indicated that the performance was substantially attenuated at an atmospheric pressure of 300 mm.Hg. In this condition a mean reduction in lever pressing of about 50 % was seen but there were considerable individual differences between animals. Further decrease of the pressure caused a complete disruption of the behavior and mortality. This suggested that the range of atmospheric pressures in which reasonable decreases of performance can be observed is rather small.

The 300 mm.Hg pressure was chosen for two reasons. First, experimental mortality was excessive when hypobaric hypoxia beneath 300 mm.Hg was used daily. Secondly, previous studies on the learning of a conditioned avoidance response in hypobaric conditions were done at 300 mm.Hg. Since dopaminergic agonists were found to be beneficial in this condition (Saligaut *et al.*, 1981a,c, 1982) it might be useful for comparative reasons to apply the same depth of hypobaric hypoxia in a model that uses a different behavioral measure.

4.3. Intracranial self-stimulation during hypoxia after repeated exposures

Behavioral adaptation was already seen after multiple exposure to normobaric hypoxia (see chapter 3). When animals perform repeatedly in a hypobaric environment, adaptation or acclimatization to low PO_2 similar to that seen at high altitudes may influence the performance after a number of exposures. The physiological changes involved in human acclimatization to low PO_2 are: increase in hemoglobin concentration of the blood, increase in pulmonary ventilation and diffusing capacity of the lung, increased vascularity of the tissues and increased ability to utilize oxygen (Guyton, 1981). A direct result of these changes is an increase of the work capacity of cardiac and skeletal muscles so that performance improves at a specific level of atmospheric pressure.

Most of the changes during acclimatization are slow. The hemoglobin concentration in humans increases from 15 g% to 22 g%, but it takes approximately a month before half of the increase is reached. The work capacity of unacclimatized persons increases from 50% (see level = 100%) to 68% after two months of acclimatization at an altitude of 17 000 Ft. These changes occur in humans that are permanently exposed to high altitudes for several days or weeks. However, it is essential with the present paradigm to investigate the influence of repeated relatively short exposure times on the performance of animals in hypobaric conditions. The next experiment was set up to examine this issue in detail.

4.3.1. Materials and methods

Nine male Wistar rats (250 ± 10 g) stereotaxically implanted in the lateral hypothalamic region of the brain were used. Housing conditions, shaping procedure and choice of the stimulation electrode were as previously described.

The training and experimental procedure were the same as in the previous experiment (see table 4.1) with the exception that the atmospheric pressure during session two was always 300 mm.Hg on hypoxia days. Performance during hypobaric hypoxia (session 2) was expressed as a percentage of the performance during session 1 (normobaric condition) on the same day.

The experiment lasted eight weeks. During the first two weeks, the training procedure (S2 at 760 mm.Hg) was used on Monday and the experimental procedure (S2 at 300 mm.Hg) was used on all other days. After these initial weeks the animals were not exposed to hypoxia for three weeks. The animals were left undisturbed in their home cage during week three and four. Retraining was given during the fifth week to familiarize the animals again with the cage and self-stimulation procedure. In the last three weeks of the experiment, the experimental procedure was used from Monday to Friday. The animals were then daily exposed to a hypobaric hypoxia (300 mm.Hg) during self-stimulation in session two.

Blood (0.5 ml) was withdrawn from the tail vein of each animal on five different days in the course of the experiment. This was done immediately after the end of the self-stimulation sessions on respectively day one (No. of exposures = 0) five (No. of exposures = 4), twelve (No. of exposures = 8), thirty-four (No. of exposures = 9) and the last day (No. of exposures = 23) of the experiment. The samples were analysed with an ABL3 acid-base laboratory (Radiometer Copenhagen).

Statistical analysis on the data was done in an analysis of variance repeated measures design. The results from the third daily session will not be presented, since performance during that session was comparable to that in first session on that same day.

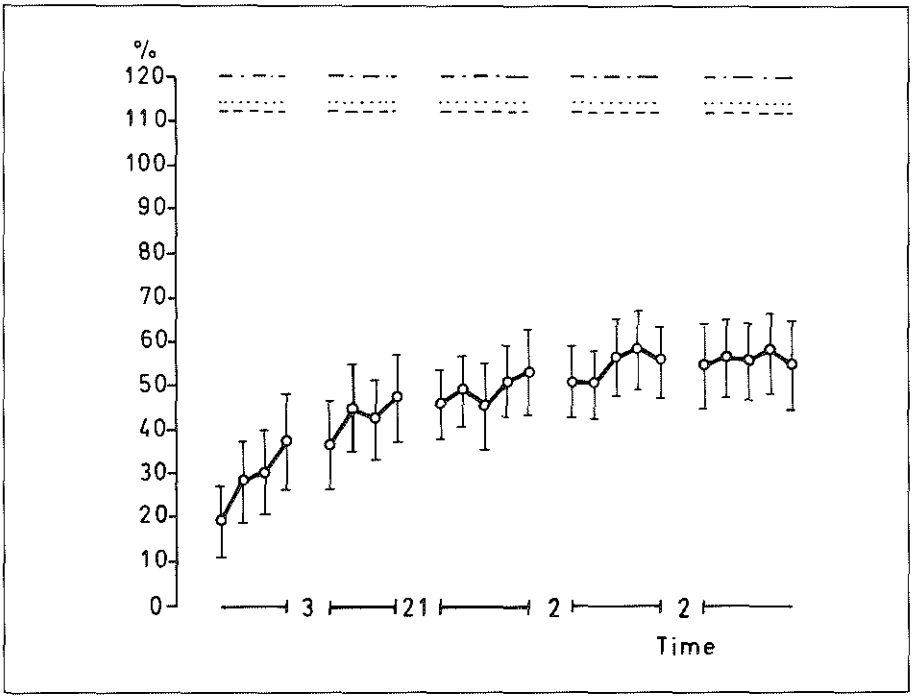

Figure 4.5. Mean (N = 9) % (± S.E.M.) of lever pressings (S1 = 100 %) at 300 mm.Hg during 23 experimental days. Each point in the figure represents a day. The numbers in the time axis represent the number of days that the animals were not exposed to hypobaric hypoxia between the two experimental days. The lines on top of the figure represent the performance during session 2 (760 mm.Hg) on three training days. The performance during hypoxia (S2) was always significantly lower (at least $p < .01$) as compared to the performance during S2 on training days.

4.3.2. Results

Figure 4.5. depicts the mean performance during the consecutive exposures to 300 mm.Hg. On all hypoxia days the performance was significantly lower (at least $p < .01$) during hypoxia (S2) as compared to the performance in normobaric conditions (S2) on training days. A gradual improvement of the performance during hypoxia was seen in the course of the experiment. The statistical analysis on all the hypoxia days indicated a significant increase

[F(22/176) = 4.03, p < .001] over time. This gradual adaptation was not interupted by the 21 days without exposure after the first two experimental weeks. However, comparisons between each of two consecutive exposures to hypoxia revealed only a significant difference [F(1/8) = 8.86, p < .05] between the performance during the fifth and sixth exposure. This suggests that the time course of the adaptation was variable between the animals.

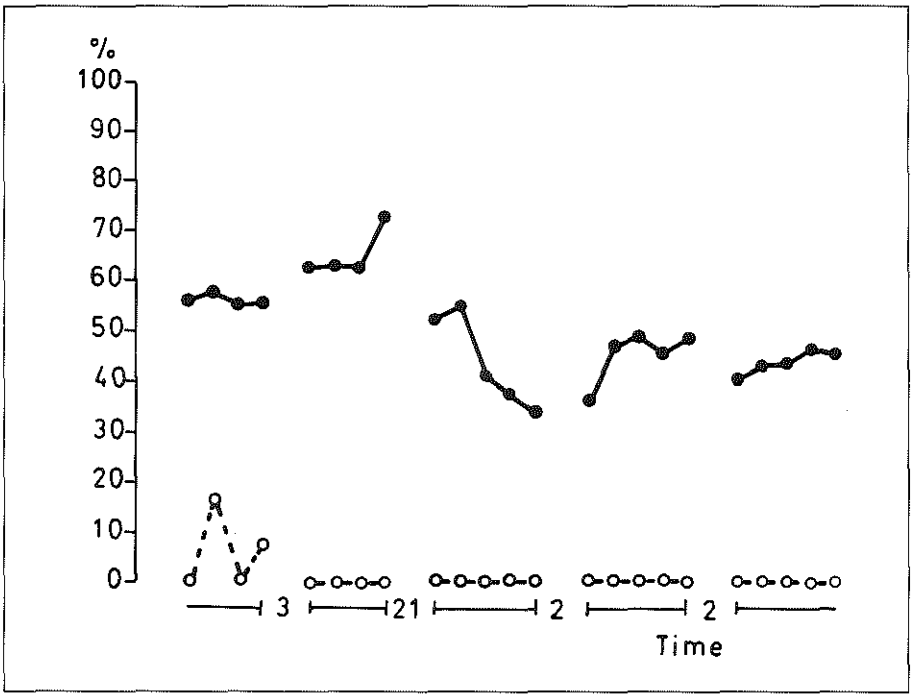

Figure 4.6. % lever pressing at 300 mm.Hg on 23 experimental days of two individual animals (Rat 7706 = broken line, Rat 7710 = full line) with good adaptation.

The differences in adaptation pattern are illustrated in figures 4.6. and 4.7. In figure 4.6. the performances of the two animals with the best adaptation are shown. As can be seen there was a clear difference in adaptation rate to reach a similar level of performance under hypobaric conditions. Adaptation was seen in six of the nine animals but both the adaptation rate and the maximal level reached were variable.

In three animals no signs of adaptation were found. As can be seen in figure 4.7. one animal did not perform at all during hypobaric exposure. Two animals performed at a specific level from the first exposure on but did not improve their performance during the subsequent exposures. In figure 4.7. the performance of one of these animals is shown.

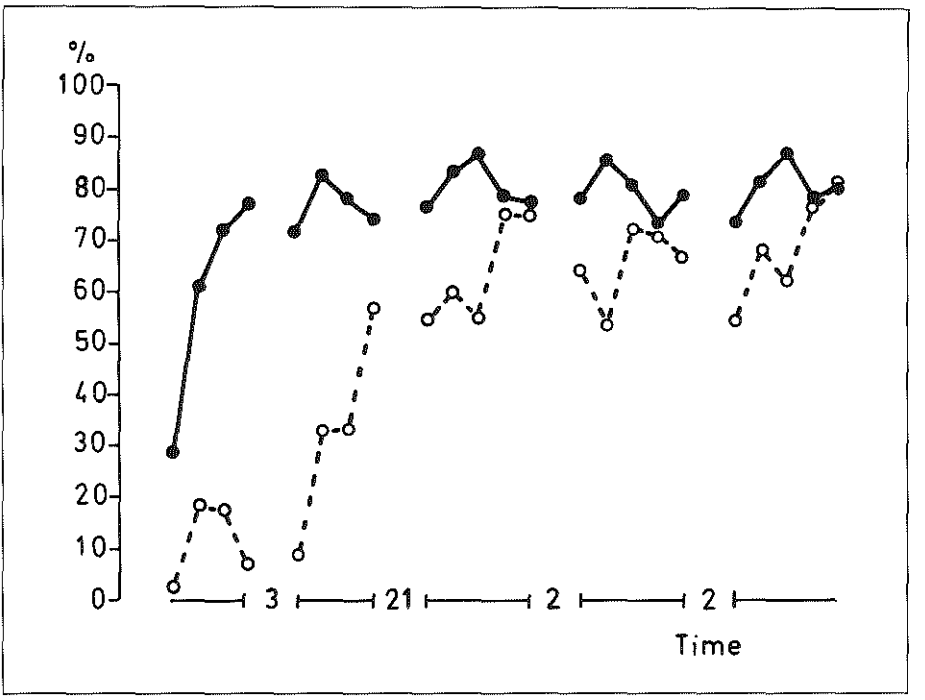

Figure 4.7. % lever pressing at 300 mm.Hg on 23 experimental days of two individual animals (Rat 7705 = full line, Rat 7711 = broken line) which did not adapt to the hypobaric exposure.

In table 4.2. the measured parameters (Hb and pH) in the different blood samples which were taken during the experiment are summarized.
No significant changes in hemoglobin (Hb) concentration or pH of the blood were found. All measured Hb concentrations including those from the animals with a good adaptation fell within the normal distribution of Hb measured in naive Wistar rats (Lewi and Marsboom, 1981).

Table 4.2.
Mean (± S.D.) Hb concentration and pH measured in five blood samples taken on different days in the course of the experiment. NE = number of exposures to hypobaric hypoxia before the sample was taken.

SAMPLE	1	2	3	4	5
NE	0	4	8	9	23
Hb G%	12.94 (± 1.54)	13.71 (± 2.34)	14.13 (± .97)	14.16 (± .83)	13.6 (± .51)
pH	7.404 (± 0.34)	7.360 (± .067)	7.400 (± .048)	7.417 (± .040)	7.409 (± .030)

4.3.3. Conclusions

Hypobaric hypoxia significantly decreased lever pressing for brain stimulation. The gradual increase of the mean performance during consecutive exposures reached a plateau after fifteen exposures. Based on the average performance of the group, adaptation to reduced atmospheric pressure seemed to be a slow process. However, averaging the individual performance data masked the differences in both magnitude and speed of adaptation between individuals. The adaptation was not related to increased hemoglobin, a known slow adaptive pathway in both humans (Dill *et al.*, 1969 ; Frisancho, 1975) and animals (Hock, 1970). In all animals in which the performance during hypobaric hypoxia improved, some adaptation was already seen during the second exposure. This observation and the fact that short exposure times (10 min.) were used suggests that the adaptation was due to increased rate and depth of pulmonary ventilation. This adaptive pathway is always operational during acute exposure to hypobaric hypoxia even after long periods of residence at high altitude (Pace, 1974).

Lever pressing is a motor act which requires physical effort. The decrease seen during hypobaric hypoxia could simply be a measurement of reduced work capacity. Work capacity is not only determined by the ambient oxygen tension but also by the efficiency with which the cardiovascular system can transport and deliver the available oxygen to the tissues. Hence, cardiovascular factors might be at the origin of the considerable differences in performance level among animals after a steady-state has been reached. The fact that the mean performance gradually decreases as a function of the decreasing atmospheric pressure (see 4.2) is an additional indication that the effects on lever pressing reflect decreased working capacity of the muscles. Inhibition of self-stimulation could also be interpreted in terms of alterations of motivation, reinforcement or disruptions of sensorimotor integrative functions.
A motivational deficit induced by hypoxia is unlikely. The initiation of self-stimulation was not affected and the rats maintained lever-pressing at a constant rate during hypoxia. This indicates that the anticipation of reward was unaffected. Further, it is difficult to conceive that an initially reduced reinforcement value of the stimulus would normalize due to adaptive processes after multiple exposure to hypoxia. Finally, it was suggested that inhibition of self-stimulation after treatment with neuroleptics was caused by a disruption of the association between the response and its consequence (Wauquier, 1976). After treatment with neuroleptics, self-stimulation is initiated but cannot be maintained. Responding therefore declines during the self-stimulation session. These patterns were not seen in our data. Therefore, it is unlikely that sensorimotor integrative functions were involved in the attenuation of self-stimulation behavior during hypobaric hypoxia. All these elements indicate that lever pressing for brain stimulation during hypobaric hypoxia cannot be used to evaluate effects upon higher central functions.

Steady-state performance can be reached during hypobaric hypoxia and consequently the model can be used in pharmacological research. However, the model can not be used to evaluate central effects of drugs. Improved lever pressing after drug treatment will indicate an increased work capacity which can be mediated by a drug effect on different mechanisms involved in the transport, delivery and utilization of oxygen. Another disadvantage of the model is related to the large differences that are seen between animals. As a consequence training of large numbers of animals will be required to obtain a group which performs at approximately the same level during hypobaric hypoxia.

In conclusion, the paradigm has only limited value for pharmacological research purposes in general and is unusable to study brain protective effects of drug.

Chapter 5
Post-Hypoxia Stimulus Discrimination in a Self-Stimulation Paradigm

5.1. Introduction

The reduction of lever pressing for brain stimulation seen during the first minutes after exposure to normobaric hypoxia (Chapter 3) could partly be due to a failure to recognize or discriminate between the relevant environmental stimuli in the experimental cage. This hypothetical explanation is strengthened by a regular observation during the drinking experiments (see Chapter 7). A delay between the onset of locomotion and the onset of drinking behavior after hypoxia was observed. Most animals started to move around the cage and even reared in the close vicinity of the drinking nipple without initiating drinking behavior. The latter could indicate that stimulus discrimination was still attenuated after postural reflexes and locomotion were normalized. To test this hypothesis a test procedure is needed in which stimulus discrimination can be evaluated independently of pure performance effects. Clincke *et al.* (1982) described such a method using stimulus discrimination in a brain self-stimulation paradigm. In this procedure rats were trained to discriminate between two electrical brain stimuli which only slightly differed with respect to pulse frequency. They found that haloperidol, a dopamine antagonist, dose-relatedly decreased performance in the system without affecting stimulus discrimination. Apomorphine, a dopamine agonist, on the other hand disrupted stimulus discrimination without attenuating normal response rates. These results clearly demonstrated that stimulus discrimination could be studied independently of the performance level.

The same procedure was used to investigate whether stimulus discrimination was disrupted in the post-hypoxic period. In this chapter two experiments will be described in which the effects of hypobaric hypoxia on stimulus discrimination were investigated.

5.2. Experiment 1 : 10-minute exposure to hypobaric hypoxia

5.2.1. Materials and methods

5.2.1.1. Animals and surgery

Adult male Wistar rats from the Janssen breeding colony, with a body weight of 250 ± 10 g were used. They were kept in individual cages under standard laboratory conditions. Food and water were always supplied *ad libitum*

Under Thalamonal® anaesthesia, single bipolar nichrome electrodes (0.254 mm ∅) were stereotaxically implanted in the lateral hypothalamic region of the brain (A: 4.6 mm; L: 1.5 mm; V: -3 mm). A jeweller's screw was placed on the frontal bone and served as an indifferent electrode. One week of recovery always preceded the training procedure.

5.2.1.2. Self-stimulation cage and equipment

The self-stimulation cage (20 x 25 x 33 cm) contained two levers mounted on opposite side walls, 6 cm above the grid floor. A swivel mounted on top of the box connected the rat with a ST-stimulator (JSI, Janssen Scientific Instruments). Stimulus delivery and data registration were controlled by a Rockwell AIM 65 microcomputer (Rockwell International Corporation) (Illustration 5.1.). Two stimuli (pulse train duration 500 msec, pulse intensity 200 μA, pulse width 4 msec) one on each lever, were available for self-stimulation and will be referred to as respectively HIGH (HS; pulse frequency 60 pps) and LOW (LS; pulse frequency 40 pps).

5.2.1.3. Training procedure

Rats were trained to discriminate between the two stimuli. During the training period they learned to obtain self-stimulation by pressing the lever on which the HIGH stimulus was available and to shift along with the position of the HS. Training sessions lasted twenty minutes and were given daily for several weeks until the discrimination was learned.

Training was given in progressive steps with increasing task complexity. During step 1 the HS was available on both levers during the whole session. During step 2 the HS was on one lever and no stimulus could be obtained from the other. No reversal was made during the sessions but the position of the HS was shifted to the opposite lever on subsequent training days. Step 3 was almost identical to step 2 since the HS was on one lever and no stimulus was given on the other. However, in step 3 reversal shifts were made during the sessions. This was done after fixed time intervals, i.e. respectively 10, 4 and 2 minutes on subsequent training days. Ultimately all shifts (2 min) were given in random sequence at the end of this training period. During step 4, the HS was on one lever and the LS was introduced with increasing steps of 5 pps on subsequent days until LS reached 40 ps. During this last training period all rèversal shifts were given in random sequence. During the whole training period each new training step was only introduced when the animals reached stable performance in the previous one. It took on average five weeks to complete the training of an individual animal. From the eight animals trained as previously described, five learned to discriminate between the two stimuli used in the experimental procedure. This means that the animals pressed the lever on which HS was available to obtain brain stimulation. Reversal shifts were reliably made along with the position of HS.

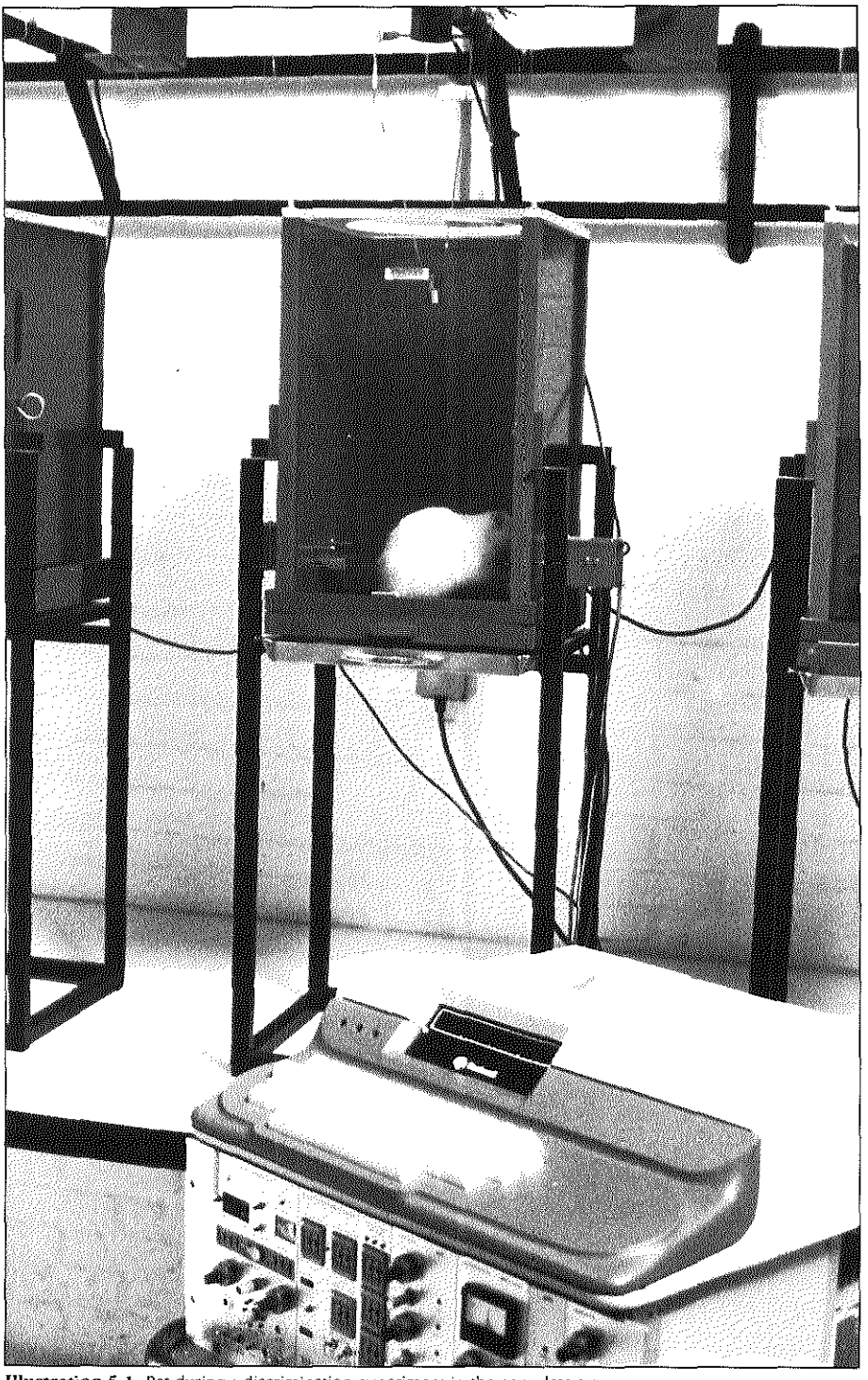

Illustration 5.1. Rat during a discrimination experiment in the complete set-up.

5.2.1.4. Experimental procedure

Experimental sessions lasted for twenty minutes. During the sessions HS and LS were simultaneously available. HS was on one and LS on the other lever. Every other two minutes HS and LS could be reversed on the opposite lever according to a randomized sequence. The randomization was restricted in such a way that each stimulus appeared an equal number of times (5) on each lever and never appeared on the same lever for more than two consecutive two-minute periods. This was done to prevent position conditioning. The number of responses on both levers and for HS and LS were registered and printed by the microcomputer.

During two weeks the animals were given daily 20-minute sessions during which they could obtain brain stimulation in the system. On Tuesday and Thursday the animals were exposed to hypobaric hypoxia (200 mm.Hg) for 10 minutes before the onset of the session. Those days will further be referred to as hypoxia (H) days. On the other days of the week the session was not preceded by hypoxia. Those days will be referred to as control (C) days.

5.2.1.5. Data transformation and statistical analysis

The level of discrimination of each animal during a specified time period was quantified by calculating a discrimation index (DI) with the following formula: $DI = \frac{RHS - RLS}{RHS + RLS}$

In the formula RHS was the number of responses (lever pressings) for HS and RLS was the number of responses for LS given during a specific time period. When the discrimination was optimal, RHS was high and RLS was low. The calculated DI then approximated 1. When the discriminaton was poor RHS and RLS were almost equal because the animals failed to make the reversal shifts and continued to press the same lever. The calculated DI then approximated zero. In extreme cases when the animal changed its preference from HS to LS, negative DI's could be observed. High negative DI's would then reflect a change in preference with the ability to discriminate. DI's were calculated for the whole experimental session (20 min) and for each consecutive 4-minute block in each experimental session.

Given the small number of animals (n = 5) available for this experiment, the statistical analyses were restricted to general analyses of variance to look for significant trends over several experimental days. Day by day comparisons were not statistically evaluated.

5.2.2. Results

5.2.2.1. Number of lever pressings

The operant behavior after the training procedure was very stable during the experimental sessions (Figure 5.1.). No significant differences were found between the total number of responses made during the six control sessions [$F(5/20) = 1.34$; n.s.]. In addition the number of responses made for the HS [$F(5/20) = 1.09$; n.s.] and for the LS [$F(5/20) = 2.44$; n.s.] turned out to be stable during the course of the experiment.

A general analysis on the total number of responses in all sessions (control and hypoxia) revealed no significant differences [F(9/36) = 1; n.s.]. A similar result was obtained for separate analysis on HS responses [F(9/36) = 1; n.s.] and LS responses [F(9/36) = 1; n.s.].

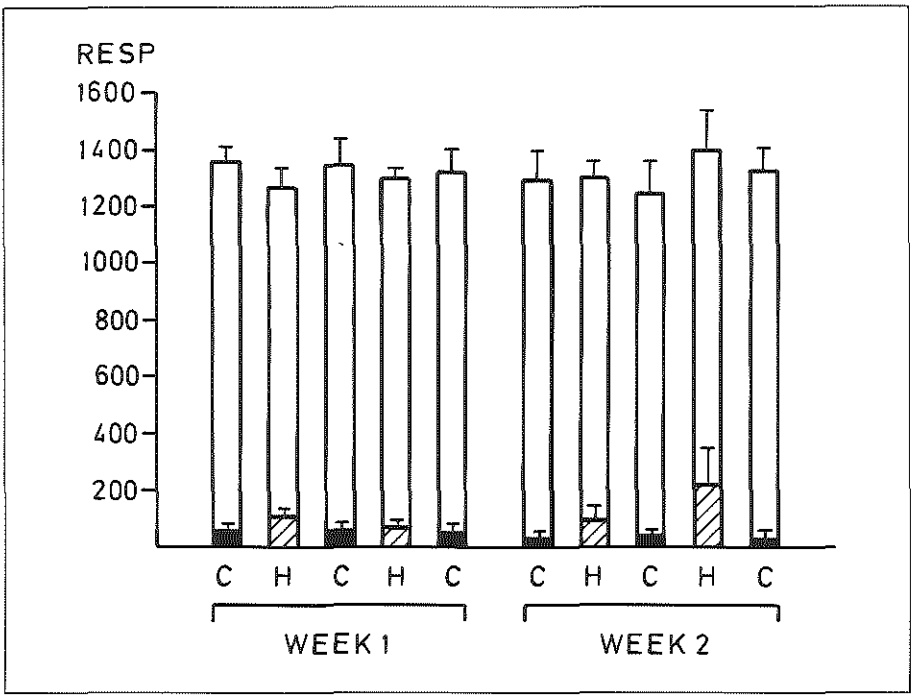

Figure 5.1. Mean (± S.E.M.) number of responses on successive C and H days. White parts of the bars are responses for HS. Black or shaded parts are responses for LS. N = 5 on each day.

A general analysis on the total number of responses on H days yielded similar results as described above. No significant differences were found over the four H days [F(3/12) = 1; n.s.]. Both separate analyses on HS responses [F(3/12) = 1; n.s.] and LS responses [F(3/12) = 1.19; n.s.] revealed no significant trends.

5.2.2.2. Level of discrimination

The DI was calculated for the whole experimental session (20 minutes) on both H and C days. These results are summarized in Figure 5.2. As can be seen there was a general tendency for the DI to be slightly lower on H days. This was confirmed by the statistical analysis on all C and H days combined [F(9/36) = 2.66; $p < .05$]. Since no significant differences could be found between the total number of lever pressings for HS and LS when the whole session was taken into account, the slight general drop in DI might have originated from reduced discrimination during the early stages of the session. Therefore, individual DI's were calculated for five consecutive 4-minute blocks of each experimental session (C and H). The mean DI was then calculated for each 4-minute block. In Figure 5.3. mean DI's for the 4-minute blocks are respresented for each H day and its preceding C days. As can be seen there was

a tendency for the DI to be lower during the first two blocks on H days. This was confirmed by a general statistical analysis (all C and H) on the DI's of the first [$F(9/36) = 3.39$; $p < .01$] and second [$F(9/36) = 2.32$; $p < .05$] block. No significant differences were found for the remaining three blocks at the end of the session.

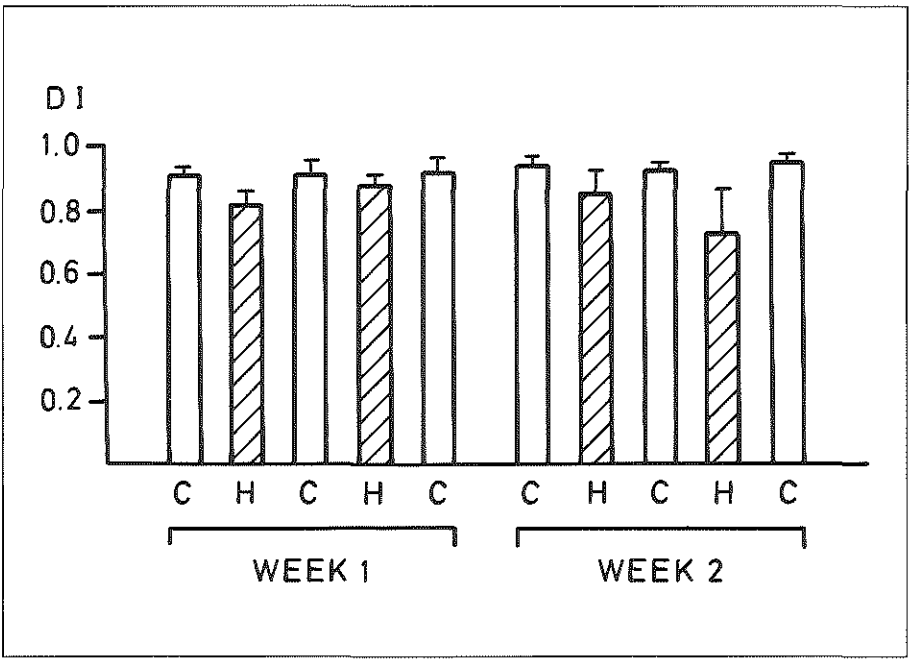

Figure 5.2. Mean (± S.E.M.) discrimination index (DI) calculated for the whole 20-minute session on C and H days. N = 5 on each day.

5.2.3. Conclusion

Although the behavioral output was normal, the results clearly indicate that subtle changes in discrimination capacity were present in the first eight minutes after the hypoxia. However, there is no evidence of any substantial discrimination deficit or impairment. If the latter were true, the DI's should have dropped to near zero in all animals. Such condition occurred in individual animals during the first and last hypoxia days but each time different animals were involved. The reduced DI's during the first eight minutes mainly originate from the fact that the animals waited somewhat longer to shift along with the HS when it moved to the opposite lever. Consequently they pressed longer for the LS and the DI drops. The results suggest that discriminative capacities can be slightly affected although motor behavior is already normalized.

The fact that the discrimination capacity was hardly affected could be related to the degree of hypoxia. To further investigate this point a second experiment was done where the degree of hypoxia was increased.

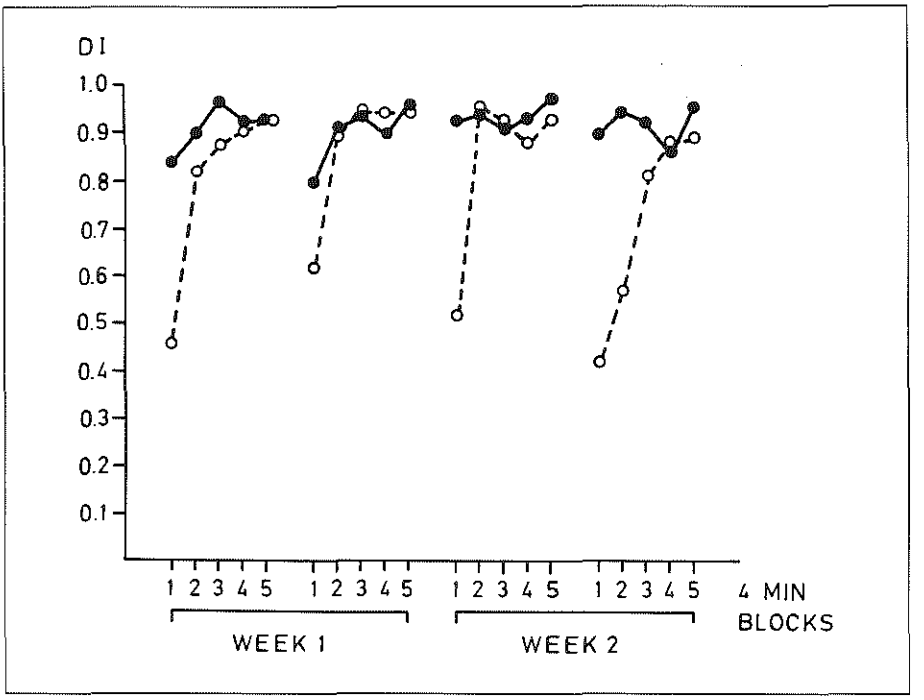

Figure 5.3. Mean discrimination index (DI) calculated for each block of 4 minutes of sessions on H days (broken lines) and their preceding C day (full lines). Successive H days go from left to right. Hypoxia is induced by exposure for 10 minutes to an atmospheric pressure of 200 mm.Hg.

5.3. Experiment 2: 20-minute exposure to hypobaric hypoxia

The degree of hypoxia could be changed through two different methods. The first option was to extend the duration of the exposure. The second option was to further decrease the pressure below 200 mm.Hg. This method was eliminated because diffuse lung bleedings occur when rats are brought to a simulated altitude with an absolute pressure of 190 mm.Hg. It was therefore decided to double the exposure time during H days in this experiment.

5.3.1. Materials and methods

The same animals that participated in the first experiment were used. During the week that followed the first experiments the animals were exposed to hypobaric hypoxia (200 mm.Hg) on Tuesday and Thursday for 20 minutes before the onset of the self-stimulation session. On the other days of the week no hypoxia was induced. Recording and data manipulation were similar as for experiment 1. No statistical analyses were made on the data of this experiment since the number of subjects was reduced to four. One animal died during the first exposure to 20 minutes of hypoxia. This illustrates that the upper limit for survival might have been reached for certain animals under these hypoxic conditions.

5.3.2. Results and discussion

Figure 5.4. depicts the mean DI's calculated for the five consecutive 4-minute blocks on H days and their preceding C day. Just as for the shorter exposure time in experiment 1, the DI's were mainly decreased during the first minutes of the sessions following the hypoxia. Although the effect seemed to be more profound after this extreme hypoxia it is clear that the capacity to discriminate between the stimuli was not abolished. During the first 4 minutes after hypoxia the general response level dropped to 70 % of the control values, but the discrimination was still reasonable under these conditions. The low mean DI observed in the first 4-minute block after the second exposure (right side of Figure 5.4.) was due to the fact that one of the animals made a completely wrong discrimination. It pressed for LS instead of HS and this resulted in a DI with negative sign (– .816). This, of course, had a profound effect on the mean, owing to the small number of animals in this experiment.

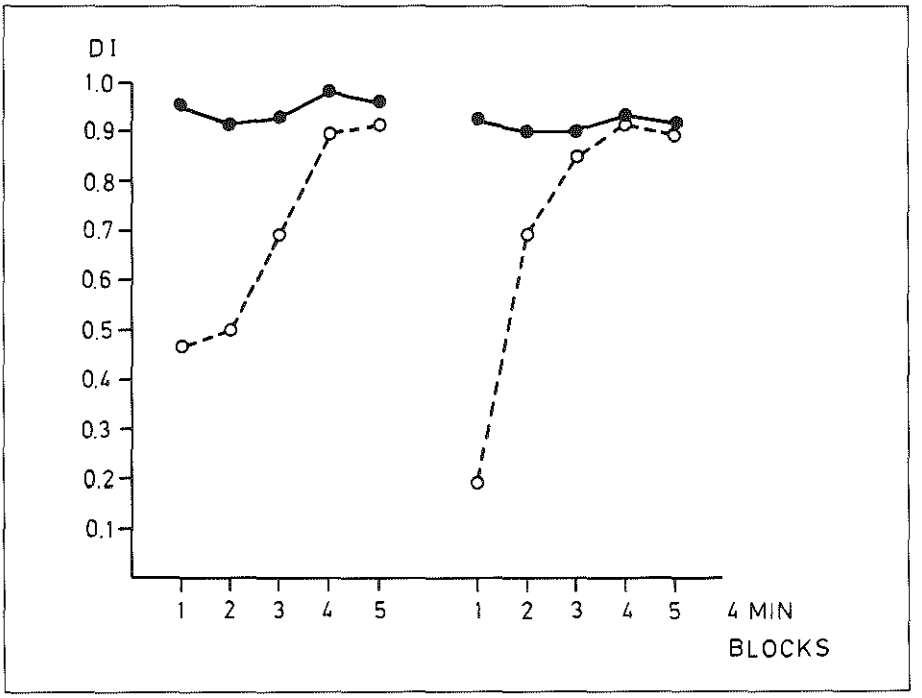

Figure 5.4. Mean discrimination index (DI) calculated for each block of 4 minutes of sessions on H days (broken lines) and their preceding C day (full lines). The left graph is the first hypoxia day and the right one the second. Hypoxia was induced by exposure to an atmospheric pressure of 200 mm.Hg for 20 minutes.

The evolution of the discrimination capacity of rats after hypoxia can perhaps better be evaluated when individual DI curves are presented as in Figure 5.5. It is clear that discrimination is completely normal in all animals eight minutes after hypoxia. The attenuation in the first minutes was variable both between the animals and within the same animal after each exposure.

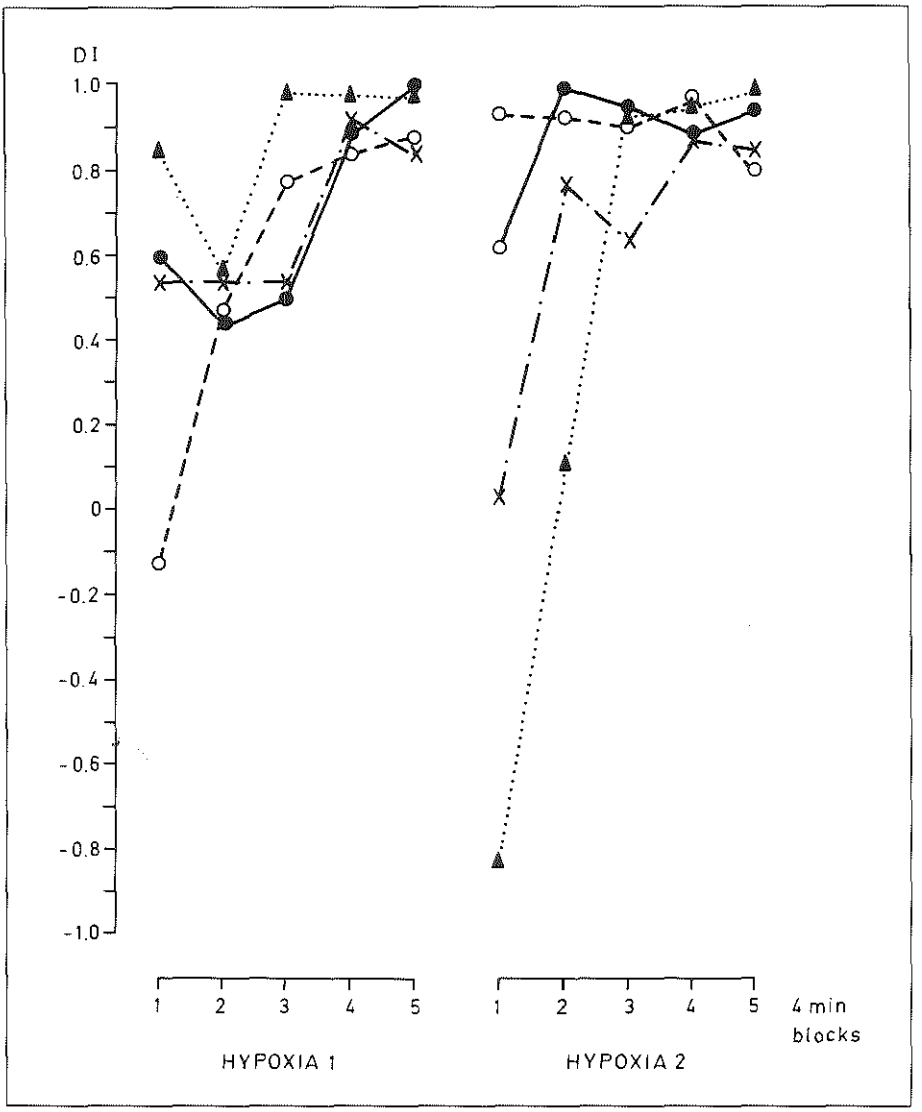

Figure 5.5. Discrimination index (DI) for each block of 4 minutes of sessions on two hypoxia days in four different animals.

5.4. General conclusions

Taking into account the results obtained in the two experiments presented in this chapter, one might conclude that stimulus discrimination is only slightly attenuated in the first minutes that follow the end of a hypoxic period. It is clear that the animals could still discriminate although the efficiency was reduced. Stimulus perception was probably not affected and the variability of the reduced discrimination efficiency between and within animals indicates that these effects might have been caused indirectly. A possible explanation could be that orientation was affected. In such condition, motor performance (e.g. lever pressing) can be perfectly normal but discrimination efficiency will be affected due to the problems to localize the lever if a reversal shift is required. This hypothesis can be tested by using a task with spatial orientation as operant behavior.

Although, it has to be recognized that the results clearly indicate that stimulus discrimination can be somewhat altered under conditions in which locomotor and learned operant behavior can be fully executed, they also imply that effects that are observed after ten minutes following hypoxia cannot be attributed to some deficiency in stimulus discrimination.

Chapter 6
Adaptation of Drinking Behavior to Restricted Drinking Times and Repeated Water Injections

6.1. Introduction

In the previous chapters we have studied the effects of hypoxia on behavior that was mainly under external stimulus control. Although lever pressing for brain stimulation and shock avoidance are adaptive behaviors in the sense that they are initiated and supported by external stimuli, both were not essential for maintenance of internal homeostasis and survival of the animals. Ingestive behaviors such as eating and drinking are typical goal-oriented behaviors involved in the maintenance of homeostasis. The main features of such behavior are that they can be initiated by internal as well as by external stimuli, and that they are complementary to visceral responses.

It is well known from the literature that ingestive behavior is suppressed during high altitude exposure and normobaric hypoxia in man (Consolazio *et al.*, 1968; Hannon, 1980) and animals (Koob *et al.*, 1970; Schnakenberg *et al.*, 1971; Krabill and Hannon, 1972; Koob and Annau, 1973, 1974; Koob *et al.*, 1974; Fregly *et al.*, 1974; Jones *et al.*, 1981; Ettinger and Staddon, 1982). Ingestive behavior involves interaction of central and peripheral mechanisms. Especially in adaptive drinking behavior the CNS plays a fundamental role in the integration of visceral and sensorimotor systems (for review of neural mechanisms involved see Swanson and Mogenson, 1981). Water, the largest component of an organism ($\pm$ 2/3), is under permanent surveillance of the CNS since considerable amounts of fluid are lost each day through water in urine and feces and through evaporation from the lungs and skin. Replacement is achieved through water resulting from oxidative processes but mainly through water available in the ingested food and by drinking water. The prompt response of the organism to hemorrhage or dehydration is a nice demonstration of the interplay between autonomic responses to adaptive behavior. The organism reacts first by an autonomic response and shunts blood to vital organs through peripheral vasoconstriction. In addition, the remaining fluid is conserved by release of antidiuretic hormone and, from the behavioral side, water is actively searched for and ingested.

The fundamental role played by the CNS in the integration of the visceral and sensorimotor systems related to adaptive drinking is illustrated by the fact that drinking behavior can be described in three sequential stages. A distinction is made between an initiation, search and consummatory phase. The initiation phase is characterized by the detection of peripheral and central deficit signals which are coordinated with environmental signals and cognitive factors. During the search phase enhanced behavioral arousal develops into an active search for water. The locomotor activity is guided by incoming salient

environmental cues and past experience with similar conditions. The actual ingestion of fluid occurs during the consummatory phase. Here the brain stem is the major control center for the motor responses, although physical characteristics of the ingested fluid may, to a large extent, determine what and how much will be consumed.

Based on these properties we concluded that adaptive drinking behavior could be useful to study how natural, intrinsic, motivated behavior is attenuated after exposure to hypoxia in control and drug-treated animals.

6.2. Adaptation of drinking behavior to restricted drinking times

To use drinking behavior as a dependent variable in an experimental set-up it is necessary to bring the animal in such a condition that both internal and external stimuli maximize the probability for drinking behavior to occur. This can be accomplished by training the animals to drink during a restricted daily session in a special drinking cage after a standard period of water deprivation. As a result of such a schedule it is expected that internal stimuli will be similar to those that occur after deprivation in a natural setting and that the duration of the initiation and search phase will be very short under such circumstances. Due to their past experience in the drinking cage, the animals will probably commence drinking within the first few seconds that water becomes available.

The aim of the first experiment was to determine the time period that the animals need to develop a new physiological balance. Stable drinking behavior has to be reached before experimental manipulations to influence drinking can be introduced.

6.2.1. Materials and methods

6.2.1.1. Animals and housing conditions

Thirty naïve adult male Wistar rats from our own breeding colony, with a mean (± S.E.M.) body weight of 250.16 (± 1.27) g were subjected to a training procedure. The animals were housed individually in standard rodent cages and kept on a 12-h day-night cycle (lights on between 7 a.m. and 7 p.m.) in a room of constant temperature (21 ± 1°C). Solid food pellets were always available *ad libitum* in the home cages.

Drinking cages were of the same size as the home cage and located in the same room. In the drinking cage the animals had access to a drinking tube filled with tap water. This drinking tube consisted of a glass burette graded to the nearest 0.5 ml and fitted with a nipple which protruded through the roof of the cage as soon as the drinking session was started (see illustration 6.1.).

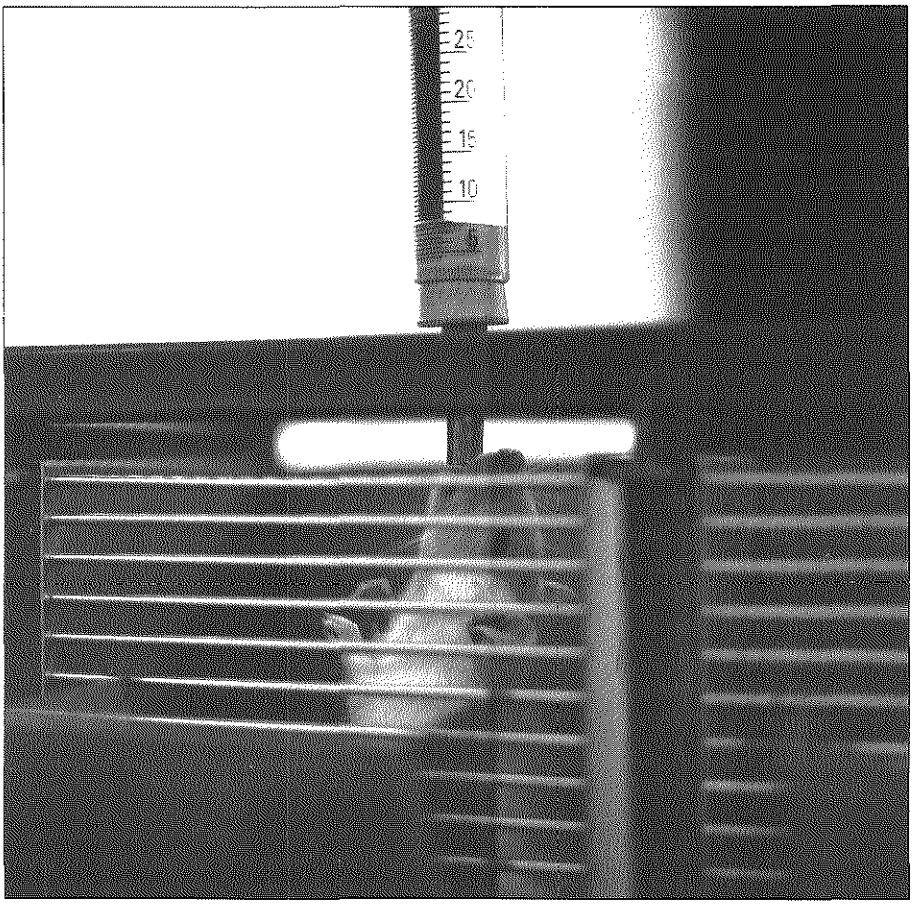

Illustration 6.1. Rat in drinking cage during training session.

6.2.1.2. Training procedure and data registration

Upon arrival in the laboratory, body weights were measured and the animals were temporarily housed for three days in the drinking cage, where they had free access to tap water from the drinking tube and to standard food pellets. Body weight and 24 h water consumption were measured daily at 11 a.m. During their first three days' stay in the drinking cage the animals could adapt to the rather unusual position of the drinking nipple. The latter could only be reached by standing on the hind limbs in an upright position.

Following the last measurement on the third day, training on a restricted time schedule for water intake began. The animals were moved to their home cages and deprived of water, but food was available *ad libitum*. The next day (24 h deprivation) body weight was measured and the rats were transferred from their home cages to their individual drinking cages where they had access to water for a 30-min period. At the end of the drinking session the animals were returned to their home cages. Food was no longer available during the drinking sessions.

All animals were trained on this schedule for 10 consecutive days. Body weight was measured daily before the onset of the 30-min drinking session. During the session, water consumption was measured at five-minute intervals. In additon water intake was registered at two-minute intervals during training days 1, 5 and 10.

6.2.1.3. Statistical analysis

Statistical differences between means were evaluated by analysis of variance (ANOVA) having repeated measurements of the factor subjects. This technique was used for simple comparisons between two means as well as for general analysis on multiple means. Analyses of trend were based on the method of orthogonal polynomials (Keppel, 1973). They were conducted on the means to isolate independent components of trend and to test them for significance.

6.2.2. Results

6.2.2.1. Body weight

The changes in body weight during the training procedure are presented in Figure 6.1. On average 10 % of the body weight was lost after the first 24-h water deprivation. It dropped from 265.77 ± 1.63 g on the last day with *ad libitum* water to 240.9 ± 1.43 g on the first training day. Subsequently a further decrease to 232.5 ± 1.44 g on training day four was observed. From that day on the trend was reversed and on the last training day it returned to 236.03 ± 2.31 g. Comparison between the mean body weights on the first and last training days indicates that the difference was no longer significant [$F(1/29) = 2.8$, n.s.]. A general analysis of the body weights over the ten training days revealed a significant trend over time (Table 6.1.).

Table 6.1.
One-way ANOVA on body weights on ten training days.

Source	SS	df	MS	F
A (days)	1838.56	9	204.28	10.21 <.001
S (subjects)	21053.43	29	725.98	
RES	5223.94	261	20.02	

The plot of mean body weights in Fig. 6.1. suggests the existence of a quadratic trend in the changes in body weights. This was confirmed by the trend analysis which is summarized in Table 6.2. Four independent significant trends could be identified. As expected the quadratic trend appeared to be the major component and accounts for more than 82 % of the main effect of factor A (days). This trend reflects the gradual adaptation of the organism to the new metabolic homeostasis that must be attained under forced conditions.

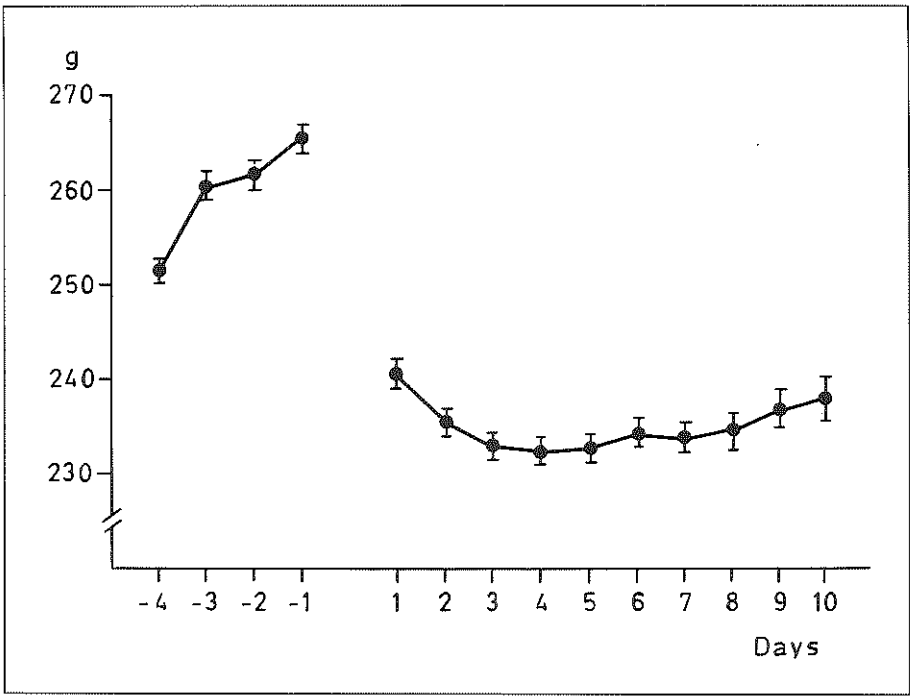

Figure 6.1. Mean (± S.E.M.) body weight (n = 30) on subsequent days with *ad libitum* water supply (-4 to -1, left part) and on ten training days with restricted 30-min drinking time (1 to 10, right part).

6.2.2.2. Daily total water consumption

The mean water intake during the three days with *ad libitum* supply and the subsequent ten training days with restricted drinking time is presented in Fig. 6.2. The mean (± S.E.M.) consumption over 24 h varies between 40.73 ± 1.27 and 35.65 ± 0.85 ml. During the first training day water consumption decreased to 12.65 ± 0.42 ml over the 30-minute period. This is approximately 33 % of the volume ingested over 24 h.

The water consumption then gradually increased over the ten training days to 20.35 ± 0.42 ml on the last day. A global analysis revealed a significant increase over the training period [$F(9/261) = 45.33$, $p < .001$]. From training day 8 on drinking behavior stabilized.
This is illustrated by the fact that water intake on day 8 (19.78 ± 0.42 ml) was no longer significantly different [$F(1/29) = 1.85$, n.s.] from the consumption on the last training day. At that time the animals consumed approximately 53 % of the volume ingested over 24 hours. The highest increase was observed during the first three training days and was then followed by a more gradual increase on subsequent days. Trend analysis (Table 6.3.) indeed identified two major components, a linear and a quadratic which together account for 91.47 % of the main effect.

Table 6.2.
Analysis of trend of mean body weights over 10 training days.

Source	SS	df	MS	F	% Main effect
Days (A)	(1838.56)	(9)			
Linear	0.45	1	0.45	< 1	
Quadratic	1513.31	1	1513.31	81.58***	82.31
Cubic	211.37	1	211.37	16.25***	11.50
Quartic	54.78	1	54.78	6.86*	2.97
Quintic	10.18	1	10.18	5.41*	0.55
Subjects (S)	21053.43	29	725.98		
A x S	(5223.94)	(261)			
Linear	3227.54	29	111.29		
Quadratic	538.01	29	18.55		
Cubic	377.17	29	13.01		
Quartic	231.44	29	7.98		
Quintic	54.43	29	1.88		
Total	28115.93	299			

*** $p < .001$; * $p < .05$

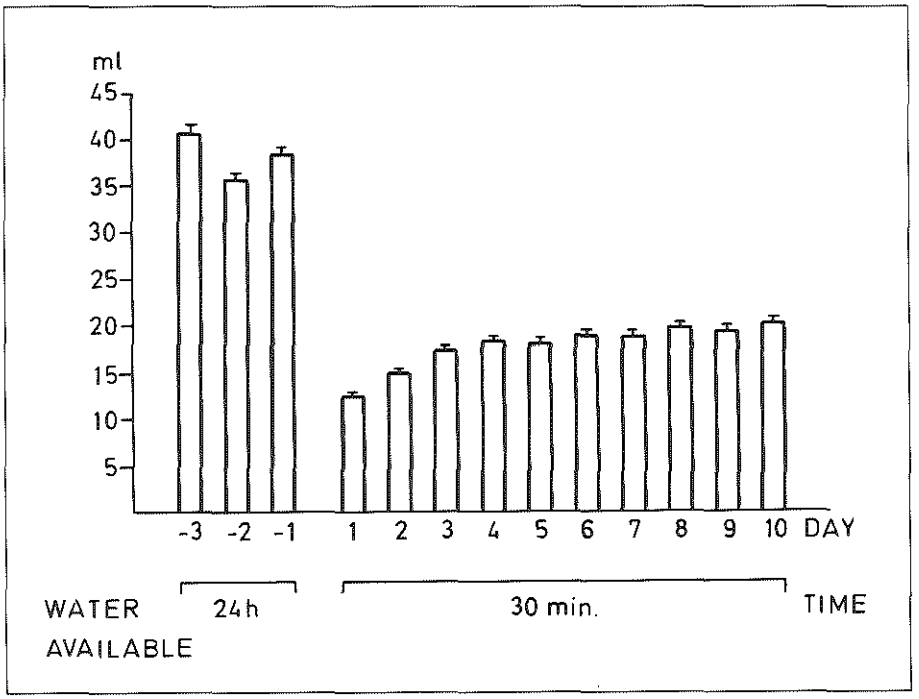

Figure 6.2. Mean (± S.E.M.) water consumption (n = 30) on subsequent days with *ad libitum* water supply (-3 to -1, left part) and on ten training days with restricted 30-min drinking time (1 to 10, right part).

6.2.2.3. Drinking pattern

The analysis of the global water consumption reveals no information about the drinking pattern of the animals when water is available during a highly restricted time. The question to be answered here is whether the increase in water consumption over the training period is due to the development of a new drinking pattern or not.

Table 6.3.
Analysis of trend of mean water intake over 10 training days.

Source	SS	df	MS	F	% Main effect
Days (A)	(1460.32)	(9)			
Linear	1131.89	1	1131.89	143.70***	77.51
Quadratic	203.89	1	203.86	81.54***	13.96
Cubic	92.80	1	92.80	23.09***	6.35
Quartic	4.13	1	4.13	< 1	
Quintic	0.25	1	0.25	< 1	
Subjects (S)	840.57	29	28.99		
A x S	(934.16)	(261)			
Linear	228.43	29	7.88		
Quadratic	72.39	29	2.50		
Cubic	116.47	29	4.02		
Quartic	123.29	29	4.25		
Quintic	101.28	29	3.49		
Total	3235.05	299			

*** $p < .001$

Drinking patterns can be studied by looking at the volumes that are ingested during specified successive time units (ml/2 min or ml/5 min) which cover the whole drinking period. Observation shows that the animals drink almost continuously during the first ten to fifteen minutes of the drinking session until they are satiated. During the second part of the session, drinking occurs in bursts scattered at random over the remaining drinking time. As illustrated in Fig. 6.3. an individual animal shows this type of drinking from the first training day on and does not essentially change it in the course of the training period. The representation of the cumulative drinking curves of training days 1, 5 and 10 (Fig. 6.4.) leads to the same conclusion since the day curves parallel each other. This type of drinking pattern results in the typical satiation curves seen when the ingested volumes of all animals are averaged and plotted for fixed time intervals (see Fig. 6.5.).
This figure clearly indicates that the increase in water intake over the training period is situated in the first half of the drinking session when drinking occurs almost uninterruptedly and the largest volumes are ingested even on the first training day.

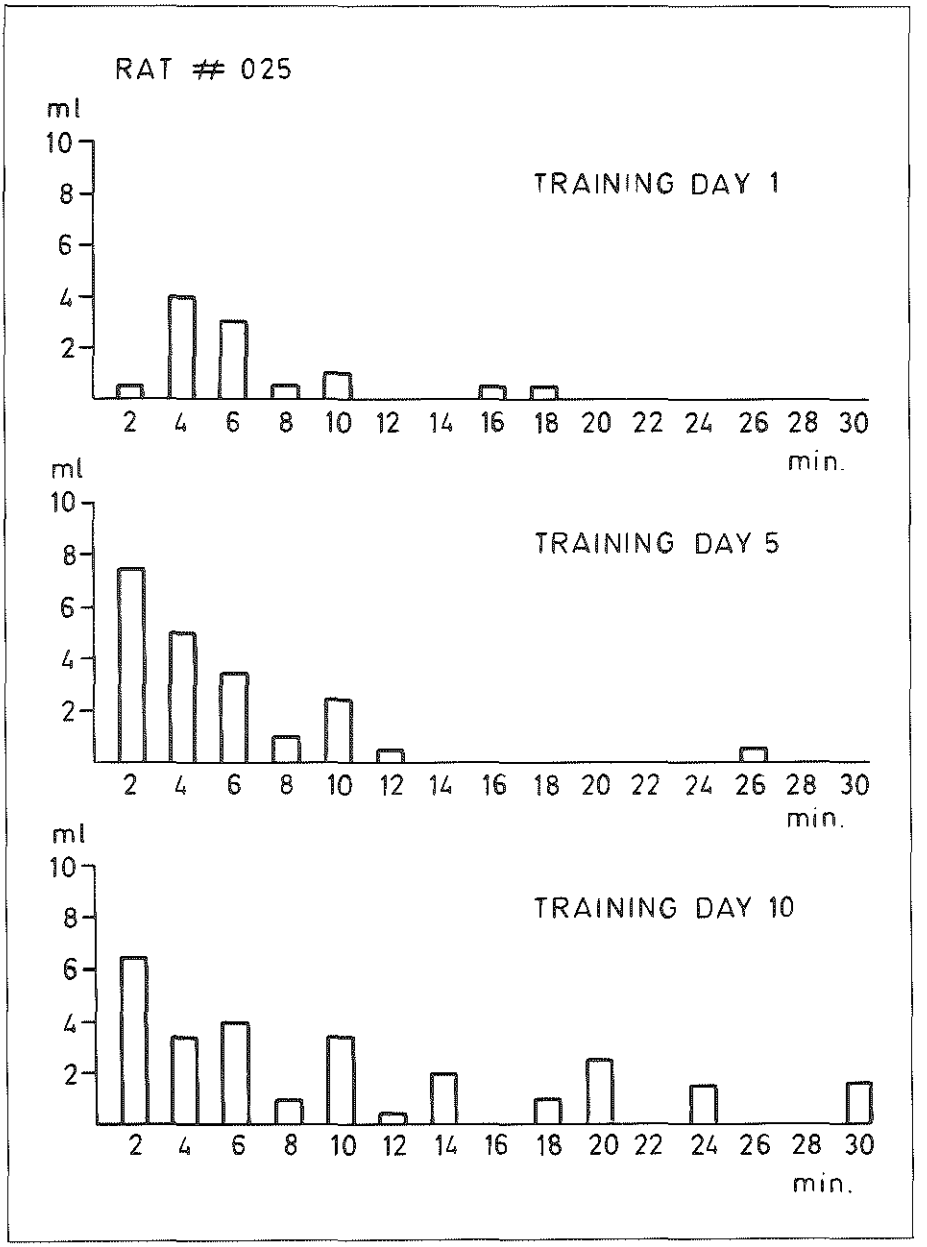

Figure 6.3. Water intake (ml) during 2-min intervals of a randomly selected animal (rat 025) on training days 1, 5 and 10.

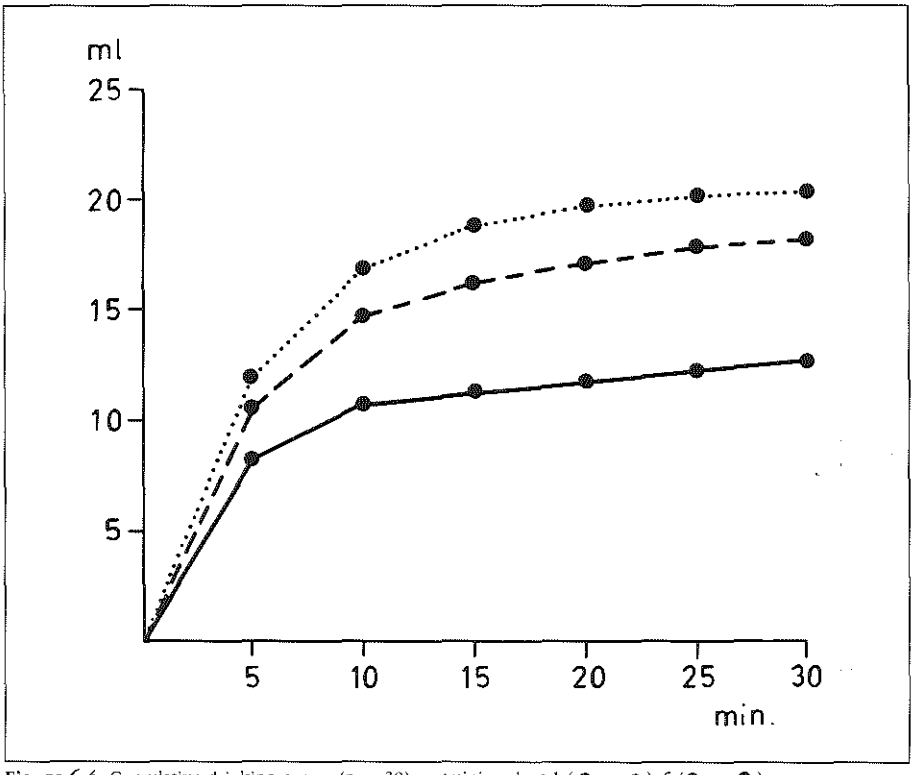

Figure 6.4. Cumulative drinking curves (n = 30) on training days 1 (●——●), 5 (●– – –●) and 10 (●• • •●).

This is statistically confirmed by comparison of the mean water intake during 5-min intervals on training days 1, 5 and 10. As can be seen in Fig. 6.6., significant increases between the intake on the respective training days are only observed in the first three 5-min periods of the drinking session. Consequently, the increase in total water consumption is not obtained by a change in the drinking pattern but merely by an increased intake in the period where drinking occurs almost uninterruptedly. This implies that trend analysis of the mean intake of successive time periods should reveal similar independent components of trend for the three training days. Table 6.4. summarizes the results of such an analysis which was calculated for both 2 and 5-min intervals (detailed results of the calculations are presented in addendum 2 at the end of the chapter).

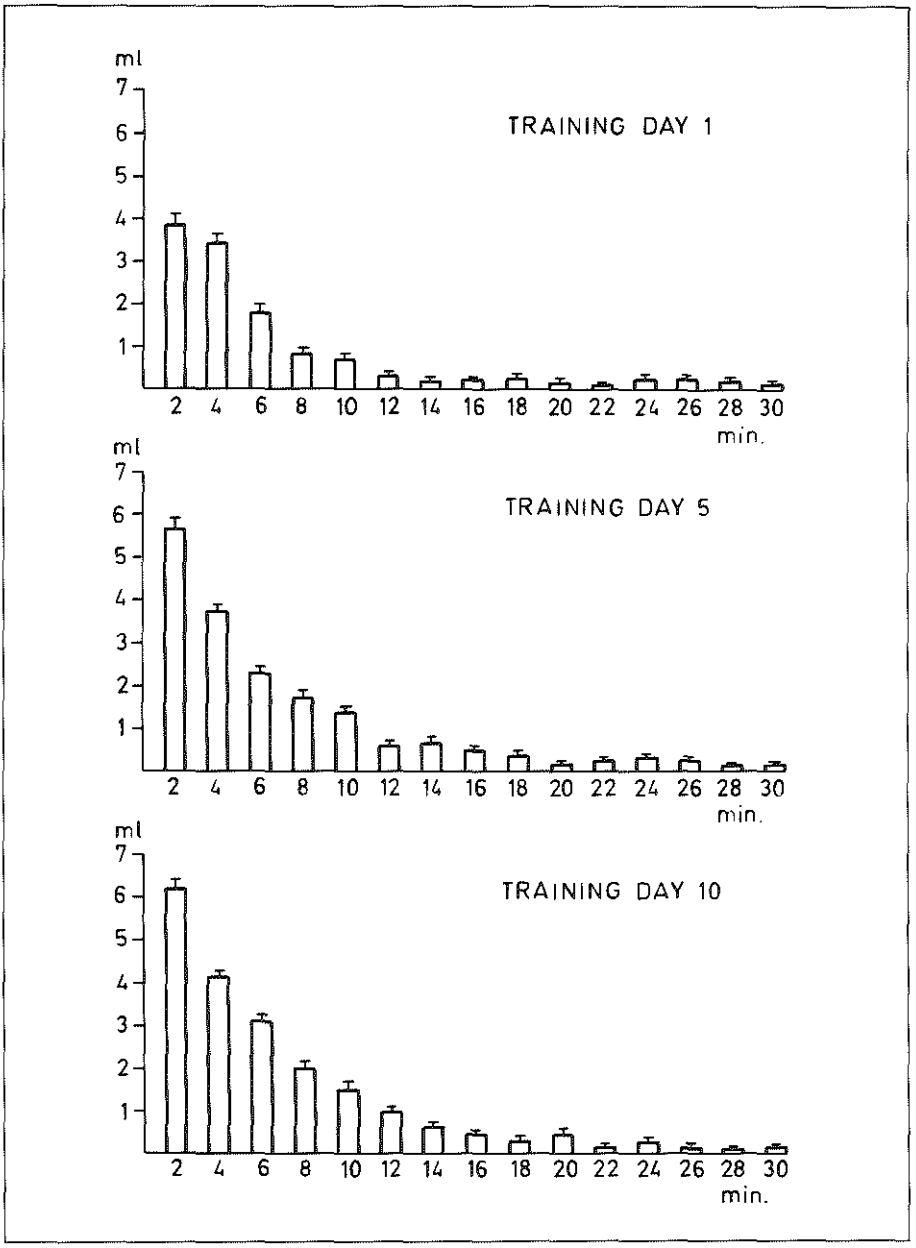

Figure 6.5. Mean (± S.E.M.) water consumption (n = 30) during 2-min intervals on training days 1, 5 and 10.

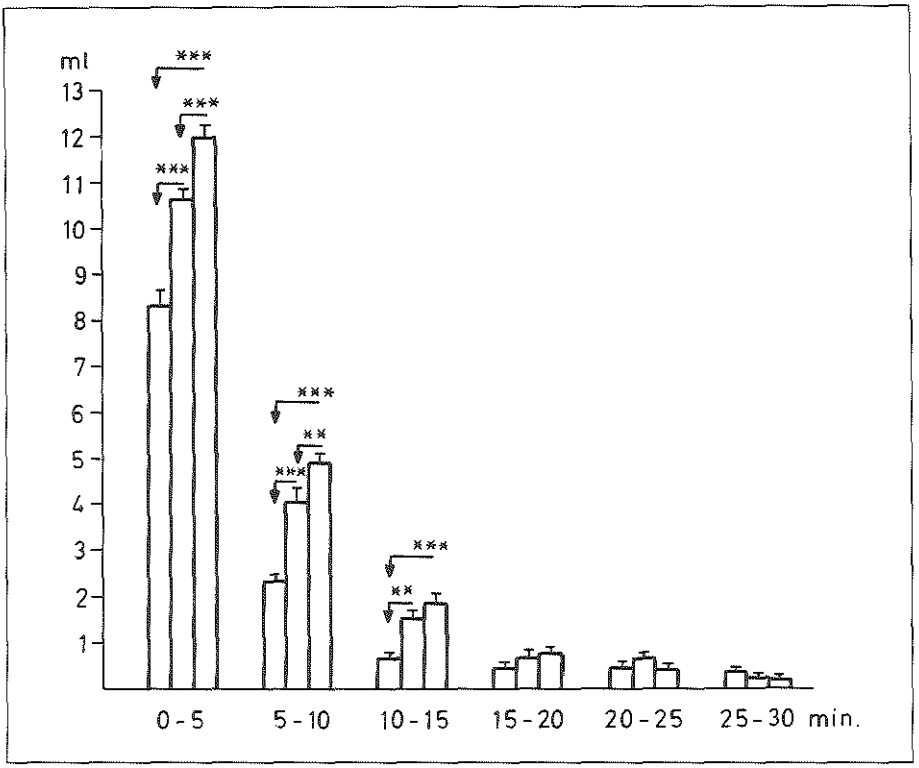

Figure 6.6. Mean (± S.E.M.) water consumption during 5-min intervals on training days 1 (left column), 5 (middle column) and 10 (right column). Significance of differences between days as indicated by arrows. *** $p < .001$, ** $p < .01$.

Table 6.4.
Analysis of trend: % main effect accounted for by major significant components.

	Day 1	Day 2	Day 10
2-min interval			
Linear (1)	57.56	65.75	69.66
Quadratic (2)	31.92	26.36	25.41
(1) + (2)	89.48	92.11	95.07
5-min interval			
Linear (1)	60.48	71.33	74.27
Quadratic	31.60	24.31	22.74
(1) + (2)	92.08	95.64	97.01

It is obvious that the major component is linear from the first training day on and that its relative importance increases over the training period. On the last training day, the curve is almost completely defined by the two major components. This type of analysis thus confirms the suggestion that the drinking pattern during restricted drinking time does not change in the course of a training period. The stability of this pattern is also illustrated by the fact that the information obtained from the separate analysis of the different time intervals offers redundant information.

6.2.3. Conclusion

We can conclude that a ten-day training period is sufficient to obtain stable drinking behavior during a restricted drinking time of 30 minutes. The fact that the animals recover from an initial drop in body weight indicates that under these circumstances they have developed a new metabolic and physiological balance. This is important, since the rats also have to change their feeding habits under the presented training regime. Eating and drinking can no longer occur in a closely alternating sequence but are now separated in time and location of occurrence.

As expected, the training procedure reduces the initiation and search phase of adaptive drinking to less than two seconds. The animals readily start drinking as soon as the water becomes available. With this very short latency time and the stable drinking pattern that is reached after a relatively short training period, the necessary conditions are met to use adaptive drinking as an independent factor in experimental conditions.

6.3. Drinking behavior after injections with water

In the next experiments we attempted to answer the question whether or not trained adaptive drinking behavior during restricted drinking times is attenuated by water injections. The answer to this question has particular relevance to the interpretation of data from experimental designs where drinking behavior is the dependent variable under study and where pharmacological treatment is one of the independent factors as part of the experimental manipulation.

Most pharmacological interventions require injections of a certain amount of fluid in which the drugs are dissolved. Such treatment is a direct interference with the fluid balance of the organism and can therefore alter the level of dehydration. Since the level of dehydration is strongly correlated with the subsequent water intake (Fitzsimons, 1972), any interference with the level of dehydration might influence the amount of water intake during restricted drinking times. In a first group of animals the effects of a single water injection given by different injection routes, at different times before the onset of the drinking session were studied. In a second group of animals the effects of daily water injections were assessed.

In principle, fluid injections should not hamper the interpretation of water intake data as long as they produce stable effects when the injections are given daily over longer time periods. If, on the other hand, such treatment disrupts the steady-state that was obtained after training, the interpretation of possible effects due to additional experimental variables (e.g. induced hypoxia) might become very hazardous. Hence, the results from the experiments with water injections will deliver crucial information with regard to the utility of drinking behavior to study the adaptation to hypoxic conditions under chronic pharmacological treatment.

6.3.1. Drinking behavior after single water injections

In the following experiment we studied the effect of single water injections administered by different routes upon the subsequent water consumption during a 30-min drinking session. In addition different time intervals between oral water injections and drinking sessions were assessed.

6.3.1.1. Materials and methods

Thirty naïve adult male Wistar rats with an initial body weight of 250 ± 5 g were subjected to the training procedure (10 days) for adaptive drinking during restricted drinking times as previously described (see 6.2.1.2.). After the last training day the trained animals were injected on four consecutive days via different injection routes and at different times before the onset of the drinking session. A standard volume (1 ml/100 g) was injected respectively p.o. -1 h on day eleven, p.o. -4 h on day twelve, s.c. -1 h on day thirteen and i.p. -1 h on day fourteen before the start of the drinking session. Water consumption was measured as described previously and statistical analyses were done with appropriate repeated measures techniques.

6.3.1.2. Results

In all animals and for all injection treatments, normal drinking patterns occurred and, consequently, typical satiation curves were observed.

A general analysis of variance of the total water intake on the last training day and after the four different injections revealed that there was a significant difference [$F(4/116) = 2.65$, $p < .05$] in water consumption after the water injections. The observed differences are presented in Figure 6.7. Two by two comparison between consumption after the different treatment conditions and the last training day indicated that this effect was due to a significant decrease in water consumption after the s.c. water injection [$F(1/29) = 5.38$, $p < .05$]. None of the other comparisons yielded a significance. The ingested water volume after the s.c. injection was on average 4.28 % lower than on the last training day.

6.3.1.1. Conclusion

The presented results indicate that the injection of water prior to a drinking session can attenuate the subsequent intake. However, the effect was only seen after the s.c. injection and not after oral or intraperitoneal injections.

An injection of 1 ml/100 g is approximately equivalent to 10 % of the volume that is ingested on control days. Hence, the observed intake reduction (4.28 % on average) is approximately 50 % lower than the infused volume. Apparently no simple one-to-one relation exists between the injected volume and the observed decrease. In fact, when individual results are checked, it appears that the intake is reduced in eighteen out of thirty animals, whilst the remaining twelve have a slightly increased water consumption. The latter finding suggests that the reduced intake after s.c. injection might be caused by factors not related to the injection route.

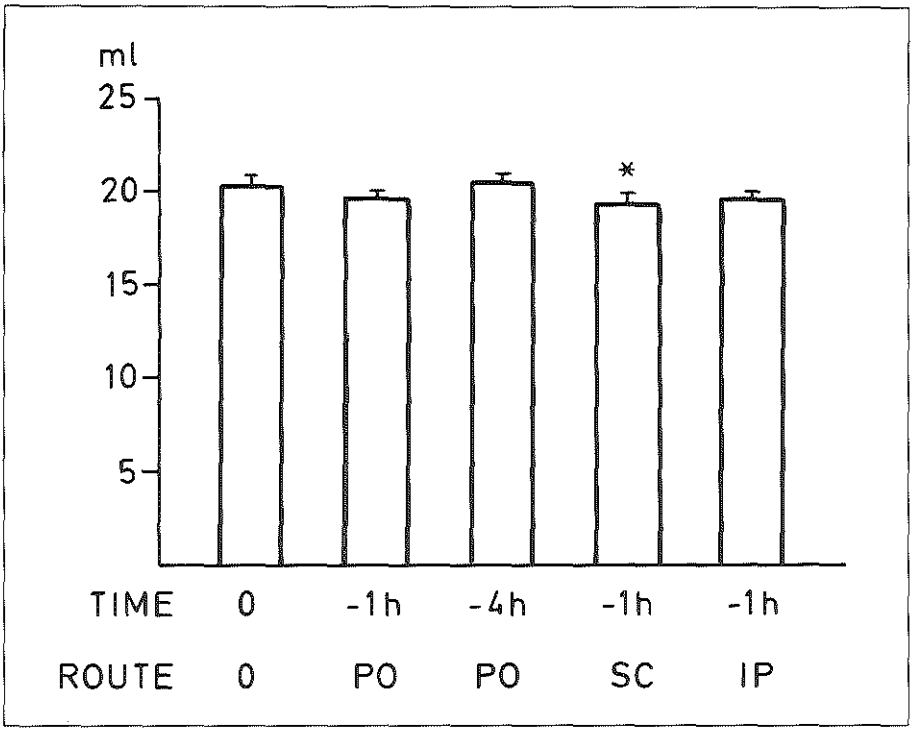

Figure 6.7. Mean (± S.E.M.) total water consumption on the last training day (left column) and after different injections of 1 ml/100 g body weight on subsequent days. Significance of differences calculated against the last training day.
* $p < .05$

Since the different injections were given on four consecutive days, the observed effect might be due to a cumulative effect of the daily injections rather than to the specific injection route applied on day three (s.c. injection). A possible explanation could be that daily injections of small amounts of water disrupt the steady-state due to cumulative effects. The precise point at which this effect is reflected in the ingested water volume might be individually variable. The next experiment was designed to further elucidate this problem.

6.3.2. Drinking behavior after chronic application of daily water injections

In the following experiment we studied the effect of a daily oral water injection on the water intake during restricted drinking times. Oral injections were chosen because a single oral injection did not attenuate the water intake (see previous experiment). An additional reason for choosing oral injections is related to the fact that some drugs cannot be dissolved and have to be administered as a suspension in water. Hence, they can only be given orally to guarantee proper distribution in the organism.

6.3.2.1. Materials and methods

Thirty naïve adult male Wistar rats with a body weight of 250 ± 5 g were trained for ten days to obtain their daily water intake during a 30-minute drinking session. From day eleven on the animals were daily injected orally one hour before the onset of the drinking session with a standard water volume of 1 ml/100 g body weight. This treatment was given for twelve consecutive days. Water intake was measured as described in 6.2.1.2.

6.3.2.2. Results

No differences in the drinking patterns were observed during the twelve treatment days as compared with the last training day. For all animals normal satiation curves were observed on all occasions.

Figure 6.8. presents the mean (± S.E.M.) water intake on the last training day and the subsequent twelve treatment days. As can be seen, considerable variability occurred once pre-session water injections were given. This was confirmed by the general analysis of variance of the total water consumption of the last training and twelve treatment days. The analysis indicates that significant differences occurred over time [$F(12/348) = 4.56$, $p < .001$]. When each treatment day was compared with the last training day only two comparisons yielded significant differences. On treatment day 3 [$F(1/29) = 5.37$, $p < .05$] and treatment day 11 [$F(1/29) = 6.02$, $p < .05$] the water consumption was significantly decreased.

Day to day variability was assessed by comparing the water intake of each treatment day with the one immediately preceding it. Five of the twelve comparisons indicated significant differences between two consecutive days. As can be seen in Table 6.5. most of these significant differences occurred in the last part of the treatment period.

For each animal the difference between the highest and lowest volume intake during the twelve-day treatment was calculated and expressed as a percentage of the highest intake. On average the difference between maximal and minimal intake was 22.7 % (± 1.63) and varied between 10.81 and 60 %. The same procedure was applied to the last two training days, where a mean difference of only 7.31 % (± 0.80) with a range from 0 to 16.67 % was found. To illustrate the instability induced by water injections, the water intake during the last three training days (steady-state) and twelve treatment days was plotted for two individual animals in Figures 6.9. and 6.10.

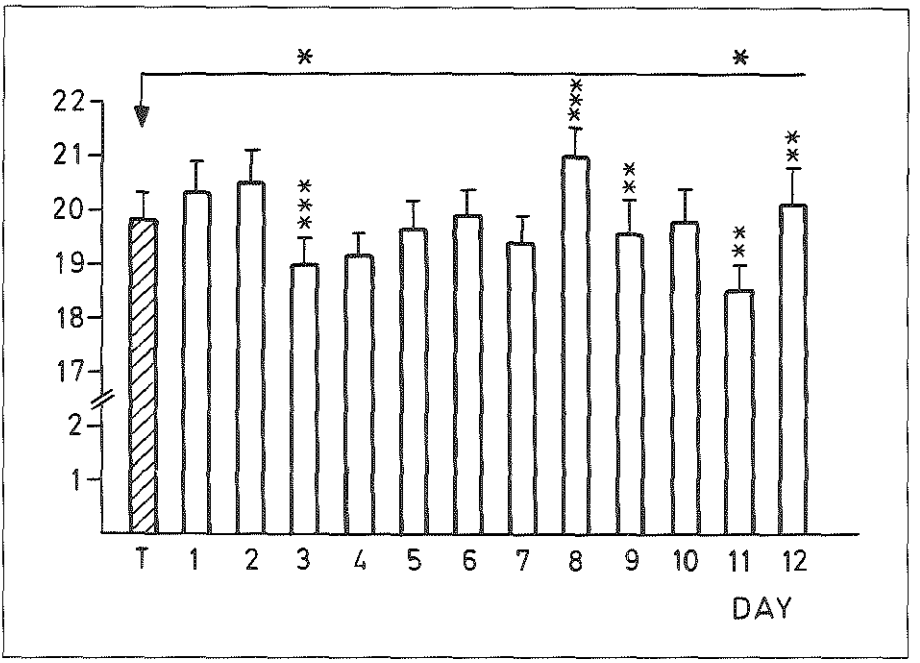

Figure 6.8. Mean (± S.E.M.) water consumption on the last training day (left shaded column) and after oral water injections (1 ml/100 g) 1 h before the drinking session on twelve consecutive treatment days. Significance of differences with last training day are indicated above the horizontal line. Significance of differences with the preceding day are indicated at the top of the columns.
* $p < .05$, ** $p < .01$, *** $p < .001$.

Table 6.5.
Mean water intake (± S.E.M.) on the last training day and twelve consecutive treatment days. Each day is compared with the preceding one.

	Mean water intake in ml ± S.E.M.	F-value df 1/29	Significance level
Training	19.85 (0.48)		
Treatment 1	20.35 (0.53)	1.56	n.s.
2	20.55 (0.56)	.11	n.s.
3	18.97 (0.51)	17.17	$p < .001$
4	19.18 (0.44)	.34	n.s.
5	19.68 (0.49)	2.08	n.s.
6	19.93 (0.50)	.30	n.s.
7	19.42 (0.54)	1.45	n.s.
8	21.00 (0.56)	17.65	$p < .001$
9	19.57 (0.56)	9.51	$p < .01$
10	19.85 (0.51)	.54	n.s.
11	18.57 (0.52)	8.67	$p < .01$
12	20.15 (0.62)	13.30	$p < .01$

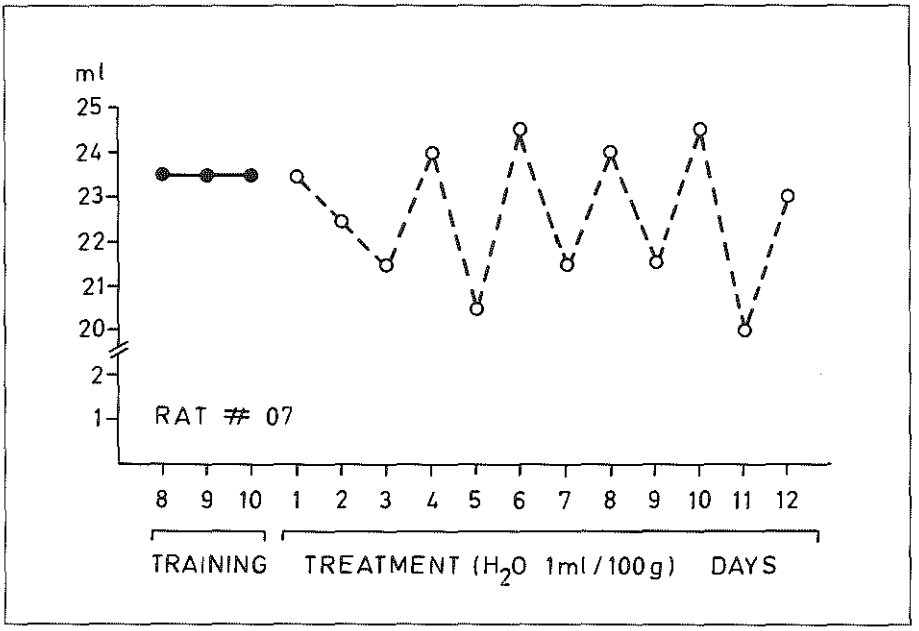

Figure 6.9. Total water consumption during the last three training days (full line) and after oral water injections (1 ml/100 g) 1 h before the drinking session on twelve consecutive treatment days (broken lines) for rat 07.

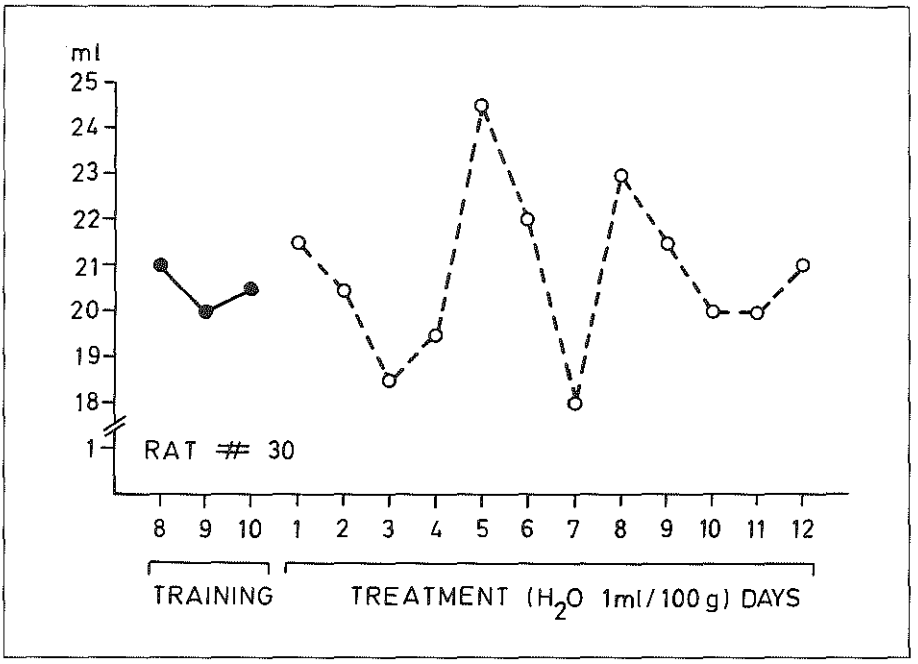

Figure 6.10. Total water consumption during the last three training days (full line) and after oral water injections (1 ml/100 g) 1 h before the drinking session on twelve consecutive treatment days (broken lines) for rat 30.

6.3.2.3. Conclusion

Although the mean water intake (measured for 30 animals) did only significantly decrease on two days when water was injected prior to the drinking session, the treatment primarily disrupted the steady-state obtained at the end of the training period. This is clearly illustrated by the day to day variability which increased with the length of the treatment period. In the last half of this period four out of six day to day comparisons indicated significant differences between the intake on subsequent days. The induced changes were not systematic and did not result in the development of a new steady-state. If this were the case one would expect a progressive decrease towards a new equilibrium in the ingested volumes. In fact, the opposite effect was observed, since the variability increased at the end of the treatment period.

The induced instability is also clearly illustrated by plotting the intake of individual animals. The speed and magnitude of the induced effect can fluctuate among animals (see Figures 6.9. and 6.10.). This fact can explain why the comparisons between the water intake during the treatment period and the intake on the last training day revealed only two significantly different points. The different onset and magnitude of the effect in the different animals will mask to a large extent the expression of the instability in mean values, especially when large numbers of animals are used as was the case in this experiment. In spite of this, the day to day comparisons provided clear evidence for the increasing disruption of the steady-state as a function of the duration of the treatment period.

The present results from the two previous experiments on pre-session water injections allow a number of general conclusions to be made with regard to their effect on steady-state drinking behavior after initial training on restricted schedules. Single water injections by different injection routes do not dramatically change the subsequent water consumption. However, if the same type of injection is given daily over a prolonged period of time (12 days), it gradually affects the water intake by rendering it increasingly unstable toward the later part of the treatment period. As such, a relatively slight interference with the water balance in deprived animals results in a disruption of the steady-state acquired after training. The onset and magnitude of the effects are variable and no systematic effects that point toward the development of a new steady-state were found within the treatment period used. A new steady-state may be obtained as was illustrated by Cooper (1979, 1982) but several months of training are required for that. This would of course make the model very uneconomical and time consuming.

Addendum 2

Table 1
Analysis of trend of water consumption during 2-min intervals on training day 1.

Source	SS	df	MS	F	% Main effect
Interval (A)	(622.93)	(14)			
Linear	358.57	1	358.57	655.83***	57.56
Quadratic	198.82	1	198.82	237.88***	31.92
Cubic	46.93	1	46.93	36.38***	7.53
Quartic	0.47	1	0.47	< 1	
Quintic	2.44	1	2.44	2.98	
Subjects (S)	10.47	29	.36		
A x S	(253.80)	(406)			
Linear	15.86	29	0.55		
Quadratic	24.42	29	0.84		
Cubic	37.34	29	1.29		
Quartic	27.21	29	0.94		
Quintic	23.72	29	0.82		
Total	(887.2)	(449)			

*** $p < .001$

Table 2
Analysis of trend of water consumption during 2-min intervals on training day 5.

Source	SS	df	MS	F	% Main effect
Interval (A)	(1078.38)	(14)			
Linear	709.05	1	709.05	105.62***	65.75
Quadratic	284.28	1	284.28	359.85***	26.36
Cubic	69.15	1	69.15	121.32***	6.41
Quartic	8.83	1	8.83	21.54***	0.82
Quintic	1.40	1	1.40	3.26	
Subjects (S)	11.21	29	.39		
A x S	(208.19)	(406)			
Linear	13.71	29	0.47		
Quadratic	22.81	29	0.79		
Cubic	16.19	29	0.57		
Quartic	11.91	29	0.41		
Quintic	12.52	29	0.43		
Total	(1297.78)	(449)			

*** $p < .001$

Table 3
Analysis of trend of water consumption during 2-min intervals on training day 10.

Source	SS	df	MS	F	% Main effect
Interval (A)	(1370.66)	(14)			
Linear	954.79	1	954.79	1591.31***	69.66
Quadratic	348.35	1	348.35	331.76***	25.41
Cubic	59.28	1	59.28	70.57***	4.32
Quartic	4.34	1	4.34	7.48*	0.32
Quintic	0.04	1	0.04	< 1	
Subjects (S)	10.37	29	.36		
A x S	(243.97)	(406)			
Linear	17.36	29	0.60		
Quadratic	30.51	29	1.05		
Cubic	24.52	29	0.84		
Quartic	16.81	29	0.58		
Quintic	17.66	29	0.61		
Total	(1625)	(449)			

*** $p < .001$; * $p < .05$

Table 4
Analysis of trend of water consumption during 5-min intervals on training day 1.

Source	SS	df	MS	F	% Main effect
Interval (A)	(1440.28)	(5)			
Linear	871.07	1	871.07	718.87***	60.48
Quadratic	455.18	1	455.18	302.97***	31.60
Cubic	106.96	1	106.96	63.29***	7.43
Quartic	7.06	1	7.06	8.51***	0.49
Quintic	.01	1	.01	< 1	
Subjects (S)	26.18	29	.90		
A x S	(166.68)	(145)			
Linear	35.14	29	1.21		
Quadratic	43.57	29	1.50		
Cubic	49.11	29	1.69		
Quartic	24.03	29	0.83		
Quintic	14.84	29	0.51		
Total	(1633.14)	(179)			

*** $p < .001$

Table 5
Analysis of trend of water consumption during 5-min intervals on training day 5.

Source	SS	df	MS	F	% Main effect
Interval (A)	(2397.18)	(5)			
Linear	1710.01	1	1710.01	1459.36***	71.33
Quadratic	592.91	1	592.91	359.40***	24.31
Cubic	102.78	1	102.78	99.19***	4.29
Quartic	1.14	1	1.14	1.64	
Quintic	.33	1	.33	< 1	
Subjects (S)	28.03	29	.97		
A x S	(185.23)	(145)			
Linear	33.98	29	1.17		
Quadratic	47.84	29	1.65		
Cubic	30.05	29	1.03		
Quartic	20.12	29	0.69		
Quintic	53.35	29	1.84		
Total	(2610.44)	(179)			

*** $p < .001$

Table 6
Analysis of trend of water consumption during 5-min intervals on training day 10.

Source	SS	df	MS	F	% Main effect
Interval (A)	(3110.18)	(5)			
Linear	2310.00	1	2310.00	1760.22***	74.27
Quadratic	707.23	1	707.23	313.58***	22.74
Cubic	90.74	1	90.74	64.81***	2.92
Quartic	2.20	1	2.20	2.74	
Quintic	.00	1	.00	< 1	
Subjects (S)	25.93	29	.89		
A x S	(202.53)	(145)			
Linear	38.06	29	1.31		
Quadratic	65.40	29	2.26		
Cubic	40.68	29	1.40		
Quartic	23.25	29	0.80		
Quintic	35.14	29	1.21		
Total	(3338.64)	(179)			

*** $p < .001$

Chapter 7
Effects of Hypoxia on Drinking Behavior

7.1. Introduction

The literature (cited in 6.1.) on the influence of hypoxia on ingestive behavior focused on the decreased intake during exposure to either hypobaric or normobaric hypoxia. Dogterom (1983) showed in the rabbit that drinking behavior was also attenuated for at least forty minutes after exposure to hypobaric hypoxia (10 minutes at 190 mm.Hg). Two main effects were seen. Initiation of drinking was postponed and water was ingested at a lower rate compared to drinking sessions which were not preceded by exposure to hypoxia. These experiments clearly indicated that drinking behavior in the post-hypoxic period was depressed. Therefore, it can be used to study the recovery of motivated goal-oriented behavior after exposure to hypoxia.

In this chapter we will describe the effects of normobaric hypoxia on drinking behavior in the rat. Exposure to normobaric hypoxia was chosen in preference to hypobaric hypoxia at atmospheric pressures under 200 mm.Hg because survival is very variable in rats when they are exposed to such extreme conditions. In addition, possible confounding effects due to rapid de- and recompression could be avoided by using normobaric hypoxia.

7.2. Materials and methods

Thirty male Wistar rats trained for 10 days on a restricted drinking time schedule (see 6.2.1.2.) were used. The experiment lasted for twelve days. Hypoxia (H) days alternated with non-hypoxia (NH) days. On NH days treatment conditions were the same as during the training period. Day 1, 3, 5, 7, 9 and 11 were H days and day 2, 4, 6, 8, 10 and 12 were NH days.

On H days the animals were exposed to a hypoxic environment just before the onset of the drinking session. Normobaric hypoxia was induced by exposing the animals for 15 minutes to an O_2/N_2 gas mixture containing 4.3 $\pm$ 0.1 % oxygen. The rats were placed in groups of two in a perspex cylinder (Ø 30 cm, height 15 cm) with separate compartments (one animal in each compartment). The cylinder was continuously flushed with the gas mixture. The composition of the air in the cylinder was permanently monitored and analysed by a Sybron/Taylor Servomex O_2 analyser OA570. Drinking sessions always started immediately after the 15-minute exposure.

The consumed water volume was measured after each 2 and 5-min intervals during the drinking session. In addition the onset of drinking (consumption of at least 0.5 ml) behavior was measured (in sec). Analysis of variance with repeated measures was used for all single and multiple comparisons. Analysis of trend was based on the method of orthogonal polynomials but restricted to the data of the 5-min interval measurements.

7.3. Results

7.3.1. Survival

Three (10 %) animals died during the first exposure to hypoxia. Post-mortem examination indicated that all three animals had marked traces of lung emphysema. Consequently, this condition probably enhanced the level of hypoxia by creating an imbalance between ventilation and perfusion. Therefore these animals died from cardiovascular collapse which was probably due to cor pulmonale.

7.3.2. Onset of drinking on H days

Onset of drinking during training and NH days occurred within two seconds. Figure 7.1. depicts the mean onset times after exposure to normobaric hypoxia on all H days. On the first H day initiation of drinking was delayed for 640.59 ± 48.94 sec. This delay then gradually decreased on the next H days to 283.70 ± 22.54 sec on H6 day. General analysis on the onset of times of the 6 H days showed a significant decrease in onset times over time [$F(5/130) = 29.73$, $p < .001$]. The greatest decrease was observed between H1 day and H2 day. It further significantly [$F(1/26) = 6.48$, $p < .05$] decreased between H2 and H3 day but then stabilized. No significant differences could be found between the onset times on H3 day and the subsequent H days.

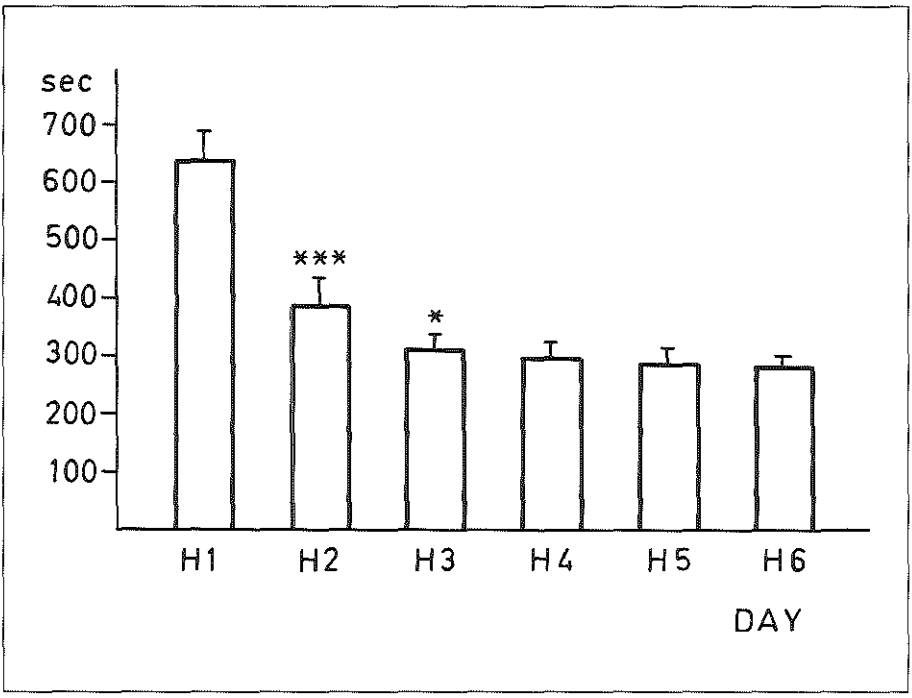

Figure 7.1. Mean (± S.E.M.) onset times (n = 27) of adaptive drinking behavior on subsequent H days. Significance of difference calculated against the preceding H day.
*** $p < .001$; * $p < .05$

7.3.3. Water consumption on H and NH days

Figure 7.2. gives a general overview of the total water consumption on the last training day and the consecutive H and NH days. A general analysis of the intake on the last training day and the six H days indicated significant differences [$F(6/156) = 142.71$, $p < .001$] in water consumption. Individual comparisons between each H day and the last training day and between each H day and its preceding NH day indicated significantly (all at $p < .001$) reduced water ingestion after exposure to normobaric hypoxia.

A general analysis of consumption on the last training day and the six NH days revealed the existence of slightly significant differences [$F(6/156) = 2.52$, $p < .05$]. However, individual comparisons between the last training day and each NH day were not significant except for the NH day between the fourth and fifth H day where a small but significant difference [$F(1/26) = 5.54$, $p < .05$] was found. This means that in general no rebound effects were seen on the days following H days. Apparently no compensation occurred for the reduced intake on H days.

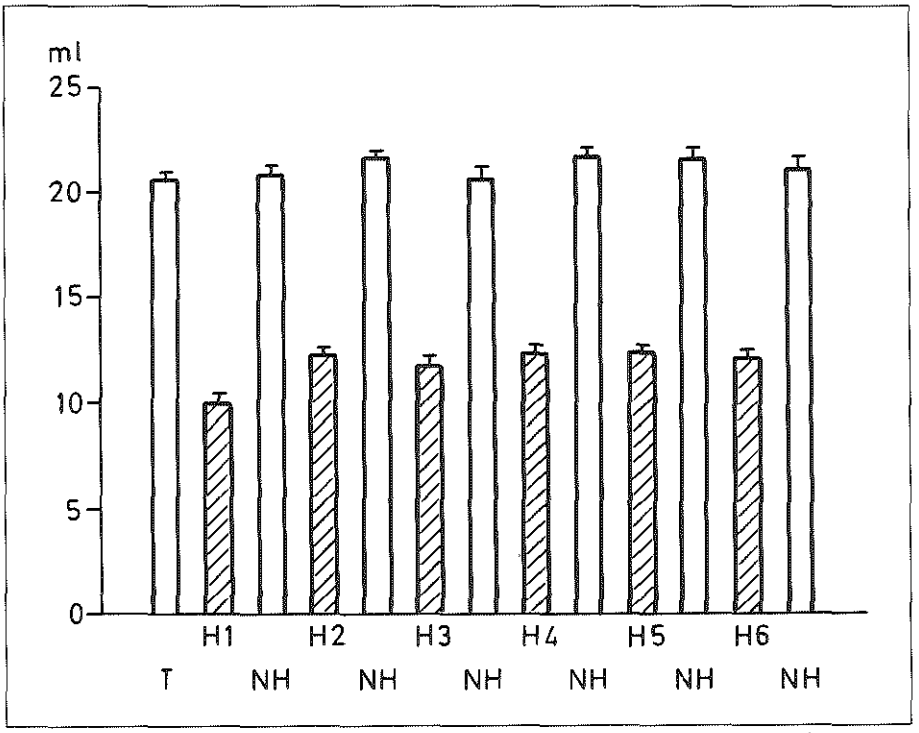

Figure 7.2. Mean (± S.E.M.) water consumption on the last training day (T) on hypoxia (H1 to H6) and on non-hypoxia (NH) days. The consumed volume was significantly lower ($p < .001$) on each H day as compared to its preceding NH day.

The reduction in water intake was the largest on the first H day. It decreased from 20.35 ± 0.42 ml on the last training day to 9.96 ± 0.41 ml on the drinking session that followed the first exposure to normobaric hypoxia. Equivalent to what was seen for the onset of drinking, an adaptation phenomenon was observed on the second day. The intake was significantly [$F(1/26) = 40.85$, $p < .001$] higher on the second H day as compared to the intake on the first H day. From the second H day on, water intake during the remaining H days stabilized around 12 ml since no significant differences could be found between the intake on the second H day and those on the subsequent H days (see Fig. 7.3.).

The delay in onset time on the first H day decreased with 50 % on the subsequent H days whereas the total water consumption only increased with 20 %. This suggests that apart from the effect on initiation there was a considerable effect on inter-draft pauses and/or ingested volume during each draft on H days.

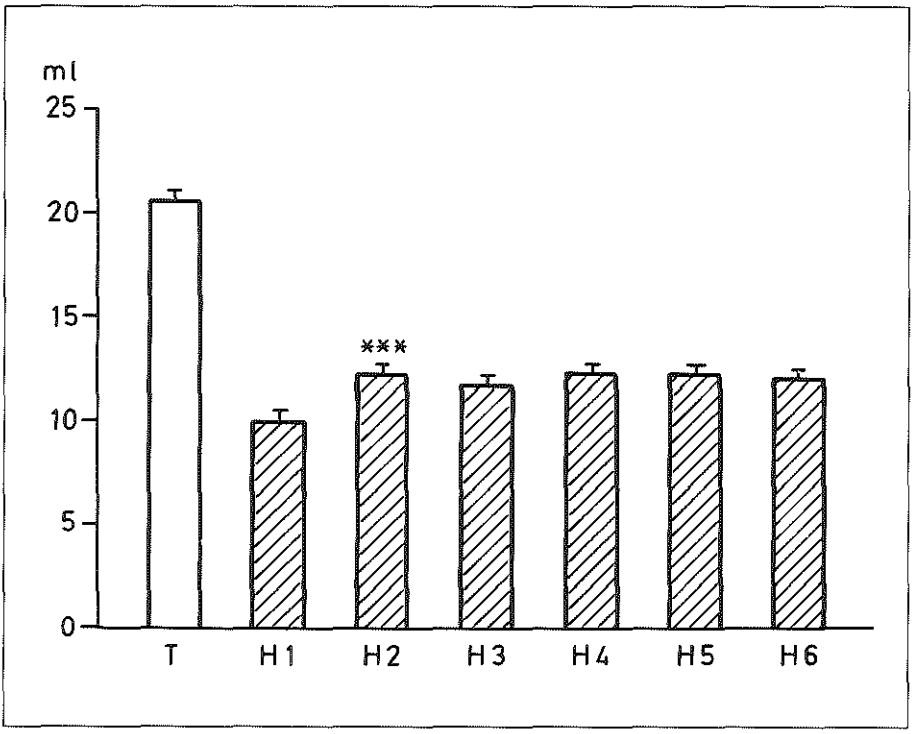

Figure 7.3. Mean (± S.E.M.) water consumption on the last training day (T) and on consecutive hypoxia days. Significance of difference calculated against the preceding H day.
*** $p < .001$

7.3.4. Drinking pattern

From the previous results it was evident that hypoxia reduced the water consumption and delayed the initiation of drinking in the post-hypoxic period. In addition the animals adapted to a certain degree to the hypoxic condition since the effects were clearly reduced after the second exposure to the hypoxic environment. A relevant question to be answered was whether or not these effects were related to the drinking pattern that the animals employed after being exposed to hypoxia.

Figure 7.4. clearly shows that the drinking pattern of an individual animal was completely different after it had been exposed to hypoxia in comparison to the pattern seen on training and NH days. Once drinking has been initiated on H days the animals started to drink in bursts and ingested only small volumes during the consecutive time intervals. When drinking commenced on H days it always did with a short draft. On NH days it did with the longest draft of the session. After repeated exposures an adaptation effect was only seen on the onset of drinking. The dynamics of drinking behavior after hypoxia were not changed.

The cumulative drinking curves represented in Fig. 7.5. are a perfect illustration that the increase in total water consumption between the first and the second H day was related to a decreased onset time. This allowed the animals to drink small amounts of water during a longer time period.

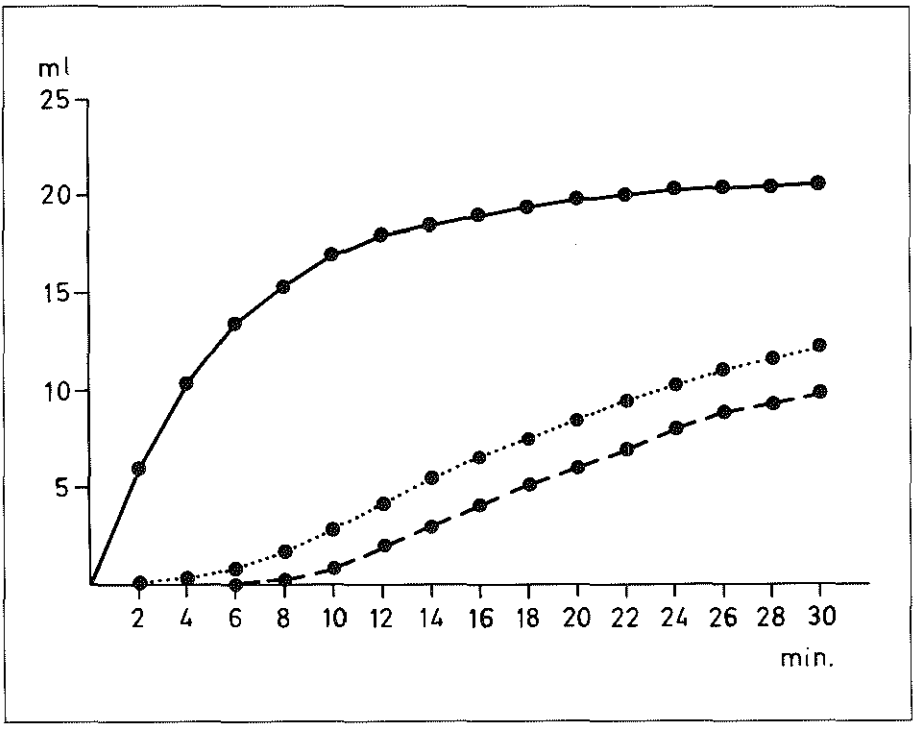

Figure 7.5. Cumulative water intake curves (n = 27) on the last training day (●——●), first hypoxia day (●– –●) and second hypoxia day (●• • • •●).

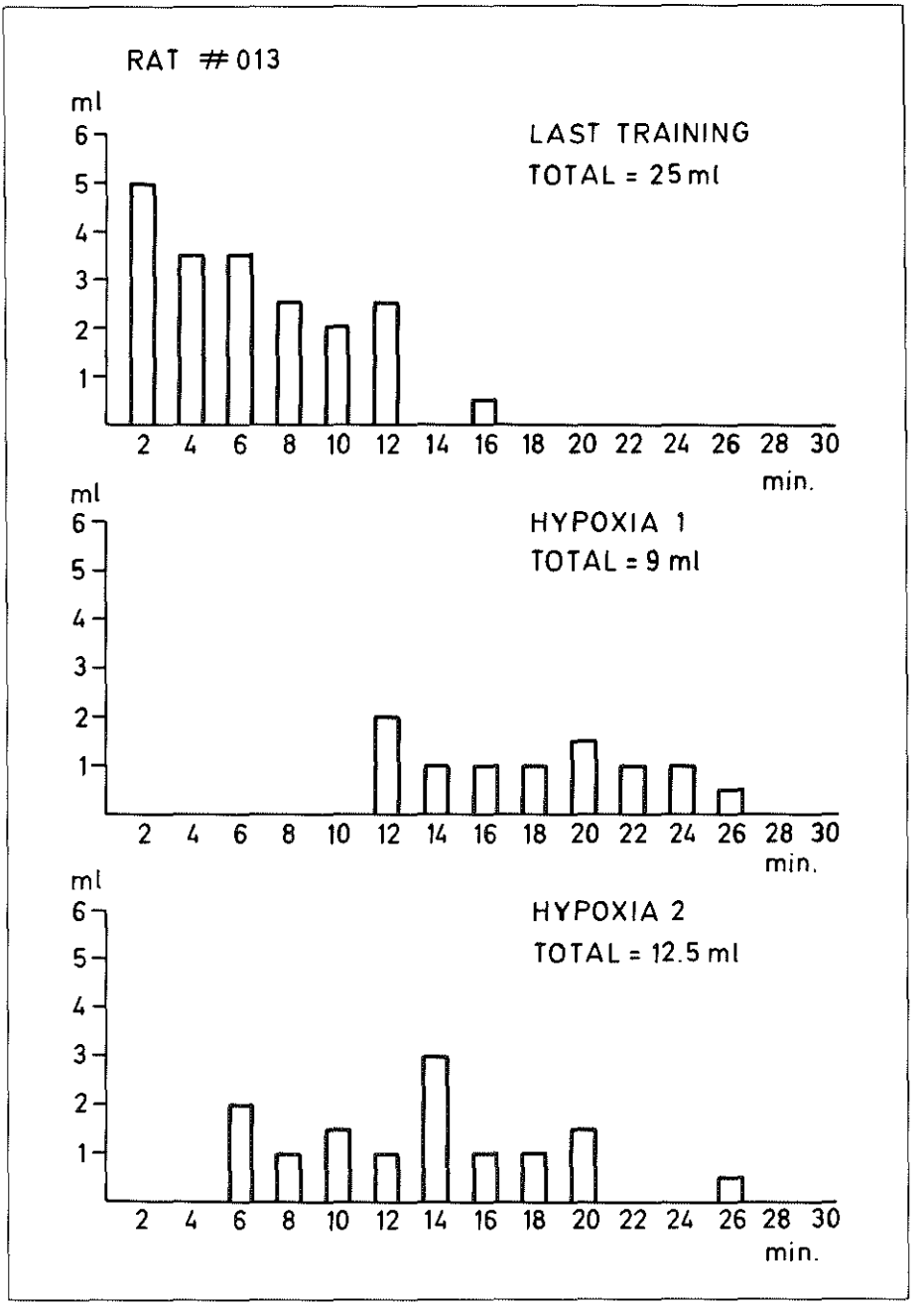

Figure 7.4. Water intake of rat 013 during 2-min intervals on the last training day, H1 day and H2 day.

The curves of H1 and H2 parallel each other perfectly. This is an indication that the typical drinking pattern following hypoxia was not changed due to a kind of adaptation. The typical satiation curves which are seen when intake is averaged over all subjects on NH days were not seen after exposure to hypoxia. The new pattern on H days resulted in a curve with gradually increasing consumption after initiation which then plateaued. This pattern reflects the almost continuous small intake during the time intervals (Fig. 7.6.).

Hypoxia induced a shift in the drinking pattern. This pattern was stable over the repeated hypoxia sessions and was not changed by adaptive processes. These observations could be confirmed by trend analysis on the mean water intake on successive five-minute intervals for hypoxia days 1, 2 and 6. Table 7.1. summarizes the results of these analyses (detailed results of the calculations are presented in addendum 3). It is obvious that the major component is no longer linear as it was for the trends on the training days (see Table 6.4.). After exposure to hypoxia the major trend was quadratic. This pattern is stable for all the hypoxic days. These results confirm that a different but stable drinking pattern was employed by animals which had been exposed to normobaric hypoxia as described previously.

Table 7.1.
Analysis of trend: % of the main effect accounted for by the major independent components.

Components	Hypoxia days		
	H1	H2	H6
Linear	38.46	4.12	4.5
Quadratic	55.66	86.79	69.01
Cubic	—	8.89	25.50
Quartic	—	—	—
Quintic	5.11	—	—

7.4. Conclusions

Like with hypobaric hypoxia (Dogterom, 1983), exposure to normobaric hypoxia had pronounced effects on adaptive drinking behavior in the post-hypoxic period. The initiation of drinking was delayed and the water consumption was significantly reduced despite the fact that the animals had sufficient time left to consume normal volumes. The reduced intake was due to a change of drinking pattern in post-hypoxic periods. All animals consumed smaller volumes of water than normal and the intake was spread over an extended number of time intervals.

The effects on the initiation phase were probably a reflection of CNS suppression shortly after hypoxia. Some recuperation seemed to be required before water deficit signals were properly integrated with external cues and drinking was initiated. This recuperation was influenced by an adaptation process which occurred between the first and second exposure to hypoxia.

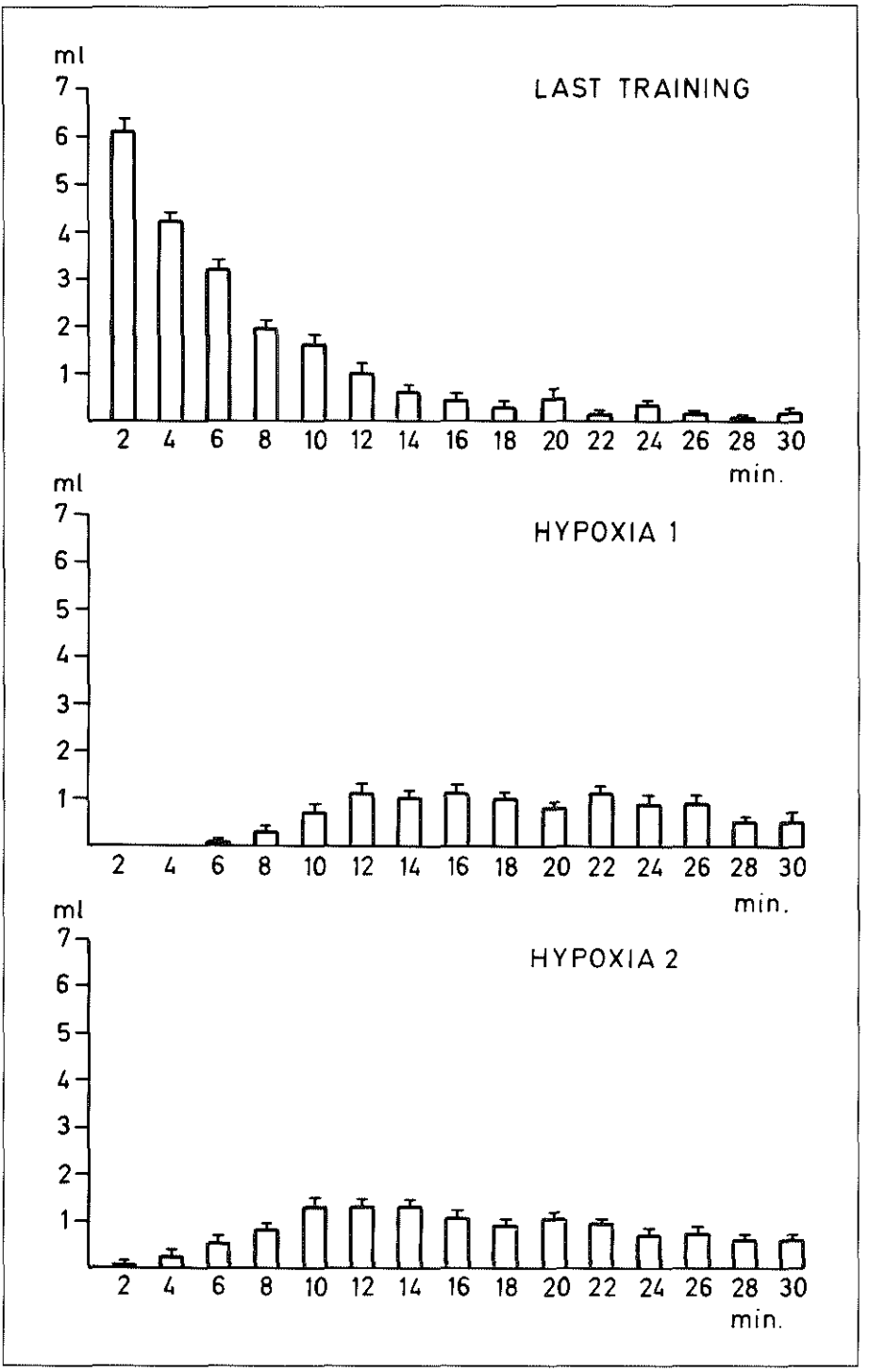

Figure 7.6. Mean (± S.E.M.) water consumption (n = 27) during 2-min intervals on the last training day, the first hypoxia day and the second hypoxia day.

Recuperation of CNS integrative functions was faster after the second exposure as indicated by the decreased latency to drink.

The nature of the adaptive process is unclear. It is probably not equivalent to the acclimatization to high altitude because the exposure time was relatively short and the adaptation did not progress after the second exposure. It could be that stress was greater during the first exposure because the animals were not familiar with the new condition. Based upon observation one can say that the panting which was seen during hypoxia was not so pronounced during the second and following exposures. Thus, the animals may have learned how to cope with a stressful situation. No direct evidence for this was available in our experiments, but the hypothesis can be tested. If the adaptation is a result of the disappearance of a novelty effect, it should be absent in animals that have experienced exposure to hypoxia before the effects on drinking are studied.

The increase of total water consumption after the second exposure was related to the faster onset since the animals had more time available to drink. During that period they constantly consumed small amounts of water and consequently the ingested volume increased. Although the post-hypoxic drinking pattern was not changed after multiple exposure, the increased water consumption could have been partly induced by slight changes in some dynamic aspects of drinking behavior. However, our measurement of the consumption during fixed time intervals was not suitable to evaluate this. Therefore, it might be better in the future to measure the number and duration of drafts, the intervals between drafts, the number of licks and the volume ingested during each draft. These parameters are more suitable to study dynamic aspects of drinking.

The drinking pattern after hypoxia suggests that the consummatory phase of drinking remained affected although the integrative function of the CNS was restored and general locomotor activity appeared to be normal. The effects on the drinking pattern were not changed by an adaptive process. With regard to the effects of hypoxia on drinking pattern there is a remarkable resemblance with the effects of acute hypoxia (hypobaric and normobaric) on feeding described by Ettinger and Staddon (1982). In their animals, feeding regulation was not affected but the feeding rate was seriously decreased during exposure to hypoxia. This means that hypoxia decreased food consumption primarily by reducing meal size. They attributed these results to an effect on "incentive" rather than to a change in "hunger" or "appetite". In other words they suggested that hypoxia mainly exerted its effects by degrading the taste of food. Such phenomena have been reported in humans by Pugh (1962). Although it is conceivable that taste is altered, sensorimotor coordination can also be an important factor. Prolonged effects on oral motor responses can occur if there is differential recovery time in different brain structures after hypoxia. A limbic structure like the amygdala is implicated in food and water intake (Fonberg, 1969) but also in oral motor responses (Hockman *et al.*, 1979). If such a structure is more sensitive to hypoxia or requires longer recuperation time it can be at the origin of the changed drinking pattern following hypoxia.
The suggestion that oral motor responses are involved is strengthened by the observation that the animals were not able to drink for extended periods of

time which they could under normal conditions. Drinking after hypoxia occurred in short burst and the rats were apparently forced to interrupt drinking. Given these clearcut and stable effects on adaptive drinking one may conclude that the same experimental paradigm might be used to study the pharmacological protection against the effects of hypoxia on CNS mediated behavior.

Two main aspects are determinant for the utility of post-hypoxic drinking behavior as a possible method for the study of protective action of drugs against hypoxia. The first deals with the possibility of inducing a steady-state performance in adaptive drinking after the induction of hypoxic conditions. The second is related to the specific necessity of injecting fluids which serve as vehicles for the pharmacological substances. This aspect is particularly relevant because it is related to a direct intervention on a physiological balance (level of dehydration) that controls the dependent variable (water consumption) under study.

The experimental evidence presented in this chapter can be used to evaluate the first aspect. As discussed earlier, normobaric hypoxia has two major effects upon subsequent adaptive drinking. Hypoxia delays the initiation of drinking and induces a specific drinking pattern that does not resemble the one observed when drinking is not preceded by hypoxia. Upon repeated application of hypoxia it was found that some adaptation occurs between first and second exposure with respect to the onset of drinking. This resulted in an increased consumption after the second exposure. However, no further improvements were found after the subsequent hypoxia sessions. This indicates that from the second exposure, hypoxia induces a steady-state with respect to its effects on initiation and global water consumption. As far as the drinking pattern is concerned, no adaptation was found after the successive hypoxia exposures. The typical post-hypoxic drinking pattern did not change after repeated exposures. We may conclude that certainly from the second exposure on, hypoxic effects on initiation of drinking, global water intake and drinking pattern are stabilized and that post-hypoxic water intake has reached a steady-state. As a consequence, the first criterion is met to consider post-hypoxic drinking as a suitable method for the investigation of protective drug effects. The obtained effects after exposure to hypoxia appear to be stable as well as reliable over time.

The experimental evidence presented in chapter 6 can be used to evaluate the second aspect. First, we have to differentiate between the different types of pharmacological studies in which drinking behavior can be used. A first line of research might investigate acute protective effects by single injections of drugs. A second approach might investigate the possible beneficial effect of pharmacological substances on adaptation to hypoxic effects. This type of research requires chronic treatment by means of daily injections of the drugs under study. For the evaluation of the protective potential of single injections, post-hypoxic drinking behavior seems to be a suitable method. As was shown in chapter 6, single vehicle injections given through different routes do not dramatically change the subsequent water intake. Apparently they do not attenuate the obtained steady-state and as such the second requirement for

application is met. This conclusion is no longer valid when one considers to use the same trained animals for multiple application of single injections on subsequent days. On economical grounds it would be attractive to start pharmacological treatment after the second exposure when a steady-state is reached. Each following exposure could then be preceded by the injection of a different drug or a different dose of the same drug. As a consequence one would enter the scheme of multiple injections that is also needed for chronic treatment to study possible effects on adaptation. This type of application however is fraught with pitfalls if one considers the effects of multiple water injections described in chapter 6. Apparently multiple injections disrupt the steady-state and render the water intake unstable. This effect seems to increase along with an increasing number of injections. Since the onset and the pace of the destabilization is to some extent different for individual animals, the effect was somewhat masked when the mean water consumption was calculated for a large group of animals. One might expect that the destabilization will be much more pronounced in the results when smaller groups are used (in pharmacological experiments 5 to 10 animals for each drug dose). The simple fact that multiple injections might increase or decrease water consumption by an average of 20 % in the course of a treatment period illustrates the complexity of interpretations of apparent drug effect results. A possible protective effect can be neutralized by the fact that the injection day coincides with a period where the water consumption is in a downward trend due to the multiple injections over days. It is of course also conceivable that the opposite situation occurs. One could easily get the impression that a non-active drug or dose of a drug protects to some extent against the effects induced by hypoxia simply because the dose is injected on a day where the water consumption is in an upward trend. Such a situation will undoubtly lead to both false positive and negative effects. From the points discussed so far we can conclude that adaptive drinking behavior is not a suitable technique to evaluate the protective effects of single drug injections when the same animals are used more than once. The same conclusions hold for experiments involving chronic pharmacological treatment to study potential increased adaptation due to drug treatment.

Adaptive drinking can be used to evaluate protective effects of drugs against hypoxic effects as long as the animals are used only once and receive a single drug injection. However, even under these circumstances some problems can arise with respect to the interpretation of the data. The drugs that are used can influence the water consumption, directly or indirectly, through specific effects on physical factors that determine to some extent the degree of fluid consumption (for example by inducing aversion or taste alterations). This would mean that independent experiments have to be done to determine the proper effects of the experimental compounds on drinking behavior before the drugs can be used to study protective effects against hypoxia.

Although adaptive drinking proved to be a very suitable method to demonstrate the prolonged effects of hypoxia on functional behavior, it appears to have only limited value for pharmacological research. The problems originating from injecting fluids in combination with the long training periods that are needed are negative aspects in pharmacological research where large numbers of compounds and dose-response relations have to be studied.

Addendum 3

Table 1
Analysis of trend on water consumption during 5-min intervals on hypoxia day 1.

Source	SS	df	MS	F	% Main effect
Interval (A)	(132.20)	(5)			
Linear	50.85	1	50.85	41.52***	38.46
Quadratic	73.58	1	73.58	56.73***	55.66
Cubic	.01	1	.01	< 1	
Quartic	1.00	1	1.00	< 1	
Quintic	6.76	1	6.76	7.20**	5.11
Subjects (S)	19.91	26	.77		
A x S	(195.72)	(130)			
Linear	31.84	26	1.22		
Quadratic	33.72	26	1.30		
Cubic	63.14	26	2.42		
Quartic	48.37	26	1.16		
Quintic	24.41	26	0.94		
Total	(847.83)	(161)			

*** $p < .001$; ** $p < .01$

Table 2
Analysis of trend on water consumption during 5-min intervals on hypoxia day 2.

Source	SS	df	MS	F	% Main effect
Interval (A)	(105.17)	(5)			
Linear	4.33	1	4.33	1.48	4.12
Quadratic	91.28	1	91.28	60.85***	86.79
Cubic	9.34	1	9.34	4.89*	8.89
Quartic	.06	1	.06	< 1	
Quintic	.16	1	.16	< 1	
Subjects (S)	22.53	26	.87		
A x S	(201.78)	(130)			
Linear	76.22	26	2.93		
Quadratic	39.07	26	1.50		
Cubic	49.68	26	1.91		
Quartic	18.67	26	0.72		
Quintic	18.15	26	0.70		
Total	(329.48)	(161)			

*** $p < .001$; * $p < .05$

Table 3
Analysis of trend on water consumption during 5-min intervals on hypoxia day 6.

Source	SS	df	MS	F	% Main effect
Interval (A)	(220.29)	(5)			
Linear	10.00	1	10.00	17.54***	4.56
Quadratic	152.19	1	152.19	122.73***	69.01
Cubic	56.17	1	56.17	43.54***	25.50
Quartic	0.10	1	0.10	< 1	
Quintic	1.83	1	1.83	2.48	
Subjects (S)	19.50	26	.75		
A x S	(147.92)	(130)			
Linear	14.70	26	0.57		
Quadratic	32.14	26	1.24		
Cubic	33.52	26	1.29		
Quartic	48.41	26	1.86		
Quintic	19.15	26	0.74		
Total	(387.71)	(161)			

*** $p < .001$

Chapter 8
Protective Effects of Flunarizine versus Verapamil on Water Intake Attenuated by Normobaric Hypoxia

In this chapter an example is given of the possibility to use adaptive drinking in pharmacological research. The text is a slightly revised copy of a publication (Clincke, G.H.C. and Wauquier, A., Protective effects of flunarizine versus verapamil on water intake attenuated by normobaric hypoxia. In: Wauquier, A., Borgers, M. and Amery, W.K.: Protection of tissues against hypoxia. Elsevier Biomedical Press, Amsterdam, 1982, pp. 287-190). The animals in this paper were used only once and received a single drug injection. Both drugs flunarizine and verapamil had no effect on the water consumption at the highest (10 mg/kg) dose tested.

8.1. Introduction

Although full and fast recovery of adaptive behavioral responses is essential for the quality of post-hypoxic life, animal models for the study of pharmacological protection against hypoxia have mainly focused on neurological outcome and prolongation of survival (1).

Drinking is an important adaptive behavioral response that participates in the regulation of body water content. The fact that all levels of the central nervous system (CNS) are involved in the initiation and execution of this behavior (2) makes it very suitable for the study of pharmacological protection against hypoxic effects on CNS mediated behavior.

Calcium plays a major role in hypoxia-induced cell damage (3, 4) and calcium antagonists have been shown to be active in a variety of models (5) and to protect against cellular damage caused by ischemic hypoxia (6). Hence, it is of interest to know whether they are able to improve post-hypoxic suppression of functional behavior under CNS control.

The effects of a single normobaric hypoxia on water intake as well as the protective effects of the Ca^{++}-entry blocker flunarizine and the reference calcium antagonist verapamil are described.

8.2. Materials and methods

Fifty six adult male Wistar rats (250 $\pm$ 10 g) were trained during 10 days to obtain water during daily 30-min sessions. The rats were transferred from their home cage to a drinking cage where they had access to a drinking tube filled with tap water. The drinking tube consisted of a glass burette graded to the nearest ml fitted with a nipple which protruded through the roof of the cage.

The animals were deprived of water with food available *ad libitum* except during the drinking sessions.

On the last training day the ingested water volume of each rat was measured each two minutes during the 30-min session. These data were taken as control values (drinking not preceded by hypoxia).

Experimental manipulations were carried out on the subsequent day. Groups of 8 rats were randomly assigned to a treatment condition and orally injected with either saline (1 ml/100 g, -1 h),flunarizine (.63, 2.5 or 10 mg/kg, -4 h) or verapamil (.63, 2.5 or 10 mg/kg, - 1 h) before exposure to a normobaric hypoxic environment (4.3 % O_2) for 15 minutes. The animals were transferred to the drinking cage immediately after the end of hypoxia and the onset of drinking as well as the water intake was measured.

8.3. Results

Hypoxia delayed the onset of drinking in saline treated animals for 822.5 ± 78.3 sec (Fig. 8.1.) and reduced the total ingested water volume to 41 ± 2.2 % (Fig. 8.2.) of the intake during the control session of the preceding day. The reduced intake was mainly due to a decrease in water consumption once drinking was initiated (Fig. 8.3.).

Verapamil was not active against the attenuated water intake. At the highest dose (10 mg/kg) tested, 3 out of 8 animals treated with verapamil died during hypoxia which possibly might be due to cardio-respiratory insufficiency.

Flunarizine significantly and dose-relatedly decreased the onset times (Fig. 8.1.) and increased the water consumption (Fig. 8.2.) as compared to saline treated animals. The latter effect, however, was due to a decreased time to initiate drinking and not to an augmentation of the drinking rate (Fig. 8.3.). The cumulative intake curve after flunarizine treatment parallels the curve after saline treatment and does not resemble the curve of non-hypoxic control days. This suggests that the dynamics of drinking behavior did not normalize although a significant improvement of initiation was seen.

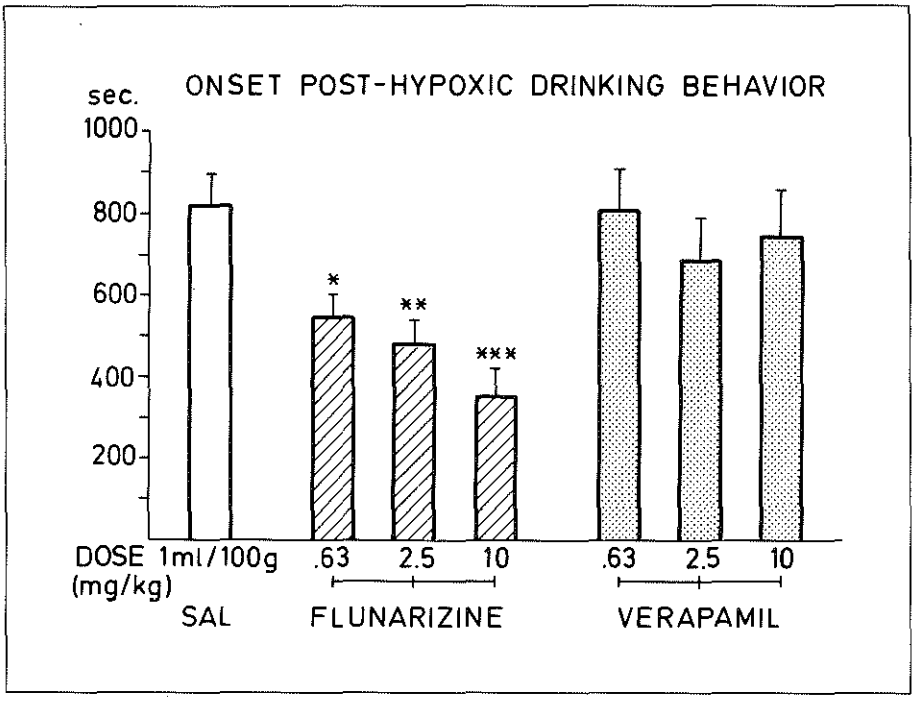

Figure 8.1. Mean (± S.E.M.) onset times of post-hypoxic drinking after different pretreatment conditions. For each dose level n = 8 except for verapamil (10 mg/kg) where n = 5. Significance of differences with saline treatment calculated with student t-test (two-tailed).
* $p < .05$; ** $p < .01$; *** $p < .001$.

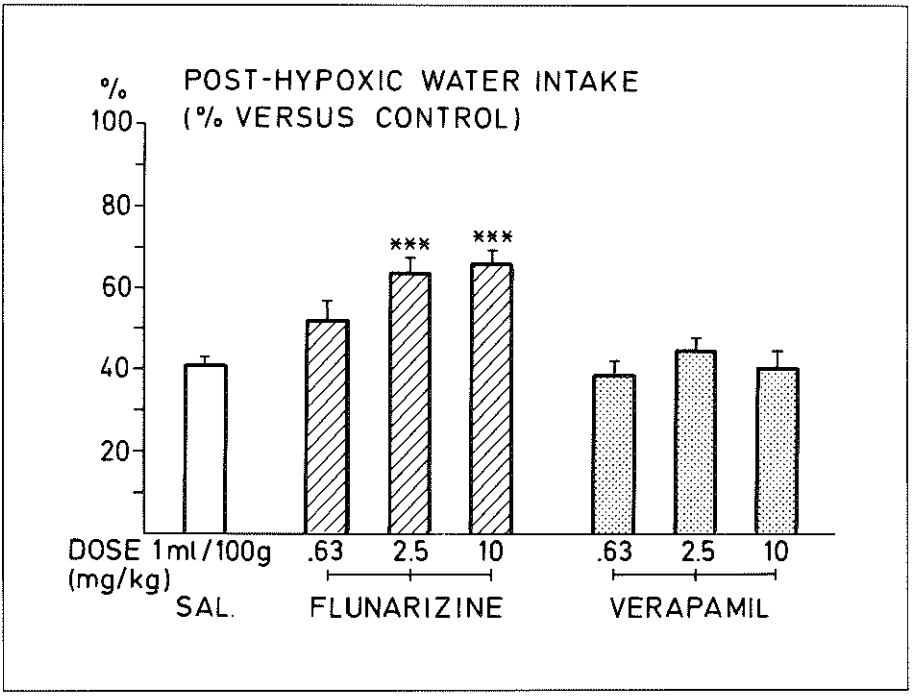

Figure 8.2. Mean (± S.E.M.) % water consumption versus control after different pretreatment conditions. For each dose level n = 8 except for verapamil (10 mg/kg) where n = 5. Significance of differences with saline treatment calculated with student t-test (two-tailed).
*** $p < .01$.

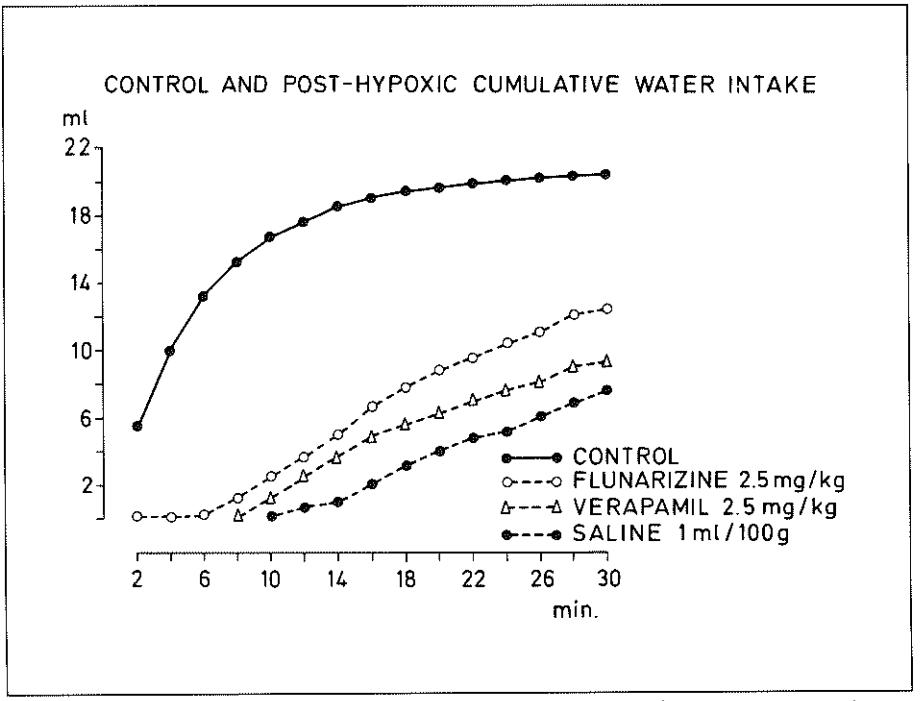

Figure 8.3. Cumulative (ml) water intake on a non-hypoxic control day (n = 56) and after hypoxia under different pretreatment conditions (n = 8 for each dose level).

8.4. Discussion

Normobaric hypoxia has differential effects on the successive phases of adaptive drinking behavior. Initiation is delayed and the consummatory phase remains attenuated once drinking has been initiated (Fig. 8.3.). Consumption itself is not exclusively dependent on central integrative mechanisms. Consumption rate and volume are determined to a large extent by multiple factors such as oral motor responses, oral stimuli and physical characteristics of the ingested fluid (2, 7, 8). Therefore it is conceivable that hypoxic effects might influence consumption through one of these mechanisms long after the CNS has sufficiently recovered to be able to detect water deficit signals and to integrate visceral and sensorimotor systems necessary for response initiation.

Although verapamil has been reported to be beneficial in ischemic heart disease (9) it fails to improve post-hypoxic recovery of adaptive behavior under CNS control. The inactivity in the present model and in basic hypoxia tests (5) may be a consequence of its low capacity to penetrate the brain.

Flunarizine has been reported to prolong survival in a variety of basic models and its protective effects against spreading depression (5, 10) and cellular damage caused by ischemic hypoxia (6) suggest a direct action at the neuronal level. The present results seem to be in line with this hypothesis. Flunarizine facilitates post-hypoxic recovery by decreasing onset times for post-hypoxic drinking but does not antagonize the effects on the consummatory phase. The latter is clearly illustrated (Fig. 8.3.) by the fact that the water intake curve after flunarizine has the same linear shape as after saline treatment. The protective effects of flunarizine seem to be located at the central level since it antagonizes the hypoxic effects on those phases of the drinking response which are exclusively under CNS control. These drug results confirm that the hypoxic effects on consumption are not due to a CNS suppression. However, further research will be needed to elucidate the exact origin of these effects.

In conclusion, although Ca^{++}-entry blockers do not by any means antagonize all hypoxic effects on adaptive drinking, they seem to preserve the brain in a condition which allows faster recovery of CNS mediated behavior provided the drug can penetrate the brain.

8.5. Acknowledgements

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8.6. References

1. Wauquier, A., Clincke, G., Ashton, D. and Van Reempts, J. (1981) In: van Hof, M.W. and Mohn, G. (Eds.), Functional Recovery from Brain Damage, Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 95-114.

2. Swanson, L.W. and Mogenson, G.J. (1981) Brain Res. Rev., 3, 1-34.

3. Farber, J.L. (1981) Life Sci., 29, 1289-1295.

4. Siesjö, B.K. (1981) J. Cer. Blood Flow Metab., 1, 155-185.

5. Wauquier, A. (1982) In: Godfraind, T., Herman, A. and Wellens, D. (Eds.), Calcium Entry Blockers in Vascular and Cerebral Dysfunctions, Westminster Publ., New York, in press.

6. Van Reempts, J., Borgers, M., Van Dael, L., Van Eyndhoven, J. and Van de Ven, M. (1982) Arch. Int. Pharmacodyn. Ther., in press.

7. Gold, R.M. and Laforge, R.G. (1977) In: Weijnen, J.A.W.M. and Mendelson, J. (Eds.), Drinking Behavior, Plenum Press, New York, pp. 247-274.

8. Mook, D.G. and Kenny, N.J. (1977) In: Weijnen, J.A.W.M. and Mendelson, J. (Eds.), Drinking Behavior, Plenum Press, New York, pp. 275-315.

9. Ferlinz, J. (1981) In: Zanchetti, A. and Krikler, D.M. (Eds.), Calcium Antagonism in Cardiovascular Therapy, Excerpta Medica, Amsterdam, pp. 92-105.

10. Wauquier, A., Ashton, D., Clincke, G. and Van Reempts, J. (1982) In: Clifford-Rose, F. and Amery, W.K. (Eds.), Cerebral Hypoxia in the Pathogenesis of Migraine, Pitman Press, Bath, pp. 139-154.

Discussion

The effects of normobaric and hypobaric hypoxia on different types of functional behavior were studied. In all paradigms a clearcut steady state effect was reached but differences in both the time course and the magnitude of the induced effects were observed. Different induction methods, and the difference between the physical requirements involved in executing a specific task may have been involved. However, these factors cannot fully account for some of the striking differences, especially when type and duration of hypoxia were kept constant.

Fifteen minutes exposure to a gas mixture containing 4.3 % oxygen mainly reduced the rate of lever pressing for electrical brain stimulation for approximately ten minutes (Chapter 3). After a similar hypoxia, initiation of drinking was delayed for an equivalent time period but the drinking pattern was altered for at least thirty minutes (Chapter 7). This was a remarkable finding since the same brain structures play a pivotal role in the control of both behaviors. Electrical stimulation of the lateral hypothalamic area of the brain not only induces lever pressing for brain stimulation but can also elicit drinking (Andersson and McCann, 1955; Mogenson and Stevenson, 1966).

The first difference between the effects seen in the two paradigms involves the initiation of behavior. In the self-stimulation paradigm, the response rate was reduced during the first ten minutes of the post-hypoxic session but the rats initiated the behavior from the first minute. In the drinking paradigm, initiation was completely blocked for at least five minutes. A slight procedure difference was probably the most important causal factor for this differential effect.

In the self-stimulation paradigm the animals received a priming stimulus as soon as they were put in the experimental cage after the exposure to hypoxia. Electrical stimulation of the hypothalamic area activates ascending and descending neuronal pathways. One of the consequences of this activation is the induction of arousal and locomotor activity (Valenstein, 1976).
Both conditions are favorable to support and enhance subsequent lever pressing. Due to the procedure, the animals received a stimulus which probably acted as an antagonist of the depressed neuronal activity. Hence, initiation was triggered externally and lever pressing subsequently rapidly recuperated in the next five to ten minutes. In the drinking paradigm no elements of the experimental procedure could directly influence the initiation. Drinking was spontaneously initiated five to ten minutes after the exposure to hypoxia.
This indicated that at that moment the brain could succesfully integrate central and peripheral cues. It is remarkable that this initiation occured at approximately the same time point where the response rate in the self-stimulation experiments approached pre-hypoxic control levels.
The difference between initiation times in both paradigms therefore seems to be primarily related to procedural differences. In fact both types of experiments suggest that hypothalamic and cortical functions normalize within ten minutes after hypoxia.

A second difference was the prolonged effects that were seen on the drinking pattern in the drinking experiments. In Chapter 7 we suggested that such an effect might have been related to differential recuperation times of different brain structures. In the case of drinking behavior one might think of the dorsomedial amygdala as a possible structure involved in such an effect (Fonberg, 1969). The amygdala plays a role in oral motor responses (Hockman *et al.*, 1979) and it has been suggested that this structure, amongst others, mediates reward (Rolls, 1972). Prolonged suppression of neuronal activity in the amygdala could therefore explain why hypoxia seems to act as a modulator of the "incentive" to eat and drink (Ettinger and Staddon, 1982).

We have not gathered direct evidence that such effect occured in our experiments. However, indications can be found in the literature that a differential effect on brain structures is a plausible working hypothesis. Selective neuronal loss which is seen after severe hypoxia (Meldrum *et al.*, 1982) clearly suggests that different brain structures respond differently to hypoxia. To our knowledge no cell damage has ever been described after the depth and duration of hypoxia used in our experiments. How then must differential effects on cell functions and recuperation times be explained?

Part of the answer to this question is certainly related to the great differences in extracellular PO_2 that have been measured in different brain structures (Carter, 1966). Even in the cortex which has a mean PO_2 of 25 mm.Hg in the grey matter, 50 % of the tissue has a PO_2 around or below 5 mm.Hg (Erdmann and Faithfull, 1981). This means that some cells or structures normally function in a high PO_2 environment whereas others are able to function in a low oxygen environment. In addition, the critical PO_2 value for neuronal function of specific cells is correlated with the level of extracellular PO_2 to which they are adapted. In *Aplysia californensis* it was shown that neurons can have an autoregulatory capacity which allows them to keep the intracellular PO_2 constant over a specific range of extracellular PO_2 values (Chen *et al.*, 1978). General decrease in extracellular PO_2 could therefore affect the function of cells which normally work in a high PO_2 environment without affecting others which already function in a low PO_2 environment during normal physiological conditions. This can only occur when changes in inspiratory oxygen concentration are able to reduce brain tissue PO_2 to a sufficiently low level. Metzger (1971) and his colleagues have shown that critical values are reached in the rat brain twenty seconds after inspiration of low oxygen gas mixtures. Based on their results we estimated that during hypoxia in our experiments brain tissue PO_2 levels were between 3 to 5 mm.Hg. This means that certain cells have been exposed to critical PO_2 levels for fifteen minutes whereas for others these conditions were still in the range where autoregulation and normal functioning was possible. Based on this differential effect one might expect differences in recuperation time after hypoxia. Consequently, prolonged effects on behavioral functions will be observed if a susceptable structure plays a key role in the execution and maintenance of that behavior.

Some of the findings of Metzger (1971) suggest that prolonged effects are not necessarily related to longer recuperation times but might be the result of the development of a secondary hypoxia. He observed that after a short period of

hypoxia (1 minute inhalation of 2.97 % oxygen) an initial increase in PO_2 was followed by an undershoot which lasted for ten minutes. These oscillations in tissue PO_2 were probably related to changes in cerebral blood flow. Hyperperfusion followed by hypoperfusion is a well known phenomenon after ischemia (Siësjo, 1981). It is interesting to note that breathing low oxygen concentrations probably triggers the same mechanisms as an ischemic attack. Such oscillations imply that in certain structures hypoxia might develop because the extracellular PO_2 decreases below critical levels long after the initial hypoxia. Hence, prolonged effects on behavioral functions are not necessarily related to longer recuperation times. They might be the reflection of functional suppression of specific structures or cells which are in an acute secondary hypoxic condition. It is plausible that alterations in perfusion are the primary cause of such secondary events.

In the preceding paragraphs we have focused on the possible origin of the differential effects that were seen in different paradigms when type and duration of hypoxia were kept constant. It is interesting to note that post-hypoxic oscillations in tissue PO_2 can also offer an alternative interpretation of the hypoxic effects on avoidance learning described in the first chapter. Reduced performance in the second daily session as compared to the first daily session was observed at least one hour after exposure to hypoxia. Such effects could be explained by assuming that they were the result of a secondary hypoxia of cells involved in retrieval mechanisms. This would agree with the observation that the hypoxic effects were more pronounced after the shortest intersession interval. In our experiments with avoidance learning exposure for one minute to pure nitrogen was used. Although some brain tissue PO_2 still exists after breathing nitrogen for such a period (Carter *et al.*, 1962), cerebral energy metabolism is dramatically disturbed (Ridge, 1972; Stefanovich, 1981). Consequently recuperation of metabolic functions might have been an additional causal factor contributing to the effects which were seen rather long after the initial hypoxia.

At this point, it is important to stress that selective effects on brain structures (primarily or secondarily induced) should only be regarded as one of the possible working mechanisms which could have been involved in a number of the models used. However, it is at least a hypothesis which can be tested in independent experiments specially designed for this purpose. As with all behavioral work it is almost impossible to obtain direct evidence on working mechanisms. Conclusive evidence can only be obtained by working at another level of analysis. This does not mean that such a working hypothesis is useless for pharmacological research. It can be used to guide the search for new compounds and to define on theoretical grounds which pharmacological profile such a new drug should have.

A recurrent finding in our experiments was a certain level of adaptation or habituation after multiple exposure to hypoxia. However, despite the almost general occurence of the phenomenon some important differences were seen depending on the time of measurement of the behavior in relation to the hypoxia. If the behavior (self-stimulation in Chapter 3; drinking in Chapter 7) was measured immediately after hypoxia some adaptation was observed mainly

between the first and second exposure. No further improvements were observed after subsequent exposures. If the behavior was measured one hour or more after the hypoxia no adaptation was seen (avoidance learning in Chapter 1). The reason for this might be that the perception of the experimental condition was slightly different immediately after the exposure to hypoxia. Then, performing after the first exposure would be equivalent to performing in a slightly changed environment. The improvement seen after the second exposure could then be attributed to the absence of a novelty effect. The fact that stimulus discrimination was only very slightly attenuated shortly after the hypoxia might indicate that altered stimulus discrimination is only occuring in a very narrow time span following hypoxia. This would explain why no adaptation was seen in the avoidance experiments when the performance was measured at least one hour after hypoxia. A different type of adaptation was observed when self-stimulation was studied during hypoxia (Chapter 4). In this paradigm a gradual improvement in the performance was seen with increasing numbers of exposures. The rate and maximal level of adaptation was different for individual animals. Although blood analysis revealed no systematic changes reminiscent of slow physiological adaptive mechanisms, this gradual adaptation probably reflected changes in respiratory and cardiovascular efficiency. No direct evidence for the exact nature of both types of habituation was found in our experiments. It is a subject wich should be studied in series of experiments specially designed for this purpose. However, independent of its origin, the occurence of adaptation phenomena has some fundamental implications for pharmacological research in the field of hypoxia.

A great number of pharmacological studies will attempt to use the same animals several times to investigate different doses and/or different drugs. It is therefore essential to stress that no drug should be given before a steady state effect has been reached after a number of exposures to hypoxia. In addition it is recommended to run an experiment of maximal duration with repeated placebo treatment. This is essential to detect interference due to repeated injections and to reveal slow adaptive processes that might develop especially when numerous exposures are planned. The results obtained after chronic treatment with water injections in the drinking paradigm (Chapter 6) are a good illustration of the necessity of such control experiments.

Adaptive phenomena are certainly not restricted to the species and tasks used in our experiments. For instance, Dogterom (1983) described similar adaptive phenomena after hypobaric hypoxia in rabbits trained in a visual discrimination task. If behavior is studied immediately after hypoxia, a high incidence of adaptation of the short type can be expected when new paradigms are used. Therefore, adaptation will always hamper to some extent pharmacological research aimed at finding protective drug effects. However, a positive aspect is the fact that adaptation can be a research issue on its own. The rather slow and gradual increase of lever pressing for brain stimulation during consecutive exposures to hypobaric hypoxia could be used as dependent variable in pharmacological experiments. A relevant question to be asked then is whether or not drug treatment can speed up adaptive processes. The only problem with

the self-stimulation paradigm is the large variability in speed and magnitude of adaptation between individual animals. It is conceivable that still significant attenuation of self-stimulation can be obtained at more elevated barometric pressure provided reinforcement schedules are changed. Inducing slow rate continuous lever pressing could reduce the physical effort required and could therefore reduce inter-individual variability. The paradigm would then be better suited to study drug effects on adaptation. In addition it might then also become possible to attribute the effects to reduced motivation rather than to a decreased work capacity of the muscles. This would open new perspectives to study centrally mediated effects with the paradigm which is impossible with the present version.

At the end of each of the respective chapters we have already discussed and evaluated the possible use of the different paradigms for pharmacological research. A comparison inevitably leads to the conclusion that avoidance learning fulfils most of the requirements of our ultimate goal: developing a model which allows to detect drugs which protect against functional deficits of cerebral origin. The potential of the paradigm to do this was illustrated in the second chapter. Although a number of the calcium entry blockers protected against mortality in survival models, only flunarizine was able to protect against the memory deficit induced by hypoxia. This indicates that with this model a further differentiation between possible candidate drugs can be accomplished. It is interesting to note that flunarizine was the only compound for which direct neuronal effects independent of circulation were demonstrated (Wauquier *et al.*, 1984). This suggests that this behavioral model might allow to distinguish between drugs with respect to their direct neuronal effects. Hence, the technique might become a powerful instrument for second level pharmacological screening; that is for differentiation between compounds that passed a first screening in survival models. Due to the problems with repeated injections drinking behavior for which some pharmacological results were presented in chapter 8 seems to be rather limited in this respect. However, this paradigm seems to have some advantages for studying fundamental aspects of brain hypoxia. The acute effect on initiation and the prolonged effect on the drinking pattern might allow some insight into the differential effects on brain structures which was suggested above. That both effects probably have a different origin is clearly suggested by the fact that flunarizine normalized to some extent initiation of drinking but could not restore a normal drinking pattern.

A final issue which is relevant to both pharmacologists and clinicians is the predictive value of the different paradigms with regard to clinical efficacy and effective doses in human. An important question is whether the observed effects in the animal models resemble those seen in humans. Some patients surviving cardiac arrest can have permanent loss of memory for events with occur both before and after the arrest (Caronna, 1979; Finkelstein and Caronna, 1978; Volpe and Hirst, 1983; Volpe *et al.*, 1983). However this does not imply that such patients are unable to learn and remember new information if enough practice is given (Cohen and Squire, 1980; Corkin, 1968). A specific memory loss with unimpaired learning was also seen in our avoidance paradigm.

The fact that cardiac arrest was not used as a method to induce hypoxia is not so essential. Hypoxia is a phenomenon which is associated with a great number of diseases. Although resemblance of induced effects can be an advantage it does not guarantee predictive value. The ultimate test is always a comparison between clinical and experimental animal results. At this moment there is insufficient clinical evidence to make such an evaluation. However, we feel that some of the paradigms tested in this thesis have great potential to detect drugs with clinical efficacy in the field of functional deficits induced by hypoxia. The fact that they can further differentiate between compounds active in survival tests already makes them useful tools for pharmacological research purposes.

Summary and conclusions

In this thesis we described the effects of hypoxia on three types of functional behavior: avoidance learning, self-stimulation and drinking behavior. Depth and induction method of hypoxia were chosen to secure maximal survival of the animals. The aim was to evaluate which behavioral paradigm could be used in pharmacological research for detection of drugs that could protect the brain against hypoxia.

In the first paradigm (Chapter 1) guinea-pigs were trained twice daily on five consecutive days to learn to avoid an electrical shock (unconditioned stimulus) upon presentation of an auditory stimulus (conditioned stimulus). In control animals the number of correct responses increased in the course of the training period. In addition, more correct responses were made during the second daily training session as compared to the first session. When the animals were exposed to hypoxia (1 min. 100 % nitrogen) immediately after each training session acquisition was not attenuated. However, the level of performance during the second session was reduced to that seen in the first session of that same day. This effect was probably due to a blockade of either the development or retrieval of an intermediate memory trace which contained new information learned in the first session. Given both the stability and the specificity of the induced effect the paradigm seemed to be suitable for pharmacological research. Four compounds; flunarizine, nimodipine, nifedipine and verapamil were tested. Three of them were known to have anti-hypoxic effects in simple survival models. Only flunarizine could antagonize the amnesic effects. Interestingly, it was also the only drug known to have direct effects on brain cells.

In the next paradigm self-stimulation behavior was used (Chapter 3-6). Rats were trained to obtain electrical brain stimulation in the lateral hypothalamus by pressing a lever. Trained animals reached an average response rate of one hundred responses in one minute. This response rate was reduced immediately after exposure to normobaric hypoxia but it normalized within ten minutes. These effects were reduced after a second exposure to hypoxia. This form of adaptation did not continue after subsequent exposures. Self-stimulation was also significantly reduced when the sessions were held under hypobaric conditions. At a barometric pressure of 300 mm.Hg a significant reduction of the response rate was observed during the whole session. A progressive slow adaptation was seen after several sessions in the hypobaric environment.

However, large individual differences were seen in both speed and magnitude of the adaptation. This form of adaptation was probably related to changes in cardiovascular functions and increase of muscle work capacity during hypoxia. The rather short duration of post-hypoxic effects and the large individual differences during performance in hypoxic conditions were problems which made the use of these models in pharmacological research difficult.

Self-stimulation was also used to investigate the effects of hypoxia on stimulus discrimination. The latter was hardly affected and the rats could perfectly discriminate between two electrical stimuli immediately after hypoxia.

In the last paradigm drinking behavior was used. During a training period rats learned to ingest sufficiently large volumes of water during a short drinking period (30 min.). After two weeks of training drinking behavior was stable. However, repeated injections with water had a destabilizing effect. When the drinking period followed immediately after exposure to hypoxia a dual effect was seen. Initiation of drinking was delayed and a completely different drinking pattern was observed. After a second exposure some adaptation was also seen. Initiation occured faster but the post-hypoxic drinking pattern was not changed due to adaptation. Due to the effects of repeated injections, this model could only be used for pharmacological research when the animals were only injected once and then exposed to hypoxia. Flunarizine significantly decreased initiation latency but could not normalize the drinking pattern (Chapter 8).

Based on the results obtained in the different experiments following general conclusions could be drawn:

1. It is possible to obtain stable effects on behavior after or during exposure to reasonable depths of hypoxia.
2. The effects on avoidance learning show that with this model the criteria are met to use this behavioral paradigm in pharmacological research aimed at finding brain protective properties of drugs.
3. It is possible to use drugs to protect against functional deficits induced by hypoxia.
4. The results obtained with the different drugs in the avoidance paradigm confirm that it is possible to further differentiate between drugs that are active in simple survival models.
5. It is not possible at this moment to evaluate the predictive value of the models for clinical application of drugs. However, both the type of effects obtained (memory disturbance) and the selectivity of the active compounds open good perspectives in this respect.
6. After repeated exposures to hypoxia either a short (behavior measured immediately after hypoxia) or a longer (behavior measured during hypoxia) type of adaptation were frequently observed. The exact nature of this phenomenon is unclear.
7. The different effects obtained with the same type of hypoxia suggest that several brain structures have a different sensitivity even if the induced hypoxia does not cause cellular damage.
8. Given the dual effect of hypoxia on drinking it seems that drinking behavior is a good paradigm to study the mechanisms and brain structures involved in the manifestation of functional deficits after hypoxia.

Samenvatting en conclusies

In dit proefschrift werden de effecten van hypoxie op drie types van functioneel gedrag, vermijdingsgedrag, zelfstimulatie- en drinkgedrag beschreven. De diepte en type van hypoxie werd zodanig gekozen dat maximale overleving van de proefdieren was gegarandeerd. De bedoeling was te evalueren welke van de gedragsmodellen geschikt kon zijn voor het farmacologisch onderzoek gericht op het vinden van stoffen die voornamelijk de hersenen beschermen tegen hypoxie.

In de eerste proefopstelling (Hfdst. 1) werden cavias twee maal per dag gedurende vijf dagen getraind om een electrische prikkel (niet geconditioneerde stimulus) te vermijden bij het aanbieden van een auditieve stimulus (geconditioneerde stimulus). In controle dieren nam het aantal juiste responsen toe in de loop van de trainingsperiode. Tevens was het aantal juiste responsen steeds hoger gedurende de tweede training dan gedurende eerste training op de zelfde dag. Wanneer de dieren blootgesteld werden aan hypoxie (1 min. 100 % stikstof) onmiddellijk na elke training werd het leren van de taak niet geremd, maar de normale verbetering van de prestatie tussen de twee dagelijkse sessies was niet meer aanwezig. Dit effect was vermoedelijk te wijten aan het feit dat hypoxie kort na de eerste training de ontwikkeling of het uitlezen van een tijdelijk geheugenspoor voorkwam. Gezien de specificiteit en de stabiliteit van de geinduceerde effecten leek deze opstelling geschikt voor farmacologisch onderzoek. Vier stoffen waarvan drie met gekende anti-hypoxische eigenschappen in overlevingsmodellen (flunarizine, nimodipine, nifedepine en verapamil) werden uitgetest in het leermodel (Hfdst. 2). Alleen flunarizine, waarvan de directe effecten op hersencellen reeds beschreven was, bleek efficient om het geheugenverlies door hypoxie tegen te gaan.

In de volgende proefopstelling werd zelfstimulatiegedrag gebruikt (Hfdst. 3-6). Ratten werden getraind om via het drukken op een hefboom zichzelf een lichte electrische stimulus in de laterale hypothalamus toe te dienen. Getrainde dieren drukten gemiddeld een honderd maal per minuut. Onmiddellijk na een periode van normobare hypoxie werd een verlaagde responsiviteit vastgesteld. Deze normaliseerde echter binnen de tien minuten.

Deze effecten waren wat gereduceerd nadat de dieren voor een tweede maal aan hypoxie werden blootgesteld. Deze vorm van adaptatie evolueerde echter niet meer na verdere hypoxiesessies. Zelfstimulatie werd ook significant gereduceerd wanneer de sessies gehouden werden gedurende hypobare condities. Bij een barometrische druk van 300 mmHg werd een significante vermindering van de responsiviteit vastgesteld. Na meerdere sessies op dit drukniveau werd een geleidelijke progressieve adaptatie vastgesteld. Er werden echter enorme individuele verschillen tussen de dieren vastgesteld. Deze vorm van adaptatie was vemoedelijk te wijten aan een beter cardiovasculair funktioneren en een verhoging van de werkcapaciteit gedurende de hypoxie. De eerder beperkte duur van de effecten na hypoxie en de individuele verschillen tijdens hypoxie maakten de opstellingen in de vorm waarin ze getest werden minder geschikt voor gebruik in farmacologisch onderzoek. Via een opstelling met zelfstimulatie werd ook nagekeken of de post-hypoxische

effecten gerelateerd waren aan een deficiëntie in de stimulus discriminatie. Dit leek niet het geval te zijn daar de proefdieren onmiddellijk na de periode van hypoxie bijna perfect in staat waren te discrimineren tussen twee electrische stimuli.

In de laatste opstelling werd drinkgedrag gebruikt. Via een trainingsperiode werden ratten geleerd om dagelijks een voldoende hoeveelheid vocht te drinken gedurende een korte periode (30 min.). Na een tweetal weken stabiliseerde het drinkgedrag. Herhaalde injecties met water hadden echter een destabiliserend effekt. Wanneer de drinkperiode onmiddellijk volgde op een periode van hypoxie werd een dubbel effect geobserveerd. Het drinkgedrag was uitgesteld en de dieren vertoonden een totaal ander drinkpatroon dan in normale omstandigheden. Na een tweede hypoxieperiode bleek zoals bij de zelfstimulatie enige adaptatie op te treden. De dieren begonnen vroeger te drinken maar aan het post-hypoxisch drinkpatroon veranderde niets. Deze opstelling bleek alleen geschikt voor farmacologich onderzoek wanneer de dieren eenmalig blootgesteld werden aan hypoxie. Met flunarizine behandelde dieren begonnen vroeger te drinken na hypoxie dan niet behandelde dieren. De stof was echter niet in staat om het drinkpatroon te normalizeren.
Op basis van de bekomen resultaten konden volgende algemene conclusies getrokken worden:

1. Het is mogelijk om met een redelijke diepte van hypoxie funktioneel gedrag op een stabiele manier te beinvloeden.
2. De effecten op leren van een vermijdingsgedrag voldoen het best aan de criteria gesteld om een gedragsmodel te gebruiken voor farmacologisch onderzoek naar de hersenbeschermende werking van farmaca.
3. Het is mogelijk om via het toedienen van farmaca bescherming te bieden tegen funktionele uitvalsverschijnselen na hypoxie.
4. De resultaten bekomen met de verschillende farmaca bevestigen dat men met het leermodel in staat is verder te differentiëren tussen stoffen die aktiviteit vertonen in overlevingsmodellen.
5. Over de predictieve waarde voor klinische toepassingen kan geen definitieve uitspraak worden gedaan op basis van de resultaten. Nochtans openen de aard van de geïnduceerde effecten (geheugenstoornissen) en de selectiviteit van de actieve stoffen gunstige perspectieven op dit vlak.
6. Wanneer een dier meerdere keren aan hypoxie wordt blootgesteld is adaptatie van het korte (gedrag gemeten vlak na hypoxie) of het lange type (gedrag gemeten tijdens hypoxie) een frequent verschijnsel waarvan de juiste oorsprong op dit ogenblik onduidelijk is.
7. De uiteenlopende effecten geïnduceerd door de zelfde vorm van hypoxie suggereren het bestaan van verschillende sensitiviteit in diverse hersenstructuren zelfs indien de diepte van de toegepaste hypoxie geen aanleiding geeft tot celbeschadiging.
8. Gezien de effecten van hypoxie op zowel initiatie als op het drinkpatroon, lijkt drinkgedrag goed geschikt voor een verdere studie van de mechanismen en de hersenstructuren betrokken in de manifestatie van funktionele uitvalsverschijnselen na hypoxie.

References

Abramson, N.S., Safar, P., Detre, K. *et al.*, Results of a randomized clinical trial of brain resuscitation with thiopental. Anesthesiology, 59 (1983) A101.

Aguilar, M.J., Gerbode, F. and Hill, J.D., Neuropathologic complications of cardiac surgery. J. Thorac. Cardiovasc. Surg., 61 (1971) 676-685.

Aldrete, J.A., Romo-Salas, F., Jankovsky, L. and Franatovic, Y., Effect of pretreatment with thiopental and phenytoin on postischemic brain damage in rabbits. Crit. Care Med., 7 (1979) 466-470.

Aldrete, J.A., Romo-Salas, F., Mazzia, V.D.B. and Tan, S.L., Phenytoin for brain resuscitation after cardiac arrest: an uncontrolled trial. Crit. Cara Med., 9 (1981) 474-477.

Allweis, C., Gibbs, M.E., Ng, K.T. and Hodge, R.T., Effects of hypoxia on memory consolidation: implications for a multistage model of memory. Behav. Brain Res., 11 (1984) 117-121.

Amery, W.K., Tissue hypoxia in health and disease. In A. Wauquier, M. Borgers and W.K. Amery (Eds.), Protection of Tissue against Hypoxia, Elsevier Biomedical Press, Amsterdam, 1982, pp. VII-X.

Andersson, B. and McCann, S.M., A further study of polydipsia evoked by hypothalamic stimulation in the goat. Acta Physiol. Scand., 33 (1955) 333-346.

Annau, Z. and Weinstein, S.A., Hypothalamic self-stimulation: interaction of hypoxia and stimulus intensity. Life Sci., 6 (1967) 1344-1360.

Annau, Z. and Weinstein, S.A., Conditioned suppression of hypothalamic self-stimulation based on low oxygen levels. Commun. Behav. Biol., 22 (1) (1968) 1-6.

Arnfred, I. and Secher, O., Anoxia and barbiturates - tolerance to anoxia in mice influenced by barbiturates. Arch. Int. Pharmacodyn. Ther., 139 (1962) 67-74.

Astrup, J., Membrane stabilization and protection of the ischemic brain. In K. Wiedemann and S. Hoyer (Eds.), Brain Protection, Morphological, Pathophysiological and Clinical Aspects, Springer Verlag, Berlin, 1983, pp. 31-37.

Astrup, J., Symon, L., Branston, N.M. and Lassen, N.A., Cortical evoked potential and extracellular K^+ and H^+ at critical levels of brain ischemia. Stroke, 8 (1977) 51-57.

Bacalzo, L.V. and Wolfson, S.K., Preferential cerebral hypothermia. Trimethamine and dextran 40 and tolerance to circulatory arrest of 90 to 120 minutes. Arch. Surg., 103 (1971) 393-397.

Baldwin, B.A. and Soltysik, S.S., Acquisition of classical conditioned defensive responses in goats subjected to cerebral ischemia. Nature, 206 (1965) 1011-1013.

Barnett, H., Paoletti, P., Flamm, E. and Brambilla, (Eds.), Cerebrovascular Diseases: New Trends in Surgical and Medical Aspects. Elsevier/North-Holland Biomedical Press, Amsterdam, 1981, 406 pp.

Baronades, S.H., Cerebral protein synthesis inhibitors block long-term memory. Int. Rev. Neurobiol., 12 (1970) 177-205.

Bell, J.A. and Hodgson, J.H.F., Coma after cardiac arrest. Brain 97 (1974) 361-367.

Belopawlovic, M. and Buchtal, A., Barbiturate therapy in cerebral ischemia. Anaesthesia, 35 (1980) 235-245.

Björk, V.O. and Hultquist, G., Brain damage in children after deep hypothermia for open heart surgery. Thorax, 15 (1960) 284-291.

Bleyaert, A.L., Nemoto, E.M., Safar, P., Stezoski, S.W., Mickell, J.J., Moossy, J. and Gutti, R.R., Thiopental amelioration of brain damage after global ischemia in monkeys. Anesthesiology, 49 (1978) 390-398.

Boismare, F., Le Poncin, M., Belliard, J.P. and Hacpille, L., Reduction of hypoxia induced disturbances by previous treatment with benserazide and L. Dopa in rats. Experientia, 31 (1975) 1190-1192.

Boismare, F., Le Poncin, M., Lefrançois, J., Hacpille, L. and Marchand, J.C., Influence des catécholamines centrales sur l'aptitude fonctionnelle du rat en hypoxie. J. Pharmacol., 8 (1977) 287-296.

Boismare, F., Le Poncin-Lafitte, M. and Rapin, J.R., Blockade of the different enzymatic steps in the synthesis of brain amines and memory (CAR) in hypobaric hypoxic rats treated and untreated with L-Dopa. Aviat. Space Environ. Med., 51 (1980) 126-128.

Boismare, F., Saligaut, C., Moore, N. and Raoult, J.P., Avoidance learning and mechanism of the protective effect of apomorphine against hypoxia. Aviat. Space Environ. Med., 52 (1981) 209-303.

Booth, D.A., Neurochemical changes correlated with learning and memory retention. In G. Ungar (Ed.), Molecular Mechanisms in Memory and Learning, Plenum Press, New York, 1970, pp. 1-57.

Branston, N.M., Symon, L., Crockard, H.A. and Pasztor, E., Relationship between the cortical evoked potential and local cortical blood flow following acute middle cerebral artery occlusion in the baboon. Exp. Neurol., 45 (1974) 195-208.

Brierly, J.B., Brain damage complicating open heart surgery: a neuropathological study of 46 patients. Proc. R. Soc. Med., 60 (1967) 858-859.

Bryant, J.H. and Thompson, R., The effect of anoxia on the acquisition of a discrimination habit. J. Comp. Physiol. Psychol., 51 (1958) 202-204.

Caillard, C., Menu, A., Plotkine, M. and Rossignol, P., Do anticonvulsant drugs exert protective effect against hypoxia? Life Sci., 16 (1976) 1607-1612.

Caronna, J.J., Diagnosis, prognosis and treatment of hypoxic coma. In S. Fahn, S. Davis and J.M. Rowland (Eds.), Advances in Neurology 26, Raven Press, New York, 1979, pp. 1-19.

Cater, D.B., The significance of oxygen tension measurement in tissue. In J.P. Payne and D.W. Hill (Eds.), A Symposium on Oxygen Measurements in Blood and Tissues and their Significance, Churchill, London, 1966, pp. 155-172.

Cater, D.B., Hill, D.W., Lindop, P.J., Nunn, J.F. and Silver, I.A., Oxygen washout studies in anaesthetized dog. J. Appl. Physiol., 18 (1963) 888.

Chen, C.F., Erdmann, W. and Halsey, J.N., The sensitivity of aplysia giant neurons to changes in extracellular and intracellular PO_2. In I.A. Silver (Ed.), Oxygen Transport to Tissue III, Plenum Press, New York, 1978, pp. 780-786.

Clifford Rose, F. and Amery, W.K. (Eds.), Cerebral Hypoxia in the Pathogenesis of Migraine. Progress in Neurology Series, Pitman, London, 1982, 210 pp.

Clincke, G.H.C., Fransen, J.F. and Wauquier, A., Drug-induced changes of brain self-stimulation using a discrimination paradigm. In F.C. Colpaert and J.L. Slangen (Eds.), Drug Discrimination: Applications in CNS Pharmacology, Elsevier Biomedical Press, Amsterdam, 1982, pp. 381-389.

Cohen, N.J. and Squire, L.R., Preserved learning and retention of pattern analyzing skills in amnesia. Science, 210 (1980) 207-210.

Consolazio, C.F., Matoush, L.O., Johnson, H.L. and Daws, T.A., Protein and water balance of young adults during prolonged exposure to high altitude (4300 meters). Am. J. Clin. Nutr., 21 (1968) 154-161.

Cooper, S.J., Benzodiazepine mechanisms and drinking in the water-deprived rat. Neuropharmacology, 21 (1982) 775-780.

Cooper, S.J. and Francis, R.L., Water intake and time course of drinking after single or repeated chlordiazepoxide injections. Psychopharmacology, 65 (1979) 191-195.

Copeland, J.G., Reitz, B.A., Roberts, A.J. and Michaelis, L.L., Hypothermic asanguineous circulatory arrest in adult dogs. Ann. Surg., 180 (1974) 728-733.

Corkill, G., Chikovani, O.K., McLeish, I., McDonald, L.W. and Youmans, J.R., Timing of pentobarbital administration for brain protection in experimental stroke. Surg. Neurol., 5 (1976) 147-149.

Corkin, S., Acquisition of motor skills after bilateral medial temporal lobe excision. Neuropsychology, 6 (1968) 255-265.

Davis, F.M., Parimelazhagan, K.N. and Harris, E.A., Thermal balance during cardiopulmonary bypass with moderate hypothermia in man. Br. J. Anaesth., 49 (1977) 1127-1132.

Denison, D.M., Ledwith, M.A. and Poulton, E.C., Complex reaction times at simulated cabin altitudes of 5000 feet and 8000 feet. Aerospace Med., 37 (1966) 1010-1013.

Dill, D.B., Horvath, S.M., Dahms, T.E., Parker, R.E. and Lynch, J.R., Hemoconcentration at altitude. J. Appl. Physiol., 27 (1969) 514-518.

Dillard, D.H., Mohri, H. and Merendino, K.A., Correction of heart disease in infancy utilizing deep hypothermia and total circulatory arrest. J. Thorac. Cardiovasc. Surg., 61 (1971) 64-69.

Dogterom, G.J., Het geheugenspoor van het konijn: consolidatie en stabiliteit. Proefschrift Erasmus Universiteit Rotterdam (1983).
Duncan, C.P., The retroactive effect of ECS on learning. J. Comp. Psychol., 42 (1949) 32-33.

Edmonds, H.L., Wauquier, A., Melis, W., Van den Broeck, W.A.E., Van Loon, J. and Janssen, P.A.J., The resuscitant action of flunarizine in a canine model of cardiac arrest. Am. J. Emerg. Med., (1984) in press.

Ettinger, R.H. and Staddon, J.E.R., Decreased feeding associated with acute hypoxia in rat. Physiol. Behav., 29 (1982) 455-458.

Farber, J.L., The role of calcium in cell death. Life Sci., 29 (1981) 1289-1295.

Finkelstein, S. and Caronna, J.J., Amnestic syndrome following cardiac arrest. Neurology, 28 (1978) 389.

Fitzsimons, J.J., Thirst. Physiol. Rev., 52 (1972) 468-561.

Fleckenstein, A., Pharmacology and electrophysiology of calcium antagonists. In A. Zanchetti and D.M. Krikler (Eds.), Calcium Antagonism in Cardiovascular Therapy, Excerpta Medica, Amsterdam, 1981, pp. 10-29.

Flohr, H., Hypoxia-induced retrograde amnesia. In: M.A.B. Brazier (Ed.), Brain Mechanisms in Memory and Learning; From the Single Neuron to Man, Raven Press, New York, 1979, pp. 277-291.

Fonberg, E., The role of the hypothalamus and amygdala in food intake, alimentary motivation and emotional reactions. Acta Biol. Exp., 29 (1969) 335-358.

Fregly, M.J., Lutherer, L.O. and Tyler, P.E., Effect of chronic exposure to cold, hypoxia and both combined on water exchange in rats. Aerospace Med., 45 (1974) 1223-1231.

Frieder, B. and Allweis, C., Transient hypoxic-amnesia: evidence for a triphasic memory-consolidating mechanism with parallel processing. Behav. Biol., 22 (1978) 178-189.

Frieder, B. and Allweis, C., Prevention of hypoxia-induced transient amnesia by post-hypoxic hyperoxia. Physiol. Behav., 29 (1982a) 1065-1069.

Frieder, B. and Allweis, C., Memory consolidation: further evidence for the four-phase model from the time-courses of diethyldithiocarbamate and ethacrinic acid amnesias. Physiol. Behav., 29 (1982b) 1065-1069.

Frisancho, A.R., Functional adaptation to high altitude hypoxia. Science, 187 (1975) 313-319.

Frost, A.M., Brain preservation. Anesth. Analg., 60 (1981) 821-832.

Gibbs, M.E. and Ng, K.T., Memory formation: a new three-phasic model. Neurosci. Lett., 2 (1976) 165-169.

Gibbs, M.E. and Ng, K.T., Psychobiology of memory: towards a model of memory formation. Biobehav. Rev., 1 (1977) 113-136.

Gibbs, M.E. and Ng, K.T., Diphenylhydantoin extension of short-term and intermediate stages of memory. Behav. Brain Res., 11 (1984) 103-108.

Gibbs, M.E. and Ng, K.T., Hormonal influence on the duration of short-term and intermediate stages of memory. Behav. Brain Res., 11 (1984) 109-116.

Gisvold, S.E., Postischemic treatment with nimodipine improves neurologic function after complete global brain ischemia in monkeys. International Symposium on Calcium Entry Blockers and Tissue Protection, Rome, 1984, Abstract.

Gisvold, S.E., Safar, P., Hendricks, H. and Alexander, H., Thiopental treatment after global brain ischemia in monkeys. Anesthesiology, 55 (1981) A97.

Giurgea, C., Lefevre, D., Lescrenier, C. and David-Remacle, M., Pharmacological protection against hypoxia induced amnesia in rats. Psychopharmacologia (Berl.), 20 (1971) 160-168.

Godfraind, T., Herman, R. and Wellens, D. (Eds.), Calcium Entry Blockers in Cardiovascular and Cerebral Dysfunctions. Martinus Nijhoff, Amsterdam, 1984.

Goldstein, A., Wells, B.A. and Keats, A.S., Increased tolerance to cerebral anoxia hypentobarbital. Arch. Int. Pharmacodyn., 166 (1966) 138-143.

Gouret, C. and Raynaud, G., Utilisation du test de la boîte a deux compartiments pour la recherche de substances protégeant le rat contre l'amnésie par hypoxie: Intérêt et limites de la méthode. J. Pharmacol., 7 (1976) 161-175.

Green, R.L., The carriage of invalid passengers by air. Br. J. Hosp. Med., 17 (1977) 32-37.

Guyton, A.C., Textbook of Medical Physiology. Sixth Edition. Saunders, Philadelphia, 1981, 1074 pp.

Halstead, W.C. and Rucker, W.B., The molecular biology of memory. In W.L. Byrne (Ed.), Molecular Approaches to Learning and Memory, Academic Press, New York, 1970, pp. 1-14.

Hannon, J.P., Nutrition at high altitude. In S.M. Horvath and M.K. Yousef (Eds.), Environmental Physiology: Aging, Heat and Altitude, Elsevier/North Holland, New York, 1980, pp. 309-327.

Harding, R.J. and Mills, F.J. (Eds.), Aviation Medicine. British Medical Association, London, 1983, 148 pp.

Hass, W.K., Beyond cerebral blood flow, metabolism and ischemic thresholds: examination of the role of calcium in the initiation of cerebral infarction. In J.S. Meyer, H. Lechner, M. Reivich, E.O. Ott and A. Arabinar (Eds.), Cerebral Vascular Disease, Vol. 3, Proceedings of the 10th Salzburgh Conference on Cerebral Vascular Disease, Excerpta Medica, Amsterdam, 1981, pp. 3-17.

Hempelmann, G., Kling, D., Lüben, V. and Bormann, B., Cerebral protection in neurosurgery, cardiac surgery and following cardiac arrest. J. Cerebr. Blood Flow Metabol. (Suppl. 1), 2 (1982) 66-71.

Hock, R.J., The physiology of high altitude. Sci. Amer., 222 (1970) 53-62.

Hockman, C.H., Bieger, D. and Wurasuriga, A., Supranuclear pathways of swallowing. Prog. Neurobiol., 12 (1979) 15-32.

Hoff, J.T. and Marshall, L.F., Barbiturates in neurosurgery. Clin. Neurosurg., 26 (1979) 637-642.

Hoff, J.T., Smith, A.L., Hankinson, H.L. and Nielson, S.L., Barbiturate protection from cerebral infarction in primates. Stroke, 6 (1975) 28-33.

Hoffmeister, F., Benz, U., Heise, A., Krause, H.P. and Neuser, V., Behavioral effects of nimodipine in animals. Arzneimittel-Forsch./Drug Res., 32 (1982) 347-360.

Hossmann, K.-A., Total ischemia of the brain. In W. Kaufmann, K.-A. Hossmann and V. Hossmann (Eds.), Brain and Heart Infarct, Springer, Berlin, 1977, pp. 107-122.

Hossmann, K.-A., Treatment of experimental cerebral ischemia. J. Cerebr. Blood Flow Metab., 2 (1982) 275-297.

Huppert, F.A., Memory impairment associated with chronic hypoxia. Thorax, 37 (1982) 858-860.

Hurwitz, D.A., Robinson, S.M. and Barofsky, I., Behavioral decrements and brain catecholamine changes in rats exposed to hypobaric hypoxia. Psychopharmacologia, 19 (1971) 26-33.

Janssen, P.A.J. and Reneman, R.S. (Eds.), Protection of the Brain from Hypoxia. J. Cerebr. Blood Flow Metabol., 2 (Suppl. 1) (1982) S1-S102.

Jones, R.M., Ter Haard, C., Zullo, J. and Tenney, S.M., Mechanism of reduced water intake in rats at high altitude. Am. J. Physiol., 240 (1981) 187-191.

Kalimo, H., Paljärvi, L., Olsson, Y. and Siesjö, B.K., Structural aspects of energy failure states in the brain. In K. Wiedemann and S. Hoyer (Eds.), Brain Protection. Morphological, Pathophysiological and Clinical Aspects, Springer-Verlag, Berlin, 1983, pp. 168.

Kamin, L.J., Retention of an incompletely learned avoidance response. J. Comp. Physiol. Psychol., 56 (1963) 713-718.

Kazda, S., Garthoff, B., Krause, H.P. and Schlossmann, K., Cerebrovascular effects of the calcium antagonistic dihydropyridine derivative nimodipine in animal experiments. Arzneimittel-Forsch./ Drug Res., 32 (1982) 331-337.

Keppel, G. (Ed.), Design and Analysis. A Researcher's Handbook. Prentice Hall, Englewood Cliffs, 1973, 658 pp.

Kolkka, R. and Hilberman, M., Neurological dysfunction following cardiac operation with low-flow, low-pressure cardiopulmonary bypass. J. Thorac. Cardiovasc. Surg., 79 (1980) 432-437.

Koob, G.F. and Annau, Z., Effect of hypoxia on hypothalamic mechanisms. Am. J. Physiol., 224 (1973) 1403-1408.

Koob, G.F. and Annau, Z., Behavioral neurochemical alterations induced by hypoxia in rats. Am. J. Physiol., 227 (1974) 73-78.

Koob, G.F., Annau, Z., Blake, W.D. and Weinstein, S.A., Hypothalamic self-stimulation, eating, and drinking: the effects of hypoxia. Commun. Behav. Biol., 5 (1970) 205-210.

Koob, G.F., Annau, Z., Rubin, R.J. and Montgomery, M.R., Effect of hypoxic hypoxia and carbon monoxide on food intake, water intake and body weight in two strains of rats. Life Sci., 14 (1974) 1511-1520.

Krabill, L.F. and Hannon, J.P., Effects of high-altitude exposure on rate of ingest a passage in rats. Am. J. Physiol., 222 (1972) 458-461.

Krieglstein, J. and Stefanovich, V. (Eds.), Stroke: Animal Models. Pergamon Press, Paris, 1982.

Lam, C.R., Geoghegan, T. and Lepore, A., Induced cardiac arrest for intracardiac surgical procedures. J. Thorac. Surg., 30 (1950) 620-625.

Lancet, Brain damage after open-heart surgery. The Lancet, 22 (1982) 1161-1164.

Laver, M.B., Buckley, M.J. and Austen, W.G., Extreme hemodilution with profound hypothermia and circulatory arrest. Bibl. Haematol., 41 (1975) 225-238.

Ledwith, F., The effects of hypoxia on shuttle avoidance in the rat. Psychon. Sci., 8 (1967) 203-204.

Levy, D.E. and Brierley, J.B., Delayed pentobarbital administration limits ischemic brain damage in gerbils. Ann. Neurol., 5 (1979) 59-64.

Lewi, P.J. and Marsboom, R.P. (Eds.), Toxicology Reference Data - Wistar Rat. Elsevier/North-Holland, Amsterdam, 1981, 358 pp.

Malone, M., Prior, P. and Scholtz, C., Brain damage after cardiopulmonary by-pass: correlations between neurophysiological and neuropathological findings. J. Neurol. Neurosurg. Psychiat., 44 (1981) 924-931.

Marshall, L.F., Smith, R.W. and Shapiro, H.M., Acute and chronic barbiturate administration in the management of head injury. J. Neurosurg., 50 (1979) 25-30.

Matthies, H., The biochemical basis of learning and memory. Life Sci., 15 (1974) 2017-2031.

Matthies, H., Learning and memory. In C. Dumont (Ed.), Advances in Pharmacology and Therapeutics, Volume 5: Neuropsychopharmacology, Pergamon, Oxford, 1979, pp. 117-135.

McGaugh, J.L., Time-dependent processes in memory storage. Science, 153 (1966) 1351-1358.

Meldrum, B., Griffiths, T. and Evans, M., Hypoxia and neuronal hyperexcitability - a clue to mechanisms of brain protection. In A. Wauquier, M. Borgers and W.K. Amery (Eds.), Protection of Tissues against Hypoxia, Elsevier Biomedical Press, Amsterdam, 1982, pp. 275-286.

Meltzer, M.L., Poor memory: a case report. J. Clin. Psychol., 39 (1983) 3-10.

Metzger, H., Erdmann, W. and Thews, G., Effect of short periods of hypoxia and hypercapnia on brain O_2 supply. J. Appl. Physiol., 31 (1971) 751-759.

Miller, J.D., Barbiturates and raised intracranial pressure. Ann. Neurol., 6 (1979) 189-193.

Michenfelder, J.D., Barbiturates for brain resuscitation: yes and no. Anesthesiology, 57 (1982) 74-75.

Michenfelder, J.D. and Milde, J.H., Cerebral protection by anesthetics during ischemia. Resuscitation, 4 (1976) 219-233.

Michenfelder, J.D. and Theye, R.A., Cerebral protection by thiopental during hypoxia. Anesthesiology, 39 (1973) 510-517.

Mogenson, G.J. and Stevenson, J.A.F., Drinking and self-stimulation with electrical stimulation of the lateral hypothalamus. Physiol. Behav., 1 (1966) 251-254.

Moseley, J.I., Laurent, J.P. and Molinari, G.F., Barbiturate attenuation of the clinical course and pathologic lesions in a primate stroke model. Neurology, 25 (1975) 870-874.

Nakanishi, M., Yasuda, H. and Tsumagari, T., Protective effect of anti-anxiety drugs against hypoxia. Life Sci., 13 (1973) 467-474.

Nielson, H.C., Zimmerman, J.M. and Colliver, J.C., Effect of complete arrest of cerebral circulation on learning and retention in dogs. J. Comp. Physiol. Psychol., 56 (1963) 974-978.

Nugent, M., Artue, A.A. and Michenfelder, J.D., Cerebral metabolic vascular and protective effect of midazolam maleate. Anaesthesiologie, 56 (1982) 172-176.

Olds, J. and Milner, P.M., Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J. Comp. Physiol. Psychol., 47 (1954) 419-427.

Pace, N., Respiration at high altitude. Fed. Proc., 33 (1974) 2126-2132.

Perutz, M.F., Haemoglobin structure and respiratory transport. Sci. Am., 239 (1978) 68-86.

Ping, F.C. and Jenkins, C.C., Protection of the brain from hypoxia: a review. Canad. Anesth. Soc. J., 25 (1978) 468-473.

Pontius, R.G., Bloodwell, R.D., Cooley, D.A. and de Bakey, M.E., Use of hypothermia in prevention of brain damage following temporary arrest of cerebral circulation: experimental observations. Surg. Forum, 5 (1954) 224-228.

Prechtl, H.F.R., Neurological findings in newborn infants after pre- and paranatal complications. In J.H.P. Jonxis, H.K.A. Visser and J.A. Troelstra (Eds.), Aspects of Prematurity and Dysmaturity, Stenfert Kroese, Leiden, 1968, pp. 303-321.

Prechtl, H.R.F. and Stemmer, G.J., The choreiform syndrome in children. Develop. Med. Child. Neurol., 4 (1962) 199-227.

Pronk, R.A.F., EEG processing in cardiac surgery. TNO, Utrecht (1982), Report R-1982-1.

Pugh, L.G.C.E., Physiological and medical aspects of the Himalayon scientific and mountaineering expedition. Br. Med. J., 2 (1962) 621-627.

Ridge, J.W., Hypoxia and the energy charge of the cerebral adenylate pool. Biochem. J., 127 (1972) 351-355.

Rockoff, M.A., Marshall, L.F. and Shapiro, H.M., High dose barbiturate therapy in humans: a clinical review of sixty patients. Ann. Neurol., 6 (1979) 194-199.

Rolls, E.T., Activation of amygdaloid neurones in reward, eating and drinking elicited by electrical stimulation of the brain. Brain Res., 45 (1972) 365-381.

Safar, P., Introduction: on the evaluation of brain resuscitation. Crit. Care Med., 6 (1978) 199-202.

Safar, P., Amelioration of post-ischemic brain damage with barbiturates. Stroke, 15 (1980) 1-9.

Saligaut, C., Moore, N., Leclerc, J.L. and Boismare, F., Dopaminergic agonists and conditioned avoidance response in normoxic or hypoxic rats. Aviat. Space Environ. Med., 52 (1981a) 19-23.

Saligaut, C., Moore, N., Boulu, R., Plotkine, M., Leclerc, J.L., Prioux-Guyonneau, M. and Boismare, F., Hypobaric hypoxia: central catecholamine levels, cortical PO_2, and avoidance response in rats treated with apomorphine. Aviat. Space Environ. Med., 52 (1981b), 166-170.

Saligaut, C., Moore, N., Daoust, M., Chretien, P. and Boismare, F., Modifications of the dopaminergic receptor sensitivity and learning of normoxic or hypobaric hypoxic rats. Arch. Int. Pharmacodyn. Ther., 251 (1981c) 80-87.

Saligaut, C., Moore, N., Chretien, P., Daoust, M., Richard, O. and Boismare, F., Interference between central dopaminergic stimulation and adrenal secretion in normoxic or hypobaric rats. Stroke, 13 (1982) 859-864.

Sara, S.J. and Lefevre, D., Hypoxia-induced amnesia in one-trial learning and pharmacological protection by piracetam. Psychopharmacologia (Berl.), 25 (1972) 32-40.

Schnakenberg, D.D., Krabill, L.F. and Weiser, P.C., The anorexic effects of high altitude on weight gain, nitrogen retention and body composition of rats. J. Nutr., 101 (1971) 787-796.

Shay, J., Does calcium influx into ischemic cells stop ADP phosphorylation? Lancet, 2 (1973) 1392.

Siesjö, B.K. (Ed.), Brain Energy Metabolism. J. Wiley and Sons, New York, 1978, 607 pp.

Siesjö, B.K., Cell damage in the brain: a speculative synthesis. J. Cerebr. Blood Flow Metab., 1 (1981) 155-185.

Silverberg, G.A., Reitz, B.A. and Ream, A.K., Hypothermia and cardiac arrest in the treatment of giant aneurysms of the cerebral circulation and hemangioblastoma of the medulla. J. Neurosurg., 55 (1981) 337-346.

Smith, A.L., Barbiturate protection in cerebral hypoxia. Anaesthesiology, 47 (1977) 285-293.

Smith, A.L., Hoff, J.F., Nielsen, S.L. and Larson, C.P., Barbiturate protection in acute focal cerebral ischemia. Stroke, 5 (1974) 1-7.

Squire, L.R., Short-term memory as a biological entity. In D. Deutsch and J.A. Deutsch, Short-Term Memory, Academic Press, New York, 1975, pp. 1-40.

Steen, P.A., Milde, J.H. and Michenfelder, J.D., No barbiturate protection in a dog model of complete cerebral ischemia. Ann. Neurol., 5 (1979) 343-349.

Steer, C.R., Barbiturate therapy in the management of cerebral ischemia. Develop. Med. Child. Neurol., 24 (1982) 219-231.

Stefanovich, V. (Ed.), Animal Models and Hypoxia. Pergamon Press, Oxford, 1981.

Stefanovich, V., Anoxic rat model. In V. Stefanovich (Ed.), Animal Models and Hypoxia, Pergamon Press, Oxford, 1981, pp. 111-123.

Stockard, J.J., Bickford, R.G. and Schauble, J.F., Pressure-dependent cerebral ischemia during cardiopulmonary bypass. Neurology, 23 (1973) 521-529.

Swanson, L.W. and Mogenson, G.J., Neural mechanisms for the functional coupling of autonomic, endocrine and somatomotor responses in adaptive behavior. Brain Res. Rev., 3 (1981) 1-34.

Theios, J. and Dunaway, J.E., One-way versus shuttle avoidance conditioning. Psychon. Sci., 1 (1964) 251-252.

Theios, J., Lynch, D. and Lowe, W.F., Differential effects of shock intensity on one-way and shuttle avoidance conditioning. J. Exp. Psychol., 72 (1966) 294-299.

Todd, M.M., Chadwick, H.S., Shapiro, H.M., Dunlop, B.S., Marshall, L.F. and Dveck, R., The neurologic effects of thiopental therapy following experimental cardiac arrest in cats. Anesthesiology, 57 (1982) 76-86.

Touwen, B.C.L., Recovery of neurologically deviant infants. In M.W. van Hof and G. Mohn (Eds.), Functional Recovery from Brain Damage. Developments in Neuroscience, Volume 13, Elsevier/North-Holland, Amsterdam, 1981, pp. 77-94.

Towart, R., Wehinger, E., Meyer, H. and Kazda, S., The effects of nimodipine, its optical isomers and metabolites on isolated vascular smooth muscle. Arzneimittel-Forsch./Drug Res., 32 (1982) 338-346.

Trojaborg, W. and Boysen, G., Relation between EEG, regional cerebral blood flow and internal carotid artery pressure during carotid endarterectomy. Electroencephal. Clin. Neurophysiol., 34 (1973) 61-69.

Valenstein, E.S., The interpretation of behavior evoked by brain stimulation. In A. Wauquier and E.T. Rolls (Eds.), Brain-Stimulation Reward, Elsevier/North-Holland, Amsterdam, 1976, pp. 557-575.

Van Belle, H., The disappearance of adenosine in blood. Effects of lidoflazine and other drugs. Eur. J. Pharmacol., 11 (1970) 241-248.

Van Harreveld, A., Effect of cerebral asphyxiation on a well established conditioned reflex. J. Comp. Physiol. Psychol., 60 (1965) 299-302.

Van Harreveld, A., Reuter, J.H. and Van Hof, M.W., Pattern discrimination after cerebral asphyxiation in the rabbit. In M.W. Van Hof and G. Mohn (Eds.), Functional Recovery from Brain Damage, Elsevier/North-Holland Biomedical Press, Amsterdam, 1981, pp. 115-119.

Van Hof, M.W., Behavioural effects of brain hypoxia in animals. In A. Wauquier, M. Borgers and W.K. Amery (Eds.), Protection of Tissues against Hypoxia, Elsevier Biomedical Press, Amsterdam, 1982, pp. 237-246.

Van Reempts, J., Borgers, M., Van Dael, L., Van Eyndhoven, J. and Van de Ven, M., Protection with flunarizine against hypoxic-ischaemic damage of the rat cerebral cortex. A quantitative morphologic assessment. Arch. Int. Pharmacodyn., 262 (1983) 76-88.

Volpe, B.T. and Hirst, W., The characterization of an amnesic syndrome following hypoxic ischemic injury. Arch. Neurol., 40 (1983) 436-440.

Volpe, B.T., Herscovitch, P., Raichle, M.E., Gazzaniga, M.S. and Hirst, W., Cerebral blood flow and metabolism in human amnesia. J. Cerebr. Blood Flow Metab., 3 (Suppl. 1) (1983) 5-6.

Wauquier, A., The influence of psychoactive drugs on brain self-stimulation in rats. In A. Wauquier and E.T. Rolls (Eds.), Brain-Stimulation Reward, Elsevier/North-Holland, Amsterdam, 1976, pp. 123-170.

Wauquier, A., Neuroleptics and brain self-stimulation behavior. In J.R. Smythies and R.J. Bradley (Eds.), International Review of Neurobiology, Vol. 21, Academic Press, New York, 1979, pp. 335-404.

Wauquier, A., Effect of calcium entry blockers in models of brain hypoxia. In T. Godfraind, A. Herman and D. Wellens (Eds.), Calcium Entry Blockers in Vascular and Cerebral Dysfunctions, Martinus Nijhoff, The Hague, 1984, pp. 241-254.

Wauquier, A. and Declerck, A.C., Neurophysiology of the hypoxic brain. In A. Wauquier, M. Borgers and W.K. Amery (Eds.), Protection of Tissues against Hypoxia, Elsevier Biomedical Press, Amsterdam, 1982, pp. 71-86.

Wauquier, A. and Rolls, E.T. (Eds.), Brain-Stimulation Reward. Elsevier/North-Holland, Amsterdam, 1976, 622 pp.

Wauquier, A., Ashton, D., Clincke, G., Niemegeers, C.J.E. and Janssen, P.A.J., Etomidat: ein barbituratsfreies Hypnotikum, antikonvulsive, antianoxische, und hirnprotektive Wirkung in Tierexperiment. In A. Opitz and R. Degen (Eds.), Anaesthesia bei Zerebralen Krampfanfallen und Intensivtherapie des Status Epilepticus, Verlaggeselischaft mbH, Ellangen, 1980, pp. 183-203.

Wauquier, A., Ashton, D., Clincke, G. and Niemegeers, C.J.E., Anti-hypoxic effects of etomidate, thiopental and methohexital. Arch. Int. Pharmacodyn. Ther., 249 (1981) 330-334.

Wauquier, A., Borgers, M. and Amery, W.K. (Eds.), Protection of Tissues against Hypoxia. Janssen Research Foundation Series, Vol. 7, Elsevier, Amsterdam, 1982, 471 pp.

Wauquier, A., Ashton, D., Clincke, G. and Van Reempts, J., Pharmacological protection against brain hypoxia: the efficacy of flunarizine, a calcium entry blocker. In F. Clifford Rose and W. Amery (Eds.), Cerebral Hypoxia in the Pathogenesis of Migraine, Pitman, London, 1982, pp. 139-154.

Wauquier, A., Ashton, D. and Edmonds, H.L., Protection against cerebral hypoxia by antimigraine drugs. In W.K. Amery, J.M. Van Nueten and A. Wauquier (Eds.), The Pharmacological Basis of Migraine Therapy, Pitman, Bath, 1984, pp. 134-148.

Wauquier, A., Fransen, J., Clincke, G., Ashton, D. and Edmonds, H.L., Calcium entry blockers as cerebral protectants. In T. Godfraind, R. Paoletti and S. Govoni (Eds.), Calcium Entry Blockers and Tissue Protection, Raven Press, New York, 1984, in press.

Weinstein, S.A. and Annau, Z., The effects of carbon dioxide on hypothalamic self-stimulation during hypoxia. Commun. Behav. Biol., 1(4) (1968) 223-229.

Weinstein, S.A. and Annau, Z., Behavioral and physiological measures of adaptation to brief repeated hypoxic exposure. Commun. Behav. Biol., 3(17) (1969) 151-157.

White, B.C., Winegar, C.D., Jackson, R.E., Joyce, K.M., Vigor, D.N., Hoehner, T.J., Krause, G. and Wilson, R.F., Cerebral cortical perfusion during and following resuscitation from cardiac arrest in dogs. Am. J. Emerg. Med., 2 (1983) 128-138.

White, R.J., Austin, P.E., Austin, J.C., Taslitz, N. and Takoaka, Y., Recovery of the subhuman primate after deep cerebral hypothermia and prolonged ischemia, Resuscitation, 2 (1973) 117-122.

Wiedemann, K. and Hoyer, S. (Eds.), Brain Protection, Morphological, Pathophysiological and Clinical Aspects. Springer-Verlag, Berlin, 1983, 168 pp.

Willoughby, J.O. and Leach, B.G., Relation of neurological findings after cardiac arrest to outcome. Br. Med. J., 19 (1974) 437-441.

Winegar, C.P., Henderson, O., White, B.C., Jackson, R.E., O'Hara, T., Vigor, D.N., Kontry, D., Wilson, B. and Shelly-Lane, C., Early amelioration of neurological deficit by lidoflazine after fifteen minutes of cardiopulmonary arrest in dogs. Ann. Emerg. Med., 12 (1983) 471-477.

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