# **Transient Hypobaric Hypoxia Improves Spatial Orientation in Young Rats**

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## Summary

To achieve a better understanding of learning and declarative memory under mild transient stress, we investigated the effect of brief hypobaric hypoxia on spatial orientation in rats. Young male Wistar rats aged 30 days were exposed for 60 min to hypobaric hypoxia, simulating an altitude of 7000 m (23 000 ft) either shortly prior to attempting or after mastering an allothetic navigation task in the Morris water maze with a submerged platform. The post-hypoxic group performed significantly better in the navigation task than the control animals (the mean difference in escape latencies was 11 seconds; P = 0.0033, two-way ANOVA with repeated measures, group × session). The experimental group also achieved a remarkably higher search efficiency (calculated as a percentage of successful trials per session), especially during the first four days following hypoxic stress (P = 0.0018). During the subsequent training, the post-hypoxic group performed better than the control animals, whilst the efficiency levels of both groups progressively converged. Spatial memory retention and recall of well-trained rats were not affected by the transient hypobaric hypoxia. These results indicate that brief hypobaric hypoxia enhances rats' spatial orientation. Our findings are consistent with several studies, which also suggested that mild transient stress improves learning.

### Key words

Spatial orientation • Learning and memory • Water maze • Hypobaric hypoxia • Stress

# Introduction

The theoretical background for studying spatial behavior includes the cognitive map theory proposed by Tolman (1948), the anatomical and physiological basis of which was suggested by O'Keefe and Nadel (1978) in their book entitled "*The Hippocampus as a Cognitive Map*". There are two basic neural forms of navigation. The first, allothesis, uses external landmarks not directly associated with the goal, recognized by vision, audition, smell or touch, to determine the position. Motor schemes are calculated by comparing angles, distances, sizes and

elevations. The second form, idiothesis, depends solely on the internal sensory inputs (i.e. proprioceptive, vestibular, kinesthetic information) and efference copies (locomotory information) calculating motor schemes by path integration (Bureš and Fenton 2000). Various mazebased paradigms including the alley maze (Tolman 1948), the radial maze (Olton and Samuelson 1976), and the water maze (Morris 1981) are commonly used to study spatial behavior in rodents.

Animal models of hypoxic-ischemic brain injury include cardiac arrest, unilateral or bilateral carotid artery ligations and exposure to high altitude (Weinachter *et al.* 

# PHYSIOLOGICAL RESEARCH

1990). The decrease of tissue oxygenation induced by hypobaric hypoxia alters many physiological and psychological processes in an elevation- and durationdependent fashion. The exposure of an organism to transient hypoxic stress activates respiratory and circulatory systems and adrenal glands, and affects neurotransmitter release and action in the central nervous system.

To reach a better understanding of the effect of hypoxic brain injury to the plasticity of the developing neural system in mammals (Trojan and Pokorný 1999), we investigated the spatial performance of rats in the Morris water maze (Morris 1981) using the altitude sickness model.

# Methods

#### Experimental subjects

We used male albino Wistar rats aged 30 days on the first day of the experiment. These animals were kept in plastic cages on natural light cycles, with free access to food and water. The animals were divided into four groups: (I) The acquisition group (N = 14) was exposed to brief hypobaric hypoxia on the first day of the experiment, shortly prior to water maze training. An eight-trial training session was given to each animal on eight successive days, in rats aged of 30-37 days. (II) The control group for the acquisition test (N = 17) received control-treatment instead of the hypoxia administered to the experimental group. (III) The retention group (N = 9)had received eight daily pretraining sessions in the water maze before hypoxic stress was administered. The memory retention test continued with another four sessions on the following four days (IV). The control group for the retention test (N = 4) received controltreatment instead of the hypoxia administered to the experimental group.

#### Hypobaric hypoxia altitude sickness model

The acute mountain sickness model used in our experiment involved hypobaric hypoxia induced by a reduction of the barometric pressure and a corresponding drop in the atmospheric oxygen pressure. The rats were exposed to hypobaric hypoxia for 60 min in an experimental chamber, simulating an altitude of 7 000 meters (23 000 feet,  $pO_2$  64 mm Hg) either shortly prior to memory test or after eight successive days of pretraining in the Morris water maze. The altitude was raised at a rate of 500 meters per minute, and reduced at

the same rate (Fig. 1). The animals were exposed to hypoxia in their breeding cages. The first memory test session was carried out 15 min after pressure had descended back to near sea level conditions.



Fig. 1. The course of the simulated altitude and equivalent barometric pressure used to induce hour-long hypobaric hypoxia.

#### Allothetic navigation task in the Morris water maze

We tested the long-term (reference) declarative memory of rats in a water maze with submerged platform (Morris 1981). The Morris water maze consisted of a circular tank, 1.8 m (6 feet) in diameter and 0.4 m deep. The water temperature was 21 °C. A 10-cm (4-inches) circular platform was submerged 1 cm below the water level in the northwest quadrant of the maze. The position of the platform remained constant throughout the whole experiment. The maze was located in a laboratory where the arrangement of furniture and other orientation points remained constant throughout the entire experiment.

The rat was placed in the water facing a wall of the tank. The starting location was changed according to a pre-defined sequence sufficiently difficult for rats to remember. The escape latency (time needed to find the platform) was recorded. Once the animal found the submerged platform, the trial ended. The session immediately continued with the next trial from the next starting location. If the animal failed to find the platform within 60 s, the experimenter guided it to the platform. In such cases, the animal was allowed to remain on the platform for 15 s and thus to orientate itself using the visual cues around the maze. When a rat found the platform by itself, the trial was scored as successful, but if a rat was led to it, the trial was scored as unsuccessful.

#### Statistical analysis

We assessed two parameters of spatial

performance in parallel: escape latency and search efficiency. A group mean was calculated from the median escape latencies of each animal's performance per session. In addition to recording the escape latency, search efficiency was calculated as a percentage of successful trials per session. Statistical analysis of the differences in both escape latencies and search efficiencies between control and hypoxic animals on successive days of testing was performed using two-way analysis of variance (ANOVA) with repeated measures (the two factors being group and session, matched by subjects). Bonferroni posttests were performed to assess daily differences.



**Fig. 2. Escape latencies for the memory acquisition test.** Numbers on the horizontal time axis indicate experimental days. The arrow shows the water maze session following immediately after the exposure of the acquisition group animals to hypoxia (on day one of the experiment). The statistical significance of the difference between the two learning curves for day one through four and for the overall difference (sessions one through eight) is shown by asterisks (\* P < 0.05, \*\* P < 0.01) based on two-way ANOVA (group × session) with repeated measures. This notation for achieved statistical significance level has been used consistently in all figures.

# Results

Does the exposure of naïve animals to transient hypobaric hypoxia shortly prior to a spatial orientation task affect their spatial memory acquisition?

We found that transient hypobaric hypoxia applied immediately prior to the first spatial learning session had a significant effect on spatial orientation in an allothetic navigation task because it enhanced the performance of 30-day-old rats (Fig. 2). The post-hypoxic group performed better in the navigation task than the control animals: the mean difference in escape latencies was 11 s. The analysis of the four sessions following the hypobaric hypoxia reveals that there is a very significant group effect on escape latency (F = 10.0; P = 0.0033). Further, we found a highly significant session effect, or "learning" (F = 47.2; P < 0.0001), no interaction between group and session (F = 0.19; P = 0.90), and a highly significant subjects matching (F = 3.2; P < 0.0001).



Fig. 3. Search efficiencies for the memory acquisition test. This figure is based on the same data as escape latencies outlined in Fig. 2. However, the curves are somewhat smoother than the vertically mirrored escape latencies curves, since the method of calculation makes them less sensitive to random fluctuations.

The experimental group also achieved remarkably higher search efficiencies (Fig. 3) in the first four days after hypoxic stress. There was again a very significant group effect (F = 11.50; P = 0.0018), a highly significant session effect (F = 55.93; P < 0.0001), no interaction between group and session (F = 1.519; P = 0.21), and a highly significant subjects matching (F = 4.104; P < 0.0001).

We have also analyzed all the eight sessions 1 through 8 to assess if the overall difference in performance still persists. During the following training sessions the post-hypoxic group still performed better than the control group, while the efficiency levels of both groups progressively converged. We found a significant group effect on the escape latency (F = 6.8; P = 0.015), a highly significant session effect (F = 71.3; P<0.0001), no factor interaction (F = 0.88; P = 0.53), and a highly significant subject matching (F = 6.2; P < 0.0001). Similarly, there was a significant group effect on search efficiency (F = 6.9; P = 0.014), a highly significant interaction (F = 3.8; P = 0.0008), and a highly significant subject matching (F = 6.6; P < 0.0001).

Although the overall learning curves obtained in

the acquisition test of experimental and control groups were markedly different, Bonferroni post-tests did not reveal any significant daily differences in spatial performance (P > 0.05; for all sessions and both measured parameters).

Does the exposure of well-trained animals to transient hypobaric hypoxia affect their spatial memory retention and memory recall?

We analyzed the escape latencies obtained during the four sessions following the hypobaric hypoxia (experimental sessions 9 through 12) and observed that hypobaric stress during a period of memory retention did not affect spatial memory retrieval in well-trained 30-day-old rats assessed by means of the escape latencies (F = 0.17; P = 0.69; Fig. 4). There was still a significant session effect (F = 3.7; P = 0.021), no interaction between group and session (F = 0.79; P = 0.51), and a highly significant subject matching (F = 7.7; P < 0.0001). Similarly, the mean search efficiency remained unaffected by exposure to hypoxic stress (F = 0.42; P = 0.53; Fig. 5). There was no session effect on search efficiency (F = 0.22; P = 0.88), no interaction between group and session (F=1.5; P=0.23), and a very significant subject matching (F = 3.3; P = 0.0035). Bonferroni post-tests did not detect any significant daily differences.



**Fig. 4. Escape latencies for the memory retention test.** The arrow indicates the water maze session immediately following the exposure of the retention group animals to the experimental hypoxia (on day nine of the experiment).

In order to address the experimental question by the analysis of "posthypoxic" sessions 9 through 12 as reported in the previous paragraph, we also analyzed the pretraining sessions 1 through 8 to assess if the groups did differ *a priori* with respect to spatial learning parameters due to population variability. Regarding the escape latency, there was no significant group effect in the pretraining period (F = 0.91; P = 0.36), a highly significant session effect (F = 40.2; P < 0.0001), no factor interaction (F = 0.86; P = 0.54) and a highly significant subject matching (F = 9.7; P < 0.0001). Similarly, regarding search efficiency, there was no group effect (F = 0.19; P = 0.67), a highly significant session effect (F = 45.2; P < 0.0001), no interaction (F = 0.26; P = 0.97) and a highly significant subject matching (F = 9.13; P < 0.0001). We conclude that the retention test experimental and control groups did not significantly differ *a priori* in their spatial performance.



**Fig. 5. Search efficiencies for the memory retention test**. The arrow again indicates the water maze session immediately following the exposure of the experimental group of rats to hypobaric hypoxia. This chart is based on the same data as the escape latencies outlined in Fig. 4.

## Discussion

Hypobaric hypoxia causes a decrease of tissue oxygenation inducing severe alterations of physiological and psychological processes in a dose- and durationdependent manner. Exposure of the organism to transient hypoxic stress activates respiratory and circulatory systems and adrenal glands, and affects neurotransmitter release and action in the central nervous system. Known metabolic changes in a hypoxia-afflicted brain include raised concentrations of lactate, glutamate, glutamine and glucose, and lowered N-acetylaspartate, myoinositol, creatine and phosphocreatine concentrations. Depending on the severity of a hypoxic insult, the morphological result of a hypoxic-ischemic brain injury may be cortical necrosis and selective neuronal loss (Šimonová *et al.* 2003) resulting in brain dysfunction.

A large number of studies have assessed

reference memory impairment after exposure to hypobaric hypoxia followed by a subsequent decrease in tissue oxygenation, both in rats (Dell'Anna et al. 1991, Shukitt-Hale et al. 1994) and in humans (Shukitt-Hale et al. 1998). The observed memory impairment is probably linked to one or more of the following pathophysiological mechanisms involved in hypoxic and post-hypoxic brain response: alteration in glutamatergic transmission (Weinachter et al. 1990), reduced hippocampal cholinergic functions (Gibson et al. 1981, Sun et al. 2002), high altitude cerebral edema (Ohashi et al. 1992, Qiao et al. 2001, Xu and Severinghaus 1998, Yarnell et al. 2000), decompression sickness (Curley et al. 1988, Sausen et al. 2001), hypoxic encephalopathy (Šimonová et al. 2003) or perhaps (in visual tasks) influenced by hypoxia-induced retinopathy (Meehan and Zavala 1982, Foulke 1985, Osada et al. 1995).

Contrary to the reference memory deterioration, which was documented by others, following hypobaric hypoxia, the results of our study show that brief hypobaric hypoxia prior to spatial memory acquisition enhances the performance of young rats in an allothetic navigation task. Our findings are consistent with several other studies which also suggest that mild transient hypoxic stress acutely improves both new memory acquisition and reference memory recall assessed by performance in memory tasks sensitive to spatial learning both in rats (Ruthrich *et al.* 1985) and humans (Schlaepfer et al. 1992).

The stimulation of neurotransmitters (namely of excitatory amino acids and dopamine) by hypoxic stress reported by others (Shukitt-Hale *et al.* 1998) could explain at least partly the observed changes in rat's cognitive performance in our experiment. Since memory improvement has also been reported after other stressful stimuli, it may be causally linked to the activation of adrenal glands involved in the acute phase or influence of corticoids in the second phase of the general stress response (Roozendaal 2000).

Methodologically speaking, both assessed parameters of spatial performance – median of the directly recorded escape latencies per session (an established measure of cognitive performance) and the search efficiency calculated as a percentage of successful trials per session (a parameter newly introduced by us) – were usually in good agreement. Occasionally, however, one of the parameters revealed differences whilst the other did not.

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