Physiology & Behavior 98 (2009) 78–84

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Keep cool: Memory is retained during hibernation in Alpine marmots

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ARTICLE INFO

Article history:
Received 11 December 2008
Received in revised form 17 March 2009
Accepted 16 April 2009

Keywords:
Marmota
Behavior
Learning, Operant conditioning
Positive reinforcement
Open field test
Familiarity detection
Locomotor activity

ABSTRACT

Hibernators display severe changes in brain structure during deep torpor, including alterations in synaptic constitution. To address a possible effect on long-term memory, we examined learning behavior and memory of the hibernator *Marmota marmota*. In two operant conditioning tasks, the marmots learned to jump on two boxes or to walk through a tube. The animals were trained during their active season. Performance improved during the training phase and remained stable in a last test, four weeks before entrance into hibernation. When retested after six months of hibernation, skills were found to be unimpaired (box: before hibernation: 258.2 ± 17.7 s, after hibernation: 275.0 ± 19.8 s; tube: before hibernation: 158.4 ± 9.0 s, after hibernation: 137.7 ± 6.3 s). Contrary to these findings, memory seemed to be less fixed during the active season, since changes in test procedure resulted in impaired test performance. Besides the operant conditioning, we investigated the animals' habituation to a novel environment by repeated open field exposure. In the first run, animals showed exploratory behavior and thus a high locomotor activity was observed (63.6 ± 10.7 crossed squares). Upon a second exposure, all animals immediately moved into one corner and locomotion ceased (7.2 ± 1.9 crossed squares). This habituation was not altered even after hibernation (6.1 ± 1.1 crossed squares). We thus conclude that long-term memory is unaffected by hibernation in Alpine marmots.

1. Introduction

Alpine marmots are highly seasonal rodents that spend half of the year in hibernation, a physiological state characterized by alternating phases of normo- and hypometabolism. In deep torpor, metabolic, respiratory and heart rate are actively reduced, followed by a drop down in body temperature to near ambient values. Thereby energy demand in winter is decreased by about 80% as compared to summer, enabling the marmots to live entirely on endogenous fat reserves during the hibernation season [1,2].

Metabolic depression during deep torpor is accompanied by profound changes within the brain. Brain blood flow [3] and metabolism [4,5] are massively reduced and no peripheral EEG can be detected [6,7]. Beyond this, length and branching of dendritic arbors as well as the number of dendritic spines are decreased in the hippocampus [8–10], thalamus and cortex [9,11] and synaptic proteins in these areas seem to dislocate from the postsynaptic density zone [12–14]. These structural changes are found to be reversed during each interbout arousal and the terminal arousal in spring, in a temperature dependent manner. Since synaptic plasticity is essential for memory formation [15,16], a possible impact of degeneration and regeneration on memory must be considered.

It has been shown that low body temperature, hypometabolism and a short photoperiod can influence learning and memory. In non-hibernators, there is evidence for impaired memory consolidation after cooling [17–20], spontaneous daily torpor [21] and at short day length [22], but already stored information seems to be unaffected. The few studies addressing the influence of hibernation on memory led to conflicting results: A study on ground squirrels, which either displayed torpor or did not show torpor following 11 days of cold exposure, demonstrated improved memory retention of the torpor group [23]; another study points to impaired memory in squirrels which had experienced a complete hibernation season, compared to a group which wintered at warm ambient temperatures [24]; furthermore, the discrimination between familiar and unfamiliar conspecifics was found to be unaffected by hibernation in one study [24], while others reported on a deficit in social recognition of unrelated individuals after hibernation [25].

To resolve the actual impact of hibernation on long-term memory, we investigated learning behavior and memory of the hibernator *Marmota marmota*. For this purpose, two common behavioral test approaches, operant conditioning and the open field test, were employed. The conditioning experiments included climbing two boxes in the correct sequence or walking through a tube, in order to obtain a food reward. The open field test was used to ascertain the habituation to a novel environment. The training was carried out during the active season of the animals and the individual performance was tested four weeks before entrance and four weeks after terminal arousal from six months of hibernation for the purpose of comparison.

2. Animals and methods

The study was performed in accordance with the guidelines of the German animal welfare act (Deutsches Tierschutzgesetz).

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doi:10.1016/j.physbeh.2009.04.013

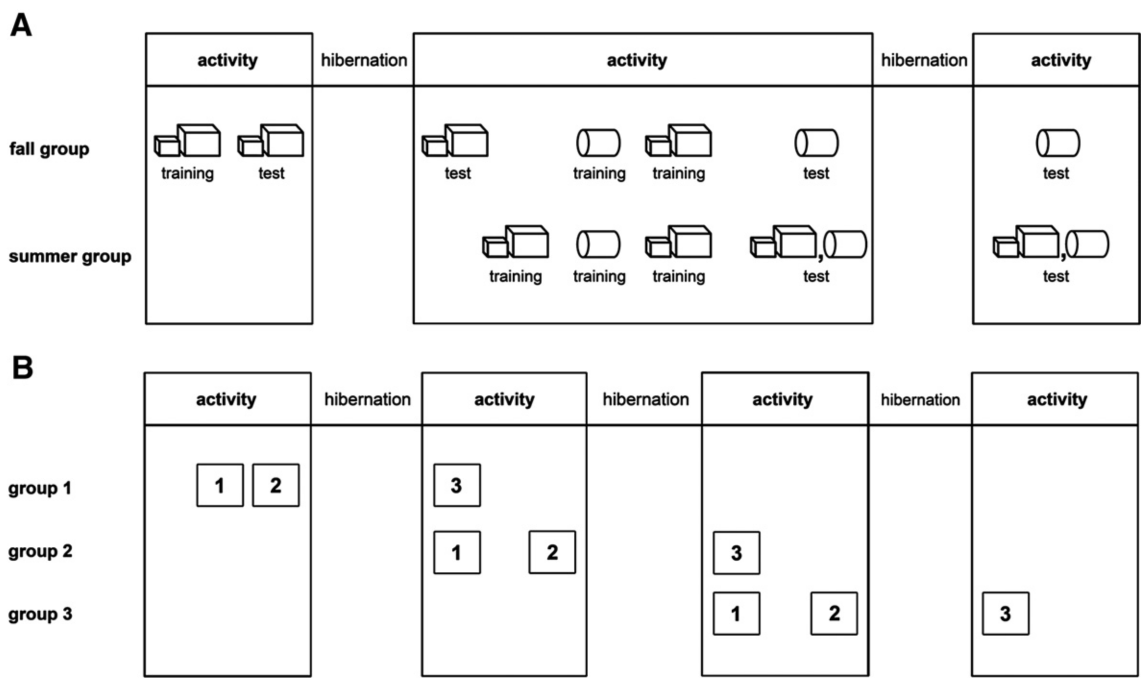

Fig. 1. Experimental time course. A: Conditioning experiments; animals were trained in the box task ( ) or the tube task ( ) during their active season and the performance was tested before hibernation in fall and after hibernation in spring. B: Open field test; 3 groups of marmots were tested in three consecutive years; the first (1) and second run (2) were carried out during the animals' active season and a third run (3) was conducted after hibernation.

2.1. Animals

The study was performed with 10 Alpine marmots bred at the Philipps-Universität Marburg, Germany. Six adult males and 4 adult females out of 3 family groups were used. Ages ranged from 2–6 years at the beginning of the study.

The marmots were housed in family groups. During the active season (April–October), the animals were kept in 12.5–35 m^2 sized pens under natural climatic and photoperiodic conditions (Marburg, Germany: 50°49 N, 09 E). Food and water was provided *ad libitum*. The diet consisted of corn, shredded soy and varying fruit and vegetables. During hibernation (November–March), the family groups were kept in standard rabbit cages ($56 \times 102 \times 60$ cm^3) in a climate chamber with an ambient temperature of 6 ± 1 °C in constant darkness and without food and water. General locomotor activity was monitored with passive infrared motion detectors, providing continuous information about the hibernation status (i.e. deep torpor, interbout arousal or terminal arousal).

2.2. Experimental time course

Prior to the main experiments, a two week long familiarization period was carried out, during which the operator spent 1 h daily within the home pens, feeding the animals and observing their behavior.

2.2.1. Conditioning experiments

The conditioning experiments were performed in two consecutive years with 8 animals in total. The first group of marmots ($n = 3$) was trained in the first task at the end of the animals active season, between August and September (in the following referred to as "fall group"). A second group of marmots ($n = 5$), which served as a naïve, age- and family group-matched control, was trained in the first task in the following summer, between June and July (in the following referred to as "summer group"). Between August and September, both the fall and summer group were trained to learn a second task. The ultimate test session of both tasks was conducted four weeks before entrance into hibernation and memory retention was examined four weeks after terminal arousal from hibernation (Fig. 1A).

2.2.2. Open field test

Data of the open field test were collected in three consecutive years with 10 animals in total. The first run (novelty training) of the initial group of marmots ($n = 3$) was conducted in fall. Novelty training of a second group of marmots, serving as naïve control animals ($n = 5$), was carried out in the following spring and a third group of marmots

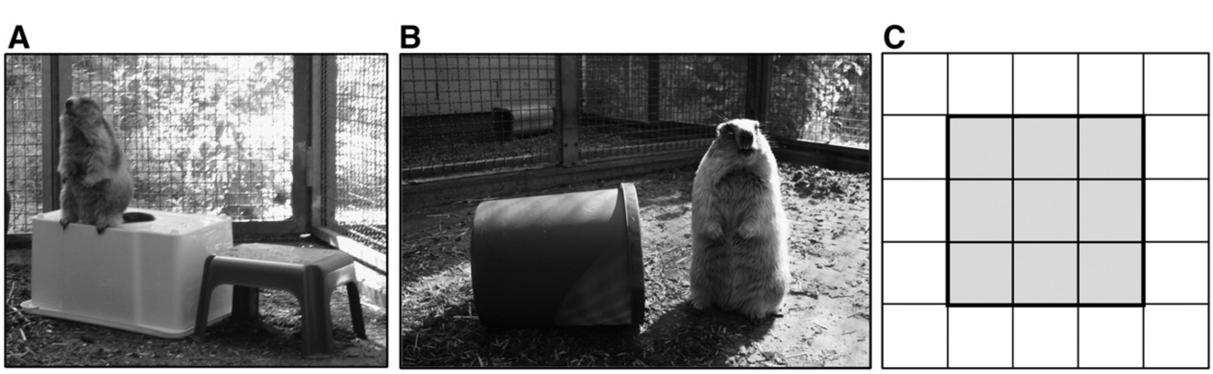

Fig. 2. Experimental set-up. A: Box task. B: Tube task. C: Open field arena; the bold line represents the border of the center fields.