Hibernation does not affect memory retention in bats

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Long-term memory can be critically important for animals in a variety of contexts, and yet the extreme reduction in body temperature in hibernating animals alters neurochemistry and may therefore impair brain function. Behavioural studies on memory impairment associated with hibernation have been almost exclusively conducted on ground squirrels (Rodentia) and provide conflicting results, including clear evidence for memory loss. Here, we for the first time tested memory retention after hibernation for a vertebrate outside rodents—bats (Chiroptera). In the light of the high mobility, ecology and long life of bats, we hypothesized that maintenance of consolidated memory through hibernation is under strong natural selection. We trained bats to find food in one out of three maze arms. After training, the pre-hibernation performance of all individuals was at 100 per cent correct decisions. After this pre-test, one group of bats was kept, with two interruptions, at 7°C for two months, while the other group was kept under conditions that prevented them from going into hibernation. The hibernated bats performed at the same high level as before hibernation and as the non-hibernated controls. Our data suggest that bats benefit from an as yet unknown neuroprotective mechanism to prevent memory loss in the cold brain.

Keywords: hibernation; memory retention; life history; *Myotis myotis*; Vespertilionidae

1. INTRODUCTION

Long-term memory can be critically important for animals in a variety of contexts [1], and yet the extreme reduction in body temperature observed in hibernating animals alters neurochemistry and may therefore impair brain function and memory retention (reviewed in Roth *et al.* [2]). Specifically, hibernating rodents experience a significant loss of synapses [3], a change in synaptic morphology and an overall loss of synaptic efficacy (reviewed in Roth *et al.* [2]). Behavioural studies on memory impairment associated with hibernation have been almost exclusively conducted on ground squirrels and provide conflicting results, including clear evidence for memory loss ([4,5]; reviewed in Roth *et al.* [2] and in Clemens *et al.* [6]). Recent work on another, large rodent—the alpine marmot—showed that these animals remember trained

skills after six months of hibernation at body temperatures of about 11°C [6]. Here, we for the first time tested memory retention after hibernation for a vertebrate outside rodents. We chose a long-lived temperate bat species, the greater mouse-eared bat (*Myotis myotis*). These bats attain nightly foraging radii up to 25 km around their roost, have to relocate small-scale feeding patches and to find and potentially classify ground-running arthropods by their faint walking sounds [7]. These bats also must return successfully to their hibernacula, summer and mating roosts over many years; bats can attain over 30 years in the wild [8]. Sustained long-term spatial memory and associative learning is probably critically important for survival for these bats. Yet, mouse-eared bats hibernate two (Portugal) to five months (central Europe) every winter to survive seasonal food shortage [9]. Their hibernation body temperature typically drops to less than 8°C [10], lower than in the marmots, such that potential decrements of brain function may be larger [6]. Based on the bats' life history and longevity, we hypothesize that maintenance of consolidated memory through hibernation is under strong natural selection in bats, and thus predict that the test bats will perform at similar level before ('pre-test') and after hibernation ('retention test') and as non-hibernated controls.

2. MATERIAL AND METHODS

(a) Animals and housing condition

Thirteen adult male greater mouse-eared bats (*Myotis myotis*) were used. Seven of the animals were captured in August 2005 near Freiburg, Germany, and six near Ruse, Bulgaria, in August 2007 under licence from the responsible authorities (Regierungspräsidium Freiburg, Germany, licence no. 55-8852.44/1095 and Ministerstvo na Okolnata Sreda i Vodita, Sofia, Bulgaria, no. 1897, 16 April 2007). The bats were kept for behavioural experiments at the Seewiesen MPI in an animal facility especially equipped for bat husbandry under licence from the Landratsamt Starnberg (no. 301c.4V-sä). They were housed in a flight cage (2.4 m length × 2.0 m width × 2.0 m height, separable into two divisions) under an inverted light regime (8 D : 16 L (darkness : light)). The bats received food (mealworms—larvae of *Tenebrio molitor*) during training, and water *ad libitum*. Their diet was also supplemented with desert locusts (*Schistocerca gregaria*) once a week and with vitamins and minerals once every four weeks.

(b) Training and testing

All bats were trained to find food in one of three arms of a plastic maze (figure 1). The maze consisted of four plastic boxes (20 × 13.5 × 10 cm), one of which was the starting box and the other three were potential feeding boxes. The boxes were connected to a large central box (24 × 16.5 × 12.5 cm) by transparent tubes (25 cm long, internal diameter 7 cm); this diameter was sufficiently large to allow the bats to crawl and turn easily. Once the bats arrived in the central box, they had to decide which of three tubes to enter (left, right or straight ahead). Each feeding box contained 10 g of mealworms; i.e. the prey-related sensory cues—smell, rustling sounds, etc.—emanating from all three feeding boxes were the same. We trained each bats to feed in only one of the boxes by closing the other two with a slide (figure 1) when a bat entered a non-rewarded maze arm. In the correct feeding box, the bats were allowed to eat two to four mealworms and then removed to start a new trial.

The bats were randomly assigned to either the hibernation group (seven bats) or the control group (six bats), about balanced between bats from Bulgaria and Germany. Half of the bats were trained to find food in the left feeding box and the other half to find food in the right feeding box (balanced between hibernation and control groups).

In an initial training step, the two non-rewarded maze arms were closed. After the bats had learned to crawl from the start box through the central box to the feeding box for food, the two other maze arms were opened. All bats entered non-rewarded arms during training; i.e. they did notice the presence of all arms. If bats entered a non-rewarded arm, access to food was denied by closing the slide and

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Figure 1. Schematic representation of the test maze.

by removing the bat from the maze. We stopped training and proceeded to conduct the pre-test when all bats choose the correct arm 10 times in the first 10 trials of a session. This was achieved after five weeks of training.

During training and experiments, the maze was cleaned with warm water and detergent between each bat to remove any odour cues. Within a session with an individual bat, the different maze arms were interchanged to remove the possibility that a bat would simply follow its own scent cues. In the wild, mouse-eared bats regularly crawl quadrupedally in roost crevices, etc. and accordingly our subjects very readily started to crawl in the test maze.

The pre-test was conducted on 12 December 2009, after 48 h of food deprivation; a duration of food deprivation which is not unnatural for temperate bats. After this pre-test, the control group was kept in a flight cage at 18–20°C, with daily access to food ad libitum as well as the possibility to fly. Each individual ate at least 5 g of mealworms per day. These conditions effectively prevented the control bats from going into hibernation.

The hibernation group was prepared for hibernation by transient exposure to low temperatures, which would simulate the onset of winter. Specifically, the bats were kept at 7°C in a climate chamber (KB53, Binder, Möhringen-Tuttlingen, Germany) for 48 h, then removed and provided ad libitum access to food. After 48 h, they went back into the climate chamber for 72 h and afterwards again received food ad libitum. They were fasted 18 h before going into the climate chamber. Hibernation started on 22 December and continued until 1 March 2010 (10 weeks). All seven bats were kept at 7°C ($\pm 0.1^\circ\text{C}$) and 80–90% humidity in a small cage (26 × 26 × 26 cm; equipped with towels for comfortable roosting) inside the climate chamber in complete darkness. Air exchange was provided by a fan. Water was accessible in the hibernation cage.

To monitor the bats' health, hibernation was interrupted twice for 3 days during which we checked each bat's condition and gave them food ad libitum. Uninterrupted cold phases without food thus lasted 17, 22 and 25 days. Measurement of body temperature was not included in our permit, but we know from other experiments that even during summer, *M. myotis* enter deep torpor (skin temperature 6–10°C) when kept at 6°C in this climate chamber (B. M. Schuller & B. M. Siemers 2009, unpublished data). We monitored the bats' behaviour via a video camera under IR illumination (owing to technical failure, only 86.7% of the second and the third session could be analysed in detail). Some bats were still quite active within the first 24 h of the three 17–25 day periods that they spent in the climate chamber and also during the last 8 h when they were woken up by slowly increasing temperature. On all other days, the bats were inactive and immobile for 94 per cent of the time, which suggests that they were in deep torpor. Some activity (crawling, slow movement inside the towels they roosted in, spreading of wings, auto-grooming) did occur in the remaining 6 per cent of the time (activity was measured in 0.5 h classes). The bats lost about 0.09 to 0.3 g of body mass per day of hibernation. This is more than the 0.05 g d⁻¹ Krzanowski [11] measured for a hibernating *M. myotis* in a Polish cave, but 7–22 times less than 2 g loss that non-hibernating *M. myotis* of our laboratory colony, kept at 18–20°C, show after 1 day without food. This substantiates that

Figure 2. (a) Average proportions of correct decisions and average time to complete a trial (b) for hibernated and control bats before (grey bars) and after (white bars) the hibernation period. Means + 1 s.d. For statistics, see text.

the bats must have been in deep torpor for most of the hibernation treatment, with exception of the first and the last day (see above), but perhaps arousing a little more often or for longer times than wild hibernating bats. After hibernation, all 13 bats were kept at 18–20°C in separate flight cage divisions for one week, fed to recover pre-hibernation body weight and then both groups were tested in the same foraging task again (retention test).

All experiments were filmed with a CCD camera (Watec, WAT-902H2 Ultimate) and recorded onto miniDV tapes (Sony DCR-TRV80E recorder). The time bats took to complete a trial (i.e. from leaving the start box to entering the correct feeding box) was scored off-line by a person unaware of the experimental condition of the bat (hibernation or control). For each bat, we scored the first 10 trials of the test session. One control bat stopped to perform after eight trials in the retention test, which were then taken as 100 per cent.

Statistical tests were calculated in SYSTAT 12.

3. RESULTS

After training, the pre-hibernation performance of all individuals was at 100 per cent correct decisions (figure 2a). All seven hibernated bats and five of the six control bats showed a correct decision in the very first retention trial (no difference between groups, Fisher exact test, $p = 0.46$). Over a series of 10 retention trials, the proportion of correct decisions was at the same high level as in the pre-test for both the hibernated bats (figure 2a; Wilcoxon signed-rank test, $Z = -1$, $p = 0.32$) and the control (i.e. non-hibernated) bats ($Z = -1$, $p = 0.32$). Importantly, the proportion of correct decisions did not differ between hibernated and control bats before (Mann–Whitney test, $U = 21$, $p = 1.0$) or after hibernation ($U = 22$, $p = 0.82$). Moreover, the average time a bat needed to complete a trial did not differ between pre- and retention tests in the hibernated (figure 2b; paired t -test, $t_6 = 1.7$, $p = 0.14$) and control bats ($t_5 = 1.6$, $p = 0.18$), nor did it differ between groups before (t -test, $t_{11} = 0.90$, d.f. = 11, $p = 0.39$) and after hibernation ($t_{11} = 0.75$, d.f. = 11, $p = 0.47$).

4. DISCUSSION

Our data demonstrate that hibernation did not affect memory retention in bats. As a cautionary note, we reiterate that uninterrupted cold phases lasted only 17, 22 and 25 days and the exact lengths of torpor bouts were unknown. As memory loss might be linked to the duration of hibernation ([6]—although their own marmot data contradict this duration hypothesis), three or four months of uninterrupted hibernation should be tested with bats in a next step.

We hypothesized that a long lifespan, high mobility and complex environments favour the evolution of effective memory protection over hibernation, because for species with such a life history and ecology, accumulation of experience over a lifetime will be especially adaptive. At the present stage, the investigated number of species is far too limited for any formal test of such a life-history hypothesis. However, it receives some qualitative support. Species of ground squirrels, in contrast to bats, live in two-dimensional environments, do not forage over large distances, do not migrate and live much shorter times (up to 4–6 years [12]) than bats (even over 30 years [8]) and indeed their memory is more affected by hibernation [13,14] than it was in our bats. Marmots again live in relatively uniform environments, but live up to 12 years [15], and they have very high memory retention [6].

In the poikilothermic snail *Lymnaea*, the retention of consolidated memory is not affected by low temperature [16], just as in marmots and as we showed here for bats. However, the process of memory consolidation (shift from short- to long-term memory) is impaired by low temperatures in *Lymnaea*. Bats make use of torpor not only during hibernation, but also on cool days during their active season. It will thus be an interesting future experiment to investigate whether bats experience a trade-off between saving energy (by becoming torpid) and consolidating newly acquired learned information (by staying warm).

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