

RESEARCH ARTICLE

Do greater mouse-eared bats experience a trade-off between energy conservation and learning?

Ireneusz Ruczyński^{1,2,*}, Theresa M. A. Clarin¹ and Bjoern M. Siemers^{1,†}**ABSTRACT**

Bats, some species of rodents and some birds are able to save energy during the summer period by decreasing their body temperature and falling into torpor. Some studies indicate that torpor prevents sleeping and causes effects similar to sleep deprivation. Impairment of processes stabilizing memory slows down learning accuracy and speed. We conducted two experiments to test whether greater mouse-eared bats, *Myotis myotis*, which commonly use torpor during the summer period, experience a trade-off between energy savings and learning abilities. We compared learning speed and accuracy in bats that were exposed to low (7°C) and higher ambient temperatures (22°C) between training and experimental sessions. Tests were conducted in experiments with food reward (food search) and without food reward (perch search). Time spent with the skin temperature above 30°C was significantly longer for bats exposed to 22°C than for those exposed to 7°C, and longer in experiments with food reward than without food reward. We observed only a very weak tendency for better accuracy and shorter search times in bats exposed to 22°C than in those exposed to 7°C. Our data indicate that memory consolidation of bats under natural conditions is not affected by daily torpor when bats are in good condition and may therefore defend against a rapid fall into torpor. We suggest that homeostatic processes connected with the circadian rhythm allow protection of the consolidation of memory for relatively simple tasks despite time spent in torpor.

KEY WORDS: Life history, Memory consolidation, Memory retention, Normothermy, Torpor

INTRODUCTION

Long-term memory is important for many animals in a variety of different contexts (Roth et al., 2010). Good memory allows for quick and effective rediscovering of food resources and hides, identification of conspecifics, etc. (Shettleworth, 2001; Stoddard et al., 1991). Storage of accurate memories is an active and costly process involving maintenance and repair of neural structures (Dukas, 1999). Therefore, animals may experience conflicts between the benefits and costs of memory retention (the keeping of memory over prolonged periods of time) (Chancellor et al., 2011; Roth and Pravosudov, 2009); for example, food-catching birds living in harsh climates have developed a larger hippocampus volume than those from milder areas, which may result in enhanced spatial memory and better survival in birds from harsh climates. A memory

is normally formed after repeated learning events (Smid et al., 2007) and sleep enhances this process (Palchykova et al., 2002; Roth et al., 2010). Sleep deprivation has negative effects on both memory consolidation [the storage of initial memory representations into long-term memory by stabilization, enhancement and integration processes (Stickgold, 2005)] and retention (Graves et al., 2003; Guan et al., 2004).

Recently, an interesting conflict between energy conservation and sleep was discovered in hibernation and torpor using mammals (for review, see Roth et al., 2010). Such mammals are able to decrease their body temperature to save energy during periods of food deficiency (Geiser, 2008). Although torpor, hibernation and sleep are all characterized by short or prolonged inactivity, they are very different physiological states (Roth et al., 2010) and are distinguished by duration, drop in body temperature and decrease of metabolic rate (Geiser, 2004; Roth et al., 2010). Both rapid eye movement (REM) sleep and slow wave sleep (SWS) are reduced during torpor and hibernation (e.g. Deboer and Tobler, 1996; Deboer and Tobler, 2000; Deboer and Tobler, 2003; Millesi et al., 2001; Trachsel et al., 1991). Animals emerging from these states spend most of their time in sleep with brain activity typical for sleep-deprived animals (Palchykova et al., 2002). This suggests that torpid or hibernating animals experience sleep deficiency (Palchykova et al., 2002; von der Ohe et al., 2007), which might lead to deficiencies in memory consolidation and retention.

The influence of torpor and hibernation on memory consolidation and retention has only been investigated in a handful of studies, primarily in rodents (Clemens et al., 2009; Mateo and Johnston, 2000; Millesi et al., 2001; Palchykova et al., 2006) but also, in one recent study, in bats (Ruczyński and Siemers, 2011). These studies tested the impact of hibernation on long-term memory and taken together provide conflicting results. Millesi et al. (Millesi et al., 2001) provide evidence of memory loss; specifically, spatial memory and memory of operant tasks were negatively affected by hibernation in European ground squirrels, *Spermophilus citellus*. Social memory, in contrast to spatial memory, showed little to no impairment in European ground squirrels or in Belgian's ground squirrels, *Urocyon beldingi* (Mateo and Johnston, 2000; Millesi et al., 2001). Contrary to the results from studies on such relatively short-lived rodents, studies on animals that live for longer and in complex environments, such as marmots and bats, suggested that their memory was not affected by hibernation at all (Clemens et al., 2009; Ruczyński and Siemers, 2011). We (Ruczyński and Siemers, 2011) postulated that the ability to protect long-term memory through hibernation depends on memory type and life history of the respective animals. We further suggested that long-lived animals and those living in complex environments have better protected memory than those that live relatively short lives and/or inhabit simple environments.

Most of these previous studies addressed memory retention over long-term hibernation. Here, we addressed a different question: is

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the consolidation (shift from short- to long-term memory) of newly acquired skills impaired by low body temperature? When an animal moves into a hibernaculum, its memory is probably already consolidated. However, animals that employ short-term torpor on a daily basis may well experience a trade-off between energy savings and memory consolidation, because the latter is expected to require a warm, active brain. The process of memory consolidation invokes the establishment of new synapses and up- or down-regulation of existing synapses. These complex cellular processes will require normothermic conditions for the biochemical machinery to work (Palchykova et al., 2006). Furthermore, torpid animals are not able to sleep in a neurophysiological sense, or may only be able to sleep at a reduced level. As outlined above, this torpor-induced sleep deprivation should counteract memory consolidation. Experiments on Djungarian hamsters, *Phodopus sungorus*, indeed indicate that torpor has a negative influence on memory consolidation and may impair object recognition (Palchykova et al., 2006). In the poikilothermic snails *Lymnaea stagnalis*, the process of memory consolidation was also impaired by quick exposure to low temperatures (Sugai et al., 2007). These results indicate that the effect of decreasing brain temperature after training could be more pronounced on memory consolidation than on memory retention.

Bats are a very interesting group of mammals in this respect. Temperate zone bats, in particular, decrease energetic costs by falling into torpor on a daily basis even during the summer period (Grinevitch et al., 1995; Turbill et al., 2003). Memory retention in the vespertilionid bat *Myotis myotis* Borkhausen 1797 has previously been shown to be not affected by long-term hibernation (Ruczyński and Siemers, 2011; Clarin et al., 2014). Here, we tested the influence of short-term daily torpor on memory consolidation in this species.

Bats are also very long-lived animals. Species of the genus *Myotis* live on average 14.9 years (Barclay and Harder, 2003) and the reported age record for *M. myotis* in the wild is 37 years (Gaisler et al., 2003). Learning, therefore, is probably crucially important in the behavioural ecology of bats (Gaudet and Fenton, 1984; Page and Ryan, 2005; Ruczyński and Bartoń, 2012). As a result, bats could be especially affected by a trade-off between memory consolidation and energy savings using torpor.

To test whether memory consolidation in bats is affected by the decrease in body temperature during daily torpor, we conducted two experiments, one requiring the bats to learn the location of food (with a food reward) and the other requiring them to learn the location of a dry perch (without a food reward). The former experiment required the bats to learn and remember a feeding site, while the latter required the bats to learn and remember an escape route from an uncomfortable situation. We tested whether learning performance depends on the duration of time during which bats stay

at high body temperatures after training. Based on the results from a rodent (Palchykova et al., 2006) and an invertebrate species (Sugai et al., 2007), and because of the expected torpor-induced sleep deprivation, we hypothesized that animals which are able to stay longer at high body temperatures after training would have a greater chance to consolidate memory and could learn faster than those that decrease their body temperatures faster, deeper and for a longer period of time after training. Specifically, we expected individuals kept at 22°C between training and experimental sessions to show faster learning of their respective tasks over consecutive days in terms of the number of correct decisions and time to complete the task than individuals kept at 7°C between training and experimental sessions.

RESULTS

Skin temperature

The duration of the daily period during which the skin temperature of the bats stayed above 30°C ($T_{\text{skin}} > 30^\circ\text{C}$) was significantly affected by experiment type ($F_{1,31} = 11.26$, $P = 0.002$) and temperature in the climate chambers ($F_{1,31} = 37.05$, $P < 0.001$). Animals kept at 22°C between sessions spent more time with T_{skin} above 30°C than animals kept at 7°C (Table 1). Bats in the perch search experiment spent less time with $T_{\text{skin}} > 30^\circ\text{C}$ than bats in the food search experiment (Table 1). The mean duration from the end of the experimental trials to the moment when T_{skin} dropped below 30°C for the first time was shorter in bats kept at 7°C compared with that in bats kept at 22°C ($F_{1,31} = 28.78$, $P < 0.001$), and shorter in bats from the perch search experiment than in bats from the food search experiment ($F_{1,31} = 9.53$, $P = 0.004$). Likewise, the duration of the longest bout with $T_{\text{skin}} < 30^\circ\text{C}$ per each day was shorter in bats participating in the food search experiment ($F_{1,31} = 30.91$, $P < 0.001$) than in bats participating in the perch search experiment, and shorter in bats kept at 22°C ($F_{1,31} = 35.88$, $P < 0.001$) than in bats kept at 7°C (Table 1). Mean skin temperature during bouts with $T_{\text{skin}} < 30^\circ\text{C}$ was lower in bats kept at 7°C, as was the minimum T_{skin} (results summarized in Table 1).

Food search experiment

Neither treatment ($\chi^2_1 = 1.12$, $P = 0.29$) nor day in training ($\chi^2_1 = 0.99$, $P = 0.32$) had an effect on how often the bats ($N = 8$ individuals per treatment group) performed a correct decision during the 2nd and 3rd day of training (Fig. 1). We did not observe differences in the proportion of correct choices made by bats kept at different temperatures during the 4th, 5th and 6th day (experimental sessions; Fig. 2). There was no difference between treatment groups ($\chi^2_1 = 1.41$, $P = 0.23$) or experimental days ($\chi^2_2 = 3.39$, $P = 0.18$). We also did not observe differences in search time between bats kept at 7 and 22°C ($\chi^2_1 = 0.34$, $P = 0.56$) or between experimental days ($\chi^2_2 = 3.13$, $P = 0.21$;

Table 1. T_{skin} measurements of bats kept in climate chambers at 7 or 22°C in the food search experiment and in the perch search experiment

	Food search experiment		Perch search experiment	
	7°C	22°C	7°C	22°C
Daily time period with $T_{\text{skin}} > 30^\circ\text{C}$ (min)	208.8±133.5	583.8±199.3	96.1±42.7	338.3±155.6
Time till $T_{\text{skin}} < 30^\circ\text{C}$ (min)	148±101	520±216	83±51	257±155
Duration of longest bout per day with $T_{\text{skin}} < 30^\circ\text{C}$ (min)	914.4±208.4	634.72±211.3	1207.2±70.5	893.3±191.2
Mean T_{skin} when $T_{\text{skin}} < 30^\circ\text{C}$ (°C)	12.4±7.5	24.2±2.0	9.0±4.8	23.6±1.7
Maximum T_{skin} (°C)	39.2	39.6	38.9	38.4
Minimum T_{skin} (°C)	6.6	22.4	6.6	22.45

Skin temperature (T_{skin}) was measured between experimental sessions during 5 days in the food search experiment and 1 day in the perch search experiment. Measurements started when the bats were placed in the climate chambers and stopped when the bats were removed from the climate chambers. Values are presented as means ± s.d.

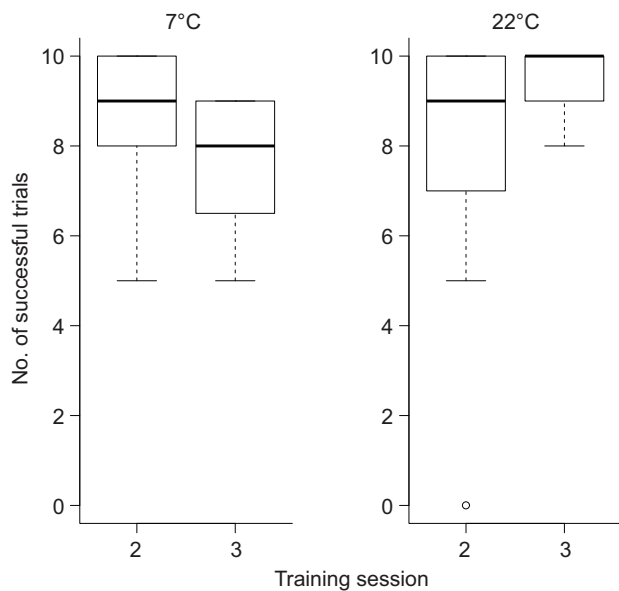


Fig. 1. Number of successful trials during an early phase of training (days 2 and 3) in the food search experiment. Only one arm of the maze was opened. Bats were exposed to 7 or 22°C in the climate chambers.

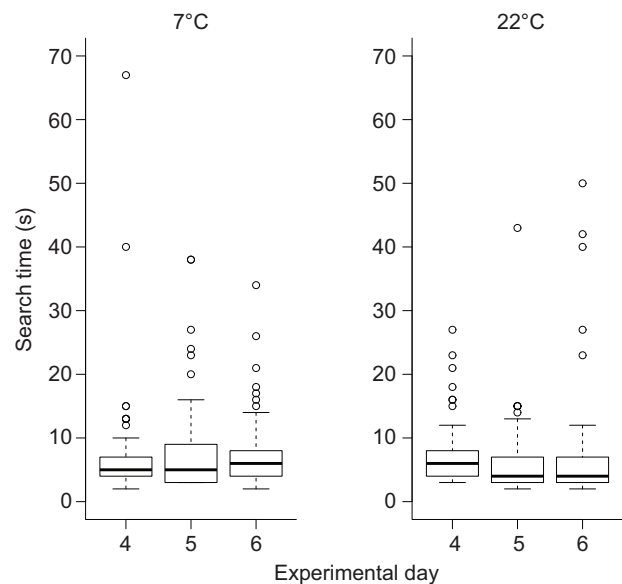


Fig. 3. Time taken to successfully complete a trial in the food search experiment. Between experiments, bats were exposed to 7 or 22°C.

Fig. 3). However, there might be a slight trend for a decrease in search time over consecutive days in the group that was kept at 22°C between experimental sessions.

Perch search experiment

We did not observe differences in the proportion of correct choices made by bats kept at different temperatures in the perch search experiment ($\chi^2=0.04$, $P=0.84$, $N=9$ individuals per treatment group). However, animals made more correct choices on the second day (consolidation test) than on the first day ($\chi^2=4.42$, $P=0.035$; Fig. 4). The time the animals took to find the dry perch did not differ between treatment groups ($\chi^2=0.37$, $P=0.54$). However, on the second day, animals found the dry perch faster ($\chi^2=11.46$, $P<0.001$; Fig. 5).

DISCUSSION

Contrary to expectations, our study showed that daily exposure to a lower temperature had no effect on the bats' learning abilities even though those bats spent less time with $T_{\text{skin}}>30^\circ\text{C}$ in comparison to bats kept at the higher temperature. This suggests that for bats living under natural conditions, daily torpor would have no significant cognitive consequence with respect to relatively simple tasks.

Although the function of sleep is not fully understood, it is known that it plays an important role in the consolidation and retention of memory (Stickgold, 2005), while torpor is a process that allows energy conservation compared with resting (Geiser, 2004). The two states of sleep and torpor are mutually exclusive. When animals decrease their body temperature, the possibility of sleep is

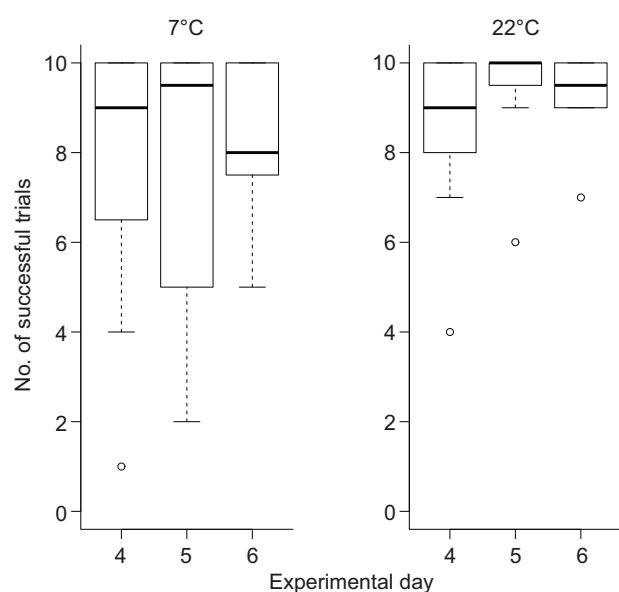


Fig. 2. Number of successful trials during the 4th, 5th and 6th day of the experiment with opened maze arms for bats exposed to 7 or 22°C.

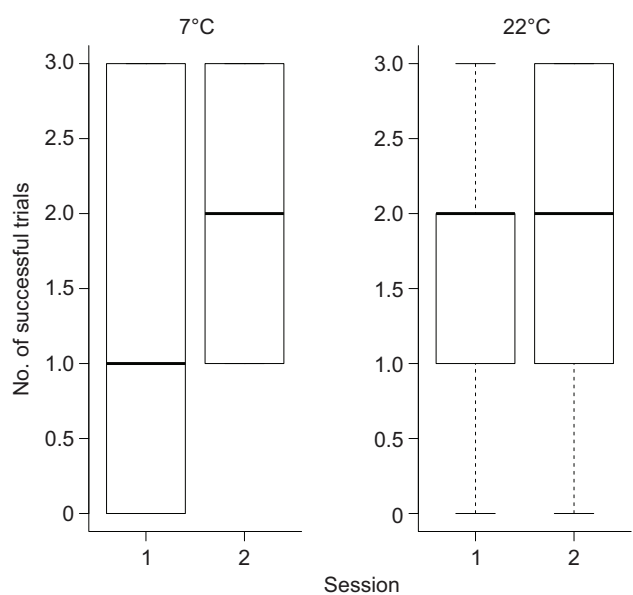


Fig. 4. Number of successful trials on both experimental days in the perch search experiment. Between experimental sessions, bats were kept at 7 or 22°C.

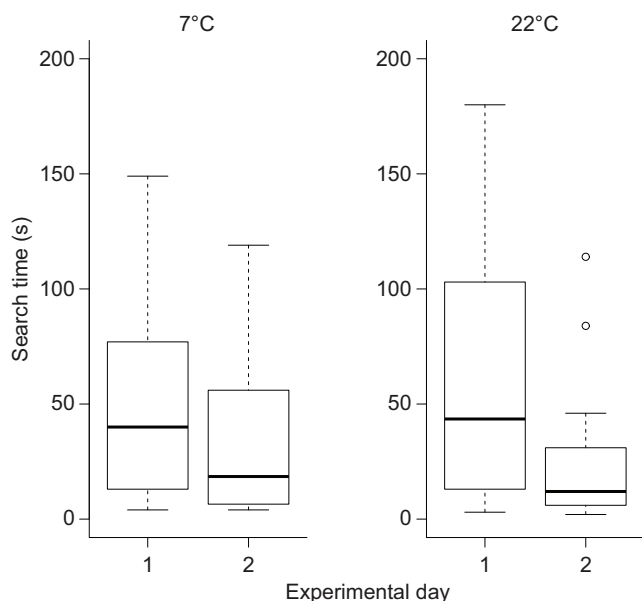


Fig. 5. Time taken to find a dry perch. Between experimental days, bats were exposed to 7 or 22°C.

suspended, and when they increase their body temperature to sleep, the costs of sustaining that high temperature increase (Roth et al., 2010; Trachsel et al., 1991). We conservatively assumed that greater mouse-eared bats (*M. myotis*) are able to sleep when their skin temperature exceeds 30°C. Djungarian hamsters spontaneously sleep when their body temperature rises above 20°C (Palchykova et al., 2002) after torpor. Unfortunately, the influence of torpor on the sleeping pattern of bats is not known (Zhao et al., 2010). Therefore, we can only speculate about the conditions necessary for bats to sleep and consolidate memory.

Our experiments of exposure to low and high temperatures did not reveal differences in the speed of learning between treatment groups, although the period of time with $T_{\text{skin}} > 30^\circ\text{C}$ was significantly different. This indicates that the duration of torpor does not play a role (or plays only a minor one) in the impairment of memory consolidation of a simple task. In other words, bats are able to avoid or minimize the conflict between energy conservation and memory consolidation. Torpor impairs object recognition in rodents in complex tasks but not in simple ones (Palchykova et al., 2006). Bats in our tasks had to associate spatial elements with food or a safe place, which may be a more complicated task than simple object recognition. Such a task demands skills of association of spatial elements with rewards. The extremely weak tendency for slower learning in bats kept at lower temperatures suggests that if torpor indeed affects learning abilities, it is happening at a very subtle level.

The lack of clear cognitive effects of the decrease in body temperature could be explained by a bat's life history. Many bat species live in complex environments and knowledge about this environment is probably important in their long life (Ruczynski and Siemers, 2011). Therefore, behavioural and physiological mechanisms should have evolved in bats to minimize the negative effects of a trade-off between energy conservation and cognitive abilities.

Negative effects of torpor may be particularly minimized when learning is associated with a food reward (which probably is the most common situation in nature). This is because digestion prevents the bats from falling into torpor quickly (Matheson et al., 2010) and thus may provide a suitable amount of time for memory

consolidation, therefore decreasing the potential effects of sleep deprivation by torpor. The subsequent fall into torpor might have no or only minimal effects on acquired skills or knowledge. The idea that recent feeding delays the onset of torpor is further supported by the fact that in both treatment groups, animals that were in the perch search experiment, which were not fed after their training session, entered torpor more quickly and spent more time with T_{skin} below 30°C than bats in the food search experiment, which received food during training and experimental trials. Nevertheless, in both treatment groups, the dynamics of T_{skin} followed natural patterns (Dietz and Kalko, 2006). Bats were active with high body temperatures for several hours after training and only subsequently decreased their body temperatures. Our data suggest that even 1–2 h during which the bats keep their body temperature high is sufficient to consolidate memory about a simple task.

We conclude that under natural situations, negative effects of torpor on cognitive ability play only a marginal role, if any, in bats. However, we suggest that starvation may amplify potential negative effects on cognitive processes in bats. We argue on a more general level that although solving cognitive problems demands high brain temperatures, a period of decreased body temperature and inactivity does not necessarily impair cognitive processes that are actively underway before and after torpor.

MATERIALS AND METHODS

Animals and housing

For our experiments, we used males and post-lactating females of greater mouse-eared bats (*M. myotis*). All animals were captured between 19 June and 15 August 2010 near Ruse in Bulgaria under licence from the responsible authorities (Ministerstvo na Okolnata Sreda I Vodita, Sofia, Bulgaria, 193/01.04.2009 and 205/29.05.2009). The bats were kept for behavioural experiments at the Tabachka Bat Research Station (Bulgaria) of the Sensory Ecology Group (Max Planck Institute for Ornithology, Seewiesen, Germany), which is run in cooperation with the directorate of the Rusenski Lom Nature Park in the district of Ruse. Before experiments began, the animals were housed in small groups in cages (50×35×40 cm length×width×height) under the natural local light regime. The bats were fed daily with 4 g of mealworms (larvae of *Tenebrio molitor*) each. Animals in the food search experiment received their food during training and experimental sessions as a reward. Animals in the perch search experiment received their food after the consolidation test (see below). All animals had access to water *ad libitum* in their cages. Additionally, bats received water after training in the perch search experiment.

Training and testing in the food search experiment

Bats (16 adult males, eight individuals in each treatment group) were hand fed and trained to eat mealworms from small bowls for 2–3 nights after they were caught. Bats were weighed before and after feeding and training. This allowed us to control for the motivation of the bats throughout the experiment. The training and testing maze (see Ruczynski and Siemers, 2011; Clarin et al., 2013) consisted of four plastic boxes (20×13.5×10 cm), one of which was the starting box, while 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 (see Ruczynski and Siemers, 2011; Clarin et al., 2013). Once the bats had 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 live mealworms so that any prey-related sensory cues – smell, rustling sounds, etc. – emanating from the three feeding boxes were the same. We trained each bat to feed in only one of the boxes. To speed up training and decrease stress for the animals, we increased the difficulty of the task over 3 days and measured learning effects over the next 3 days. Training and experiments were conducted during the natural activity hours of the bats (1 h after sunset until 1 h before sunrise), as follows.

On day 1, bats were placed in the starting box and subsequently allowed to explore the maze and all of its boxes for 15 min. In each box there were three mealworms, so the bats were motivated to visit different boxes.

On day 2, again, the bats were placed into the starting box and could crawl to the centre box. Here, only one arm was opened ('correct') while the two other arms were closed by slides ('wrong'). Each terminal box (three boxes) was baited with around 10 g of mealworms. When a bat went into the correct terminal box, it could eat mealworms. This procedure was repeated 10 times. During the first four trials, the bats received four mealworms per trial and in the remaining six trials, they received two mealworms. If a bat turned back, stopped for a longer period of time (ca. 3 min) or successfully found and ate mealworms, it was removed from the maze for 1–3 min before the next trial started. The bats did not receive additional food after training.

On day 3, the procedure from day 2 was repeated. However, bats received four mealworms during the first three trials and two in the remaining seven trials. During the last (10th) trial, the bats received additional mealworms, but not more than 40–45 mealworms in total during one training session, corresponding to ca. 4 g of mealworms. All mealworms were consumed in the maze. The number of mealworms eaten was noted after each trial.

On days 4, 5 and 6, all maze arms were opened. If a bat chose a wrong arm, it was punished by closing the arm with a slide before the bat entered the terminal box. After an unsuccessful trial, the bat was removed and after a short break, the next trial started. During the last (10th) trial, the bats received additional mealworms in the maze when necessary.

The bats were assigned to either the group kept at 7°C ($\pm 0.1^\circ\text{C}$; eight bats) or that at 22°C (eight bats). The temperature in caves used by *M. myotis* is around 15°C during the summer period (B.-M. Clarin, personal communication). We chose lower and higher temperatures for keeping the bats, in order to differentiate between conditions that could affect the duration of daily torpor. Half of the bats were trained to find food in the left terminal box and the other half to find food in the right terminal box (balanced within group). After each training or experimental session, the maze was cleaned with water and detergent to remove possible 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.

After training and experiments, bats were placed in plastic boxes (1.3 l; one for each individual, with numerous holes ensuring air exchange between the box and ambient air) and, 30 min after training, they were transported to climate chambers (KB53, Binder, Möhringen-Tuttlingen, Germany). Bats spent around 22 h per day in the climate chambers. The animals were removed from the climate chambers 1 h before experiments began and transported to the experimental room. Within this 1 h, the bats increased their skin temperature to similar levels in the control (22°C) and experimental groups (7°C).

Air exchange between the boxes and ambient air was fan assisted. Water was accessible in the boxes *ad libitum*. All experiments were filmed with a CCD camera (Watec, WAT-902H2 Ultimate) and recorded on miniDV tapes (Sony® DCR-TRV80E recorder). The time that 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 blind to the experimental condition of the bat (kept at 7 or 22°C).

Training and testing in the perch search experiment

For this second experiment, 18 post-lactating females were caught early in the morning when returning from foraging. Training started the night after the bats were caught. Until training started, bats had *ad libitum* access to water. Animals were not fed until the end of the consolidation test (see below). Naive bats were placed in the experimental setup at the starting point. The bottom of the arena was covered by water to a depth of 0.5 cm. A dry perch was hidden behind a plastic wall. The perch was only accessible by crawling around the plastic wall either on the right side or on the left side. On the other side, access to the dry perch was blocked by another plastic wall. If a bat made a wrong decision and crawled around the wall on the wrong side, it was not taken out of the arena, but allowed to turn around and further explore the arena. To analyse the number of correct decisions, we scored only the first choice an animal made when put into the arena. When

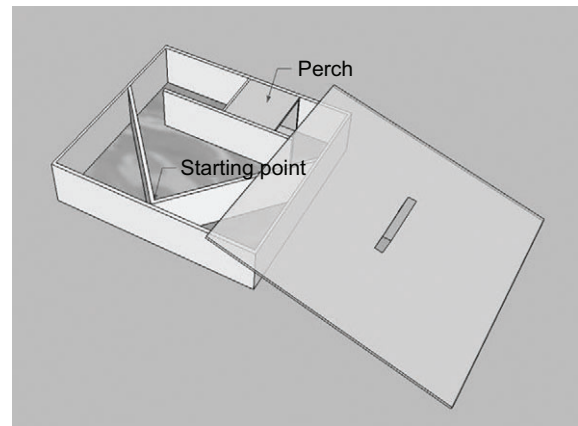


Fig. 6. Experimental setup used in the perch search experiment. Here, the dry perch is only accessible from the right side.

analysing the searching time, we counted the total time an animal needed to find the dry perch. Bats had 3 min to find the dry perch (Fig. 6). If the bat did not find the dry shelter, it was removed from the maze and dried for around 1 min in soft paper. When a bat found the perch, it was allowed to stay there for 3 min. Afterwards, the bat was removed and a new trial started. Every bat had three trials within a training session (one night). Bats were dried in soft paper after training and transported to climate chambers 15 min after finishing the last trial. Bats were divided in two groups (nine individuals in each treatment group); one was kept at 7°C while the other was kept at 22°C. In each group, five bats had to crawl around the plastic wall on the right side and four bats had to crawl on the left side to reach the dry perch. Bats stayed in the climate chambers for 22.5–23 h. For the consolidation test, bats were removed from the climate chambers 1 h before the experiment and kept in small boxes in the experimental room (temperature between 22 and 25°C). Bats were weighed 10–15 min before the test and were checked to see if they were cold or warm to avoid starting experiments with bats still in torpor. The consolidation test was conducted in the same way as the training. Bats were fed after the last trial.

Measurement of T_{skin}

All bats were tagged with iButtons ETL1 (Dallas). The iButton mass was reduced to 1–1.3 g by removing the metal capsule and covering it with rubber shrink tube (van Marken Lichtenbelt et al., 2006; Robert and Thompson, 2003). We used iButtons even though they produce ultrasound, which potentially may disturb bats (Willis et al., 2009), because all bats were exposed to the same acoustic stimulus and the climate chambers produced continuous noise to which the bats habituated quickly. Loggers were glued to the skin in the interscapular region using skin adhesive (Hanfred Sauer GmbH, Labboch, Germany) at least 24 h before the food search experiment began and at least 12 h before the perch search experiment. Skin temperature was measured every 5 min. We compared the duration of time during which the bats had a T_{skin} above 30°C, because higher brain temperature favours the possibility of sleeping and memory consolidation (Palchykova et al., 2002; Roth et al., 2010). We used differences in the time spent with $T_{\text{skin}} > 30^\circ\text{C}$ as an indicator of the possibility of potential memory consolidation. For comparison, we used only the times during which the bats were in the climate chambers. iButtons were taken off after experiment ended and before the bats were released.

Statistical analysis

All statistical analysis was conducted in R Version 2.15.2 (R Development Core Team, 2012). For computation of mixed effects models, we used the lme4 package (Bates et al., 2012). To compare the time spent with $T_{\text{skin}} > 30^\circ\text{C}$ and to compare maximum bout duration with $T_{\text{skin}} < 30^\circ\text{C}$, we ran linear models containing both experiment type (food search versus perch search) and treatment (7°C versus 22°C) as fixed factors. From the perch search experiment, we only had one measurement per individual. For the

food search experiment, we had five measurements per individual. To achieve more balanced data-sets, we analysed mean duration with $T_{\text{skin}} > 30^{\circ}\text{C}$ for each individual in the food search experiment. To compare the proportion of correct decisions during one session, we ran generalized linear mixed effects models for binomial data with day and treatment as fixed factors and individual as random factor. To analyse search times, we computed linear mixed effects models with day and treatment as fixed and individual as random factors. All data on time, except for those of maximum torpor bout duration, were log-transformed to achieve equal variance and a distribution close to the normal distribution. All P -values reported were derived from log-likelihood ratio tests of the minimal adequate model and a model that differs from the minimal adequate model in not containing the fixed effect in question. All box-and-whisker plots show median, 25th percentile, 75th percentile, minimum and maximum. Outliers have values at least 1.5 times the interquartile range (IQR) larger than the 75th percentile or 1.5 times the IQR smaller than the 25th percentile.

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Competing interests

The authors declare no competing financial interests.

Author contributions

I.R., B.M.S. and T.M.A.C. conceived and designed the experiments. I.R. performed the experiments. I.R. and T.M.A.C. analyzed the data. B.M.S. contributed reagents/materials/analysis tools. I.R., B.M.S. and T.M.A.C. contributed to the writing of the manuscript.

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