

RESEARCH ARTICLE

Retaining memory after hibernation: Performance varies independently of activity levels in wild grey mouse lemurs

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Abstract

Hibernation, a hypometabolic state associated with low body temperature and reduced metabolic and activity rates, represents one adaptation to harsh seasonal environmental conditions. As a consequence of hypometabolism, energetically costly neuronal processes also ought to be reduced. Since active neuronal pathways are prerequisites for learning and memory, and because previous studies revealed variable patterns, it remains unclear whether and how hibernating animals retain memories, however. Here, we investigated the effect of seasonally reduced activity on memory retention in 36 wild grey mouse lemurs (*Microcebus murinus*). Data from activity loggers confirmed that female grey mouse lemurs entered hibernation during the cool dry season, whereas males exhibited episodic bursts of activity throughout the austral winter. Thus, compared to males, we predicted females to show lower memory retention of visual and spatial stimulus–reward associations learned before hibernation. In contrast to our prediction, all individuals performed worse in the post-hibernation testing session in both types of tests, compared to the pre-hibernation learning session, and males ($N = 11$) performed even worse than females ($N = 14$) in the post-hibernation testing session. Although females ($N = 9$) equipped with activity loggers tended to be less active than males ($N = 4$), sex-specific activity levels were unrelated to interindividual differences in memory retention. Hence, the post-hibernation decrease in performance of grey mouse lemurs may reflect a more general disability to retain stimulus–reward associations than a lack of memory retention due to seasonal hypometabolism, as suggested for some species of bats or squirrels.

KEYWORDS

activity, cognition, discrimination learning, primates, seasonal hypometabolism, spatial memory

1 | INTRODUCTION

Entering a hypometabolic state is one strategy to escape harsh environmental conditions, such as extensive droughts, cold spells or

food shortages (Lyman et al., 1982). Two hypometabolic states are distinguished. Daily heterothermy or torpor lasts for up to 24h, whereas hibernation (also referred to as long-term torpor or estivation) persists in several bouts that may last several consecutive

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weeks, usually on a seasonal basis, while the circadian clock seems to be suppressed (Geiser & Ruf, 1995; Mohr et al., 2020). In both hypometabolic states, several processes in an animal's body are affected, resulting in lowered body temperatures and reduced metabolic and activity rates (Ruf & Geiser, 2015). Notably, energy expenditure during hibernation is reduced to 1%–10% of basal metabolic rates, allowing an animal to survive periods of limited access to resources (Dausmann et al., 2004; Geiser, 1988; Geiser & Ruf, 1995; Lyman et al., 1982; Mohr et al., 2020; Ruf & Geiser, 2015). As a consequence, energetically costly physiological functions, such as mobility, digestion or neuronal processes, are reduced. The reduction in neuronal activity, in particular, is thought to impede learning and memory since the brain requires a constant energy supply to maintain neuronal pathways (Lukas & Campbell, 2000; Mink et al., 1981). Moreover, hibernating mammals have smaller relative brain sizes than non-hibernating mammals, presumably due to the temporary reduction in energy supplies (Heldstab et al., 2018). Yet, it remains largely unclear whether and how hibernating animals retain memories during hibernation, also because of contradictory results of previous studies.

Studies on ground squirrels (*Sciuridae*) either reported lower or similar memory retention in hibernating individuals as compared to non-hibernating conspecifics (McNamara & Riedesel, 1973; Millesi et al., 2001; Zhao et al., 2004). Furthermore, patterns of memory retention appear context-specific. While spatial memory was reduced, social memory was retained in hibernating versus non-hibernating European ground squirrels (Millesi et al., 2001). Studies on greater mouse-eared bats (*Myotis myotis*) and alpine marmots (*Marmota marmota*) reported no negative effect of hibernation on memory retention (Clemens et al., 2009; Ruczynski et al., 2014; Ruczynski & Siemers, 2011). However, Bechstein's bats (*Myotis bechsteinii*) failed to remember a previously learned association following hibernation (Hernández-Montero et al., 2020). Thus, there is heterogeneity across various mammals and contexts in memory retention across hibernation. Essentially, these studies differed in the duration of the (induced) hibernation and also in the nature of the learned association (Hernández-Montero et al., 2020). Furthermore, all but Bechstein's bats were tested in captivity. Thus, it is difficult to draw general conclusions from these heterogeneous studies.

Several lineages of basal primates also undergo hibernation, despite their (sub-)tropical habitats, including dwarf (*Cheirogaleus* spp.) and mouse lemurs (*Microcebus* spp.) in Madagascar, bushbabies (*Galago moholi*) in Africa and slow lorises (*Nycticebus* spp.) in Southeast Asia (Blanco et al., 2018; Dausmann et al., 2004; Ruf et al., 2015). In contrast to the more “traditional” temperate/arctic hibernators, which are adapted to long predictable cold seasonal habitats, tropical hibernators are opportunistic and “thermolabile” (Dausmann & Warnecke, 2016). Southern lesser bushbabies undergo daily or multiday torpor occasionally (Nowack et al., 2010), pygmy slow lorises (*Nycticebus pygmaeus*) hibernate for 26–62 h, whereas some Malagasy lemurs hibernate for up to several months (Blanco et al., 2018; Dausmann et al., 2004; Fietz & Ganzhorn, 1999; Ruf et al., 2015), albeit with pronounced variation among sympatric

species, as well as among populations and individuals within species (Blanco et al., 2018; Kobbe et al., 2011).

In grey mouse lemurs (*Microcebus murinus*), adult females in central western Madagascar enter hibernation for several months, whereas adult males only enter bouts of daily torpor (Rasoazanabary, 2006; Schmid, 1999; Schmid & Kappeler, 1998). This sex difference could be driven by different reproductive strategies because the brief annual mating season ensues immediately after females emerge from hibernation. Males might be caught in an arms race that puts a premium on timely male physiological readiness for scramble competition polygyny (Eberle & Kappeler, 2004; Schmid & Kappeler, 1998). Hibernating females do not have higher survival rates than non-hibernating males (Kraus et al., 2008). Thus, the ultimate explanation for this sex difference in seasonal activity, which is also found among some ground squirrels (Healy et al., 2012; Michener, 1992), remains obscure but is likely related to reproductive advantages.

However, this uncommon sex difference in activity patterns offers a rare opportunity to investigate variation in memory retention in relation to variable hibernation patterns under natural conditions. To this end, we conducted a visual and spatial discrimination task before mouse lemurs reduce their activity levels and females enter hibernation. We recorded individual variation in (in-)activity with the help of activity loggers and re-captures. After females emerged from hibernation, we retested the same individuals to assess memory retention of the learned stimulus–reward associations. We predicted no sex difference in learning of the association tasks before hibernation, as previously reported as part of another study (Henke-von der Malsburg et al., 2021). Instead, considering the assumed reduced neurological processes during hibernation, we predicted variation in memory retention after hibernation in accordance with sex-specific activity levels during the dry season. Specifically, we expected hibernating females to show worse memory retention than non-hibernating and more active males.

2 | MATERIAL AND METHODS

2.1 | General procedure

We tested the performance of 36 (22 females, 14 males; Table 1) wild grey mouse lemurs in two association learning tasks under short-term captivity in Kirindy Forest, Western Madagascar, between 2017 and 2019. Capture and testing procedures were approved by the Ministry of the Environment, the Mention Zoologie et Biodiversité Animale Université d'Antananarivo and the CNFEREF Morondava. Further, we evaluated our sample following the STRANGE framework (Social background; Trappability and self-selection; Rearing history; Acclimation and habituation; Natural changes in responsiveness; Genetic make-up and Experience) for animal behaviour research (Webster & Rutz, 2020).

Mouse lemurs are solitary foragers but form sleeping groups with conspecifics during the day (Eberle & Kappeler, 2006), thus

TABLE 1 Total sample sizes per sex and task, as well as sample sizes of mouse lemurs equipped with activity loggers in pre-hibernation learning and post-hibernation testing sessions

	N females	N females equipped with activity loggers	N males	N males equipped with activity loggers	Total N tested
visual discrimination	12	8	8	4	20
spatial discrimination	10	8	6	1	16
Pre-hibernation learning	22	15	14	5	36
visual discrimination	8	4	5	2	13
spatial discrimination	6	5	6	1	12
Post-hibernation testing	14	9	11	3	25

experiencing similar social backgrounds and rearing histories. We captured individuals using Sherman live traps, which we baited with small pieces of banana. Grey mouse lemurs show a generally high “trap-happiness” (Kraus et al., 2008), which may minimize a potential bias due to trappability. We briefly restrained newly captured individuals with .6 μ l ketamine (50mg/ml) per 1 g body mass to mark them with a subdermally implanted PIT tag (Trovan, Usling, Germany). We took standard morphometric measures, such as body mass, bitygomatic breadth, body and tail length, as well as tail circumference from all individuals that were captured for the first time in the respective field season (Eberle & Kappeler, 2002; Henke-von der Malsburg et al., 2021). In addition to these targeted captures for experimental animals, we conducted monthly captures to assess population dynamics and general activity levels (Eberle & Kappeler, 2002). We housed subjects in individual cages in a closed room at the research station, where we performed the experimental tests at night under red light conditions. Individuals were fed daily with small insects and banana pieces after testing and had ad libitum access to water. After a maximum of three nights, we released individuals at their capture site at dusk (Henke-von der Malsburg et al., 2021). Nine subjects had to be recaptured to complete the pre-hibernation learning sessions because they failed to learn the tasks within three nights. In general, grey mouse lemurs show a weak stress response toward the capture procedure (Hämäläinen et al., 2014) and acclimate easily to the testing environment (Henke-von der Malsburg et al., 2021).

We focused on adult individuals that already participated in other experimental tasks in previous studies (Henke-von der Malsburg et al., 2021; Huebner et al., 2018) to reduce a potential bias toward greater general testing experience, although all subjects were naïve to the discrimination tasks of the present study. We only tested individuals that were motivated and voluntarily participated in the experiments (Henke-von der Malsburg et al., 2021; Henke-von der Malsburg & Fichtel, 2018). To account for potential self-selection, we controlled for individual variation in exploration and neophilia that may affect discrimination performance or memory (Dougherty & Guillette, 2018; Griffin et al., 2015; Guido et al., 2017). The discrimination tasks required a brief habituation phase that all individuals passed. We conducted pre-hibernation learning sessions at the end of the rainy season

around April when female mouse lemurs increase body mass while males retain a constant body mass (Dammhahn & Kappeler, 2012; Schmid & Kappeler, 1998). We conducted post-hibernation testing sessions between August and November as soon as we recaptured the same subjects.

To relate an individual's body condition to its experimental performance in cognitive tests, we calculated an individual's body mass index (BMI), dividing its body mass [g] by bitygomatic breadth [mm] (Henke-von der Malsburg et al., 2021). Additionally, we calculated the change in tail circumference [mm] between the end of the rainy and the end of the dry season. This difference provides a proxy for the body fat used during the dry season since mouse lemurs store most of their fat in their tail (Fietz, 1998; Schmid, 1999). Finally, to control for potential age effects on cognitive performance, we estimated an animal's age as the difference between the testing date and its presumed birth date (January 1 of the year in which they were first captured; Eberle & Kappeler, 2002). Subjects ranged from .30 to 8.41 years of age (mean: 1.89 ± 2.07 years).

To measure the activity levels of mouse lemurs, we equipped $N = 21$ subjects ($N = 15$ females, $N = 6$ males; Table 1) with collars containing a geolocator (GDL3pam, Swiss Ornithological Institute, Sempach, Switzerland; Liechti et al., 2013; Meier et al., 2018). We removed the collars when we conducted the post-hibernation memory retention test session.

2.2 | Experimental tests

Experimental testing started between 6:00p.m. and 7:00p.m. under red light conditions, when subjects were active and motivated, and ended when their motivation decreased (Henke-von der Malsburg et al., 2021). All experimental sessions were videotaped (Sony HDR-CX 240) and later analysed in BORIS (version 5 or higher; Friard & Gamba, 2016). Most of the subjects ($N = 32$) already participated in other experiments of earlier or parallel studies, thus, being familiar with the housing conditions (Henke-von der Malsburg et al., 2021; Huebner et al., 2018). The remaining $N = 4$ individuals were naïve to the testing environment but habituated quickly to the testing conditions. We tested individuals either in a visual discrimination ($N = 20$) or spatial discrimination

($N = 16$; Table 1) task. All individuals were naïve to these specific tasks.

2.2.1 | Visual discrimination

For the visual discrimination learning task, we used an apparatus consisting of a plate with four tubes that differed in shape and pattern of the form surrounding the rotating lid at their front part (Figure 1(a)) (Henke-von der Malsburg et al., 2021). During an initial training session, we removed the lids so that mouse lemurs could easily access the food rewards (small pieces of banana) in each of the tubes. After six trials in which the animals ate all four food rewards, we started with the actual pre-hibernation discrimination learning.

In the learning phase, we baited only one of the forms, serving as S^+ that was pseudo-randomized between subjects. Throughout an experimental session of 15 trials, we changed the position of the S^+ at least every third trial in a pseudo-randomized way, that is, the S^+ form was either on the left (position 1 or 2) or right side of the plate (position 3 or 4; Figure 1(a)) for the respective half of the trials. We cleaned the whole apparatus with 70%-ethanol before each experimental session and after every third trial to remove potential odour cues.

We continued the pre-hibernation discrimination learning until the subjects achieved an 80% learning criterion, choosing the S^+ -form for at least 24 out of 30 trials in two consecutive sessions without prior manipulation of other forms (Henke-von der Malsburg et al., 2021). To ensure the subjects learned to associate the S^+ with the food reward, we performed an additional testing session of 15 trials that we subsequently used to assess the pre-hibernation criterion performance.

The post-hibernation testing was performed several months later without prior training, but using the same S^+ as in the pre-hibernation discrimination learning task. As before, we conducted

15 trials per session, with randomly changed positions of the S^+ and cleaning the testing apparatus after every third trial with ethanol. We used the first post-hibernation testing session for assessing the post-hibernation retention performance. In case the subjects did not perform on an 80%-performance level (min. 12 correct trials) in this session, we continued with the testing similarly to the pre-hibernation discrimination learning until the subjects achieved the original 80%-learning criterion (re-learning).

We counted the number of correct and incorrect trials for each session and added up the number of errors per trial. An error was defined as manipulating one of the three non-rewarded forms. We calculated a retention score by dividing the post-hibernation retention performance (number of correct trials) by the pre-hibernation criterion performance. Additionally, we calculated the difference in errors between the first post-hibernation testing session and the last pre-hibernation learning session.

A total of $N = 20$ individuals ($N = 12$ females, $N = 8$ males; Table 1) participated in the visual discrimination learning task. Four females and three males were not recaptured after the hibernation period. These individuals were, hence, excluded from the analyses. Of the remaining eight females and five males, four females and two males were equipped with activity loggers. Except for one female, all recaptured individuals had to be trained to re-learn the visual discrimination after hibernation as they did not perform on an 80%-performance level during the first post-hibernation testing session.

2.2.2 | Spatial discrimination

For the spatial discrimination learning task, we used a plus maze consisting of four uniform arms with a removable end box that could be closed and which contained a small lid in which we placed a food

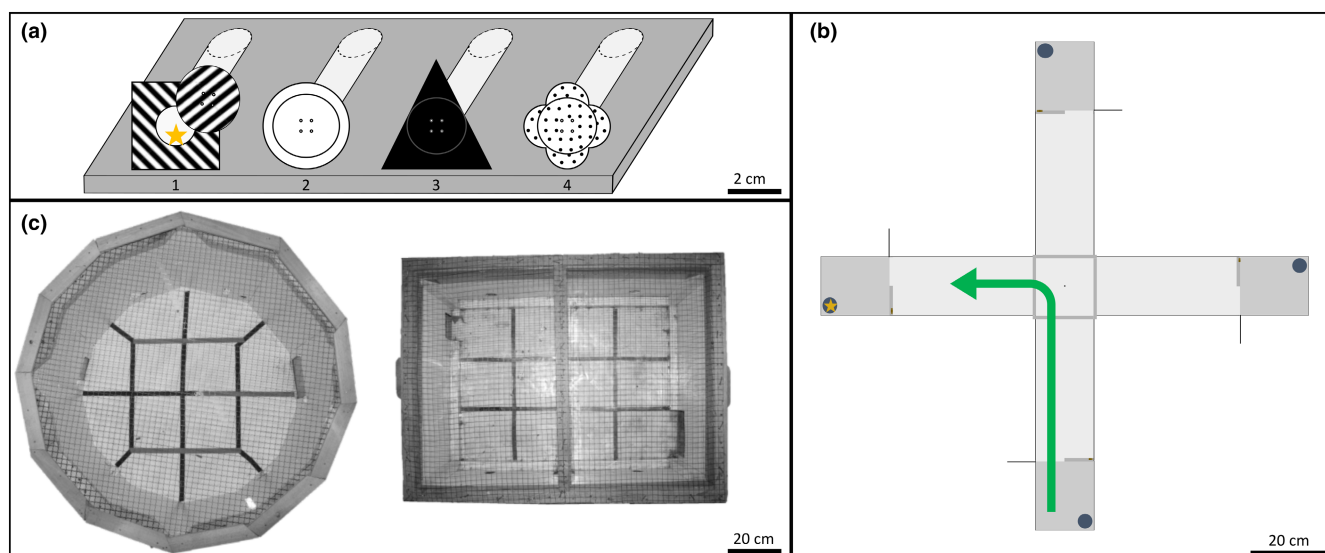


FIGURE 1 (a) apparatus used for the visual discrimination experiment. In this example, the striped square would be the visual stimulus that should be associated with the food reward (star). Numbers indicate the positions of the tubes. (b) plus maze used for the spatial discrimination experiment. The arrow indicates a correct path in the case for the food reward (star) being positioned in the left end box. (c) arenas used for the open field and subsequent novel object test (novel objects not shown).

reward (small piece of banana; [Figure 1\(b\)](#); Henke-von der Malsburg et al., 2021). After an initial familiarization trial in which the mouse lemurs could extract the food reward out of each of the three end boxes (the fourth end box was the start box from which we released the animal into the maze, therefore not containing a food reward), we proceeded with the pre-hibernation discrimination learning. Here, we baited only one of the three endboxes placed at a predetermined S^+ position pseudo-randomized between subjects. To control for odour cues, we placed a piece of banana peel at the end of each arm out of reach for the animals. We rotated the endboxes throughout the session to ensure that the animals would not simply follow odour cues emerging from the end boxes. Additionally, we cleaned the maze before a session and after each third trial to remove potential odour cues (Henke-von der Malsburg et al., 2021). The rest of the testing procedure remained the same as in the visual discrimination task, that is, per session we conducted 15 trials, and subjects were tested until they reached a learning criterion of 80% correct trials.

We counted the number of correct and incorrect trials for each session and added up the number of errors per trial. We defined a trial as correct when the subject did not enter one of the other arms before reaching the food reward in the S^+ box at the end of the S^+ arm. An error was defined as entering one of the three non-rewarded arms of the maze.

A total of $N = 16$ individuals ($N = 10$ females, $N = 6$ males; [Table 1](#)) participated in the spatial discrimination learning task. One female did not reach the learning criterion, and three females were not recaptured after the hibernation period. These individuals were excluded from further analyses. Of the remaining six female and six male mouse lemurs, five females and two males were equipped with activity loggers. Two females and two males did not perform at an 80%-performance level in the post-hibernation retention session and were, thus, retrained until they achieved the original learning criterion.

2.2.3 | Personality tests

To control for potential personality effects, we estimated individuals' exploration and neophilia using an open field test and a novel object test. For the open field test, we transferred subjects to one of two arenas (cylindrical or rectangular base; [Figure 1\(c\)](#)) which they could explore freely during a 5-min test duration. We used the duration individuals spent locomoting during these 5 min as our measure for *exploration*. Afterwards, we introduced a novel object (plastic Snoopy or metallic toy car) into the arena and recorded the animals' responses toward the novel object. As our measure for *neophilia*, we used the number of contacts individuals made with the novel object during the 5-min test. To estimate the repeatability of these variables, we conducted the two tests twice; once in the pre-hibernation learning phase and once in the post-hibernation retention phase with alternating open field arenas and different novel objects (Henke-von der Malsburg et al., 2021). Both variables were repeatable (*exploration*: Intraclass correlation coefficient [ICC] = .182; *neophilia*: ICC = .314; ICCest, Wolak, 2015).

2.3 | Morphometric measures and activity

To compare variation in fat storage between the sexes, we applied a Mann–Whitney U test on the difference in tail circumference between the last capture before and the first capture after the hibernation period. To assess general activity levels, we compared recapture rates between both sexes from before the onset of the hibernation period (April) until the emergence of hibernation (October) using proportion tests (“prop.Test”) applied to the monthly capture rates.

To measure the activity levels of a subset of the subjects ($N = 21$), we used geolocators (GDL3pam) provided by the Swiss Ornithological Institute that we attached to a harness serving as a collar. The logger weighted a maximum of 4% of an individual's body mass (mean = $3.29\% \pm .42\%$, min = 2.41%, max = 4.14%). To ensure the adequate attachment of the logger without harming the animal, we briefly restrained the animals with ketamine when attaching the collar and afterwards surveyed the individuals for a minimum period of 30h in their housing cages. Additionally, we controlled the collar's fit every time we recaptured an individual and had to remove only one collar due to minor health problems.

Although we are convinced that the collars did not affect the animals' survival in the forest, because many other individuals have been previously equipped with a radio collar (Dammhahn & Kappeler, 2008; Eberle & Kappeler, 2002; Eberle & Kappeler, 2004; Lühns et al., 2009), we only recaptured $N = 13$ individuals after the hibernation period. Mouse lemurs generally face high mortality rates from a variety of raptors, carnivorans, snakes and another lemur species, especially during the hibernation season (Goodman et al., 1993; Kraus et al., 2008; Rasoloarison et al., 1995), so the failure to recapture collared individuals was not larger than the corresponding proportion of un-collared animals.

The activity loggers recorded the static and dynamic acceleration along the z-axis of the sensor once per minute. To reduce memory space, only the mean of an acceleration sample (*pitch*) and the sum of the absolute differences between consecutive data points (*activity*) were stored (Liechti et al., 2013). The *pitch*, representing the static acceleration, consists of 32 successive measurements at a frequency of 10 Hz and reflects the relative position of the logger to its z-axis. *Activity*, representing the dynamic acceleration, reflects the change of position of the logger. Hence, if *activity* is equal to zero, the collar was in a static position, while it has moved when *activity* is smaller or larger than zero. Although the discrete values of the *pitch* do not necessarily reflect the position of the mouse lemur, the differences between data points, that is, *activity*, reflect whether the mouse lemur was moving or not.

We calculated an individual's activity threshold to define its daily activity state (active vs. inactive). Therefore, we first examined the maximum nighttime (from 3:00 p.m. to 7:00 a.m.) and daytime *activity* measure. Second, we examined an activity threshold as the 95%-quantile of the daytime *activity* (i.e. the resting period of mouse lemurs) over the recorded period. We visually verified this threshold to classify a reasonable amount of daytime activity as inactive states (ESM, [Figure S2](#)). The recorded *activity* below this threshold

was assumed to be random noise resulting from a movement of the collar only. Above this threshold, we consider the recorded *activity* as body movement that is most likely when the animal was actively moving. We noted a daily activity state as active if the maximum *activity* per night was above the activity threshold. If the maximum *activity* per night was below this threshold, we scored the daily activity state as inactive. We counted the number of active and inactive days between the final pre-hibernation criterion session and the first post-hibernation retention session. We compared sex-specific activity levels by applying a t-test on the number of inactive days between the test sessions.

2.4 | Statistical analyses

We conducted all analyses in R (version 4.0.0, R Core Team, 2020), using multivariate (mixed) models to examine variation between pre-hibernation and post-hibernation performances, variation in the difference between these performances and variation in pre- and post-hibernation learning speeds.

To achieve easier interpretable coefficients, we scaled all covariates to a mean of zero and a standard deviation of 1 (Schielzeth, 2010). For all models, we first tested the overall effect of the fixed factors by comparing the full model to its null model comprising only the intercept. For the individual predictors, we then derived respective test statistics and *p*-values using the function “drop1.” We used significance levels of .05. Depending on the model's error structure, tests of significance were either based on an analysis of variance (ANOVA) with an F-test, or likelihood ratio tests (LRT). We either extracted the overall effect sizes (R^2) directly from the model's summary output or calculated Nagelkerke's R^2 . To control for model validity and stability, we checked Cook's distance, DFBetas, DFFits (function “dffits”) and variance inflation factors and controlled for overdispersion, variance inflation factors (Fox & Weisberg, 2011), and for normality and homogeneity of residuals (plotting a qq-plot for the residuals and a scatterplot between residuals and fitted values), where appropriate. No influential cases or apparent deviations from the respective model's assumptions were detected.

2.4.1 | Variation between pre-hibernation and post-hibernation performances

We calculated two models to examine variation between pre-hibernation and post-hibernation performances. To model the success rate as the relation between correct and incorrect trials, we set the combination (using the “cbind” function) of respective numbers as the response in a generalized linear mixed model with a binomial error structure (family = “binomial”) and logit link function (BMM; package “lme4”; Bates et al., 2015). To model the number of errors, we log-transformed the error count and calculated a linear mixed model with Gaussian error distribution (Bates et al., 2015). In both models, we set

sex in interaction with the task (visual vs. spatial) and the session (pre-hibernation vs. post-hibernation) in interaction with the task as fixed factors. As random factors, we used the individual ID.

2.4.2 | Variation in differences between post- and pre-hibernation performances

We first examined variation in differences between post- and pre-hibernation performances as a function of the experimental task by setting the deviation in the performance score (retention score vs. difference in error counts) as response and the task (visual vs. spatial) as a fixed factor in a linear model with Gaussian error distribution.

Second, to examine the effects of an individual's activity, sex, age, BMI change, personality, as well as the days between tests on the deviation between the pre-hibernation criterion performance and the post-hibernation retention performance, we calculated separate linear models with Gaussian error distribution per task (visual or spatial) and performance score (retention score or difference in error counts). To reduce model complexity, we calculated individual models with (i) sex and days between tests, (ii) age and BMI change, (iii) exploration and neophilia and (iv) the number of inactive days between the post- and pre-hibernation test sessions as respective fixed factors.

2.4.3 | Variation in pre-hibernation and post-hibernation learning speed

Finally, we examined variation in pre- and post-hibernation learning speeds. We first examined differences in learning speed between sexes, testing phases and experimental tasks. To this end, we calculated a linear mixed model with Gaussian error distribution and the number of trials conducted (log-transformed) as the response variable (package “lme4”; Bates et al., 2015). We set sex and the interaction between the testing phase (pre- vs. post-hibernation) and the experimental task (visual vs. spatial) as fixed factors and used the individual ID as a random factor.

Second, we examined variation in the pre- and post-hibernation learning speed in response to individual characteristics by calculating separate linear models per testing phase and task with the number of conducted trials (log-transformed) as the response variable. As fixed factors, we set (i) the mean age and the mean BMI in the respective testing phase, or (ii) exploration and neophilia.

3 | RESULTS

3.1 | Variation between pre-hibernation and post-hibernation performances

The model evaluating variation in the success rate between test sessions and experimental tasks differed significantly from its

null model ($X^2_{[5,25]} = 140.52, p < .001$). All predictors significantly influenced the success rate (sex*session: $LRT = 10.13, df = 3, p = .001$; session*task: $LRT = 9.27, df = 3, p = .002; R^2 = .94$; Table 2; Figure 2(a)). Males achieved higher success rates than females in the pre-hibernation criterion session, but lower success rates in the post-hibernation retention session. For both sexes, success rates in the pre-hibernation criterion session were higher than those in the post-hibernation retention session. Success rates in the post-hibernation retention session were higher in the spatial discrimination than in the visual discrimination task.

The model evaluating variation in error counts significantly differed from its null model ($X^2_{[5,25]} = 56.40, p < .001$; Table 2). Sex in interaction with the test session significantly influenced the error count ($LRT = 5.61, df = 3, p = .018$), while the test session in interaction with the experimental task did not ($LRT = .77, df = 3, p = .382; R^2 = .68$; Table 2; Figure 2(b)). Males made more errors in the post-hibernation retention session than females. Both sexes made more errors in the post-hibernation retention session than in the pre-hibernation criterion session.

3.2 | Variation in differences between post- and pre-hibernation performances

3.2.1 | Experimental task

The model evaluating variation in the retention score between the visual and spatial discrimination task, that is, the number of correct trials in the post-hibernation retention session in relation to the number of correct trials in the pre-hibernation criterion session, significantly differed from its null model ($F_{[1,23]} = 14.81, p < .001; R^2 = .39$). In the spatial discrimination task, subjects had a higher retention score than in the visual discrimination task (estimate = $.305 \pm .08, t\text{-value} = 3.85$). The model evaluating variation in error counts between pre-hibernation criterion session and

post-hibernation retention session did not significantly differ from its null model ($F_{[1,23]} = 1.46, p = .240; R^2 = .06$). The difference in error counts did not differ between the visual and spatial discrimination (estimate = $-3.34 \pm 2.77, t\text{-value} = -1.21$).

3.2.2 | Individual characteristics

None of the models evaluating variation in the retention score in response to individual characteristics (sex and number of days between testing, age and BMI change, exploration and neophilia) did differ from their respective null models in the visual discrimination (sex+ days: $F_{(2,10)} = 2.99, p = .096, R^2 = .25$; age+ BMI: $F_{(2,10)} = 1.21, p = .337, R^2 = .03$; exploration+neophilia: $F_{(2,10)} = .45, p = .647, R^2 = -.10$), or in the spatial discrimination (sex+ days: $F_{(2,9)} = 1.93, p = .201, R^2 = .14$; age+ BMI: $F_{(2,9)} = .79, p = 0.482, R^2 = -.04$; exploration+neophilia: $F_{(2,9)} = 2.55, p = .133, R^2 = .22$).

Similarly, none of the models evaluating variation in the error count did differ from their respective null models in the visual discrimination (sex+ days: $F_{(2,10)} = 3.18, p = .085, R^2 = .27$; age+ BMI: $F_{(2,10)} = 1.70, p = .232, R^2 = .10$; exploration+neophilia: $F_{(2,10)} = .99, p = .405, R^2 < 0$), or in the spatial discrimination (sex+ days: $F_{(2,9)} = 1.73, p = .231, R^2 = .12$; age+ BMI: $F_{(2,9)} = .87, p = .453, R^2 = -.02$; exploration+neophilia: $F_{(2,9)} = .70, p = .520, R^2 = -.06$). Hence, the assessed individual characteristics and the days between pre- and post-hibernation test sessions did not explain variation in memory retention of visual and spatial stimulus reward associations.

3.2.3 | Morphometric measures and activity

The Mann-Whitney U test comparing the change in tail circumference between the sexes confirmed that females lost significantly more body mass than males across the hibernation period ($U = 117.5$,

TABLE 2 Statistics evaluating variation in success rate and error score in response to sex, session, and task

Model response	Predictor	Estimate \pm se	Test statistic	p-value
Success rate	Intercept	F, visual, post: $.49 \pm .22$	2.25	-
	sex*session	M: $-.78 \pm .29$	-2.65	.001
		pre: $2.28 \pm .40$ M:pre: $1.85 \pm .63$	5.63 2.94	
session*task		pre: $2.28 \pm .40$	5.63	.002
		spatial: $1.61 \pm .30$	5.28	
		pre:spatial: $-1.74 \pm .56$	-3.11	
Error count	Intercept	F, visual, post: $2.15 \pm .22$	9.92	-
	sex*session	M: $.62 \pm .28$	2.24	.018
		pre: $-1.52 \pm .29$ M:pre: $-.88 \pm .37$.29 .37	
session*task		pre: $-1.52 \pm .29$	-5.20	.382
		spatial: $-.42 \pm .27$	-1.52	
		pre:spatial: $.31 \pm .37$.83	

Abbreviations: F: Female, M: Male, pre: Pre-hibernation criterion, post: Post-hibernation retention, visual: Visual discrimination, spatial: Spatial discrimination, SE: standard error

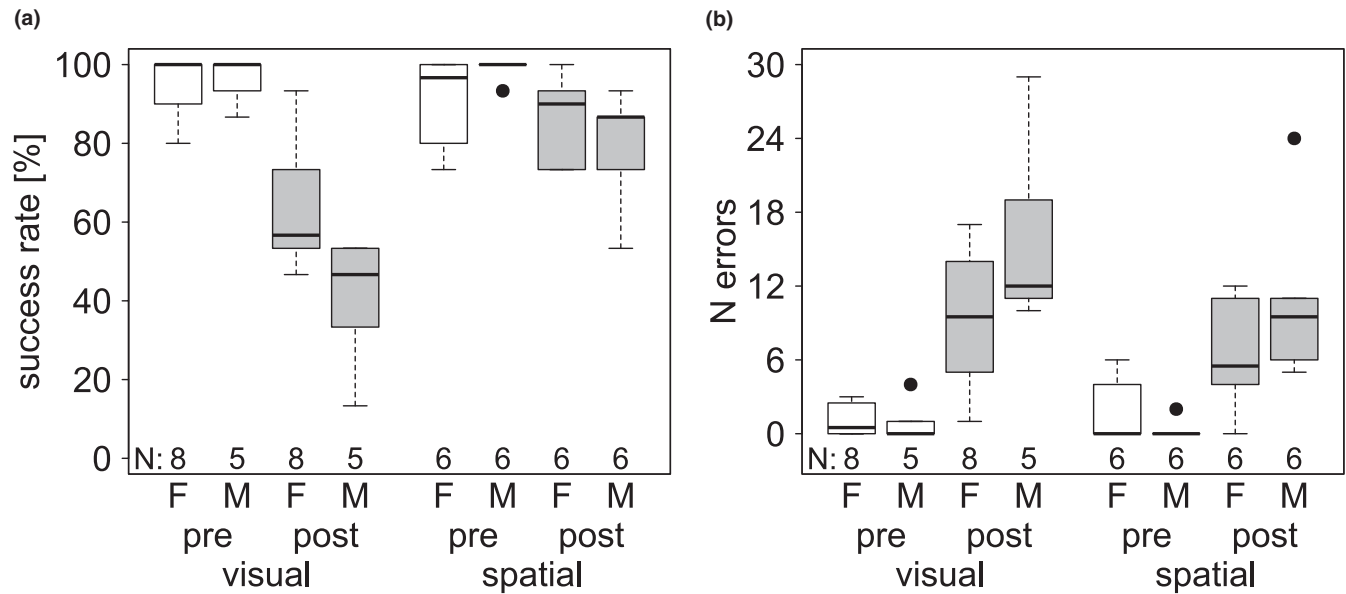


FIGURE 2 Success rates (a) and error counts (b) per sex (F: Females, M: Males), session (white: Pre-hibernation criterion, grey: Post-hibernation retention) and task (visual and spatial discrimination). Sample sizes are given below each boxplot.

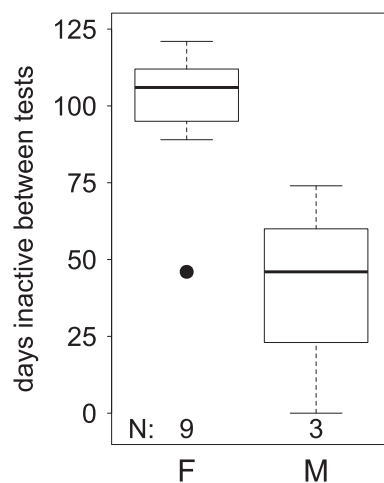


FIGURE 3 Activity levels between pre-hibernation criterion session and post-hibernation retention session per sex. Sample sizes are given below boxplots.

$p = .028$). The proportion tests comparing recapture rates between sexes across months confirmed that females were less active than males in the dry season, as they were significantly less often recaptured in August ($p = .001$), but not in April ($p = .677$), May ($p = 1$), September ($p = .695$), or October ($p = .238$; ESM, Table S1). The t-test comparing activity levels (number of inactive days between the two test sessions) between sexes was not significant, with a trend towards females being more inactive than males ($t_{[2,51]} = 2.60$, $p = .096$). On average, female mouse lemurs were inactive for 99.44 ± 22.56 days, while male mouse lemurs were inactive for only 40.00 ± 37.36 days (Figure 3).

The models evaluating variation in the retention score in response to the activity level did not differ from their respective null models in the visual discrimination ($F_{[1,4]} = .93$, $p = .390$; $R^2 = -.01$;

ESM, Figure S3A) or in the spatial discrimination ($F_{[1,4]} = .10$, $p = .766$; $R^2 = -.22$; ESM, Figure S3B). Similarly, the models evaluating variation in the difference in error counts in response to the activity level did not differ from their respective null models in the visual discrimination ($F_{[1,4]} = 2.83$, $p = .168$; $R^2 = .27$; ESM, Figure S3C) or in the spatial discrimination ($F_{[1,4]} = .35$, $p = .589$; $R^2 = -.15$; ESM, Figure S3D). Thus, our activity measure did not explain variation in memory retention of visual and spatial stimulus-reward association tests.

3.3 | Variation in pre-hibernation and post-hibernation learning speed

Twelve (7 females, 5 males) of 13 individuals in the visual discrimination and four (2 females, 2 males) of 12 individuals in the spatial discrimination task did not perform on an 80% learning criterion in the post-hibernation retention session. They were retrained until re-learning the stimulus-reward association.

The model evaluating variation in learning speed in response to sex, the testing phase and the experimental task significantly differed from its null model ($X^2_{[4,25]} = 34.02$, $p < .001$, $R^2 = .61$). Sex did not predict variation in learning speed (LRT = .63, $df = 1$, $p = .426$), but the testing phase (pre- vs. post-hibernation) in interaction with the task predicted variation in learning speed (LRT = 5.38, $df = 3$, $p = .02$). Mouse lemurs needed more trials to accomplish the pre-hibernation learning phase than the post-hibernation re-learning phase (estimate = $.27 \pm .13$, t -value = 2.05). They also learned the spatial discrimination faster than the visual discrimination in the pre-hibernation learning phase (estimate = $.44 \pm .19$, t -value = 2.35) and even faster in the post-hibernation re-learning phase (estimate = $-.68 \pm .16$, t -value = -4.28; Figure 4).

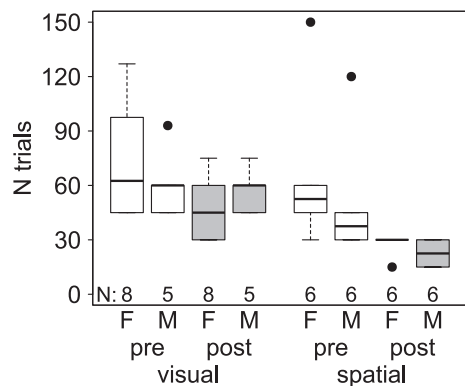


FIGURE 4 Number of trials conducted in the pre-hibernation learning phase (white) and the post-hibernation re-learning phase (grey) per sex and task.

The models evaluating variation in learning speed in the pre-hibernation testing phase in response to individual characteristics did not differ from their respective null models in the visual discrimination (age+BMI: $F_{(2,10)} = .16, p = .852, R^2 = -.16$; exploration+neophilia: $F_{(2,10)} = 1.64, p = .241, R^2 = .10$), or in the spatial discrimination task (age+BMI: $F_{(2,9)} = 3.80, p = .064, R^2 = .34$; exploration+neophilia: $F_{(2,9)} = .39, p = .690, R^2 = -.13$). Similarly, the models evaluating variation in learning speed in the post-hibernation testing phase in response to individual characteristics did not differ from their respective null models in the visual discrimination (age+BMI: $F_{(2,10)} = .42, p = .673, R^2 = -.11$; exploration+neophilia: $F_{(2,10)} = 1.46, p = .277, R^2 = .07$), or in the spatial discrimination (age+BMI: $F_{(2,9)} = 1.27, p = .328, R^2 = .05$; exploration+neophilia: $F_{(2,9)} = 2.27, p = .159, R^2 = .19$). Hence, the assessed individual characteristics did not explain variation in learning speed of visual or spatial stimulus–reward association tests.

4 | DISCUSSION

In this study, we investigated variation in memory retention in relation to sex-specific seasonal energy-saving strategies for the first time in a wild primate species. Overall, grey mouse lemurs performed worse in the post-hibernation retention session than in the pre-hibernation learning session, and their performance was worse in the visual discrimination compared to the spatial discrimination task. Individual characteristics (age, personality) did not explain this variation, thus suggesting a low sampling bias concerning self-selection (Webster & Rutz, 2020). Estimated activity levels tended to be lower for female mouse lemurs, but males exhibited worse memory retention than females, especially in the visual discrimination task. Our study, therefore, contributes another example of a stimulus-dependent lack of memory retention across hibernation. In contrast to our prediction, we found no evidence for superior memory retention in males despite higher activity levels during the hibernation period. Several factors may explain this set of results.

First, the failure to find evidence for the predicted sex difference in memory retention is not due to a sex difference in average activity levels. Several previous studies in this population and captivity evidence that female grey mouse lemurs hibernate during the cool dry season while males remain more active (Fietz, 1998; Perret, 1992; Perret & Aujard, 2001; Rasoazanabary, 2006; Schmid, 1999; Schmid, 2001; Schmid & Kappeler, 1998). Interindividual variation in energy-saving strategies has also been well-studied in another mouse lemur species. Reddish-grey mouse lemurs (*M. griseorufus*) exhibit extreme individual flexibility in heterothermy, with some individuals exhibiting irregular short torpor bouts, regular daily torpor, prolonged torpor of a few days, or hibernation over several weeks, depending on their individual condition and fat reserves (Kobbe et al., 2011). The accumulation of sufficient seasonal body fat was the crucial factor determining the thermal behaviour of reddish-grey mouse lemurs. In grey mouse lemurs, torpor use is also dependent on body condition and ambient temperature (Vuarin et al., 2013). While we lack this detailed information about individual variation in activity, several lines of evidence (variation in tail circumference, recapture rates, average activity measures) indicate that our study females were less active than males across the cool dry winter months.

Second, male mouse lemurs may not have retained the previously learned associations because they lost ecological salience and the corresponding memory was overwritten as they encountered new cognitive challenges across the period females spent hibernating. Females, in contrast, were not confronted with as many new cues between the two testing phases, which may proximately explain their better retention performance compared to males. There are no studies of sex differences in memory retention in other species with sex-biased hibernation strategies, such as *Callospermophilus lateralis* (Healy et al., 2012) or *Urocitellus richardsonii* (Michener, 1992). To evaluate the hypothesis that processing new information hampers memory formation of earlier associations, especially when repetitions become less frequent, this notion could be tested experimentally in study systems where inactivity patterns can be controlled experimentally, such as salamanders (Wilkinson et al., 2017).

Third, by using more than one learning task and by controlling for various aspects of the behaviour and physiology of the mouse lemurs, our study offers several preliminary conclusions about the various intrinsic and extrinsic determinants of learning and memory. Because we were unable to include body temperature recordings in our study, the duration and depth of torpor may have varied among individuals, which, in turn, might affect neuronal functions and memory retention. We also had no control over the proportion of individuals available for re-testing, but our final, smaller sample size was similar to that in most previous studies on this topic and yielded robust results. Nonetheless, follow-up studies that improve these aspects would be welcome.

Fourth, the spatial stimulus–reward association was faster learned and better retained than the visual stimulus–reward association. The plus maze may have offered an ecologically more relevant learning task for mouse lemurs than the visual discrimination task because spatial cues are used daily to relocate gum trees or

sleeping sites, whereas visual cues may play a less salient role in their daily foraging behaviour. In a previous study, we also found mouse lemurs to learn another spatial stimulus–reward association faster than a visual stimulus–reward association (Henke-von der Malsburg et al., 2021), suggesting that the extent to which a stimulus–reward association is learned and subsequently memorized may depend on the nature of the stimulus and its salience for the study species. In addition, interference-based forgetting, in particular retroactive interference (Crossley et al., 2019), may have interfered with memory formation or expression, especially in males that were exposed to more stimuli after the initial testing session. The intensity of the learning process may also be relevant, as suggested by a study of alpine marmots, where high memory retention might have resulted from a very long training phase that may have consolidated the stimulus–reward contingency into long-term memory (Clemens et al., 2009). Further, neither the time delay between the two testing sessions nor the individual condition or personality variation had any statistical effect on the pre-post hibernation difference in test performance. Thus, our study had a high internal, but perhaps a low external validity. However, the use of the selected experimental tasks allows for more valid interspecific comparisons on this topic, as most previous studies also used some spatial or association learning task (Hernández-Montero et al., 2020).

Finally, our non-invasive study of wild animals cannot contribute any insights into the neurophysiological processes underlying the observed patterns of learning and memory formation in hibernating mammals. Hibernation affects neurophysiological processes, as dendritic arbours retract and more than half of the synapses get lost (von der Ohe et al., 2007). These negative effects contrast the effects of brumation on amphibians or reptiles. Brumation is an energy-saving strategy in poikilothermic amphibians and reptiles that parallels hibernation in homeothermic mammals. Under brumation, brain activities are also reduced but, following the decreasing environmental temperature, poikilotherms cool down much more rapidly (Wells, 2007). The rapid cooling causes inhibition of enzymatic activities, which inhibits de novo syntheses of proteins, leads to apoptotic cell death, and, therefore, hampers memory retention on the one hand (Cerrri et al. 2009). On the other hand, neural connections may be “frozen,” which consolidates memory as suggested for two species of salamander (Kundey et al., 2018; Wilkinson et al., 2017). Pronounced memory loss may negatively affect survival, specifically when animals rely on food storage or the depletion of previous food patches. Therefore, a counteractive mechanism preventing memory loss should be adaptive (Roth et al., 2010). Hibernation in ground squirrels is periodically interrupted by such arousals, after which the animals fall into sleep-like states of inactivity before re-entering hibernation (Daan et al., 1991). During arousal, the animal's core temperature increases, and thereby brain activities are resumed, leading to the re-growth of neuronal dendritic arbours and reconstruction of neuronal connections (Daan et al., 1991; Strijkstra & Daan, 1997). Laboratory studies on mice and ground squirrels suggested such arousals as the main driver for memory retention (Nowakowski et al., 2009; Weltzin et al., 2006). While we lack respective information on mouse lemurs,

closely-related dwarf lemurs (*Cheirogaleus* spp.) compare to several species of ground squirrels in electroencephalographic and metabolic patterns during hibernation. Similar to the ground squirrels, the dwarf lemurs expressed monotonous ultra-low-voltage EEG during hibernation bouts, which are interrupted by periods of sleep after spontaneous arousal (Blanco et al., 2016). Given the increasing use of mouse lemurs in studies of cognition, genetics and physiology (Ezran et al., 2017; Fichtel, 2022; Lum et al., 2021), future studies may also offer opportunities for exploring their brain activity around hibernation.

5 | SUMMARY AND CONCLUSIONS

Our study offers the first insights into memory retention in hibernating wild primates. Exploiting a rare sex difference in seasonal torpor use, we predicted better memory retention in male grey mouse lemurs, who only exhibit short, sporadic torpor bouts. We found that all individuals performed worse in a visual and spatial association learning task after hibernation, but, in contrast to our prediction, females were slightly better at retaining previously learned associations after hibernation than males. The reasons for failed memory retention following hibernation remain unclear, but it appears unlikely that it was primarily driven by sex-specific seasonal hypometabolism.

AUTHOR CONTRIBUTIONS

JHM, CF and PMK conceived the study. JHM was primarily responsible for data collection and data analyses. All authors contributed to manuscript writing.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Supportive data are available on FigShare (Henke-von der Malsburg et al., 2022). R-scripts will be made available upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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