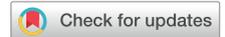Small Mammal Research

Behavioral patterns of laboratory Mongolian gerbils by sex and housing condition: a case study with an emphasis on sleeping patterns

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ABSTRACT

The behavioral patterns of Mongolian gerbils (Meriones unguiculatus) housed individually and in same-sex groups (siblings) were characterized. Gerbils were continuously video-recorded 24 hours (day 1) and 120 hours (day 5) after housing conditions were established (no environmental enrichment was implemented). Video samples totaling 2016 minutes were scored to obtain measures of maintenance (drinking, sleeping, grooming, and eating), locomotor (jumping and rearing), communication (foot stomping), and stereotyped behaviors (gnawing bar and digging), which were compared across housing conditions and sex. Irrespective of sex or housing, gerbils dedicated between 65 and 75% of the day to maintenance behaviors; more than 50% of this time was dedicated to sleep. Time allocated to other behavioral states—for example, bar gnawing, digging, and eating—remained below 5% of the observation time. A comparison of day 1 and day 5 only indicated an increase in water consumption. Housing only affected water-drinking time during day 1, with individual gerbils spending more time drinking than grouped animals. Independent effects of sex were only observed for sleep and the overall behavioral category of maintenance during day 5. Further exploration of sleep patterning across different periods of the day was conducted. Distribution of sleeping was less systematic during day 1 than during day 5, most likely related to habituation to the new housing conditions. During day 5, regardless of sex or housing, gerbils showed the lowest levels of sleeping at the start of the dark cycle (18:00-20:59 hours) and the highest around midnight (00:00-02:59 hours). Although these findings require future replication with more robust groups of animals, they overall support the notion that captive gerbils are crepuscular—that is, more active during light-to-dark transitions—and suggest that changes in housing produce disruptions of activity-sleeping patterns and water consumption of adult gerbils.

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Introduction

Meriones unguiculatus (Mongolian gerbil) is a popular animal model in a wide range of research areas, such as gastric diseases (Backert et al., 2016; Heimesaat et al., 2014; Kiga et al., 2014; Wogensen et al., 2015), ischemic brain injury (Ahn et al., 2016; Fujita et al., 2013; Schwentker, 1963; Starkey et al., 2007), epilepsy (Cakir et al., 2016; Heo and Kang, 2012; Kayacan et al., 2016; Oh et al., 2013), auditory functioning (Galazyuk and Hebert, 2015; Huet et al., 2016; Šušić and Maširević, 1986; von Trapp et al., 2017), animal and veterinary sciences (Baumans and Van Loo, 2013; Grant, 2014; Hocker et al., 2017), psychobiology (Caras and Sanes, 2017; Costantini et al., 2015; McCarty, 2017; Rico et al., 2016), and animal cognition (Jiwa et al., 2010; Pérez-Acosta et al., 2016; Weinert et al., 2007). Despite *M. unguiculatus*' long history, current popularity, and versatility as an animal model (more than 50 years Rosenthal, 1994), a review of the literature

indicates the lack of a single comprehensive and detailed source of information regarding its behavioral patterns in laboratory conditions (Hurtado-Parrado et al., 2015). Instead, partial and heterogeneous descriptions of behavioral aspects in different domains (e.g., social, sexual, and parental) can be found throughout the vast and dissimilar literature. Aware of this situation, we recently developed a standardized and comprehensive catalog of the behavior of Mongolian gerbil in captivity and under natural conditions (Hurtado-Parrado et al., 2015). We currently use this catalog in a series of studies aimed at systematically characterizing the behavior of gerbils across diverse experimental procedures [e.g., memory, learning, and emotion tasks (Hurtado-Parrado et al., 2017; Rico et al., 2016)] and laboratory settings (e.g., lighting cycles and food restrictions). Here, we present the results of a field-test of this catalog, in which we characterized the behavioral patterns of fourteen Mongolian gerbils (seven males and seven females) housed in two laboratory housing conditions—that is, individual and same-sex groups. To that aim, we video-recorded these animals 24 and 120 hours after arrangement of the corresponding housing conditions (day 1 and day 5) and measured nine behaviors belonging to four relevant categories; namely, maintenance (drinking, sleeping, grooming, and eating), locomotor (jumping and rearing), communication (foot stomping), and stereotyped activities (gnawing bar and digging). The high prevalence of sleeping behavior observed during the study (gerbils allocated more than 50% of the observation time to this state) led us to conduct further analyses aimed at analyzing the distribution of this behavior across different segments of the day.

Behavioral evidence is important when assessing and making decisions regarding laboratory animal welfare (Beaver, 2010a,b). We expect that the behavioral methodology described here and the preliminary findings we offer could be used to inform and study further the management and well-being of M. unguiculatus in captivity, including investigating relationships between different behavioral dimensions and normal and pathological biological functions (Von der Behrens, 2014).

Case study

Gerbils

The behavior of 14 ten-week (75-80 grams) experimentally naïve Mongolian gerbils (M. unguiculatus) obtained from the Colombian National Institute of Health (Bogotá, Colombia) was analyzed in this case study (seven male and seven female). Animals were non-specific pathogen free and were housed in Tecniplast® (Italy) polycarbonate cages ($42 \times 20 \times 20$ cm), which contained dust-free wood shaving bedding, and were kept in an animal room under a 12-hour light/dark cycle (lights on at 6:00 a.m.). Tap water and standard rodent pellets (LabDiet 5001®) were available ad libitum. No environment enrichment was included. The room temperature was maintained at 23°C ($\pm 3°$) with 55% ($\pm 5\%$) relative humidity.

All animals were weaned at week 3 and housed in groups of four individuals of the same sex. On week 10, four males (M) and four females (F) were randomly chosen and housed individually in separate cages (hereon labeled I-M1, I-M2, I-M3, I-M4, and I-F1, I-F2, I-F3, and I-F4, respectively). Same-sex groups of three subjects (three male and three female) were housed in cages labeled G-M and G-F, respectively.

All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (Panksepp et al., 2017) and were approved by the Institutional Animal Care and Use Committee (IACUC) at Fundación Universitaria Konrad Lorenz (FUA-019-12).

Instruments and materials

Video recordings

Each cage was recorded with two separate video cameras (Video Balun®—SC-M202), which were arranged to provide a single image that would cover the entire cage using software TechSmith® Camtasia 8.3 (see Figure 1 for a sample screenshot).

According to recommendations on nonintrusive illumination for rodents during dark cycles (Bayne and Turner, 2013; Waiblinger and Konig, 2004), red LED light, delivered through the railings of the rack, was used during the 12-hour dark cycle (18:00-05:59 hours) to improve the quality of the video recordings. Measurement inside the cages indicated that the intensity of white light during the light cycle (06:00-17:59 hours) was between 41 and 50 lux, whereas the intensity of red light during the dark cycle (18:00-05:59 hours) was between 21 and 30 lux.

JWatcher + video 1.0

Video files were manually scored and analyzed using the JWatcher + video 1.0, which is a freeware available at http://www.jwatcher.ucla.edu (University of California & Macquarie University) (Blumstein and Daniel, 2007). Necessary files for scoring the videos and analyzing the data using the JWatcher (global and focal behavioral definition files and focal analysis file) are available from the Open Science Framework repository http://doi.org/10.17605/OSF.IO/6VNGQ.

Procedure

Video recordings and behavioral sampling for scoring and analyses

Two of the three animals housed in groups (cages G-M and G-F) were randomly selected for marking via superficial shaving of the head and back or the sides. A focal-animal sampling approach was followed (Altmann, 1974). Continuous video recording of all configured cages (I-M1, I-M2, I-M3, I-M4, I-F1, I-F2, I-F3, I-F4, G-M, and G-F) started the day after the animals were assigned to their corresponding housing condition (individual or group housing). Recordings obtained between the 00:00 and 23:59 hours of the first and fifth day after housing condition assignment were scored and analyzed (hereon labeled day 1 and day 5). Three-minute segments were randomly chosen for each hour of the 24 hours of video recordings for each subject—that is, 5% random sampling. This resulted that in group cages (GM and GF), the sampling for each of the three animals occurred independently—for example, the video segment randomly chosen for subject 1 of the female-group cage (FM) during the first hour of observation of day 1 was minute 24-27, for subject 2, it was minute 26-29, and for subject 3, it was minute 40-43. This sampling process resulted in a selection of 672 videos of 3-min length each (48 videos per subject)—that is, a total of 2016 minutes of video-recording used for scoring and analysis.

The frequency and duration of maintenance (drinking, sleeping, grooming, and eating), locomotor (jumping, rearing, and foot stomping), and stereotyped behaviors (gnawing bar and digging) occurring in each video were manually scored using JWatcher + video 1.0. An average 95% interobserver agreement was established (Johnston and Pennypacker, 2009). Detailed definitions of each of these nine target behaviors were based on those compiled by Hurtado-Parrado et al (2015) and can be found in Table 1.

Statistical analyses

Mean percentage of observation time was calculated for drinking, sleeping, grooming, eating, bar gnawing, rearing, and digging. Only in the case of jumping, the average rate per minute was calculated (the duration of each instance of this response was
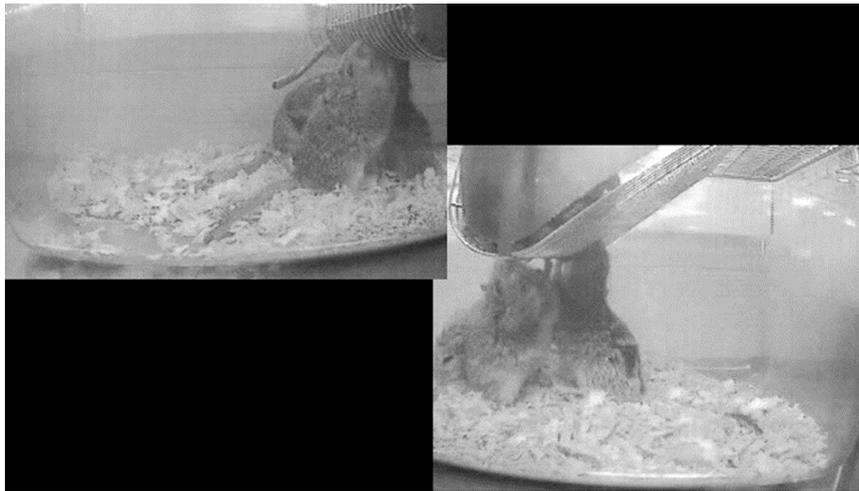
Figure 1. Sample screenshot of the video recordings produced for each cage during the study. Two separate cameras were arranged to provide a single image that covered the entirety of the cage.

considered negligible). A mean percentage of time allocated to maintenance and stereotyped behavioral categories was obtained by aggregating the data on the corresponding individual behaviors. All these calculations were conducted separately for the two days of observation—that is, day 1 and day 5. Wilcoxon signed-rank tests

Table 1
Ethogram based on the study by Hurtado-Parrado et al. (2015)

Category/Behaviors	Definition
Maintenance	
Drinking	The animal took water into its mouth by licking the spout that was fitted in the laboratory bottle.
Sleeping	The animal had no body movement, its eyes were shut, and it was in one of the following postures: alone or in contact with other conspecifics (only for group cages), sitting with its head tucked down between the rear legs, resting on its back with legs in the air, or lying on one side of its body.
Grooming	Episodes in which the animal licked, bit, scratched, nibbled, and/or rubbed its own body regions (using the mouth, forepaws, and/or hindpaws).
Eating	The animal brought its snout in contact with the laboratory chow that was available in a feeding tray located on top of the cage.
Stereotyped behavior	
Digging	The animal made rapid back and forth movements with its front paws, which alternated with movements of its hind legs that were directed toward the floor, wall, or corner. These bouts of digging were composed of more than seven consecutive scratches with the front legs that were typically followed by or interspersed with hind leg kicks.
Gnawing bar	The animal grasped between its teeth one of the metal bars of the lid located on top of the cage and moved its mouth up and down this bar while chewing. The animal was in a rearing posture and, usually, one hind leg was raised slightly and intermittently.
Locomotor	
Rearing	The animal was standing upright on its hind legs with a straight back and both of its front paws were off the floor and may have been or not in contact with the wall of the cage.
Jumping	The animal pushed itself off the ground with the hind legs in a "jump-like" vertical movement.
Communication	
Foot stomping	The animal rhythmically pounded the substrate with rapid movements of one or both hind feet in brief repetitive bursts of approximately 5 or 6 movements.

were used to compare measures of each observed behavior and global behavioral category on day 1 versus day 5. Effect size (r) was calculated for relevant cases using the following formula $r = Z/\sqrt{N}$ (Rosenthal, 1994).

Distribution of normality for factors, sex and housing, was assessed using the Shapiro-Wilk test, which indicated normality in more than 80% of the cases[*]. Accordingly, two-way ANOVAs were implemented to test the effects of sex and housing on each of the behavioral measures for day 1 and day 5. The magnitude of the effect was evaluated using the eta-square (η^2). Foot stomping was not observed during any of the observations; thus, no data on this behavior or the corresponding behavioral category (communication) were analyzed.

Considering the high prevalence of sleeping observed during the study, further analyses were conducted. Data for each day of observation (day 1 and day 5) was segmented in eight 3-hr bins (all with the same number of 3-min segments), and the mean percentage of time allocated to sleeping on each of these bins was calculated. Two-way Friedman ANOVAs on the amount of time that the gerbils spent sleeping across these eight periods of the day were implemented separately for housing condition (individual and group) and sex (male and female). These analyses were conducted for both days of observation (day 1 and day 5). Between-subject factors were sex and housing, and the repeated measures factor (within-subjects) corresponded to the eight 3-hr bins. A P-value below 0.05 was considered statistically significant. Pairwise comparisons were conducted when needed, and $P < 0.01$ level of significance was adopted to reduce type-I error.

Results

Across the two days of observation (day 1 and day 5), all the animals spent more than 50% of the time sleeping and allocated less than 10% to the other scored behaviors, irrespective of sex (male and female), housing condition (individual and group), or day of observation (day 1 and day 5). Regarding the behavioral categories, gerbils allocated between 65% and 75% to maintenance-related behavior (drinking, sleeping, grooming, and eating), followed by 4% to 6% allocated to stereotype-related behavior (gnawing bar and digging), and less than 2% allocated to locomotor behavior.

Regarding jumping, which was measured only in terms of rate, gerbils overall displayed a low rate of this behavior, which typically remained below 1/minute. No instances of foot stomping were observed during the study (see Tables 2 and 3).

Wilcoxon signed-rank tests were used to compare measures of each observed behavior and each global behavioral category on day 1 versus day 5. No statistically significant differences were found by sex (male and female) or housing condition (group or individual housing) on any of the measures ($Ps > 0.05$). Wilcoxon tests were also conducted without considering sex and housing condition, showing only a significant difference in percentage of time drinking water ($Z = -2.132$, $P = 0.031$); namely, all gerbils increased their time allocated to drinking water from day 1 ($M = 0.000$) to day 5 ($Median = 1.028$) with a moderate effect size ($r = -0.403$).

Each behavior and global category was then analyzed by sex, housing, and day of observation (see Table 2 for day 1 and Table 3 for day 5). A two-way ANOVA on day 1 data indicated a significant effect of housing (Table 2) on the percentage of time that gerbils spent drinking water. The time allocated to drinking by individual gerbils was significantly higher ($M = 1.006$, $SD = 0.666$) than that of gerbils housed in groups ($M = 0.0$, $SD = 0.0$; see Figure 2A). A large effect was observed for this difference ($\eta^2 = 0.537$). No sex differences or interactions between housing and sex were observed for any of the other behaviors during the first 24-hr period of observation. In addition, no differences due to sex, housing, or interaction between these factors were observed for the global behavioral categories (see Table 2).

Two-way ANOVAs on day 5 data indicated significant sex differences in the amount of time that the gerbils spent sleeping and their rate of jumping (Table 3), with a large-size effect for both factors ($\eta^2_{sleeping} = 0.374$; $\eta^2_{jumping} = 0.417$). Regarding sleep, males allocated significantly more time to this behavior ($M = 69.7$; $SD = 3.961$; see Figure 2C) than females ($M = 60.84$; $SD = 7.972$). Regarding jumping, males showed lower rates ($M = 0.037$; $SD = 0.064$) than females ($M = 0.108$; $SD = 0.098$). A significant difference in jumping related to housing condition was also identified (Table 3), for which a large effect was observed ($\eta^2 = 0.512$). Rate of jumping of individual gerbils was significantly lower ($M = 0.032$, $SD = 0.04$; see Figure 2B) than that of gerbils housed in groups ($M = 0.129$, $SD = 0.106$). A housing-sex interaction on jumping was found (see Figure 2B), with a large effect size for this factor ($\eta^2 = 0.40$), which indicated that grouped female gerbils had a significantly higher rate of this behavior than the individually housed females and grouped and individual males ($Ps < 0.05$; see Figure 2B). Neither sex nor housing differences, or an interaction between these factors, were identified for any of the other observed behaviors.

Regarding differences across behavioral categories during day 5, two-way ANOVAs indicated a large effect of sex in maintenance activities ($\eta^2 = 0.494$) and other behaviors ($\eta^2 = 0.412$). No effects of housing or sex-housing interaction were found. Male gerbils allocated higher percentages of time to maintenance behavior ($M = 78.83$; $SD = 7.154$; see Figure 2D) as compared to female gerbils ($M = 68.32$; $SD = 5.581$).

Table 2
Statistical overview of the sex differences and the effect of housing on each observed behavior and global behavioral categories of Mongolian gerbils during day 1

| | | | Housing | | Two-way ANOVA | | | | | |
| | Total | | Individual | Group | Housing | | Sex | | Housing*Sex | |
Behavior	M	SD	M ± SD	M ± SD	$F_{(1, 13)}$	P	$F_{(1, 13)}$	P	$F_{(1, 13)}$	P
Bar gnawing	2.502	2.784	2.897 ± 2.458	1.975 ± 3.334	0.321	0.584	0.211	0.656	0.645	0.44
Male	2.782	3.467	2.617 ± 3.05	3.003 ± 4.683						
Female	2.221	2.143	3.177 ± 2.132	0.946 ± 1.639						
Digging	2.344	2.481	1.705 ± 1.96	3.193 ± 3.02	1.149	0.309	0.689	0.426	0.456	0.515
Male	1.835	2.464	1.600 ± 1.651	2.150 ± 3.723						
Female	2.852	2.582	1.815 ± 2.491	4.236 ± 2.381						
Drinking*	0.575	0.711	1.006 ± .666	0 ± 0	11.591	0.007*	0.168	0.691	0.168	0.691
Male	0.644	0.823	1.127 ± .792	0 ± 0						
Female	0.505	0.639	0.8853 ± .608	0 ± 0						
Eating	1.253	1.389	1.608 ± 1.634	0.78 ± .901	1.084	0.322	0.469	0.509	0.002	0.967
Male	1.528	1.866	1.897 ± 2.422	1.035 ± 1.003						
Female	0.978	0.724	1.319 ± .371	0.524 ± .909						
Grooming	5.07	4.663	6.486 ± 5.065	3.182 ± 3.624	2.129	0.175	0.504	0.494	3.02	0.113
Male	6.155	5.468	9.257 ± 4.949	2.019 ± 2.838						
Female	3.985	3.803	3.715 ± 3.859	4.346 ± 4.552						
Rearing	1.173	1.08	1.255 ± 1.213	1.064 ± .972	0.094	0.765	1.185	0.302	0.054	0.822
Male	1.522	1.373	1.666 ± 1.677	1.331 ± 1.159						
Female	0.824	0.597	0.8451 ± .4191	0.7979 ± .8967						
Sleeping	59.99	15.10	53.74 ± 14.83	68.32 ± 11.81	3.784	0.088	0.464	0.511	1.356	0.271
Male	58.06	9.482	55.55 ± 10.791	61.405 ± 8.109						
Female	61.92	19.89	51.935 ± 19.703	75.235 ± 11.817						
Jumping	0.058	0.068	0.073 ± .086	0.0378 ± .029	0.765	0.402	0.043	0.841	0.000	0.99
Male	0.062	0.079	0.073 ± .104	0.0423 ± .036						
Female	0.054	0.062	0.069 ± .08	0.0333 ± .0288						
Behavioral category										
Maintenance	66.89	13.2	62.84 ± 14.08	72.28 ± 10.7	2.187	0.17	0.197	0.667	4.032	0.072
Male	66.39	8.347	67.837 ± 9.83	64.461 ± 7.37						
Female	67.39	17.54	57.856 ± 17.321	80.106 ± 6.979						
Locomotion	1.173	1.08	1.255 ± 1.213	1.064 ± .972	0.094	0.765	1.185	0.302	0.054	0.822
Male	1.522	1.373	1.666 ± 1.677	1.331 ± 1.159						
Female	0.824	0.597	0.8451 ± .419	0.7979 ± .896						
Stereotyped	4.864	2.884	4.605 ± 2.762	5.168 ± 3.276	0.103	0.755	0.052	0.823	0.045	0.836
Male	4.618	2.863	4.2175 ± 1.522	5.153 ± 4.512						
Female	5.074	3.116	4.992 ± 3.885	5.183 ± 2.542						

M, Mean; SD, Standard Deviation. N = 14.
* Two-way ANOVA indicated a significant main effect of housing (P < 0.05).

Table 3
Statistical overview of the sex differences and the effect of housing on each observed behavior and global behavioral categories of Mongolian gerbils during day 5

Behavior	M	SD	Housing Individual M ± SD	Housing Group M ± SD	Two-way ANOVA Housing $F_{(1, 13)}$	Housing P	Sex $F_{(1, 13)}$	Sex P	Housing*Sex $F_{(1, 13)}$	Housing*Sex P
Bar gnawing	2.658	3.397	2.926 ± 4.077	2.301 ± 2.542	0.1	0.759	0.703	0.421	0.248	0.629
Male	3.558	4.397	4.247 ± 5.37	2.638 ± 3.472						
Female	1.759	1.984	1.604 ± 2.301	1.965 ± 1.938						
Digging	2.863	3.056	3.997 ± 3.666	1.288 ± .662	2.808	0.125	0.544	0.478	0.116	0.741
Male	2.201	2.908	3.126 ± 3.726	0.967 ± .747						
Female	3.472	3.292	1.815 ± 2.491	4.236 ± 2.381						
Drinking	1.357	1.289	1.806 ± 1.483	0.754 ± .735	2.46	0.148	0.654	0.438	0.788	0.396
Male	1.042	0.917	1.239 ± 1.05	0.781 ± .832						
Female	1.672	1.607	2.38 ± 1.779	0.728 ± .81						
Eating	2.748	3.253	3.074 ± 4.018	2.314 ± 2.126	0.177	0.683	1.952	0.193	0.062	0.808
Male	1.455	1.148	1.588 ± 1.31	1.279 ± 1.138						
Female	4.04	4.209	4.558 ± 5.484	3.35 ± 2.607						
Grooming	2.785	2.833	2.775 ± 2.383	2.798 ± 3.593	0.000	0.989	1.545	0.242	0.014	0.91
Male	3.803	3.582	3.874 ± 2.615	3.709 ± 5.312						
Female	1.766	1.46	1.675 ± 1.786	1.887 ± 1.253						
Rearing	2.03	2.332	2.575 ± 2.929	1.303 ± 1.014	0.931	0.357	0.088	0.773	0.772	0.4
Male	2.308	3.037	3.349 ± 3.84	0.9206 ± .709						
Female	1.752	1.534	1.8 ± 1.916	1.686 ± 1.276						
Sleeping*	65.27	7.594	63.82 ± 7.732	67.2 ± 7.642	0.955	0.352	5.977	0.035*	0.592	0.459
Male	69.7	3.961	69.391 ± 3.233	70.111 ± 5.563						
Female	60.84	7.972	58.252 ± 6.806	64.304 ± 9.474						
Jumping	0.0742	0.087	0.032 ± 0.4	0.129 ± .106	10.509	0.009*	7.147	0.023*	0.6.68	0.027*
Male	0.0397	0.064	0.031 ± .053	0.05 ± .088						
Female	0.108	0.098	0.033 ± .031	0.208 ± .041						
Behavioral category										
Maintenance	73.58	8.231	71.48 ± 6.299	76.38 ± 10.203	2.042	0.184	9.77	0.011*	0.191	0.671
Male	78.83	7.154	76.09 ± 4.913	82.498 ± 9.065						
Female	68.32	5.581	66.867 ± 3.42	70.27 ± 8.122						
Locomotion	2.03	2.332	2.575 ± 2.929	1.303 ± 1.014	0.931	0.357	0.088	0.773	0.772	0.4
Male	2.308	3.037	3.349 ± 3.84	0.9206 ± .709						
Female	1.752	1.543	1.8 ± 1.916	1.686 ± 1.276						
Stereotyped	5.495	3.181	6.924 ± 3.089	3.59 ± 2.309	4.148	0.069	0.081	0.782	0.07	0.796
Male	5.759	3.682	7.374 ± 3.742	3.606 ± 2.74						
Female	5.231	2.864	6.474 ± 2.779	3.574 ± 2.413						

M, Mean; SD, Standard Deviation. N = 14.
* Two-way ANOVA indicated a significant main effect of housing (P < 0.05).

Sleeping behavior

Figure 3 shows the percentage of time that gerbils spent sleeping across eight different 3-hr periods (bins) of the two days of observation (i.e., day 1 and day 5). These data are separately represented by housing (panel A; individual vs. group housing) and sex (panel B; male vs. females).

Sleeping patterns of individually housed gerbils

A nonparametric two-way Friedman's ANOVA on individual-housing data showed significant differences across bins of both days of observation—χ^2 (15) = 43.51; P < 0.001. We adopted P < 0.01 level of significance for multiple comparisons across bins to reduce Type-I error. Table 4 shows statistically significant pairwise comparisons for housing and sex factors and for day 1 and day 5 of observation (comparisons between bins of day 1 and day 5 were considered irrelevant and thus were not included in the analysis).

Pairwise comparisons across bins for day 1 showed significant differences between 00-03 hours (M = 70.5; SD = 25.58) and 21-24 hours (M = 26.82; SD = 26.21) and between 12-15 hours (M = 76.84; SD = 24.65) and 21-24 hours (see Table 4). These results indicate that individual gerbils on the first day spent more time sleeping during the hours that followed midnight and noon and were more active during the hours before midnight. On day 5, percentages of sleep during bins 18-21 hours (M = 33.39; SD = 18.26) and 21-24 hours (M = 25.56; SD = 27.33) were significantly lower when compared to the rest of the time segments (Ps < 0.01), excepting 06-09 hours (see Table 4). Similar to day 1, on day 5, individual gerbils spent more time sleeping throughout most of the periods of observation, starting after midnight, and increased their activity during the first part of the night (i.e., after 06:00 hours) up until midnight.

Sleeping patterns of gerbils housed in groups

A two-way Friedman's ANOVA showed statistical differences among bins for gerbils housed in groups - χ^2 (15) = 48.15; P < 0.001. Pairwise comparisons for day 1 (see Table 4) indicated that somewhat similar to individual gerbils, grouped gerbils spent significantly more time sleeping during the bin 00-03 hours (M = 90.29; SD = 12.98) than during bins 15-18 hours (M = 29.47; SD = 22.06) and 18-21 hours (M = 27.03; SD = 33.26). However, contrary to what was observed in individual gerbils, grouped animals spent significantly more time sleeping during the hours before midnight, namely 21-24 hours (M = 88.27; SD = 15.36) than during afternoon (15-18 hours) and early evening (18-21 hours).

In day 5, the sleep pattern of grouped gerbils during the hours before midnight (bin 21-24 hours) changed, becoming to a certain extent similar to that observed in individual gerbils. That is, grouped gerbils spent significantly less time sleeping during late evening hours (21-24 hours; M = 27.51; SD = 24.71) as compared to most of the morning bins—that is, 00-03 hours (M = 91.64; SD = 13.57), 03-06 hours (M = 77.05; SD = 19.26), and 06-09 hours (M = 94.95; SD = 8.50).
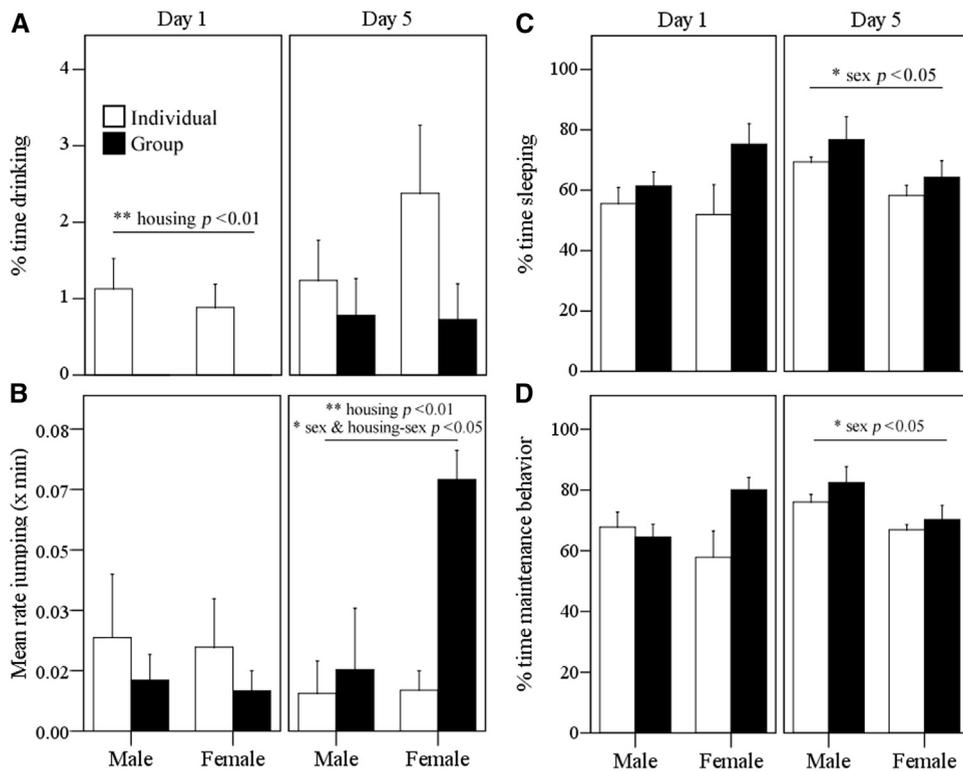
Figure 2. Distribution of gerbils' drinking, sleeping, jumping, and maintenance behaviors. Panels A, C, and D show mean percentage of time subjects allocated to drinking water, sleeping, and maintenance behavior, respectively. Panel B shows mean rate of jumping (responses per minute). Data for day 1 and day 5 and for male and female gerbils are presented separately. White bars represent gerbils housed individually, and dark bars, gerbils housed in groups. Error bars indicate ± SEM.

Sleeping patterns of male gerbils

Gerbils also exhibited sex-dependent changes in the time spent sleeping across different periods of the day. A two-way Friedman's ANOVA on male data showed significant differences across the eight 3-hr bins—χ^2 (15) = 59.51; $P < 0.001$. Pairwise comparisons for day 1 (see Table 4) indicated significantly higher percentages of time sleeping during the hours following midnight (00-03 hours; $M = 86.76$; $SD = 15.04$) and noon (12-15 hours; $M = 83.92$; $SD = 18.99$) than during the first hours of the night (18-21 hours; $M = 19.47$; $SD = 25.02$). In day 5, a more marked difference in the periods of rest and activity was observed. Male gerbils spent significantly more time sleeping (around 80% of the observation time) across the periods that started in midnight (bin 00-03 hours) until the end of the afternoon (bin 15-18 hours), excepting the 09-12 hours period, than during the first half of the night—that is, bin 18-21 hours ($M = 38.99$; $SD = 30.30$) and bin 21-24 hours ($M = 37.1$; $SD = 21.9$).

Sleeping patterns of female gerbils

A two-way Friedman's ANOVA showed significant differences for females across the different periods of observation—χ^2 (15) = 26.98; $P = 0.029$; however, different to males, those differences were only observed in day 5 (i.e., percentages of time sleeping did not vary significantly across day 1). The period with lowest percentages of sleeping (below 30%) was between 21 and 24 hours ($M = 15.68$; $SD = 25.21$), which significantly differed to other periods during the day with more time allocation to sleeping, including the first hours after midnight (00-03 hours; $M = 84.47$; $SD = 20.13$), and the period comprised between the end of the morning ($M = 69.49$; $SD = 18:36$) throughout all afternoon—that is, 12-03 hours ($M = 72.06$; $SD = 13.53$) and 15-18 hours ($M = 71.28$; $SD = 26.81$).

Sleeping patterns by sex and housing condition

Figure 4 shows the percentage of time that female and male gerbils spent sleeping across the eight 3-hr periods (bins) of the two days of observation (i.e., day 1 and day 5) comparing housing condition (individual vs. group housing). An analysis of these data by both sex and housing factors showed significant differences in the percentage of time sleeping across the different periods of observation.

Two-way Friedman's ANOVA showed no differences in the time that individually housed females allocated to sleep across the periods of observation—χ^2 (15) = 17.82; $P = 0.272$. Conversely, the same test showed that male individual gerbils did differ in their daily distribution of sleep—χ^2 (15) = 42.03; $P < 0.001$. Pairwise comparisons across 3-hour bins for day 1 (see Table 4) indicated that individual males had significantly more activity during the first part of the night—that is, bins 18-21 hours ($M = 34.08$; $SD = 24.26$) and 21-24 hours ($M = 26.41$; $SD = 24.04$)—than during the first hours after midnight (00-03 hours; $M = 89.59$; $SD = 16.16$), early morning (06-09 hours; $M = 62.37$; $SD = 23.42$), and the first part of the afternoon (12-15 hours; $M = 89.18$; $SD = 19.91$), which were periods with higher percentages of sleeping (above 60% of the observation time). These animals showed a more differentiated pattern of sleep-activity in day 5, with higher percentages of sleep during the first hours of the day, starting at midnight—bins 00-03 hours ($M = 91.44$; $SD = 13.05$) and 03-06 hours ($M = 95.5$; $SD = 6.33$)—than during the first part of the night—that is, bins 18-21 hours ($M = 20.56$; $SD = 9.2$) and 21-24 hours ($M = 31.94$; $SD = 25.39$).

Regarding group housing, only females showed significant differences in the percentages of time allocated to sleep throughout the periods of observation—χ^2 (15) = 27.77; $P = 0.023$. Pairwise comparisons for day 1 (see Table 4) indicated that grouped females spent significantly more time sleeping during the late evening (21-
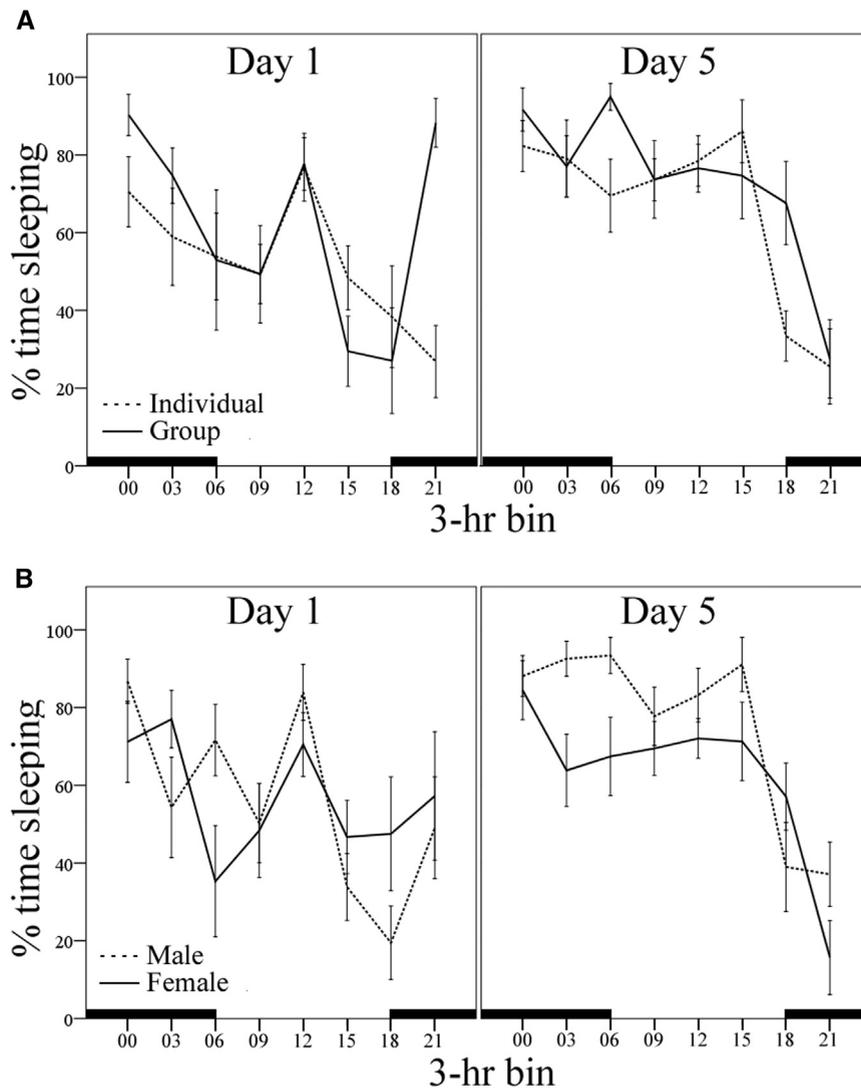
Figure 3. Percentage of time that gerbils spent sleeping across eight 3-hr bins of the two days of observation (i.e., day 1 and day 5). Panel A shows a comparison by housing conditions (individual and group housing). Panel B shows a comparison by sex (male and female). Error bars indicate ± SEM; solid horizontal dark bars on bottom indicate the dark periods of the light cycle (lights off between 18 and 06 hours); Table 4 shows statistically significant pairwise comparisons across bins.

24 hours; $M = 97.26$; $SD = 3.65$) and the first hours after midnight (00-03 hours; $M = 97.58$; $SD = 3.43$) than during the early morning (06-09 hours; $M = 21.87$; $SD = 37.89$). This pattern changed in day 5, as higher percentages of sleep were observed during the first hours after midnight (00-03 hours; $M = 99.62$; $SD = 0.34$) and early morning (06-09 hours; $M = 90.67$; $SD = 11.2$), than during late evening (21-24 hours; $M = 11.02$; $SD = 19.09$).

Discussion

The purpose of the present case study was to characterize the behavioral patterns of male and female Mongolian gerbils housed under two laboratory conditions—that is, individual and same-sex group housing. To that aim, maintenance (drinking, sleeping, grooming, and eating), locomotor (jumping and rearing), communication (foot stomping), and stereotyped behaviors (gnawing bar and digging) were measured and analyzed 24 and 120 hours after the subjects were assigned to their corresponding housing conditions (day 1 and day 5 of observation, respectively).

Of the 48 hours of video recordings obtained per animal, 5% was scored via a sampling process aimed at covering each hour of the

two days of observation (day 1 and day 5). This strategy resulted in the random selection of 672 videos of 3 min each for scoring (48 video samples per animal, one per each hour of the two days), which totaled 2016 minutes of video recording. Notwithstanding the important effort entailed in observing and scoring that amount of video time, future studies not only should test larger groups of animals but also could include more samples and/or larger percentages of time to test the accuracy of our measurements. This includes testing if using as unit of analysis cages, instead of individuals housed in groups, is a more robust approach that controls for confounding variables such as particular intracage dynamics. In addition, more days of the week and/or various weeks could be analyzed to test for possible seasonal effects—for example, behavioral variations related to different moments of the week or the month that follow the assignment of the animals to their corresponding housing arrangements.

Our results showed that animals, irrespective of sex, housing, or day of observation, dedicated between 65% and 75% of the observation time to maintenance behaviors, of which, the majority was dedicated to sleep (above 50%). Gerbils engaged in other behavioral states—bar gnawing, digging, drinking, eating, grooming, and

Table 4
Statistically significant pairwise comparisons across bins for housing and sex factors and for day 1 and day 5 of observation

	χ^2	df	P	Bin 1 hr-hr	Bin 2 hr-hr	Statistic	SE	Sig
Housing								
Individual	43.51	15	<0.001					
Day 1				00-03	21-24	6.25	2.38	0.009
				12-15	21-24	6.25	2.38	0.009
Day 5				00-03	18-21	6.812	2.38	0.004
				00-03	21-24	7.625	2.38	0.001
				03-06	18-21	6.375	2.38	0.007
				03-06	21-24	7.188	2.38	0.003
				09-12	21-24	6.562	2.38	0.006
				12-15	18-21	6.562	2.38	0.006
				12-15	21-24	7.375	2.38	0.002
				15-18	18-21	8.062	2.38	0.001
				15-18	21-24	8.875	2.38	<0.001
Group	48.15	15	<0.001					
Day 1				00-03	15-18	7.917	2.74	0.004
				00-03	18-21	7.75	2.74	0.005
				15-18	21-24	-8.25	2.74	0.003
				18-21	21-24	-8.083	2.74	0.003
Day 5				00-03	21-24	10.833	2.74	<0.001
				03-06	21-24	7.5	2.74	0.006
				06-09	21-24	10.833	2.74	<0.001
Sex								
Male	59.51	15	<0.001					
Day 1				00-03	18-21	8.071	2.545	0.002
				00-03	15-18	6.714	2.545	0.008
				12-15	18-21	7.357	2.545	0.004
Day 5				00-03	21-24	8.5	2.545	0.001
				00-03	18-21	8.286	2.545	0.001
				03-06	21-24	8.643	2.545	0.001
				03-06	18-21	8.429	2.545	0.001
				06-09	18-21	9.143	2.545	<0.001
				06-09	21-24	9.357	2.545	<0.001
				12-15	21-24	7.214	2.545	0.005
				12-15	18-21	7.000	2.545	0.006
				15-18	21-24	8.786	2.545	0.001
				15-18	18-21	8.571	25.545	0.001
Female	26.98	15	0.029					
Day 5				00-03	21-24	9.5	2.545	<0.001
				09-12	21-24	7.429	2.545	0.004
				12-15	21-24	7.071	2.545	0.005
				15-18	21-24	6.643	2.545	0.009
Individual—Male	42.03	15	<0.001					
Day 1				00-03	21-24	9.5	3.367	0.005
				06-09	18-21	9.00	3.367	0.008
				12-15	21-24	8.75	3.367	0.009
Day 5				00-03	18-21	9.75	3.367	0.004
				03-06	18-21	9.75	3.367	0.004
				15-18	18-21	11.75	3.367	<0.001
				15-18	21-24	10.5	3.367	0.002
Group— Female	27.77	15	0.023					
Day 1				00-03	06-09	10.833	3.887	0.005
				06-09	21-24	10.167	3.887	0.009
Day 5				00-03	21-24	13.167	3.887	0.001
				06-09	21-24	10.167	3.887	0.009

$P < 0.01$ level of significance was adopted to reduce type-I error. Nonsignificant comparisons are not presented (e.g., individual—female or group—male).

rearing—less than 2% of the time and displayed average rates of locomotor behavior (jumping) below 1 per min. A comparison of day 1 and day 5 across all the behavioral measures only indicated a significant increase in the percentage of time that gerbils dedicated to drinking water from day 1 to day 5. Worth noting is that the only communicative behavior considered for analyses—foot stomping—was not detected in any of the animals during the study.

Independent effects of sex were only observed for sleeping behavior and the overall behavioral category of maintenance during day 5. Males allocated more time than females to sleep and, more generally, to maintenance activities. In addition, an interaction

between housing and sex was observed in jumping, with grouped female gerbils having a significantly higher rate of this behavior than the other groups.

The developmental stage of the animal, the type of rodent species, and the type of behavioral test have been some of the main factors analyzed in social-isolation studies. It has been demonstrated that social deprivation during neonatal, postweaning, adolescent, and adult stage has distinct neurochemical and behavioral consequences. Although long-term effects of this variable have been reported on the behavior of rats, they have not been observed in mouse or gerbils (Einon et al., 1981)

In the present case study, housing had an independent effect only on the amount of water consumed during day 1, with individual gerbils spending more time drinking than grouped animals. These findings are consistent with previous research that has reported similar effects of housing on water consumption (Krohn et al., 2011). More generally, they are also consistent with studies on polydipsia phenomena (Porter and Bryant, 1978), which have attributed changes in gerbils' water consumption to slight environmental variations (Vanderweele, 1974). However, the present study provides additional information that suggests that this effect is acute/transitory; in particular, the fact that differences were only observed during day 1—that is, immediately following housing arrangements. Future research may not only explore this effect more systematically but also could approach it from a comparative perspective because previous research has reported that water consumption of gerbils and rats is similar (Polioudakis and Roper, 1977).

Studies on social isolation of adult gerbils show that the effects of this manipulation depend on the sex of the subjects and the housing condition prior to the isolation. For instance, anxiety and stereotyped behavior are affected when a male or female gerbil is isolated from a same-sex group/pair or an opposite-sex cage mate (Hendrie and Starkey, 1998; Shimozuru et al., 2008). Although the effects of social isolation on the behavior of gerbils have been primarily detected using experimental tests [e.g., open field, elevated plus-maze and social-interaction test (Hendrie and Starkey, 1998; Pickles et al., 2012; Starkey and Hendrie, 1998)], this is the first report on the effect of housing on the behavior of this species recorded continuously inside their home cages. It seems that variables such as the age at which the social isolation was carried out, the housing condition before isolation (same-sex vs. opposite-sex cage mate), and the context of observation (e.g., home cage vs. novel environment in behavioral tests) could have contributed to the reduced effects of the factors studied in the present study.

Another indicator of alteration in the behavior of animals is stereotyped behavior (repetitive responses that lack functionality) (Wiedenmayer, 1997a). It has been proposed that these behaviors originate early during development and vary between subjects. Although they have been related to low cognitive performance (Waiblinger and König, 2007) or abnormal behavior (Wiedenmayer, 1997b), there is a debate about the abnormality of this type of behavior. In the present case study, the percentage of time devoted to stereotyped behaviors (gnawing bar and digging) was overall very low and did not seem to depend on sex or housing condition. Notwithstanding the incidence of stereotyped behavior in this study was low, previous research has reported that the complexity of the environment could trigger this type of behavior (Waiblinger and König, 2007; Wiedenmayer, 1997b). Thus, other conditions potentially interesting and relevant for future exploration are environmental enrichment and stress conditions in housing, for example, work time, frequency of bed change, or vocalizations derived of procedures, which could affect the gerbils' ethogram considering they are highly sensitive to environmental changes (Klaus et al., 2000).
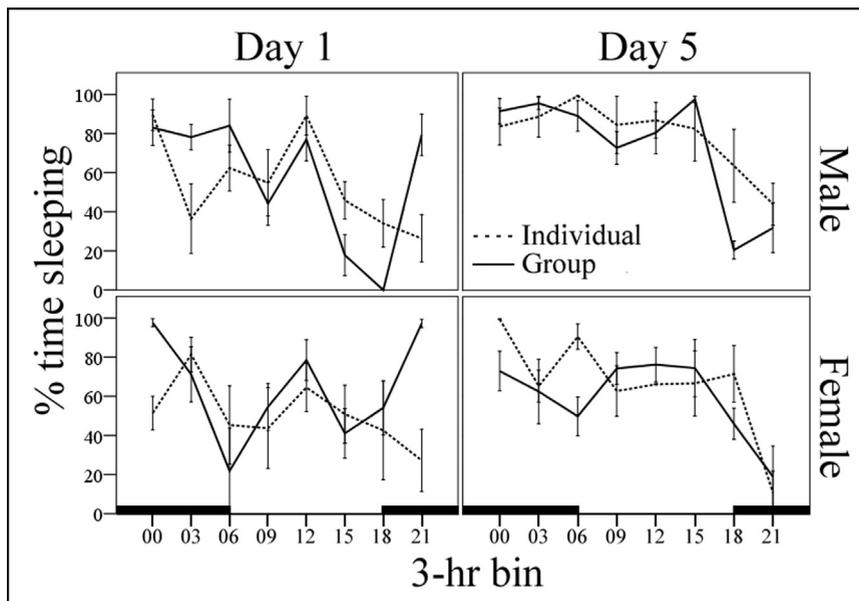
Figure 4. Percentage of time that male and female gerbils spent sleeping across eight 3-hr bins of the two days of observation (i.e., day 1 and day 5). Dashed lines indicate individual housing, and solid lines, group housing. Error bars indicate ± SEM; solid horizontal dark bars on bottom indicate the dark periods of the light cycle (lights off between 18 and 06 hours); Table 4 shows statistically significant pairwise comparisons across bins.

Although the effects of housing conditions have been widely studied in mice and rats (Fontoura-Andrade et al., 2017; Jirkof, 2015), reports for gerbils are limited. Yet, considering that the Mongolian gerbil is a highly social species that lives in colonies in the wild (Agren et al., 1989), individual housing was expected to have affected its behavioral patterns. For instance, it has been demonstrated that individual housing produces increments in anxiety, social investigation, and aggression in males (Schwentker, 1963; Starkey and Hendrie, 1998), and deficits in social behavior of females (Pickles et al., 2012). We found that gerbils housed individually, compared to those housed in groups, spent more time drinking while reduced the time allocated to sleep. Although this is the first report on the effects of social isolation on maintenance behaviors of gerbils, our results are at least consistent with the observation that isolated housing increases consumption of water in rats (Hawken et al., 2013) and ethanol in mice (National Research Council [U.S.]. Committee for the Update of the Guide for the Care and Use of Laboratory Animals and Institute for Laboratory Animal Research [U.S.], 2011). A dysregulation of the hypothalamic-pituitary-adrenal axis function due to social stress has been the proposed mechanism responsible for the increments in drinking behavior (Butler et al., 2014). Future research could extend and supplement these interpretations with physiological analyses, for instance, heart rate, which has been used as an indicator of anxiety related to housing conditions in rats (Azar et al., 2011; Sharp et al., 2014) and has not been explored in gerbils to date.

The high prevalence of sleep observed in the gerbils of our study (near 50% of the observation time) is consistent with that reported by Šusić and Maširević (Subiros et al., 2016), who found that their subjects allocated approximately half of the observation period to this behavior. Our further exploration of the data related to this behavior indicated a patterning of sleeping across different periods of the day.

Distribution of sleeping time was overall less systematic during day 1 than during day 5, most likely related to habituation of the animals to the housing conditions. Observed differences between male and female at this point suggest this habituation process varies by sex.

During day 5, regardless of sex or housing condition, gerbils showed the lowest levels of sleeping at the start of the dark cycle (18:00-20:59 hours) and the highest around midnight (00:00-02:59 hours). These results are consistent with those obtained by Weinert et al. (Weichbrod et al., 2018) who reported in gerbils a peak of daily activity near the light–dark transition. All together, these findings support Šusić and Masirević's notion that the gerbil in captivity is a crepuscular animal, being more active during transitions between light and dark (Subiros et al., 2016).

Differences in distribution of sleeping time across the time bins analyzed were more consistent during day 5 than during day 1 (see Table 4). This finding once again suggests a regularization of the gerbils' behavioral patterns—in this case, sleeping—days after variations in housing conditions have occurred. Although an analysis of the interactions between housing and sex on distribution of sleep indicated limited effects, the patterning of time allocation to this behavior still appeared to be dependent on sex and housing. Animals housed individually showed more differences across the analyzed time bins of day 5; similarly, males during day 5 seemed to have more marked variations in time allocation to sleep than females.

Lack of relevant research on factors that affect gerbils' sleep patterning across day periods precludes comparisons of our findings. However, our results are consistent with those of studies conducted with rats in which an interaction between sex and social isolation has been observed—for example, Hong et al. (2012) reported higher activity levels in isolated females than in male rats. Sex is a crucial variable in determining the neuroendocrine and behavioral response to social isolation (Ehlers et al., 1993); thus, it seems that similar to rats, female gerbils' sleeping-activity patterns are more sensitive to housing conditions than males.

Social housing of captive animals has been recognized over the last decade as a critical factor for improving animal welfare (Baumans and Van Loo, 2013). Although housing in groups is a common practice for gerbils kept in captivity, some procedures such as surgeries, monitoring, food, and substance-intake control require individual housing. Our results suggest that short-term individual housing in adult gerbils is associated with disruptions of activity-sleeping patterns and water consumption. These

alterations should be considered before exposing gerbils to experimental tests to assess their potential confounding effects.

Acknowledgments

Conflict of interest

The authors declare they have no conflict of interest.

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