Journal of the American Association for Laboratory Animal Science
Vol 51, No 2
March 2012
Pages 170–176

The Effect of Different Working Definitions on Behavioral Research Involving Stereotypies in Mongolian Gerbils (*Meriones unguiculatus*)

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Many sources of variation in animal experiments are related to characteristics of the animal or its husbandry conditions. In ethologic studies, observational methods can also affect interexperimental variation. Different descriptions for a behavior can lead to divergent findings that may be incorrectly attributed to other factors if not recognized as stemming from a classification dissonance. Here we discuss 2 observational studies in Mongolian gerbils (*Meriones unguiculatus*). The first study describes how data vary when 2 different working definitions are used for stereotypic digging: WD_{mor}, a definition based on a morphologic description of the behavior, and WD_{12}, a definition that relies mainly on a duration criterion of digging bouts (greater than 12 s). The total duration and number of stereotypic bouts were 22.0% and 63.1% lower, respectively, when WD_{12} was applied compared with WD_{mor}. However, strong correlations existed between data generated by WD_{mor} and WD_{12}, indicating that the 2 definitions yielded qualitatively similar results. The second study provides the first report that laboratory gerbils develop stereotypic behavior that is characterized by alternating bouts of digging and bar-gnawing. Of the 1685 stereotypy bouts investigated, 9.1% comprised both stereotypies, 87.6% consisted of digging only, and 3.3% consisted of bar gnawing only. Working definitions that neglect combined stereotypies can result in considerable underestimation of stereotypic behavior in Mongolian gerbils.

Abbreviations: WD_{mor}, working definition for stereotypic digging developed after morphologic description of nonstereotypic digging; WD_{12}, working definition for stereotypic digging that accommodates a 12-s cutoff for duration; WD_D, working definition for stereotypic digging developed by ad libitum sampling of observations of caged laboratory gerbils; BG, bar gnawing; D, digging; DBG, digging and bar gnawing.

When comparing results from behavioral studies on the same species conducted by different authors, several aspects of the experimental setups can be possible sources of variation. Factors that have been investigated for their potential influence on behavior can be classified into 4 main categories: animal-related elements, housing conditions, and environmental and observational factors. Animal-related elements include source (inhouse bred or shipped) and strain (genetic makeup) of the subjects, as well as sex, age, early experience, and the inherent stage of behavioral development.[5,20,26,31,35,36,39,42,45] Housing conditions include degree of contact with human caretakers or experimenters, individual or group-housing, cage size and type, amount and type of bedding, feeding and watering regimens, and absence or presence and type of environmental enrichment.[2,9,13-15,18,30,33,41,43,44,48,51,54] A third type of factors relates to ambient characteristics of the animal room or stall (macroenvironment) and, more importantly, of the cage (microenvironment): the presence and perception of noise, the lighting schedule and how it coincides with other external cues that affect biological clocks, ambient temperature, humidity, and ventilation.[3,10,11,16,29,37,47,48,57] Finally, individual characteristics of the observer and decisions regarding behavioral data collection, such as recording and sampling method, time of day of observation, the number of and consistency between observers, and the amount of detail with which the ethogram is composed might directly affect the consistency and quality of the collected data and, eventually, the outcome of the experiment.[17,18,21,22]

Although intralaboratory research generally is standardized to a great extent, much of the standardization can be lost when research groups study similar phenomena under slightly different conditions that involve one or more of the aforementioned sources of variation.[6] Comparing results from different studies is hampered by a number of factors. First, an adequate list of experimental details may not be reported. In other cases, the details may be reported but are not or perhaps for methodologic reasons cannot be taken into account by other groups. Similarly, working definitions for behaviors are the cornerstone of ethologic experiments, and the use of even slightly different descriptions of the same behavior by different scientists could lead to different findings. Because this divergence in results is often not recognized as stemming from a classification dissonance, the differences could falsely be attributed to other factors. Although use of different definitions for the same behavior will logically lead to different results, documenting the magnitude of such differences improves the accuracy of reporting (sometimes even simply by creating the opportunity to permit comparison) how one's own findings fit into the overall knowledge base.

Received: 05 Nov 2010. Revision requested: 07 Dec 2010. Accepted: 13 Aug 2011.
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When studying stereotypies, a methodologic problem lies in the fact that a definition must be created before it is known what exactly constitutes the stereotypic behavior.[32] Consequently, this definition must be adapted as data become available and should be regarded as a working definition: a clear and unambiguous tool to study a certain stereotypy that should be corrected as understanding about the concept increases. The characteristics of stereotypies have been summarized as follows: (1) the movement must be morphologically identical, (2) it must be repeated regularly, and (3) the whole behavior must seem purposeless or aberrant.[32] But several important questions arise from these requirements. First, exactly how morphologically identical do movements need to be and, as a consequence, when are they sufficiently different to be classified as 2 distinct behaviors? Second, how many times must a behavior be repeated to be classified as a stereotypy, and should a minimal duration be assigned? Finally, how do we ascertain that the behavior has no obvious function? Such questions have already been raised, reviewed and discussed extensively by other authors.[23,25] However, scientific data on how the use of different working definitions affect classification of the same stereotypy are scarce. Here we systematically investigate this issue with regard to stereotypic digging in laboratory Mongolian gerbils (*Meriones unguiculatus*).

Digging behavior in Mongolian gerbils has been observed under natural conditions and in the laboratory.[1,38] In the wild, the resulting burrows function primarily to protect animals from predators and temperature fluctuations. In addition, digging movements (in the wild or under artificially generated conditions in the laboratory) have been noted in the context of territoriality, grooming, and foraging–exploring.[1,50] In the laboratory cage, digging is described by many authors as rapid, alternating forelimb movement against the cage floor, wall, or corner, but it is rarely identified as a stereotypy.[8,40] A detailed working definition for stereotypic digging in Mongolian gerbils was first described as digging behavior lasting longer than 12 s.[50] Shorter instances were considered nonstereotypic digging. This definition was based on observations of the differential development of digging in young gerbils (17 to 37 d of age) according to location in the cage. At cage edges and in the corners, digging increased consistently after day 24. At 37 d of age, digging bout duration never exceeded 12 s in the middle of the cage, whereas the maximal duration of a stereotypic digging bout at the edges and in the corners lasted 50 s.[50] For adult animals of unreported age, the duration for digging in corners increased slightly to 60 s.[51] Furthermore, the appearance of a gerbil digging at the cage edges and in the corners became more fixed with increasing age, in that the animal held its legs more to the side, with its ears laid back and its eyes closed, throughout a digging bout. The working definition that emphasizes the duration threshold of 12 s[50] has been adopted by other research groups.[45,50-52] A study of gerbils until 37 d of age showed that stereotypic digging can be suppressed most when an opaque artificial burrow system, consisting at minimum of a tunnel and a chamber is provided in the cage,[45,52] suggesting that the motivation to perform stereotypic digging in the laboratory cage is related to the need to retreat into a burrow.

In rodents, the desire to escape from the cage has been proposed to underlie the development of another stereotypic behavior, that is, bar gnawing, which consists of chewing on bars of the cage lid.[19,31,54] In Mongolian gerbils, bar gnawing is seen first around the age of 18 d and increases considerably after weanling gerbils are separated from their parents at the age of 35 d, but this rise is less pronounced if the dam has already given birth to a new litter.[45,54] Our own casual observations suggest that some gerbils adopted a behavioral sequence of alternating bouts of stereotypic digging and bar-gnawing whereas others did not.

Our aim here is 2-fold: (1) to compare quantification of stereotypic digging behavior using 2 working definitions, one derived from the identification of normal digging behavior and the other the established definition that relies on duration;[50] and (2) to provide the first account of a combined digging–bar gnawing stereotypy. In addition, the implications of the use of the duration-based definition[50] on the quantification of this combined stereotypy are examined.

Materials and Methods

Different gerbils were used in each of the studies described below, the husbandry conditions remained the same. Gerbils were group-housed (2 or 3 animals of the same sex per cage) after weaning (at 30 to 35 d after birth). The cage environment before and after weaning was identical. Makrolon type IV cages with a galvanized wire lid on top (55 × 33 × 20 cm, Bio-Services, Schaijk, The Netherlands) contained wood shavings (Gold Mix, Carfil, Turnhout, Belgium), and cage enrichment was provided in the form of paper tissue (Mini-Tork , Tork, Guildford, Australia), good-quality grass hay, and chew blocks, all offered ad libitum. The chew blocks were homemade by cutting and dividing branches from apple and cherry trees. Pellets of rodent diet (2016 Teklad Global 16% protein, Harlan, Horst, The Netherlands) and tap water were available ad libitum. All gerbils were kept under a 14:10-h light:dark cycle, with lights off between 0900 and 1900. Room temperature averaged 20 ± 1 °C, and relative humidity values ranged between 30% and 60%. Continuous ventilation of the animal room was provided. Gerbils were housed and treated according to the regulations stipulated by the Council of the European Communities.[5]

Effect of different working definitions on quantification of the digging stereotypy. ***Animals and husbandry.*** For this study, 17 Mongolian gerbils (2 female, 15 male; age: mean, 254 ± 24 d; range, 81 to 318 d) were used. These gerbils had been bred (F_1 generation) from 5 pairs (age, 8 wk) purchased in 2007 from the outbred SPF RjTub:MON stock (Elevage Janvier, Le Genest-St-Isle, France). Breeding occurred within the context of a project evaluating neurobiochemical markers for stereotypic behavior, which was approved by the Ethical Committee of the Faculty of Veterinary Medicine at Ghent University (EC 2006/053).[28]

Working definition. The first working definition (WD_{mor}) was derived from preliminary observations carried out for a total of 14 h on 4 gerbils, housed in a glass terrarium (50 × 30 × 35 cm) bedded with 6 kg peat mixed with 350 g sawdust. Casual observations indicated a clear difference between the behavior of a gerbil digging while creating a tunnel (nonstereotypic digging) compared with when this behavior was not related to burrowing (stereotypic digging). One characteristic was the lack of regularly repeated hindleg kicks, which in nonstereotypic digging served to remove substrate after it had been loosened by the forelimbs. Incomplete motor patterns have been recognized previously as a characteristic in some stereotypies.[32] Therefore, the purpose was to use this information to accurately describe nonstereotypic digging and, from this information, to derive a new definition of a stereotypic digging bout. During the preliminary study, a total of 296 digging bouts (3115 s) leading to the progressive creation of burrows and 62 (830 s) other digging bouts were evaluated. The working definition of a nonstereotypic digging bout was determined as "performance of 4 to 7 foreleg scratches followed by 1 or 2 hindleg kicks." Subsequently, the definition for stereotypic digging bouts was coined "digging behavior comprising more than 7 scratches with the front legs that are potentially, but not necessarily, followed by or interspersed with hindleg kicks." The second definition (WD_{12}) considered digging to be stereotypic when it lasted longer than 12 s.[50]

In the current study, stereotypic digging was quantified by using both WD_{mor} and WD_{12}. When the sequence of digging was interrupted for longer than 1 s because the gerbil paused or expressed any other behavior, the bout was considered to be terminated.

Behavioral observations. Video footage of the gerbils was collected for 6 d over an 8-d period, starting in late February 2008. Each gerbil was observed under dim red light (PF 712E, 1.3 lx, Philips, Andover, MA) for 4 h, starting at the beginning of the dark period (0900). Black and white CCTV cameras (WV BP-150 and WV BP-550, Panasonic, Osaka, Japan) and VCR (AG-6124 and AG-6730, Panasonic) were used for videorecording. For identification purposes, the fur of the gerbils was marked by clipping. Behavior observations were obtained by using continuous recording and focal animal sampling. In total, 68 h of data were analyzed.

Statistical analysis. The total number, total duration (s), frequency (no. of bouts per hour), and average duration (s) of stereotypic digging bouts were calculated using both working definitions. The data were entered in SAS (version 9.1.3, SAS Institute, Cary, NC) and tested for normality by using the univariate procedure. Subsequently, a paired *t* test investigated the extent of the difference between data assessed by using WD_{mor} and WD_{12}. Pearson correlation test statistics were calculated to explore the association between data for each parameter.

Combined stereotypy digging: bar gnawing. *Animals and husbandry.* For this study, 24 Mongolian gerbils (13 female, 11 male; age: mean ± SEM, 226 ± 4 d; range, 187 to 243 d) were observed. These gerbils were bred (F_1 generation) from 5 pairs (age, 8 wk) of gerbils purchased in 2004 from the outbred SPF RjTub:MON stock (Elevage Janvier). Breeding occurred within the context of a project regarding biotelemetry monitoring during the development of stereotypic behavior; this protocol was approved by the Ethical Committee of the Faculty of Veterinary Medicine at Ghent University (EC 2003/019).[27]

Subjects were housed from weaning (30 to 35 d after birth) in the same colony room and experienced identical environmental conditions as described for the previous study.

Behavioral observations. Video footage of the gerbils was collected for 10 d over an 18-d period during May 2005 by using the same methods as mentioned for the previous study. Analysis of the videos occurred in 2008 by another observer than that for the earlier study. The working definition for a digging stereotypy (WD_D), developed by casual behavioral observations in caged laboratory gerbils by using the ad libitum sampling technique, was "rapid digging with front legs, interspersed with hindleg kicks. These movements can be made while the gerbil is hunched or while standing upright against the cage wall. Because of the smooth cage surface, slipping of the hind legs followed by quick restoration of balance toward the upright position often occurs." Bar-gnawing was defined as "the behavior where the gerbil grasps a bar from the food hopper between its teeth and moves its mouth up and down this bar while chewing. The posture of the gerbil is upright, and usually one hindleg is raised slightly and intermittently." A total of 96 h of data were analyzed.

Statistical analysis. To explore the characteristics of a combined stereotypy, the duration of the intervals between every possible sequence of digging (D) and bar-gnawing (BG) bouts was calculated and a frequency distribution constructed using Excel 2003 (Microsoft, Redmond, WA). Subsequently, descriptive statistical analysis was performed, which included the combined DBG stereotypy. A behavioral bout typically is viewed as terminated when the animal engages in a different behavior or when the current behavior is interrupted for longer than 1 s.[12,44,55] As a result, sequences of digging and bar-gnawing spaced 1 s or less apart can be regarded as belonging to the same bout. However, most D–D and all BG–BG intervals lasted at least 1 s. Therefore, the combined stereotypy (DBG) was defined as the alternating occurrence of stereotypic digging and bar-gnawing with intervals equal to or less than 1 s.

To assess whether a relationship existed between the amount of digging and bar-gnawing expressed in combined stereotypy bouts, the total duration of both behaviors within such bouts was calculated and a Pearson correlation test conducted. Finally, to examine the effect of the established WD_{12} on the quantification of the combined stereotypy, the descriptive statistical analysis was repeated by using results generated after applying WD_{12}.

Results

Effect of different working definitions on quantification of the digging stereotypy. Table 1 summarizes the results of the paired *t* test, including the difference of the group means, when comparing WD_{12} with WD_{mor} to characterize the digging stereotypy in Mongolian gerbils. Use of WD_{12} decreased the total duration of stereotypic digging bouts by 22.0% and the total number of bouts by 63.1% compared with WD_{mor}. Consequently, the average bout duration of the WD_{12} data doubled.

To investigate whether a gerbil performing frequent stereotypic digging as identified by WD_{mor} is still recognized as such according to WD_{12}, the Pearson correlation test statistic (Pearson *r*) was calculated (Table 1). All parameters showed strong, positive correlation between data from WD_{mor} and WD_{12}.

Combined stereotypy: digging and bar gnawing. The total number of intervals was 2060 (range, 0 to 9134 s), of which 1569 consisted of digging followed by digging (D-D), 227 of digging followed by bar-gnawing (D-BG), 221 of bar-gnawing followed by digging (BG-D), and 43 of bar-gnawing followed by bar-gnawing (BG-BG; Figure 1). Only 4 D-D intervals lasted 1 s, whereas all other D-D occurrences were at least 2 s in duration. Similarly, no BG-BG intervals of less than 2 s were observed. In contrast, 175 D-BG intervals and 71 BG-D intervals were 1 s or less in duration (Figure 1). In all D-BG or BG-D intervals lasting longer than 1 s, bar-gnawing consisted of an isolated bout or represented the start or end of a bout in which an alternating sequence of bar-gnawing and digging was expressed at least once and with less than 1 s in between.

All 24 gerbils expressed stereotypic digging ($n = 24$), but not all engaged in bar-gnawing ($n = 13$) or the combined stereotypy ($n = 13$). Nine of the 13 bar-gnawing subjects expressed the combined stereotypy, whereas the remaining 4 did not. In comparison, 4 gerbils displayed bar-gnawing solely as part of the combined stereotypy and did not engage in this behavior separately throughout the 4-h observation period. During a DBG bout, a gerbil (repetitively or not) alternated between the 2 stereotypic behavioral elements (D and BG) and—in the case of a sequence of digging followed by bar-gnawing—twisted its thorax after digging to reach the bars of the food hopper. After bar-gnawing, the gerbil subsequently shifted its weight back to the previous position, either to start another digging bout, thereby repeating the stereotyped cycle, or to initiate a different behavior, thereby interrupting the cycle.

Table 1. Influence of working definitions (WD_{mor} and WD_{12}) on descriptive parameters (mean ± SEM) for the digging stereotypy in Mongolian gerbils (*Meriones unguiculatus*)

	WD_{mor}	WD_{12}	t(23)	Mean WD_{12} – mean WD_{mor} (%)	Pearson r
Total duration (s)	1553.92 ± 327.78	1212.00 ± 264.81	3.89[a]	−22.00	0.9914[a]
Total number	110.41 ± 22.16	40.76 ± 7.99	3.89[a]	−63.08	0.9264[a]
Frequency (no. of bouts per hour)	34.16 ± 6.78	12.54 ± 2.46	3.95[a]	−63.29	0.9254[a]
Average bout duration (s)	12.84 ± 1.35	25.78 ± 1.60	−11.25[b]	100.78	0.7969[a]

WD_{mor}, working definition developed after morphologic description of nonstereotypic digging; WD_{12}, working definition that accommodates a 12-s duration cutoff.
[a]$P = 0.001$
[b]$P < 0.0001$

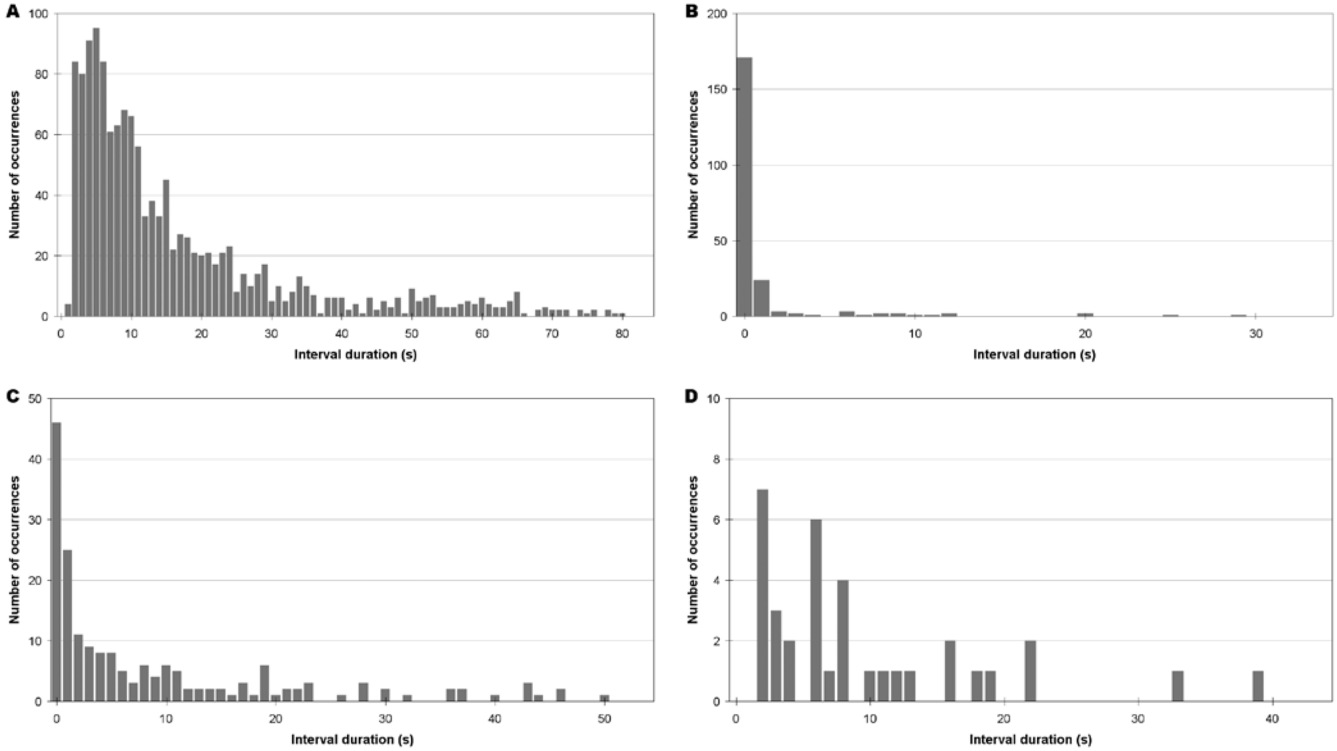

Figure 1. Frequency charts of intervals for digging (D) and bar-gnawing bouts (BG) in laboratory Mongolian gerbils. 1(A) D–D intervals (n = 1569). (B) D–BG sequence intervals (n = 227). (C) BG–D sequence intervals (n = 221). (D) BG–BG sequence intervals (n = 43).

Of the total 1685 stereotypic bouts, which lasted a total of 41596 s (23.1% ± 3.7% of the time the animals were active during observations), the digging stereotypy (D) occurred 1476 times (87.6% of the total number of stereotypic bouts; 17.3% ± 3.1% of the active time), bar-gnawing (BG) 55 times (3.3%; 0.8% ± 0.3%) and the combined stereotypy (DBG) 154 times (9.1%; 5.0% ± 1.4%).

On average, DBG bouts lasted 61.0 ± 5.1 s, and the average time the gerbils spent bar-gnawing or digging during a combined stereotypy bout was 26.3 ± 2.9 s and 18.9 ± 2.6 s, respectively. During a DBG bout, bar-gnawing was seen 1.3 ± 0.07 (range, 1 to 10) times and digging 1.4 ± 0.1 (range, 1 to 9) times. Gerbils started 96.8% of DBG bouts by digging ($n = 149$) and 3.2% by bar-gnawing ($n = 5$), whereas they terminated 11.00% of the bouts ($n = 17$) while digging and 89.0% while bar-gnawing ($n = 137$; Table 2) The association between digging and gnawing within DBG bouts showed a significant, but weak, positive correlation: $r = 0.30$ ($P = 0.0002$).

Finally, the effect of using WD_{12} compared with WD_D to quantify the amount of digging in the combined stereotypy was investigated. Compared with WD_D, data from WD_{12} discarded as much as 40.4% of stereotypic digging bouts (Figure 2). Consequently, the number (WD_{12}, $n = 78$; WD_D, $n = 154$; a decrease of 49.4%) and total duration (WD_{12}, 5162 s; WD_D, 9724 s; a decrease of 46.91%) of DBG bouts decreased with the use of WD_{12}, although the average duration of a combined stereotypy bout remained essentially unaffected (WD_{12}, 66.18 ± 5.18 s; WD_D, 61.01 ± 5.14 s).

Discussion

To quantitatively investigate to what extent different working definitions for the same behavior influence the experimental outcome in laboratory Mongolian gerbils, we compared results from using a working definition for stereotypic digging derived from observations of normal, apparently functional digging (WD_{mor}) with the only detailed definition available in literature (WD_{12}).[50] Remembering that WD_{12} discards digging bouts lasting less than 12 s, the difference between data from WD_{mor} and WD_{12} for the various parameters is based mainly on omission of short stereotypic bouts. When WD_{12} was used, the

Table 2. Combined stereotypy bouts expressed as numbers of bouts that start and end with digging or bar-gnawing

Starts with	Ends in: Digging	Ends in: Bar gnawing	Total
Digging	15	134	149
Bar gnawing	2	3	5
Total	17	137	154

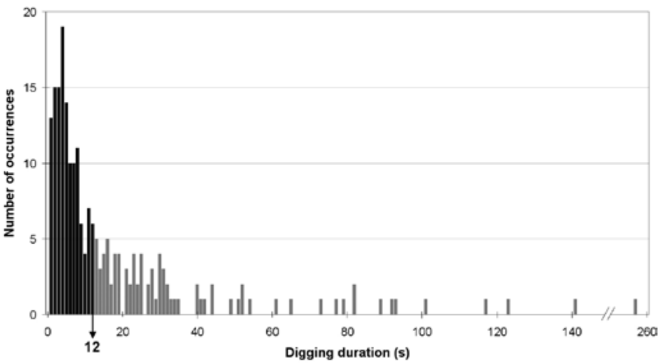

Figure 2. Frequency graph of the duration of digging bouts that were part of a DBG (digging and bar-gnawing) bout in laboratory Mongolian gerbils. Black bars indicate digging bouts of 12 s or less; grey bars indicate digging bouts longer than 12 s.

total duration, total number and frequency decreased, whereas the average digging bout duration increased compared with that from WD_{mor}. Adopting WD_{12} led to a 22% decrease in the total duration of stereotypic digging, meaning that over one fifth of the total duration of stereotypic digging consisted of short (less than 12 s) digging bouts. This drop in duration corresponded to a 60.00% decrease in the total number of stereotypic bouts and a 2-fold increase in average bout duration when the data were analyzed using WD_{12} compared with WD_{mor}.

Most of the studies using WD_{12}[50] have been performed in young gerbils, but at least one report has been made in adults.[51] However, in the current observational studies on adult gerbils, the visual distinction between nonstereotypic digging (performed in the appropriate substrate and related to burrowing) and stereotypic digging was made easily. The working definition WD_{mor} is, like WD_{12}, based on morphologic properties of the behavior and the posture of the stereotyping animal, but it differs most from WD_{12} in that WD_{mor} lacks a cutoff duration. Our results involving adult gerbils showed that 60% of the digging stereotypy bouts were shorter than 12 s while being 'locally fixed.'[52] They also were morphologically identical to stereotypy bouts of longer duration. Together, these observations suggest that, compared with a definition like WD_{mor}, WD_{12} represents an underestimation of stereotypic digging when quantitatively investigating this behavior in adult animals.

Although the differences in data between WD_{mor} and WD_{12} are considerable, the degree of association among the different parameters remained high. Absolute values aside, it is expected that WD_{12} would allow valid comparison of parameters between study populations. Similar to those of other studies, absolute values of behavioral parameters might differ, yet general conclusions would remain the same.[18]

The use of 2 different working definitions, especially when they mainly differ in the requirement of a minimum duration for a behavior (as was the case for WD_{mor} compared with WD_{12}), may not be problematic if the user recognizes the meanings of different outcomes. This conclusion assumes that the pattern of stereotypic digging develops and then maintains the same pattern of expression throughout the adult life of the gerbil.[4] Further analysis of the data from our second study revealed that 13 of the 24 gerbils consistently alternated gnawing and digging. Based on frequency analysis results, we propose that some gerbils develop a combined stereotypy in which short bouts of digging and bar-gnawing alternate. As a result, adopting WD_{12} in studies of gerbils with established stereotypies would result in discarding short bouts that might be part of a DBG sequence. This would profoundly affect the number of occurrences of a DBG cycle and the total and average duration of bouts, as was shown in our second study comparing WD_{12} and WD_D. As stated previously, differences in absolute values do not necessarily lead to different conclusions, but one must be aware of them when comparing population means for a given species and behavior between experiments.

In our second study, out of 1685 stereotypic behavior bouts, only 55 consisted of stand-alone bar-gnawing (3.3%; 0.8% ± 0.3% of the active time), whereas the combined stereotypy (DBG) was almost 3 times more frequent (9.14%; 5.03% ± 1.36% of the active time). The digging stereotypy, however, was displayed the most often (87.6%; 17.3% ± 3.1% of the active time). Based on literature data, the same appears to be true in young gerbils for digging and bar-gnawing (younger than 37 d) .[53,54] Gerbils develop such stereotypies before 30 d of age. As the animals become older, the behavior patterns become more established and are increasingly expressed. However, the current study showed that, with increasing stereotypy age and apparent behavioral rigidity, some variability becomes introduced by fusing behavioral elements into a stereotypic cycle.

The combination of 2 or more behavioral elements in a stereotyped cycle has previously been reported (for example, in tethered sows and caged mink[7,24]). Why the gerbils develop a behavior that combines 2 behavioral elements remains to be elucidated.[51] Much like the digging stereotypy, the combined stereotypy is locally fixed; that is, it is seen only in cage corners and, more specifically, the corners nearest to the food hopper.[50] The equal proximity to the cage corner and the food hopper may, in the combined stereotypy, have facilitated switching from digging to bar-gnawing without interruption or change of location in the cage. Our understanding of the motivation behind the adoption of a combined stereotypy in gerbils and whether and how the behavioral patterns become established stereotypies continues to develop. One author mentions that rigidification of behavior is spotted most easily in appetitive behavior patterns.[29] Digging and bar-gnawing represent such appetitive behaviors. The environment provides triggers for certain behaviors (in this case, digging and bar-gnawing) but cannot provide for completion (that is, creation of a tunnel or chewing through cage bars). Although expression of stereotypies often implies a decrease in behavioral variation, our study lends support to the idea that captive animals seem to produce complexity in a simple or barren environment.[29] However, unlike a previous observation that subjects under such conditions develop new motor patterns,[29] gerbils in the current study increased complexity by combining existing patterns. Unlike the studies involving sows[7] or mink,[24] gerbils from the current study expressed no more than 2 fixed behaviors (digging and bar-gnawing), and neither did they introduce varied behavior during a stereotypic behavior sequence. Furthermore, 4 of the 13 gerbils that expressed the combined stereotypy in our study did not display bar-gnawing as a stand-alone behavior during the observation period. Although bar-gnawing is the least expressed stereotypy when compared with stand-alone stereotypic digging or the combination of both, this result could indicate that rather than introducing variability, the pattern of behavior is becoming more rigid, with fewer elements appearing as stand-alone behaviors. An ontogenetic approach could plot the time course of behavioral development, including stereotypic behavior.[34] This design allows determination of whether stereotypic behavior, which is inherently rigid in performance, becomes increasingly invariant in some gerbils or instead remains flexible after the developmental stage.

Although we examined limitations to the established definition of stereotypic digging, WD_{12}, we cannot recommend a choice between WD_{mor} and WD_D, because the definitions were used to examine video footage from separate studies. Future research should address whether WD_{mor} has added value over WD_D, given that WD_{mor} requires time-consuming manual counting of forelimb and hindlimb movements for each digging bout.

In conclusion, we have shown that using different definitions for the same behavior (stereotypic digging in the Mongolian gerbil) leads to different results. Differences were seen mainly as different absolute values, whereas relative associations (as indicated by correlations) were strong. Nonetheless, use of the WD_{12} definition caused many digging bouts to be classified as nonstereotypic, yet the appearance of both types of bouts suggested little reason to consider them different. The consistent expression of a sequential combination of digging and bar-gnawing in half of the sampled gerbils also demonstrates the effect of using a working definition that is based on a cutoff duration. When WD_{12} was used, digging bouts of 12 seconds or less were considered to be nonstereotypic, thereby reducing the number and duration of combined stereotypy bouts.

Acknowledgments

We thank Drs C Wiedenmayer, E Waiblinger, H Würbel, and G Mason for providing additional insights regarding stereotypic behavior (particularly in gerbils) over the past years; K Verschueren for critical comments on the manuscript; R Van Gansbeke for logistical support; and S Merciny (lumadox.be) for editing tables and graphs and formatting graphs according to JAALAS style.

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