

Environmental Influence on Development, Behavior, and Endocrine Morphology of Gerbils

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CLARK, M. M. AND B. G. GALEF, JR. *Environmental influence on development, behavior, and endocrine morphology of gerbils.* PHYSIOL. BEHAV. 27(5) 761-765, 1981.—Gerbils reared under standard laboratory conditions grow and develop more rapidly, achieve sexual maturity when younger, and are less reactive to stimulation than gerbils reared in environments providing access to a tunnel-like shelter. Gerbils reared in open cages have lighter adrenal glands, heavier pituitary glands, and heavier reproductive organs than gerbils raised with access to shelter. Comparison of gerbils reared in cages providing access either to opaque or transparent shelter with animals maintained in open cages either on a 12-hr light-dark cycle or in constant darkness revealed that different aspects of environments providing shelter affected different characteristics. Opportunity to move from an open area to a sheltered one increased behavioral reactivity. Reduced exposure to illumination affected developmental rates, reproductive organ and pituitary gland weights. Both reduced exposure to illumination and access to a shelter affected adrenal gland and body weights. These data suggest that expression of the phenotype typical of domesticated gerbils requires deprivation of a variety of stimuli normally experienced by burrow-dwellers during ontogeny.

Behavior Development Endocrine morphology Gerbils Dark rearing Domestication

THE results of recent studies in our laboratory indicate that the behavior, morphology, and rate of development of Mongolian gerbils can be profoundly influenced by the nature of the physical environment in which gerbils mature [4, 5, 6]. Comparison of the phenotype of domesticated gerbils reared either in tunnel systems constructed by their parents or in cages providing access to a tunnel-like shelter with that of gerbils reared in standard laboratory cages reveals a variety of differences which parallel those often attributed to the process of domestication resulting from artificial selection in captive environments [2]. As can be seen in Table 1, which summarizes the results of our previous studies [4, 5, 6] of the effects of rearing environment on behavioral and morphological characteristics, rearing in cages providing access to shelter has been found to enhance avoidance responses to sudden stimulation, delay maturation, reduce both absolute and relative reproductive organ weights, reduce absolute, but not relative pituitary gland weights, enhance relative, but not absolute, adrenal gland weights and reduce body weight.

Animals maturing within a burrow system or in laboratory cages which provide access to a tunnel-like shelter are exposed to a variety of types of stimulation different from those experienced by conspecifics reared in standard laboratory environments [4, 7, 9]. In particular, the degree of exposure to light and the presence of opportunities to flee to a place of concealment differ dramatically between animals reared in

standard laboratory cages and those reared in environments providing shelter.

The results of our previous studies, undertaken to determine those aspects of shelter-rearing responsible for producing a wild phenotype, indicate that experience of moving from an open area to one providing concealment suffices to enhance behavioral reactivity in domesticated Mongolian gerbils. Neither rearing in darkness nor rearing in isolation from human handlers had similar effects [4]. The present experiment was undertaken to determine whether the same feature of the shelter rearing environment which produces enhanced reactivity is sufficient to produce wild-type rates of development and wild-type morphologies.

METHOD

Animals

Subjects were 64 litters of Mongolian gerbils (*Meriones unguiculatus*) born to sixteen multiparous breeding pairs acquired from Tumblebrook Farm (Brookfield, MA). Breeding pairs were housed in polypropylene cages (35×30×15 cm) lidded with 1.27 cm hard-ware cloth and carpeted with a thin layer of wood-chip bedding. The colony was maintained on ad lib Purina Laboratory Chow and water in a temperature controlled colony room illuminated on a 12-hr light-dark cycle.

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TABLE 1
EFFECTS OF REARING WITH ACCESS TO A
TUNNEL-LIKE SHELTER

Category	Measure	Opaque Shelter vs 12-hr L/D Open
Behavior	Seizures	*
	Flight	‡
	Concealment	‡
	Footthumping	*
Development	Eye-opening	§
	Sexual Maturity	§
Reproductive Organ Weights	Uteri	§ (§)
	Ovaries	§ (§)
Endocrine Gland Weights	Testes	§ (n.s.)
	Pituitary (Females)	§ (n.s.)
	Pituitary (Males)	§ (n.s.)
	Adrenals (Females)	n.s. (†)
Body Weight	Adrenals (Males)	n.s. (†)
	Females	¶
	Males	¶

Signs in parentheses refer to organ weights relative to body weight (data from [3, 4, 5], and unpublished observations).

*Increases or accelerates, $p < 0.05$.

†Increases or accelerates, $p < 0.01$.

‡Increases or accelerates, $p < 0.001$.

§Decreases or retards, $p < 0.05$.

¶Decreases or retards, $p < 0.01$.

n.s. = not significant.

Each of the 16 breeding pairs reared, in counterbalanced order, one litter in each of the four environments described below.

Rearing Conditions

Within 24 hr of birth, each litter and its parents were assigned to one of four rearing conditions, differing in the opportunity they provided for flight to shelter and the access which they provided to a darkened area. Two groups of subjects were reared in translucent cages (35×30×15 cm) providing no access to shelter (open cages). One of these groups was maintained in a colony room on a 12-hr light-dark cycle (*Group 12-hr L/D Open*) and the other in constant darkness (*Group 24-hr Dark Open*). Animals in the latter group were exposed to light for 10 min/day to prevent retinal degeneration [15].

The two remaining groups of subjects were maintained in cages (35×30×15 cm) providing access to a shelter (a 28×15×9 cm enclosure with a 5×5 cm entrance hole) in a colony room maintained on a 12-hr light-dark cycle. The shelters available to subjects in one of the shelter-reared groups were constructed of transparent Plexiglas (*Transparent-Shelter Group*) and those available to subjects in the second shelter-reared group were constructed of 0.63-cm plywood (*Opaque-Shelter Group*).

Subjects remained undisturbed in their respective rearing environments until behavioral testing and subsequent autopsy at 61 days of age except for inspection for eye-opening twice daily from 15 to 22 days of age (day of birth equals Day

1) and inspection for vaginal opening of female subjects on Day 30, 39, 46, 53 and 60 postpartum.

Testing

Four subjects from each of the 16 litters reared in each of the four environments were tested for their response to sudden visual stimulation. Half of the subjects from each of the four rearing environments (16 males and 16 females) served as Experimental subjects and the remainder (16 males and 16 females) as Control subjects in the test situation.

The apparatus and test procedures used in behavioral testing are described in detail in Clark and Galef [4,5]. In brief, subjects' response to presentation of a novel visual stimulus (a Latex mask of a human face) was determined by observation. Individual subjects were placed in a 1.2×1.2×0.9 m enclosure, the wooden floor of which was bare except for a 0.3×0.3×0.15 m refuge in one corner of the enclosure which provided a potential place of concealment. Subjects in both the Experimental and Control Groups were allowed to become familiar with the enclosure and any seizures (Grade 1 through Grade 5 as described by Loskota *et al.* [11]) occurring during the familiarization period were recorded.

Immediately following the period of familiarization with the enclosure, Experimental subjects were presented with the visual stimulus for 15-sec. During the 2-min period following stimulus presentation, the experimenter recorded each Experimental subject's latency to enter the refuge, total time spent in concealment and instances of footthumping. Control subjects were treated identically to Experimental subjects except that they were not presented with a visual stimulus at the end of the familiarization period.

Autopsy

Within 1-hr of completion of behavioral testing each experimental and control subject was sacrificed by anesthetic overdose and weighed. Adrenal and pituitary glands, testes or ovaries and uteri were then excised and weighed.

Comparisons

Effects of access to shelter on phenotype were assessed by comparing data collected from subjects reared in the 12-hr L/D Open condition with that of subjects reared in the Transparent-Shelter condition.

Effects of darkness on phenotype were assessed in two ways: (1) by comparisons of data collected from subjects reared in 12-hr L/D Open and 24-hr Dark Open conditions and (2) by comparison of data of subjects reared in Transparent-Shelter and Opaque-Shelter conditions.

To avoid reporting repeated statistical tests on the same data, measurements of endocrine morphology of subjects serving as Experimental and Control subjects during behavioral testing was analyzed separately and data from each subgroup was used only once in any statistical comparisons reported. All p values reported are 2-tailed.

RESULTS

Behavior

The main results of behavioral testing of Experimental and Control subjects from the four rearing conditions are presented in Figures 1A–D, which show respectively:

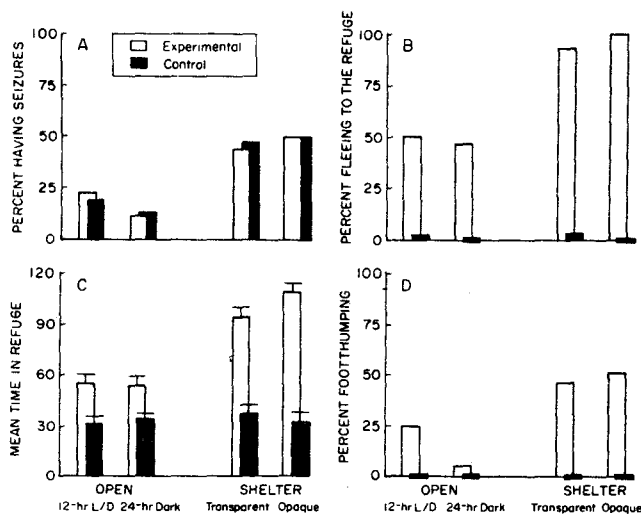


FIG. 1. Responses of Experimental and Control subjects, reared in four environments, (A) Percentage of subjects having seizures prior to stimulus presentation, (B) Percentage of subjects fleeing to the refuge during the 3-sec following stimulus presentation, (C) Mean time spent in the refuge during the 2-min following stimulus presentation, (D) Percentage of subjects footthumping in the 2-min following stimulus presentation.

(Fig. 1A) the percentage of subjects exhibiting seizure activity during the familiarization period.

(Fig. 1B) the percentage of subjects reaching shelter within 3-sec of stimulus presentation. Subjects entering the refuge within 3-sec of stimulus presentation (or, in the case of Control subjects, in the 3 sec following the moment when stimulus presentation would have been initiated) were assumed to have fled in response to presentation of the stimulus. A 3-sec criterion has been found previously to distinguish subjects responding to the stimulus from those randomly entering the refuge [4].

(Fig. 1C) the mean number of seconds spent by subjects in the refuge during the 2-min observation period.

(Fig. 1D) the percent of subjects footthumping during the 2-min observation period.

The present data confirm our previous finding that the feature of the sheltered environment responsible for the enhanced behavioral reactivity to stimulation of gerbils reared in environments providing access to shelter is the opportunity to move from an open area to an enclosed one, rather than the reduced exposure to illumination provided by Opaque-Shelter [4]. Comparison of the behavior of subjects reared in the Transparent-Shelter condition with that of subjects reared in the 12-hr L/D Open condition reveals that the former animals convulsed more frequently during the exploration period, $\chi^2=4.80, p<0.05$, fled more frequently in response to stimulus presentation, $\chi^2=12.7, p<0.001$, spent more time in concealment (Student's *t*-test, $t(62)=4.28, p<0.001$, and footthumped with higher frequency, $\chi^2=3.91, p<0.05$, than the latter. Both comparison of the behavior of subjects reared in 12-hr L/D Open with that of subjects reared in 24-hr Dark Open and comparisons of the behavior of subjects reared with access to transparent shelter with that of subjects reared with access to opaque shelter revealed

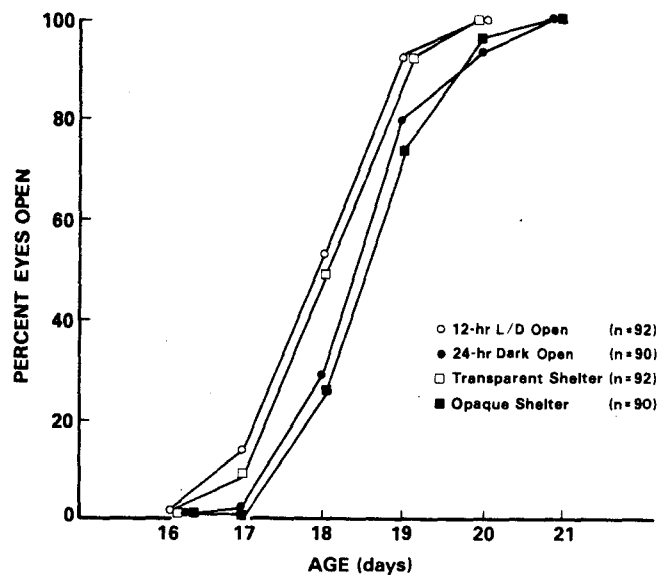


FIG. 2. Percentage of subjects from the four rearing conditions with both eyes fully open.

only minimal effects of increased exposure to darkness during otogeny on reactivity to stimulation.

Further evidence of the importance of access to shelter, but not of exposure to darkness, in enhancing behavioral reactivity is to be found in comparisons of the behaviors of subjects reared in darkness (24-hr Dark Open Group) with that of subjects reared with access to opaque shelter. On each of the four measures, the latter animals exhibited greater reactivity than the former (all *p*'s < 0.001).

Development

Figures 2 and 3 show, respectively, the age at which eye-opening (defined as the age at which both eyes were first observed fully open) and vaginal-opening occurred in subjects reared in the four environments.

Examination of Figs. 2 and 3 reveals that rates of development in gerbils, unlike behavioral reactivity, was affected by the amount of illumination to which subjects were exposed, and not by access to shelter, during ontogeny.

Eye-opening occurred earlier in animals reared in open cages on a 12-hr light-dark cycle than in subjects reared in open cages maintained in constant darkness (Kolmogorov-Smirnov test, $D_{max}=0.263, p<0.01$). Eye-opening also occurred earlier in animals maintained with access to a transparent shelter than in subjects maintained with access to an opaque one ($D_{max}=0.282, p<0.01$). Similarly, vaginal opening occurred at an earlier age in female gerbils reared on a 12-hr light-dark cycle than in females reared in constant darkness ($K_D=15, p<0.05$) and at an earlier age in females reared with access to transparent shelter than in females reared with access to an opaque shelter ($K_D=14, p<0.05$).

Examination of Figs. 2 and 3 and statistical tests reveal

TABLE 2

MEAN BODY WEIGHT (g) AND MEAN ABSOLUTE (mg) AND RELATIVE (mg/100 g) WEIGHTS OF THE REPRODUCTIVE ORGANS AND ENDOCRINE GLANDS OF FEMALE AND MALE GERBILS

Measure	Rearing Condition							
	Open				Shelter			
	12-hr L/D		24-hr Dark		Transparent		Opaque	
Body weight (♀)	59.5±1.5		52.5±1.5		54.8±0.8		51.7±1.5	
Body weight (♂)	71.9±1.4		60.2±1.6		66.1±1.8		62.5±1.6	
Uteri	94.2±11.2 (155.2±18.2)		39.3± 4.2 (73.7± 6.6)		76.0±16.9 (137.1±29.2)		46.3± 4.4 (79.2± 6.5)	
Ovaries	21.3± 1.7 (34.2± 2.6)		14.4± 1.0 (27.2± 1.5)		17.2± 1.2 (31.4± 1.5)		14.3± 0.7 (27.7± 1.0)	
Testes	885.4±14.7 (1231.4±21.0)		799.1±28.2 (1327.4±34.1)		868.3±20.9 (1323.6±35.2)		810.0±16.8 (1296.0±24.5)	
Pituitary (♀)	2.8± 0.1 (5.0± 0.2)		2.5± 0.1 (4.6± 0.2)		2.8± 0.2 (5.1± 0.3)		2.4± 0.1 (4.7± 0.2)	
Pituitary (♂)	2.8± 0.1 (4.0± 0.2)		2.4± 0.1 (4.0± 0.2)		2.6± 0.1 (4.1± 0.3)		2.4± 0.2 (3.9± 0.2)	
Adrenals (♀)	23.0± 0.5 (40.1± 1.1)		23.6± 0.7 (45.3± 1.7)		24.5± 0.5 (45.8± 1.8)		24.3± 0.9 (47.0± 1.0)	
Adrenals (♂)	26.1± 0.6 (36.4± 0.9)		26.6± 0.7 (44.1± 0.9)		27.3± 0.7 (41.8± 1.3)		27.7± 1.0 (44.2± 0.8)	

Numbers in parentheses refer to relative organ weights. Measures of variance are S.E.M.'s.

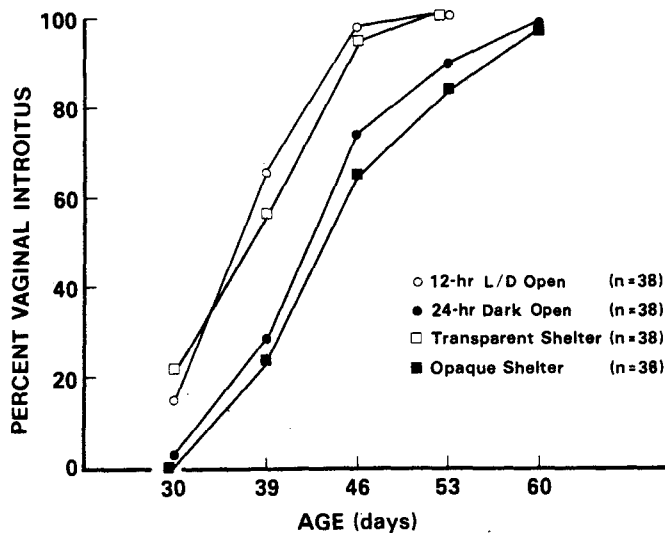


FIG. 3. Percentage of female subjects from the four rearing conditions exhibiting vaginal opening.

minimal effects of the presence of shelter on either age at eye-opening (Fig. 2, Group 12-hr L/D Open vs Group Transparent Shelter, $D_{\max}=0.087$, $p=n.s.$) or age at vaginal opening (Fig. 3, Group 12-hr L/D Open vs Group Transparent Shelter, $K_D=3$, $p=n.s.$).

Weights of reproductive organs. As might be expected, given the efficacy of reduced exposure to illumination in delaying vaginal introitus and the insufficiency of experience of shelter to do so, reproductive organ weights were markedly affected by exposure to illumination and not by experience of shelter. The relevant data are presented in Table 2 which shows both absolute and relative organ weights of subjects reared in the four environments.

As can be seen in Table 2, and as statistical tests confirm,

development of the reproductive organs of both male and female subjects was inhibited by rearing in environments providing reduced illumination (12-hr L/D Open vs 24-hr Dark Open and Transparent-Shelter vs Opaque-Shelter, all paired t -tests t 's (15) >2.16 , all p 's <0.05) but not by rearing in environments providing access to shelter (12-hr L/D Open vs Transparent-Shelter, both t 's (62) <1.20 , both p 's >0.20).

Endocrine gland weights. Reference to Table 1 reveals a complex pattern of effects of shelter-rearing on pituitary and adrenal gland weights. Shelter-rearing both decreased absolute pituitary weights (leaving their magnitude relative to body weight unchanged) and increased relative adrenal weights (leaving their absolute magnitude unaltered).

As can be seen in Table 2, and as statistical tests confirm, amount of illumination experienced during ontogeny had no effect on pituitary gland weights relative to body weight (12-hr L/D Open vs 24-hr Dark Open and Transparent-Shelter vs Opaque-Shelter, all paired t 's (15) <1.43 , all p 's >0.10), but had effects on absolute pituitary gland weights (12-hr L/D Open vs 24-hr Dark Open, both paired t 's >2.47 , both p 's <0.05 ; Transparent-Shelter vs Opaque Shelter, η paired $t=2.14$, $p<0.05$; δ paired $t=1.84$, $0.05<p<0.10$). The opportunity to flee to shelter had no reliable effect on either absolute or relative pituitary gland weights.

As can also be seen in Table 2, none of our environmental manipulations affected absolute adrenal gland weights (12-hr L/D Open vs 24-hr Dark Open and Opaque-Shelter vs Transparent-Shelter, all paired t 's (15) <0.60 , all p 's >0.20 ; 12-hr L/D Open vs Transparent Shelter, both t 's (62) <0.55 , both p 's >0.20), while relative adrenal size was increased by both the opportunity to flee to shelter (both t 's (62) >2.80 , both p 's <0.02) and prolonged exposure to darkness during maturation (12-hr L/D Open vs 24-hr Dark Open, both paired t 's (15) >2.45 , both p 's <0.05).

Thus, the effects of shelter-rearing on pituitary gland weights (summarized in Table 1) can be attributed to the effects of the reduced illumination which shelter-reared subjects experience, while the effects of shelter-rearing on adrenal gland weights (summarized in Table 1) can be attributed both to the reduced illumination and to the opportunity to flee to cover provided by sheltered environments.

Body weight. Body weight, like relative adrenal gland

size, was affected by both the opportunity to flee to shelter (12-hr L/D Open vs Transparent-Shelter, both t 's (62) > 2.23, both p 's < 0.05) and by reduced exposure to illumination during ontogeny (12-hr L/D Open vs 24-hr Dark Open, both paired t 's (15) > 4.28, both p 's < 0.001).

DISCUSSION

Comparison of descriptions of wild Mongolian gerbils provided by Soviet field workers with descriptions from North American laboratories of domesticated gerbils reveals a number of differences in phenotype typical of the effects of domestication on rodents. Wild Mongolian gerbils are said to be "timid and unapproachable" ([8], p. 10), while their domesticated counterparts are gentle and curious [16]. Members of wild populations grow less rapidly [10], achieve sexual maturity at a later age [10], have larger adrenal glands (relative to body weight) [1], and may have larger reproductive organs than individuals reared in captivity. (It is difficult to calculate useful indices of reproductive organ weights in free-living gerbils because of the extreme seasonal variability they exhibit [1].)

Such differences in phenotypes of wild and captive individuals are frequently interpreted as resulting from the process of artificial selection acting on captive populations over many generations [2, 13, 14]. Unfortunately, gerbils examined in the period closest to their introduction into captivity were maintained in cages providing access to shelter [12], while later reports describe gerbils maintained in cages lacking sheltered areas [16, 17]. The results of the present experiment suggest that the reported differences in phenotype may be explained as easily in terms of differences in maintenance conditions as in terms of the action of artificial selection.

Phenotypic characteristics produced by shelter rearing (such as enlarged adrenal glands, reduced reproductive organs and enhanced reactivity) are frequently reported in animals subject to environmental stressors [3, 14, 18]. It is

therefore somewhat surprising to find that rearing in shelter, which more closely approximates natural circumstances than does rearing in open cages, produced phenotypes normally associated with maintenance under stressful conditions. The present data suggest that hyper-emotionality, adrenal hypertrophy and gonadal hypotrophy may be produced by non-stressful environments, though the mechanism underlying such effects remains obscure.

The results of the present experiment further indicate that no single feature of a rearing environment providing access to a tunnel-like shelter suffices to produce the full range of phenotypic characteristics typical of wild gerbils. Rather, rearing with access to a darkened shelter provides a complex of experiences which differentially affect the behavior, development, and morphology of shelter residents. Movement from an open-area to an enclosed one enhances behavioral reactivity and relative adrenal gland size, reduces body weight and leaves rates of development, reproductive organ and pituitary gland weights unaffected. The reduced exposure to light experienced by gerbils reared in tunnel-like shelters fails to affect behavioral reactivity but has profound effects on rates of development and a range of morphological features.

Our data indicate that expression of the phenotype typical of domesticated gerbils requires deprivation in captivity of a variety of types of stimulation normally experienced by burrow-dwelling gerbils during ontogeny. Evidence of effects of artificial selection in captivity on the phenotype of domesticated gerbils is not yet available.

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