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Cholinergic Mechanisms in Scent Marking Behavior by Mongolian Gerbils *(Meriones unguiculatus)*

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YOSHIMURA, H. *Cholinergic mechanisms in scent marking behavior by Mongolian gerbils* (Meriones unguiculatus). PHARMAC. BIOCHEM. BEHAV. 13(4) 519–523, 1980.—The Mongolian gerbil reared in Japan manifested scent marking behavior in the similar behavioral topography as described by foreign researchers. The frequency of marking was affected by early isolation housing; isolated male gerbils exhibited a higher frequency of marking than aggregated males. The marking behavior was suppresed by scopolamine (1.5 mg/kg, IP), whereas methylscopolamine was ineffective. In order to elucidate the possible neural mechanisms, acetylcholinesterase (ACh-E) and choline acetyltransferase (Ch-Ac) activities were measured in 8 discrete areas of the brain (the cortex, striatum, amygdala, hypothalamus, midbrain, hippocampus, olfactory bulbs, and pons plus medulla oblongata). There was, however, no signficant difference between isolated and aggregated gerbils in either ACh-E or Ch-Ac activity in any of the brain areas. These results suggest that other neural events, such as changes in the ACh turnover rate or in ACh release, may participate in the manifestation of scent marking behavior.

Scent marking behavior   Mongolian gerbils   Acetylcholinesterase   Choline acetyltransferase

THE Mongolian gerbil *(Meriones unguiculatus)* has recently been introduced into physiological and behavioral research [23,25]. The most evident behavioral characteristic of the gerbil is the manifestation of scent marking behavior: the gerbil marks low-lying objects in the environment by rubbing them with a midventral sebaceous scent gland. In general, scent marking in mammals has been considered an important form of social communication associated with territory, dominance, and aggression [18,22]. As extensively studied by Thiessen *et al.* [25,30], the scent marking behavior in gerbils is classified as a species-common response closely related to territoriality. However, the Mongolian gerbil used in the United States originates from a few mating pairs which were sent from Japan in 1954 [23] so that the animals are closely inbred. The question which arises is whether or not there exists a generality in the behavioral characteristics between gerbils reared in Japan and in the United States. Unfortunately, little work on scent marking behavior in gerbils has been done in Japan.

Since the experimental procedure for quantitative measurement of the behavior was developed [25,26], considerable efforts have been made to elucidate the regulating mechanisms of scent marking. There are at least two major factors in the manifestation and maintenance of marking response in gerbils— androgen from the testes and olfactory input. Castration in males leads to a striking reduction in marking frequency and gland size, whereas both are restored by replacement therapy with either testosterone propionate peripherally [4, 23, 25] or testosterone implanted into the preoptic area of the hypothalamus [24]. On the other hand, olfactory bulbectomy in gerbils completely eliminates the scent marking behavior [2,27]. Thus, it is conceivable that marking behavior is controlled by central mechanisms.

Recent research has indicated that both marking and agonistic behaviors in gerbils are affected by social factors [15, 24, 32], such as individual housing or high population density. In mice [31] and rats [13,14], isolation-induced physiological and behavioral changes have been defined generically as an "isolation syndrome," based on the view that individual housing constitutes a "stressful situation" in terms of social deprivation. However, Brain [6,7] disputed whether isolated housing represents "stress." He assumed that an isolated mouse shares many characteristics of a territorial dominant mouse. This is supported by the evidence that a decrease in population density increases territoriality and dominance [8], and that the adrenocortical activity, which are considered an index of stress, does not correlate with the isolation-induced behavioral change [1,5]. Interestingly, it has been reported that plasma corticosterone levels and adrenal gland weight does not correlate to either emotionality or agonistic behavior in gerbils following prolonged isolation [24]. On the other hand, increasing evidence indicates that the central cholinergic system may play an important role in the manifestation of isolation-induced behavioral changes in mice [10,17] and rats [21,34]. There is, however, little information concerning the neuro-regulating mechanisms of scent marking behavior in gerbils following isolation housing.

This study was conducted to investigate whether or not the brain cholinergic system participates in the manifestation of scent marking behavior following early isolation housing, and to confirm whether the Mongolian gerbil reared in Japan manifests scent marking response in the same behavioral topography as those of gerbils in the United States.

METHOD

Animals

The Mongolian gerbils *(Meriones unguiculatus)* used in the experiment were born and raised in our laboratory. Originally, our laboratory stock was obtained from the gerbil colony of the Institute of Medical Science at the University of Tokyo. All animals had free access to food and water. Their cage floors were covered with wood shavings. All animals were handled once per week for cage cleaning and replenishment of food and water. The temperature was maintained at 23±1°C, and the light cycle was automatically controlled (light on at 7:00 a.m., off at 7:00 p.m.).

Apparatus

The open-field apparatus was constructed of gray Plexiglas (the size of the floor was 60×60 cm, and enclosed by walls 46 cm high), except for a clear side panel which allowed observation from a lateral view. The floor was removable for ease of cleaning. Six pegs, made from clear Plexiglas (2.5 cm long, 1.2 cm wide, and 0.6 cm high), were attached to the floor at regular intervals. The open-field apparatus was illuminated by an incandescent bulb (25 W) mounted 60 cm above the floor, and the intensity of illumination was controlled at approximately 250 lux on the center of the field. There was no other illumination in the test room.

Behavioral Studies

Gerbils were reared from weaning to adulthood in two different housing conditions—an isolated and aggregated group. All the animals were weaned between 23 and 25 days after birth. Each male gerbil in the isolated group (n=8, from 4 different litters) was housed in a 30×19×13 cm metal cage with a wire mesh top. The aggregated animals (8 males and 12 females, from 4 different litters) were housed in a 42×25×15 cm polycarbonate cage with a wire mesh top, one litter per cage (4 to 6 animals/cage). After 30 days in these experimental conditions, behavioral testing was begun only on the males. In this experiment, females were not tested because marking behavior is rarely observed in females [33], and also in order to eliminate the problem of endocrine change during estrous cycles. Each male was placed in the open-field apparatus and observed for 10 min using a video monitor system, and, at a later time, the frequency of marking and of rearing were scored. The tests were carried out from 9:00 a.m. to 11:00 a.m. Marking behavior was assessed by counting the number of ventral rubs to the pegs; this response was characterized by a slight spreading of the hind legs, coupled with a lowering of the abdomen over the pegs. The floor and pegs were cleaned after each trial with a dilute alcohol solution. The behavioral testing was repeated at 60 and 120 days after weaning. At the end of the behavioral observation all animals were sacrificed by decapitation and the brain cholinergic enzyme activities were determined.

Pharmacological Studies

Eight male gerbils were housed individually from weaning to adulthood. Apparatus and behavioral testing were identical to those described above. At approximately 120 days of age, each gerbil was tested for marking behavior without injection. Then the following treatments were conducted on four consecutive days: saline, scopolamine, saline, and methylscopolamine. As methylscopolamine rarely crosses the blood-brain barrier, this drug was used in order to distinguish scopolamine's peripheral action from its central and peripheral anticholinergic action. Scopolamine hydrobromide (Merck) and methylscopolamine nitrate (Merck) were dissolved in 0.9% saline and injected intraperitoneally at a dose of 1.5 mg/kg (1 ml per 100 g of body weight). The injection-test interval was 30 min for both drug and vehicle.

Biochemical Studies

The brain was quickly removed and 8 regions—cortex, amygdala, striatum, hypothalamus, midbrain, hippocampus, olfactory bulbs, and pons plus medulla oblongata—were separated on an ice-cold glass plate according to the general protocol in rats described by Glowinski and Iversen [12]. For the enzyme assays, tissues were homogenized in 5 mM Tris buffer containing 0.2% Triton X-100. Acetylcholinesterase activities were determined at 37°C [20] by the spectrophotometric method of Ellman *et al.* [9] using 10^{-5} M of iso-OMPA as an inhibitor of cholinesterase. Choline acetyltransferase activities were determined by the radiochemical micromethod of Fonnum [11] using (^{3}H)-acetyl Coenzyme A as a substrate. Protein content was measured according to the method of Lowry *et al.* [9].

Statistical Analysis

The statistical evaluation of behavioral data was performed by means of analysis of variance (unweighted-means solution), and differences between the groups were evaluated by means of the two-tailed t-test. Difference in biochemical data between the groups were evaluated by means of the two-tailed t-test.

RESULTS

The incidence of marking behavior increased gradually with length of the isolation period; after 120 days of isolation all isolated gerbils exhibited marking behavior. When the isolated male gerbil was placed in the open-field apparatus, the gerbil exhibited high exploratory behavior and rapidly approached one of 6 pegs on the floor. After sniffing of the peg for a few brief moments, the gerbil lowered his abdomen with a slight spreading of the hind legs and then rubbed the ventral sebaceous gland over the peg. The marking response to the pegs was performed repeatedly. Usually the gerbil deposited his sebum on all of the pegs during 10 min test period. The aggregated male gerbil manifested the marking behavior in the same behavioral pattern as the isolated gerbil, but the frequency of marking was less. As shown in Table 1, there was no significant difference between the aggregated and the isolated gerbils in the proportion of animals showing marking. Analysis of variance revealed a significant difference in the frequency of marking between the aggregated and isolated groups, $F(1,11)=13.44$, $p<0.01$, while the performance did not change significantly across testing days, $F(2,22)=1.21$. By contrast, no significant difference in the frequency of rearing was found between the groups, $F(1,11)=0.01$; whereas the subject's performance changed significantly across testing days, $F(2,22)=6.83$, $p<0.01$. On the other hand, the body weight of gerbils had a tendency to increase during the experimental period, $F(2,22)=35.98$, $p<0.01$, and there was a significant differ-

TABLE 1

EFFECT OF EARLY ISOLATION ON THE OPEN-FIELD BEHAVIOR IN THE MALE MONGOLIAN GERBIL

Days after weaning	Aggregated group (n=7)	Isolated group (n=6)	Significance*
30 days			
incidence of marking	86%	67%	
frequency of marking	5.1 ± 3.5	10.7 ± 5.2	n.s.
frequency of rearing	84.6 ± 9.2	97.7 ± 6.9	n.s.
body weight (g)	61.0 ± 1.6	60.0 ± 2.1	n.s.
60 days			
incidence of marking	86%	83%	
frequency of marking	4.3 ± 1.1	15.6 ± 4.0	$p<0.05$
frequency of rearing	92.0 ± 8.7	86.6 ± 8.7	n.s.
body weight (g)	67.3 ± 2.9	75.3 ± 2.0	$p<0.05$
120 days			
incidence of marking	86%	100%	
frequency of marking	3.4 ± 1.0	28.5 ± 2.3	$p<0.01$
frequency of rearing	65.9 ± 5.6	65.5 ± 4.7	n.s.
body weight (g)	74.0 ± 1.0	84.5 ± 3.2	$p<0.01$

Each value is shown as mean ± SE. The open-field behavior was tested for 10 min, and the tests were carried out from 9:00 a.m. to 11:00 a.m. *Significance was evaluated by two-tailed t-test comparing isolated group with aggregated group.

ence between the aggregated and isolated groups, $F(1,11)=7.12, p<0.05$.

The effects of anticholinergic drugs on the marking behavior are shown in Table 2. Intraperitoneal injection of scopolamine (1.5 mg/kg) significantly suppressed the marking behavior as compared with either saline ($t=3.15, p<0.02$) or non-treatment ($t=5.66, p<0.01$). The frequency of marking recovered completely 24 hr after the injection of the drug. Methylscopolamine at the same dose did not suppress marking behavior. The evidence suggests that the suppressive effect of scopolamine is due to its central anticholinergic property, because methylscopolamine does not cross the blood-brain barrier easily.

The results of the studies on the activity of acetylcholinesterase and choline acetyltransferase in 8 discrete brain areas are shown in Table 3. Although the central anticholinergic drug suppressed marking behavior, no difference in either synthetic or catabolic enzyme activities was found between the isolated and aggregated animals in any of the brain areas determined in this study.

In the present experiments, 3 isolated males and one aggregated male were discarded from the data because they manifested a convulsive seizure during the first testing in the open-field apparatus. Contrary to the observation in early isolated gerbils by Berg *et al.* [3], there was no significant difference in the incidence of seizure between isolated and aggregated animals.

DISCUSSION

The present study indicates that Mongolian gerbils available in Japan possess a similar behavioral topography of ventral scent gland marking to those described previously [2,25]. This tends to support the view that scent marking behavior is a species-common response in *Meriones unguiculatus* [25,30]. It has been reported that both isolation and social competition facilitate scent marking behavior [16,29]; whereas high-population density decreases the behavior [15]. The present data are consistent with previous reports showing that social isolation of gerbils from weaning to adulthood results in a higher marking frequency. Interestingly, isolated males shows a striking post-pubertive increase of marking frequency (about 183%), while aggregated

TABLE 2

EFFECTS OF ANTICHOLINERGIC DRUGS ON MARKING BEHAVIOR IN THE MALE MONGOLIAN GERBIL

Treatments	Frequency of Marking	Significance*
None	22.4 ± 5.7	
Saline	21.0 ± 3.9	n.s.
Scopolamine (1.5 mg/kg, IP)	8.0 ± 4.8	$p<0.01$
Saline	21.4 ± 3.7	n.s.
Methylscopolamine (1.5 mg/kg, IP)	22.9 ± 4.7	n.s.

Each value is shown as mean ± SE (n=7).
*Comparison between treatment and non-treatment (two-tailed paired t-test).

TABLE 3

BRAIN ACETYLCHOLINESTERASE AND CHOLINE ACETYLTRANSFERASE ACTIVITIES IN THE MONGOLIAN GERBIL AFTER EARLY ISOLATION

Brain Areas	Acetylcholinesterase activity*		Choline acetyltransferase activity†	
	Aggregated group (n=7)	Isolated group (n=6)	Aggregated group (n=7)	Isolated group (n=6)
Cortex	40.72 ± 0.60	41.68 ± 1.13	40.83 ± 1.54	41.46 ± 2.08
Striatum	560.69 ± 24.78	516.15 ± 24.24	224.10 ± 14.16	214.28 ± 13.16
Amygdala	104.09 ± 4.39	109.25 ± 5.56	107.37 ± 4.83	113.85 ± 5.10
Hypothalamus	125.99 ± 1.95	122.48 ± 3.32	62.87 ± 2.25	64.60 ± 1.75
Midbrain	151.56 ± 4.69	149.82 ± 5.28	105.52 ± 2.39	111.66 ± 3.39
Hippocampus	61.87 ± 0.94	64.44 ± 1.33	40.33 ± 1.66	44.72 ± 2.33
Olfactory bulbs	43.14 ± 1.37	41.48 ± 0.72	24.76 ± 1.05	25.93 ± 1.45
Pons + Medulla Oblongata	128.33 ± 6.20	127.09 ± 7.34	138.14 ± 7.15	142.38 ± 9.65

Each value is shown as mean ± SE.

*nmoles acetylthiocholine hydrolyzed/min/mg protein; †nmoles acetylcholine synthesized/hr/mg protein.

males which were housed with littermate females do not show any such change.

Spencer *et al.* [24] found that plasma corticosterone levels and adrenal gland weight, which were considered an index of stress, did not correlate with the behavioral change in gerbils following prolonged isolation. The mechanisms in the brain associated with marking behavior, however, are uncertain. Present data indicate that only a centrally active anticholinergic drug suppresses the marking. These results suggest that activation of the brain ACh receptor is necessary to manifest the marking behavior. Biochemical analysis, however, demonstrated no significant difference between aggregated and isolated gerbils in either acetylcholinesterase or choline acetyltransferase activities in any brain area. Essman has found that isolated mice showed a significant elevation in free cortical ACh and a significant reduction in cortical bound ACh as compared with aggregated mice [10]. I have also previously found that rats which manifested the mouse-killing behavior following prolonged isolation showed higher ACh content in the diencephalon without any change of brain ACh-E activity, as compared with aggregated animals [34]. In these species, interestingly, scopolamine suppressed the isolation-induced aggressive behavior [17,34]. Therefore, it would appear that the determination of the brain neurotransmitter level, including turnover rate or release of acetylcholine, assists in an understanding of isolation-induced behavioral changes in rodents. Whether the increased marking frequency following isolation housing in gerbils was due to the change in ACh availability or to other neural mechanisms, remains a question for future study.

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