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Spontaneous behavior of the gray short-tailed opossum (*Monodelphis domestica*) in the elevated plus-maze: comparison with Long-Evans rats

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Abstract. We observed the spontaneous behavior of a laboratory marsupial - the gray short-tailed opossum (*Monodelphis domestica*) - in the elevated plus-maze (EPM) during six consecutive sessions and compared it with the behavior of Long-Evans rats. During the first exposure to the maze both species spent most of the time in the enclosed arms but opossums showed much higher frequency of entries into the open arms and stayed there longer. On the third and subsequent days opossums reduced their entries into the open arms and spent more time on the central square, where unlike rats they frequently groomed their lower belly and hind legs. During the last sessions they started spending more time in the enclosed arms. It is concluded that probably opossums, like rats show a stable anxiety evoked by open space. However, in the rat anxiety prevails over motivation to explore a new environment, while in the opossum it is initially at equilibrium with curiosity which habituates slower than in the rat. Results are discussed in the context of different ecology of the gray opossum that actively searches and hunts quickly moving insects. Thigmotaxic behavior, while strong in both species, dominates spontaneous behavior of the rat, but not opossum.

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INTRODUCTION

The gray short-tailed opossum (*Monodelphis domestica*, herein referred to as the gray opossum – Fig.1) is a marsupial mammal native to the tropical part of South America. This species was introduced as a laboratory animal only in the last twenty years (Kraus and Fadem 1987). All laboratory bred gray opossums are descendants of a few animals captured in 1978 and a few years later. Adult males weigh 90-110 g and females 65-100 g. Gray opossum easily breeds in the laboratory conditions. Females do not have a pouch (marsupium). Pups are born after about 14-days gestation at a very early stage of development, which was the main reason of introducing this species as a model of developmental research. After birth pups attach themselves to mother's teats for about 28 days and are further nursed and protected until weaning at about 50 days after birth. Gray opossums reach sexual maturity at approximately 18-20 weeks of age and live for about three years. This species has been an object of developmental (Molnar et al. 1998, Huffman et al. 1999), anatomical (Turlejski et al. 1997, Molnar et al. 1998, Djavadian et al.1999), physiological (Poran et al. 1993 a,b), sexual (Fadem 2000) and behavioral studies (Kimble and Whishaw 1994, Fadem et al.1997).

In the wild, gray opossums are solitary animals living in stone crevices, holes in wood or human dwellings that are most active during first hours of the evening. They are omnivorous but their main sources of food are insects and small vertebrates that are actively searched for and hunted (Hunsaker and Shupe 1977, Kraus and Fadem 1987, Kimble 1997). Otherwise, little is known about behavior of the wild gray opossum. Laboratory investigations of the social behavior of males and females of different ages showed an increase of social aggression after puberty, when they start to defend vigorously their territories (Hunsaker and Shupe 1977, Fadem and Corbett 1997). Aggression is manifested by fights between males, while females show more threat behavior, including open-mouth postures and screeching vocalizations (Fadem and Corbett 1997). There is only one report on the formal testing of behavior of the gray opossum in a standard apparatus. Kimble and Whishaw (1994) tested opossums in the Morris water tank and eight-arm radial maze and found that they are not able to find the hidden platform in the tank, but instead constantly circle along its walls. Although they learned to locate the visible platform, their performance in this task was much poorer than that of the rats. Strong thigmotaxic preference (tendency to move along the walls) was proposed as an explanation of this behavior of the gray opossum. Their performance in the radial maze without walls was much better, but they were using different search strategies than rats and making more errors. Recently this species is used in an increasing number of laboratory investigations that demand knowledge of its behavior. Therefore, we decided to describe its behavior in a further set of standard laboratory tests, starting from the elevated plus-maze test (EPM), which is an important tool in pharmacological research (Pellow et al. 1985, Pellow and File 1986, File 1993, Treit et al. 1993).

Fig. 1. Gray short-tailed opossum in its living cage.

The EPM test was first introduced by Montgomery (1955) as a tool of testing the interplay of two opposing drives: anxiety elicited by staying in the open space, and the drive to explore a new environment (or curiosity - Berlyne 1966). Some authors postulate involvement of two more factors: the general level of locomotor activity of the animal (Espejo 1997a, Ramos et al. 1997) and decision making or risk evaluation (Rodgers and Johnson 1995, Weiss et al. 1998). The EPM maze is built of two open arms and two arms enclosed by walls that are connected by a central square. The whole apparatus is placed at some height above the floor. Spontaneously behaving rats and mice avoid entering its open arms and prefer to stay in the enclosed arms, as they allow for thigmotaxic behavior (moving close to the walls), which is a natural habit of rodents (Barnett 1963). The preference for the enclosed arms is not reduced by previous experience in the absence of evident danger (e.g. after previously confining rats for some time to the open arms - Bertoglio and Carobrez 2000). During the second and subsequent exposures to the EPM preference for the enclosed arms was still increasing (rats: Treit et al. 1993, Gonzalez and File 1997, Bertoglio and Carobrez 2000; mice: Espejo 1997b). Therefore, while curiosity and motivation to explore the apparatus decreases with consecutive exposures, the anxiety evoked by entering the open arms and staying in them was stable. For this reason EPM is in wide use as a test of the level of anxiety in rodents and of the effects of anxiolytic or anxiogenic drugs (Handley and Mithani 1984, Pellow et al. 1985, File 1993, Treit et al. 1993, Gonzalez and File 1997). The most frequently used (negative) measures of anxiety are the percentage of entries into the open arms and percentage of time spent in these arms. EPM was also used to study the factors of age and sex in the behavior of rats (Silva et al. 1996).

Rodents manifest their balance of curiosity and anxiety in the EPM test not only by the pattern of entries to various parts of the maze, but also by changes of the whole repertoire of behavior, like sniffing the walls and floor, rearing and grooming (Anseloni and Brandao 1997, Espejo 1997a). In mice the frequency and duration of grooming decreases in subsequent trials, whereas locomotor activity, sniffing and rearing remain at the same level (Espejo 1997b). It is not yet known whether the pattern of spontaneous behavior motivated by an interplay of anxiety and curiosity that is observed in the elevated plus-maze is characteristic only for rodents or is similar in other mammals as well. Therefore, in the present preliminary investigations we decided to compare the pattern of behavior of the gray opossums and Long-Evans rats using the same EPM apparatus and experimental design.

METHODS

Subjects

All animals were experimentally naive at the beginning of the study. Seven gray opossums (four males and three females) were taken from the breeding colony of the Nencki Institute of Experimental Biology. Animals were 9 months old at the beginning of experiment. Males weighed 100-110 g and females 65-75 g. They were kept in an animal room with controlled environment: day/night cycle 14:10 (lights on 8.00 a.m.), temperature 25(± 2)°C and humidity (50-60%) and housed individually in standard plastic rats' cages (46 x 23 x 19 cm) supplied with a small house-box inside. Animals had free access to food and water. They lived on a diet of cat food in dry pellets and mashed meat supplemented with fruits and vitamins.

Eight rats of the Long-Evans strain, five months old at the beginning of experiment, were bred in the Institute of Oncology, Warsaw. Four males weighing 280-310 g and four females weighing 220-280 g, were housed four per cage (60 x 45 x 19 cm) with free access to water and standard rat chow. Development of the gray opossums is much slower than that of the rats and they do not reach sexual maturity before they are 6-7 months old, and therefore the relative (physiological) age of our rats and opossums was similar.

All experimental procedures were conducted with the approval of the Ethics Committee of the Nencki Institute of Experimental Biology and were in accordance with The NIH Guide for the Care and Use of Laboratory Animals.

Apparatus

The EPM apparatus was made of plywood painted white. All four arms were 50 x 10 cm. The two open arms were opposite to each other and perpendicular to the closed arms that were flanked by 40 cm high plywood walls. The four arms were attached to a 10 x 10 cm square platform forming the hub of the plus-shape. The apparatus was elevated 70 cm above the floor of the sound-isolated cabin (2.5 m x 2.7 x 2.6 m) and lit by a dim light (<50 lux) emitted from a light source placed directly above the maze. A TV camera mounted vertically over the maze recorded the behavior of animals for a later analysis. The time counter of the video recorder was used as a timer with which we measured duration of various elements of behavior.

Procedure

All animals were observed during six consecutive sessions in the EPM. Sessions started at 10 a.m. with the opossums followed by rats. All animals were carried to the experimental room in their home cages. They were friendly and could be handled without gloves. At the beginning of each session they were placed on the central square of the maze facing one of the open arms and then left for 5 min to freely explore the apparatus, while the camera registered their activity. The maze was cleaned with ethyl alcohol after each animal to weaken chemosensory cues for the exploratory behavior. After the first session there was a 48 h delay, and then the next five sessions proceeded daily.

Measures and their analysis

During analysis of the videotaped records of the experimental sessions classical parameters of the EPM test were calculated. These were the total time (s) spent in the 1.open arms, 2.enclosed arms, 3.central square and the number of entries into the 1.open arms, 2.enclosed arms and 3.central square. An entry was counted when an animal placed all four paws in an arm or square. Duration of other types of behavior and the number of such episodes were also recorded. Registered were: rearing – taking the upright posture with forelegs moving in the air or placed against the wall of the enclosure; grooming – licking and scratching of the head or forelegs and long-lasting cleaning of fur of the lower belly and/or hind limbs which was specific for opossums. Defecation scores were noted after every session. Both species were sniffing in the apparatus but accurate evaluation of this activity was not possible in our setup.

Statistical comparisons were carried out using two way analysis of variance (ANOVA; Groups x Sessions, with repeated measures on sessions). The independent variables of groups were sex (male vs. female) or species (opossums vs. rats). Dependent variables were: the percentage of entries into the open arms relative to the total of entries into any arm ((open/total) x 100) in the six sessions; percentage of time (in seconds) spent in the open arms relative to the total time spent in the maze ((open/300) x 100); and percentage of time spent in the central square relative to total time spent in the maze ((centre/300) x 100). All post-hoc analyses were done with the Tukey test for unequal N or HSD test. Statistical analysis was performed with STATISTICA 5. Student *t*-test for independent values was used where appropriate.

RESULTS

Sex factor

During the first session male and female opossums entered the open arms with a similar frequency (45.25% ± 2.69% and 40.96% ± 0.96% respectively, Fig. 2). This frequency was changing in the following sessions. Two-way ANOVA (sex x sessions, 2 x 6) with repeated measures on the second factor applied to analyze percentage of entries into the open arms, showed no significant main effect of sex ($F_{1,5}$ = 1.77; $P>0.05$), but a significant main effect of sessions ($F_{5,25}$ = 7.80; $P<0.001$) and no significant interaction ($F_{5,25}$ = 2.59; $P>0.05$). All opossums entered the open arms more frequently during the first and second session than in the subsequent sessions (Tukey test; $P<0.05$). Similar analysis for rats showed no significant main effect of sex ($F_{1,5}$ = 0.94; $P>0.05$) or session ($F_{5,30}$ = 2.33; $P>0.05$) and no significant interaction ($F_{5,30}$ = 0.48; $P>0.05$).

Time spent in the open arms by all four groups of animals was consistent with the number of entries into the arms. Two-way ANOVA on the percentage of time spent in the open arms for male and female opossums and for sessions (2 x 6, with repeated measures on sessions) showed no significant effect of sex ($F_{1,5}$ = 0.40; $P>0.05$) but a significant effect of sessions ($F_{5,25}$ = 10.50; $P<0.001$). Interaction of those factors was not significant ($F_{5,25}$ = 2.03; $P>0.05$). Time spent in the open arms during the second and all subsequent sessions was significantly shorter than during the first session (Tukey test, $P<0.05$). ANOVA of analogous rat results showed no significant main effect.

Percentage of time spent on the central square was similar in males and females of both species. ANOVA for male and female opossums or rats and sessions (2 x 6,

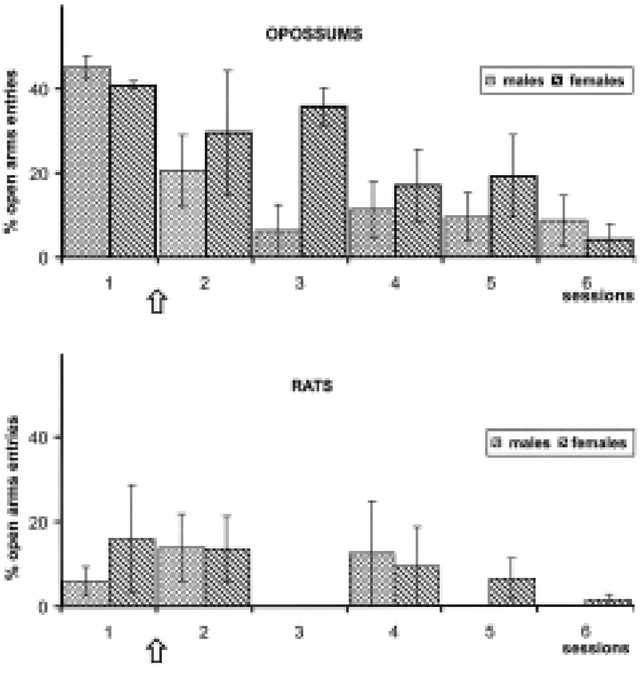

Fig. 2. Mean (± SEM) percentage of entries into the open arms of the elevated plus-maze of the male and female opossums (top) and rats (bottom) in consecutive sessions. Arrow indicates the 48 hours interruption between the first and second session.

repeated measures on the last factor) showed no significant sex differences in either opossums ($F_{1,5} = 0.018$; $P>0.05$) or rats ($F_{1,5} = 3.0$; $P>0.05$). The effect of sessions was also not significant.

Species factor

Figure 3A shows the percentage of entries into the open arms during successive sessions by the opossums and rats. Results for males and females were joined, as the effect of sex was not significant. Two-way ANOVA (species x sessions, 2 x 6, repeated measures on the last factor) for the percentage of entries showed that opossums entered the open arms more frequently than rats ($F_{1,13} = 5.91$; $P<0.05$). The main effect of sessions ($F_{5,65} = 8.40$; $P<0.0001$) and the interaction term ($F_{5,6}5 = 3.02$; $P<0.01$) were also significant. Post hoc tests showed that during the second session (after two-days interruption) opossums entered the open arms with the same frequency as during the first session ($P<0.05$). During further sessions this frequency decreased in opossums, whereas it remained stable in rats, and therefore in the fourth session the percentages of open arms entries in the two species were almost equal (post hoc test $P>0.05$).

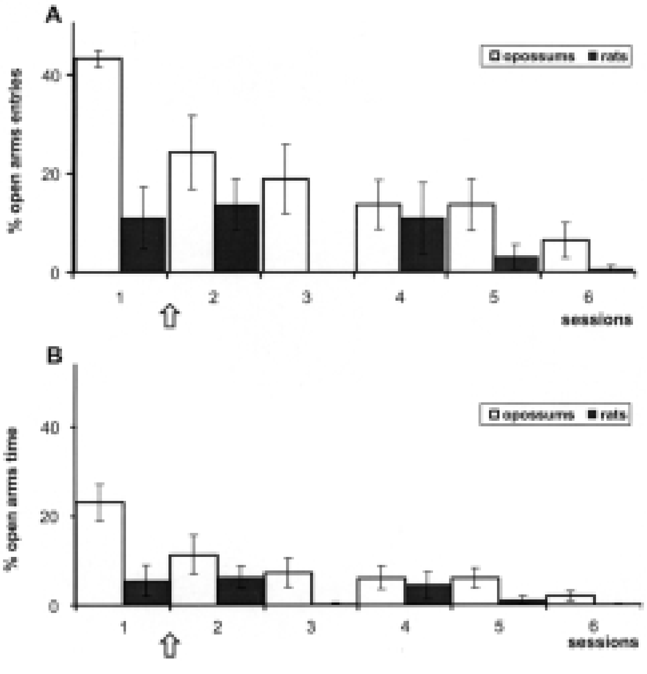

Fig. 3. Mean (± SEM) percentage of entries into the open arms of the elevated plus-maze (A) and of the time spent there (B) by the gray opossums and rats during consecutive sessions. Arrow indicates the 48 h interruption between the first and second session.

Opossums spent more time in the open arms than rats (Fig. 3B). Two-way ANOVA (species x sessions, 2 x 6, repeated measures on the last factor) showed a significant main effect of species on the percentage of time spent in the open arms ($F_{1,13} = 6.0$; $P<0.01$) and significant main effect of sessions ($F_{5,65} = 9.74$; $P<0.001$) as well as significant interaction ($F_{5,65} = 3.86$; $P<0.001$). Post hoc tests revealed that time spent in the open arms of EPM during both first and second sessions was significantly different from that in the following sessions ($P<0.05$). During the first session opossums spent more time in the open arms than during the subsequent sessions and than rats did during all sessions (post hoc tests $P<0.05$).

ANOVA for species and sessions (2 x 6, repeated measures on the sessions) showed that percentage of transitions through the central square was not different in the opossums and rats ($F_{1,13} = 0.38$; $P>0.05$). Their locomotor activity decreased on subsequent days ($F_{5,65} = 3.10$; $P<0.01$). Although both species were passing across the central square with the same frequency, opossums spent more time there than rats (Fig. 4). The ANOVA (species x sessions, 2 x 6, repeated measures on the last factor) showed significant main effect of species ($F_{1,13} = 10.89$; $P<0.01$) but not of sessions ($F_{5,65} = 0.98$; $P>0.05$) and no significant interaction ($F_{1,13} = 0.38$; $P>0.05$).

Locomotor activity of opossums and rats, as measured with the percentage of entries into the enclosed arms was not significantly different when analysed for species ($F_{1,13} = 0.28$; $P>0.6$) or for sessions ($F_{1,13} = 1.15$; $P>0.05$), with no significant interaction ($F_{5,65} = 0.72$;

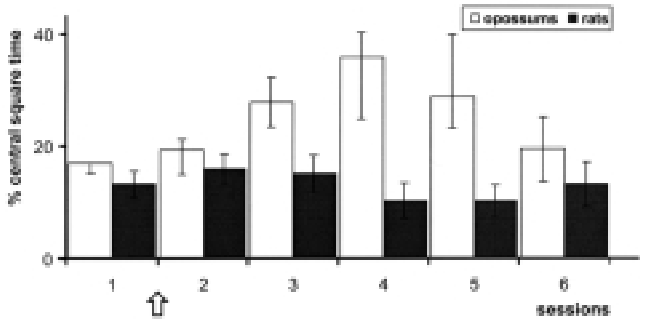

Fig. 4. Mean (± SEM) percentage of time spent on the central square of the elevated plus-maze by the gray opossums and rats during consecutive sessions. Arrow indicates the 48 h interruption between the first and second session.

$P>0.05$). However, the time spent in the enclosed arms was significantly shorter in the opossums than in the rats. Two-way ANOVA (species and sessions, 2 x 6, repeated measures on the last factor) showed a significant main effect of species ($F_{1,13} = 10.34$; $P<0.01$), but not of sessions ($F_{5,65} = 1.5$; $P>0.189$) and no significant interaction ($F_{5,65} = 1.3$; $P>0.05$).

Other measures of behavior

The mean number of grooming episodes in the opossums and rats (0.86 ± 0.13 and 1.04 ± 0.16 respectively) and their duration in seconds (12.7 ± 2.48 and 14.0 ± 1.42) remained similar on the subsequent days, which was shown by the *t*-test ($t_{10} = 0.88$, NS; $t_{10} = 0.42$, NS). Opossums were grooming in both enclosed arms and the central square, while rats did it in the enclosed arms (with the exception of one rat that was grooming also in the center). In the opossums grooming of the head and forelegs was often short and followed by prolonged cleaning of fur of the lower abdomen and hind limbs, while rats never displayed such behavior.

Unlike the number of groomings, number of rearings changed in successive sessions. ANOVA for species and sessions (2 x 6, repeated measures on the last factor) showed a significant effect of sessions ($F_{5,65} = 4.85$; $P<0.001$), with no significant main effect of species ($F_{1,13} = 0.32$; $P>0.05$) and no significant interaction ($F_{5,65} = 0.81$; $P>0.05$). Post hoc tests showed that the number of rearings was higher during the first session than in the following ones ($P<0.05$).

Defecation scores were higher in rats than in opossums. Two-way ANOVA for species and sessions (2 x 6, with repeated measures on last factor) showed a significant main effect of species ($F_{1,13} = 6.46$; $P<0.02$). The main effect of sessions ($F_{5,65} = 1.76$; $P>0.05$), as well as the interaction term ($F_{5,65} = 0.81$; $P>0.05$) were not significant.

DISCUSSION

Rats investigated in our elevated plus-maze apparatus showed typical anxiety-driven behavior (Montgomery 1955, Pellow et al. 1985, Pellow and File 1986). The traditionally examined indicator of anxiety, the percentage of entries into the open arms, showed that their anxiety did not decrease with experience. At the same time the number of entries into the enclosed arms, which is a measure of general activity in the EPM (Espejo 1997a), did not change over sessions. The same was true for the percentage of time spent in the enclosed arms. Therefore, the main results obtained in our research in the elevated plus-maze are similar to the results of other authors. One difference that we noted is that the two days of interruption after the first session had no effect on the percentage of open arms entries in the second session, contrary to the results reported by Bertoglio and Carobrez (2000). However, a relatively low number of rats in our experiment could have been a factor decreasing chances of proving more subtle changes. Frequency of rearings steadily decreased in the rats, which could have been an indication of a decreasing exploratory activity in the elevated plus-maze (Espejo 1997b). Number and duration of grooming episodes, as well as defecation scores were stable throughout the study. Overall, behavior of our rats may be interpreted as driven by stable anxiety, prevailing over much weaker and decreasing curiosity.

Results of various experiments showed that the main source of anxiety of rats or mice in the elevated plus-maze is the open space (Treit et al. 1993, Fernandes and File 1996), as neither the height of elevation of the EPM apparatus over the floor nor the presence of a low rim on the open arms had an effect on the anxiety of rats. Accordingly, in our experiments rats avoided not only open arms but also the central square. Closeness of walls reduces anxiety, and therefore rats and mice tend to stay by the wall or spontaneously move near it in the open-field test, especially during the first exposure or when the open space is well-lit and/or without obvious cues (Nasell et al. 1998, Devan et al. 1999). This tendency was named "thigmotaxis" (Barnett 1963). In consecutive sessions the level of anxiety decreased and animals gradually started visiting the central part of the field (Archer 1973) which indicates that novelty of the place may elicit both fear and curiosity. In the water maze thigmotaxis decreased when animals learned to locate the platform (Whishaw and Jarrard 1995). In general, behavior of rats in various experimental setups was consistent with their natural tendency to thigmotaxic behavior and with the preference for staying in the enclosed space.

Kimble and Whishaw (1994) showed that gray opossums do not form spatial memory in the Morris water maze in the place navigation task and their learning of the cued navigation task is much poorer than that of the rats. Gray opossums were constantly circling along the walls of the tank. In the eight–arm radial maze without walls they performed much better, but they used different strategies than rats. The poor performance of the gray opossums in the water tank was explained by their thigmotaxic tendency that in this species is presumably even stronger than in the rat, and does not allow opossums to leave the wall of the tank. Our informal observations of those opossums that escaped during handling in the animal room confirms their strong tendency to move along the walls and into narrow spaces in a situation perceived as dangerous or evoking strong fear (like an attempt to catch them). In spite of that, behavior of the gray opossums in the EPM differed markedly and in an unexpected way from that of the rat, as it was not based on the thigmotaxic tendency. Gray opossums made much more entries into the open arms than rats and stayed there longer. This difference was very distinct during both first and second session. On the subsequent sessions, percentage of time spent in the open arms gradually decreased but even during the last session it was higher than in the rats. This spontaneous behavior of the opossum puts in question the explanation of their behavior in the Morris water maze with their unusually strong thigmotaxic tendency. An alternative explanation may be, that staying in the Morris tank is very much aversive (or anxiogenic) for opossums, that are poor swimmers (Kimble and Whishaw 1994) so they concentrate on finding a way out of it rather than on finding a place of relative safety inside the tank.

Still another possible explanation is a different (or lower) cognitive capacity of the gray opossum, that does not allow it to memorize the position of platform or choose a strategy bringing only partial gain. The report of Kimble and Whishaw (1994) was based on the rats' competence to create spatial maps related to remote landmarks or proximal cues. Rats use visual, auditory or olfactory cues for allothetic orientation, whereas the movement generated internal cues (somatosensory or vestibular) for idiothetic orientation, when visible etc. cues are not available or cannot be used for spatial orientation (Bures et al. 1997, Fenton et al.1998). Hearing in the gray opossum is comparable to that of the rat (Frost and Masterton 1994) and the capacity of its visual system is not yet known, but most probably of the same range of acuity. Olfactory perception that is extensively used in the chemosensory exploratory behavior (Poran et al. 1993a,b) may be the most important. Absence of olfactory cues in the water maze could have been one of the reasons of opossums' failure of spatial learning at the level typical for rats (Kimble and Whishaw 1994).

Unlike rats, opossums switched their activity in the consecutive sessions from the open arms to the central square. During the fourth session they were spending as much time there as in the enclosed arms. This position is frequently associated with risk-evaluation or decision-making processes (Rodgers and Johnson 1995, Weiss 1998). It may be also an equivalent of the natural behavior of watching for prey at a place that in the same time allows for convenient observation of the environment and relative safety. At the central square opossums frequently groomed their fur, especially of the hind limbs. This behavior may indicate that they stayed in this place in spite of the anxiety that they experienced (Espejo 1997b). However, relation of various patterns of the opossums' behavior to the level of anxiety (or fear) may be clarified only in future behavioral and pharmacological investigations.

Comparisons of behavior of the wild and laboratory rats allows for rejection of hypotheses explaining differences between the behaviors of gray opossum and laboratory rat based on a lower degree of domestication of the former, as the wild rats avoid entering open space even more than the laboratory rats (for review see Boice 1973).

In conclusion, we found that avoidance of the open space in the elevated plus-maze is a less dominant behavioral feature of the gray opossum than of the laboratory rat. If thigmotaxis is as strong or stronger feature in the opossum than in the rat, then in the plus-maze test it is evidently counterbalanced by another, exploration-related or prey-searching-related motivation. Such motivation in the Morris tank does not exist, and then the thigmotaxic tendency of the gray opossum in response to fear-inducing situations may be fully revealed. The strong exploratory drive in the gray opossum is most probably a consequence of species-specific adaptations to active hunting of small, quickly escaping prey. This different balance of avoidance of endangering situations and exploration for food resources should be reflected in the differences of wiring of the brain of the gray opossum and rat, which remains to be investigated. Results of the elevated plus-maze test show that the gray opossum may be a suitable model for investigating the exploratory and hunting behavior in mammals, while situations evoking strong negative motivation may evoke mostly rigid escape responses.

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