The open field (OF) test is frequently used in the investigation of conflicting motivations, the anxiety driven freezing or thigmotaxy and curiosity driven spontaneous investigation of the unknown objects. This test has been devised for the study of rodents behavior (Henderson 1969). If rodents are left in an open, unknown space, the anxiety-driven behavior (thigmotaxy, avoiding the open space) dominates for a long time over curiosity or investigative drive (Geyer et al. 1986). Although they start to investigate new objects after a substantial delay, they start to feel less anxious in that space only after several exposures, but even then they investigate objects shortly and show thigmotaxy (Pisula et al. 2012). This tendency is much more prominent in the wild, than in the laboratory rats (Hughes and Boice 1973).

Species belonging to other mammalian orders, particularly opossums, have been also studied in the OF (Wesierska et al. 2003). However, there is a question if in species other than rodent the OF test evokes the same set of motivations as in rodents and what is the balance of anxiety and curiosity. The answer to these questions is partially negative, as the results of our earlier investigations of the laboratory opossum showed. What more, general level of activity is another factor, rarely taken into account in standard OF tests (Meyza et al. 2009).

During last 40 years the gray short-tailed opossum became a new laboratory animal (VandeBerg et al. 1997, Nicholls et al. 1999, Ley 2002, Samollow 2008, Grabiec et al. 2009). It belongs to the superorder of Marsupials that shared the last common ancestor with Placentals more than 130 millions years ago (Ji et al. 2002). Despite having some primitive features, e.g.:
Open field activity, opossums and rats

absence of placenta or corpus callosum in the central nervous system (CNS), marsupials have evolved to inhabit various ecological niches, paralleling adaptations of eutherian mammals.

The growing interest in the Monodelphis opossum as a laboratory species resulted in several studies of its behavior, including comparative studies using opossums and rodents. Kimble and Whishaw (1994), using the 8-arm radial maze showed that opossums learn poorly and have poor spatial memory. These results were not repeated and as concerning the water maze were not confirmed in our laboratory (unpublished results). It is possible that the differences depend mainly on different metabolic rate and different inborn strategy of food seeking. In our previous studies we compared behavior of the opossums and rats in the elevated plus-maze and in the open field, including their response to new objects (Wesierska and Turlejski 2000, Wesierska et al. 2003). Using free exploration paradigm we have also compared reactions to novelty in the opossums, laboratory (Wistar) rats and wild (WWCPS) rats (Pisula et al. 2012). We conclude, that opossums are very inquisitive and active, showing also lower level of anxiety in the new environment than rats and investigating new objects with a shorter delay and for a longer time.

In all these tests opossums expressed also a higher rate of locomotor activity and lower latency to exploration of the novel object than rats. At the start of the test or when facing new arrangement, opossums are anxious. However, they avoided the unknown open space for a shorter time, directed their activity towards the central area of the open field earlier than rats, explored the new environment longer and explored new objects after a shorter delay and for a longer time than rats. Therefore, unlike in rats, in the opossum curiosity-driven exploration prevailed over anxiety. This feature is probably a part of their specialized insect-seeking behavior requiring a higher level of activity and risk-taking than collection of the plant food. Therefore the first goal of the present experiments was to extend results of previous experiments using different version of the OF test.

Another problem we tried to address was the intraspecies heterogeneity and variability of responses. Researchers investigating behavior of animals have to cope with the individual variability of their responses. The extent of behavioral variability may depend on the investigated species, strain or line, masking the influence of experimental factors. This variability is partially genetically defined, but dependent also on epigenetic factors (Alter et al. 2008). In rats selection for different levels of activity or other behavioral traits resulted in development of specific lines, like Roman high and low reactive rats (for review see Steimer and Driscoll 2003). Individual variability of rats’ responses to novelty allowed for dividing rats into two different phenotypic groups, with high and low locomotor activity (Kabbaj et al. 2000). Interestingly, measurement of the locomotor response to novelty allowed for prediction of their response to other behavioural tasks. For example, rats exhibiting high locomotor response to a novel environment displayed less anxiety-like behavior in the elevated plus maze and explored the light environment more in the light-dark box than rats showing low locomotor response to novelty (Kabbaj et al. 2000). DeFries and coauthors (1978) selected mice for 30 generations on the basis of their behavior in the open field, which resulted in developing marked differences in the mean activity between the low-active

![Fig. 1. Locomotory activity of the opossums and rats. (A) Total distance traveled during the two-hour session by different groups of animals (mean ± SEM, in cm); (B) proportion of session time (in percents) spent on locomotion (mean ± SEM). The sign * indicates statistically significant difference between LA (white bars) and HA (black bars) groups of the same species. This explanation refers to all graphs.](image-url)
and high-active lines. However, there is still behavioral variability in each selected line.

Problems concerning variability of the rate of spontaneous locomotor activity are similar. We wanted to compare the extent of variability in Wistar rats and laboratory opossums and to investigate how the most and least active groups differ in various parameters of activity. Our question was, if the variability of locomotor activity in the OF within the population of the opossums that were selected and domesticated for much shorter time was greater than in the long-time selected Wistar rats.

**METHODS**

**Animals**

Sixty male Wistar rats and 24 Monodelphis opossums (12 males and 12 females) were used for the registrations. The rats were bred in the animal house of the Gdansk Medical University and were 3 months old at the beginning of experiments. They were kept three per standard rat cage with free access to water and food (rat pellets). Opossums were bred in the animal house of the Nencki Institute of Experimental Biology, Warsaw and were one year old, i.e. they were fully mature adults. Opossums are solitary animals therefore they were kept individually in standard rat cages equipped with a small hiding place. They were fed dry food for kittens, canned meat for cats and fresh fruits. The care and treatment of animals were in accordance with the guidelines for laboratory animals established by the National Institute of Health. Experiments were approved by the Local Ethics Committee for Animal Experimentation in Gdansk. After finishing registrations, all animals were used for other experiments.

**Apparatus**

All animals were tested individually in the open field apparatus, where their activity has been continuously recorded with a videocamera. The apparatus consisted of a white plastic box with the floor size 50 × 50 cm and walls 50 cm high. The central part of the open field was defined as a centrally placed imaginary circle of the radius of 15 cm, thus occupying about 28% of the box floor area. An array of the infrared light-emitting diodes was placed under the floor and an infrared digital camera was placed 150 cm above the floor of the box. The camera recorded the entire course of experiment at the speed of 24 frames per second. The specialized computer software (VideoTrack ver. 2.0; ViewPoint) analyzed the record on-line, defined the contour of the animal, positioned its center of gravity within the field and tracked its displacements. Results of this tracking (direction, distance and speed of movement) were recorded every 5 seconds. These recorded data were later used for analysis of the path length, speed of movement, distinguishing the place where the activity took place (center or periphery.

Fig. 2. Exploration of the center of the field during the session. (A) Number of entries from periphery to the center; (B) percentage of session time spent on activity in the center; (C) the total length of paths across the center of the field. Other explanations as in Fig. 1.
of the field). Finally, the program drew within the contour of the field the total track the animal walked during the session, coding the speed of movement with the track color. As the point drawing pathway has been placed in the center of gravity of the animal, therefore the drawn paths never touched walls.

**Experimental procedure**

Each registration lasted 2 hours and took place between 5:30 pm and 7:30 pm, encompassing the lights-off hour at the animal house in the light-dark cycle (at 6:30 pm). The floor of the experimental box has been

Fig. 3. Drawings of the total paths traveled by exemplary animals from different groups. Computer-drawn tracking of the center of gravity of the investigated animals showing total paths traveled by a representative member of the LA and HA groups of rats and opossums during the 2-hour session in the open field. Green color – slow movements (3.0–6.3 cm/s); red color – fast movements (>6.3 cm/s). Note the large difference between the LA and HA groups. Rats of both groups avoided the center of the field (A, B), while opossums penetrated the whole field (C, D).
illuminated at the level of 85–95 lx (depending on the place).

Selection of animals showing the extremely low and high levels of the locomotor activity has been conducted off-line, after the program drew and evaluated the whole path traveled by each animal during the session. On the basis of the length of pathway, the eight most active and eight least active rats were selected out of the 60 investigated to form the highly active (HA) and low active (LA) groups. Due to the scarcity of opossums, their groups (8 highly active and 11 low active) were selected out of the 24 investigated opossums of both sexes. There were no differences in locomotor activity between female and male opossums. The higher number of animals in the LA group was caused by almost identical scores of four animals at the upper limit of the LA group. They were all included into the LA group. After selection, both sexes were equally represented in the HA (4 males and 4 females) and LA (5 males and 6 females) groups.

The following parameters of the spontaneous locomotor activity were calculated off-line for each consecutive 30 seconds of recording: (1) total distance traveled by an animal (in cm); (2) percent of time the animal spent on the locomotor activity; (3) number of incursions into the central part of the experimental box; (4) percent of time spent in the central part of the experimental box; (5) the speed of movement, classified as either immobility, slow or rapid movements. On the basis of experiments with rats and mice, producers of the Viewpoint apparatus defined slow movements as conducted with the speed of from 3.0 to 6.3 cm/s. Trajectory of these movements was marked in green on the screen. Slower movements (usually centered at one point and reaching only a few cm outside that point) were classified as sitting immobile. Movements with the speed faster than 6.3 cm/s were classified as rapid and marked in red.

Behavioral differences between groups were analyzed with the statistical software (StatSoft, Inc. 2010). Significance of differences between two groups was tested using the non-parametric t-Student test or Mann-Whitney test. Multiple group analyses were made by analysis of variance (ANOVA) followed by post hoc Holm-Sidak tests with species and groups as factors. The level of significance was set at $P<0.05$. Unless stated otherwise the data are given as the mean ± SEM

RESULTS

Locomotor activity

The longest distance traveled by a rat during the two-hour session was 12 317 cm and the shortest 694 cm. Therefore, there was 17.7 time-fold difference in the investigated group of Wistar males. Rats selected to the HA group traveled on average 9 685 ± 745.2 cm, while the LA rats traveled on average 1 036 ± 82.7 cm, which makes a 9.4-fold difference (Fig. 1 A). Statistical analysis showed that the difference in the mean traveled distance between the HA and LA rats was highly significant ($P<0.0001$).

The longest and shortest distance traveled by opossums were 49 924 cm and 1 525 cm, respectively (33-fold difference). Therefore opossums showed a much higher variability of their locomotor activity in the OF. Opossums selected to the HA group traveled significantly longer distance ($26 573 ± 3 540.9$ cm) than those selected to the LA group ($7 060 ± 1 184.8$ cm).
The 3.8-fold difference between the means was relatively smaller than in the rats.

Nevertheless, two way ANOVA showed a strong statistical difference between species ($F_{1,34}=39.3$, $P<0.001$), between LA and HA groups ($F_{1,34}=59.4$, $P<0.001$) and between species and groups ($F_{1,34}=8.8$, $P=0.006$). It is worth to noticing, that the level of activity of the LA opossums was still closer to the HA than LA rats (Fig. 1A).

Opossums explored the OF in locomotion for a significantly larger proportion of the session time than rats (Fig. 1B). The mean proportion of the session time spent on the locomotor activity was 15.5 ± 1.7% for all rats and 29.0 ± 1.5% for opossums. Kruskal-Wallis one way analysis of variance on ranks showed that there was a significant difference between the two species in the average time spent on locomotor activity ($H=30.7$ with 3 degrees of freedom, $P<0.001$). The average proportions of time spent on locomotor activity by the HA and LA groups of opossums and rats are shown in Figure 1B. On average, the HA rats spent 28 ± 2.4% of the session time exploring the open field, while the proportion of time spent on activity by the LA rats was over tenfold lower (2.5 ± 0.2%). This difference between the HA and LA groups of rats was statistically significant ($P<0.001$). Similar, but weaker behavioral differences were observed in the selected groups of opossums (Fig. 1B). The HA group explored the open field for a longer time than the LA group (45 ± 3% vs. 13 ± 2% of the total time). This almost four-fold difference was statistically significant ($P<0.001$).

Spatial distribution of the locomotor activity

The HA rats entered the central area of the open field 15 times more frequently than the LA rats (415 ± 82 vs. 27 ± 12 times, Fig. 2A) and remained there for 6.8 ± 1.8% of the session time, while the LA rats remained in the center for only 0.7% of the session time (Fig. 2B). There is a statistically significant difference between these results ($P=0.002$).

Some of the LA rats crossed the central part only a few times during the whole session. As a result the HA rats traveled more than twelve times longer distance across the central part of the open field than the LR rats (1 577 ± 302 cm vs. 125 ± 36 cm, Fig. 2C).

We counted the numbers of crossings of the open field central area during whole experimental session for both groups of opossums. The difference was only two-fold (589 ± 104 times vs. 241 ± 41 times, Fig. 2A).

Distance traveled across the center of the field by the HA opossums was about four times longer than that traveled by the LA opossums (5 702 ± 930 cm vs. 1 497 ± 276 cm, $P<0.001$, Fig. 2C). However, proportion of the session time spent in the center by the HA opossums was only about twice longer (20.5% vs. 11.6% Fig. 2B). This indicates that the HA opossums moved relatively faster when traveling across the central part of the OF than when they traveled closer to walls and faster than LA opossums (Fig. 4).

Both groups of opossums penetrated the whole floor of the open field apparatus without any sign of avoidance of the center (Fig. 3). All these measures and observations show that opossums from both the HA and LA group very actively explored the whole open field apparatus and did not avoid its central area, while rats showed a marked thigmotaxy. The difference in the level of defensive reactions between the investigated HA and LA groups of rats was much stronger than the difference between the HA and LA groups of opossums.

Speed of movement

The spatial distribution of movements, as presented in Figure 3A–D, was analyzed above. In the Figure 3A–D the speed of the animals’ movements were coded on the trajectories by colors, the green color indicating slow locomotor movements (faster than 3.0 and slower than 6.3 cm/s), while the red color indicates fast movements (over 6.3 cm/s).

The LA rats made infrequent, high-speed dashes between corners, therefore their average speed of movement (5.7 cm/s ± 0.2, Fig. 4A) was slightly higher than the speed of movements of the HA rats (4.8 ± 0.1 cm/s) that did not dash this frequently. This difference was statistically significant ($P<0.003$).

The opposite tendency has been observed in the opossum. Those belonging to the HA group moved faster than opossums from the LA group (8.3 ± 0.5 cm/s vs. 6.3 ± 0.4 cm/s, Fig. 4A). Therefore, trajectories of the HA opossums are dominated by the red color, indicating fast movements, while in the trajectories of LA opossums there is more paths marked green, indicating movements slower than 6.3 cm/s (Fig. 3 C–D). The same was true about the speed of movement of the opossums in the center of the OF (Fig. 4B).
on real values of the speeds of movement showed a
tendency on the margin of significance ($P=0.066$).

**DISCUSSION**

Here we report the results of analysis of the sponta-
neous locomotor activity of the opossums and Wistar
rats during two-hour session of the open field test.
Analysis of the recorded activity revealed species-
specific differences. Opossums were generally more
active: they spent more time on locomotor activity,
moved faster and traveled longer total distance during
the session. Moreover, opossums spent proportionally
more time in the central part of the open field and
crossed the center more frequently than rats, therefore
showing higher level of locomotor activity and lower
level of anxiety.

There were no sex-related differences in the path
length or spatial characteristics of activity in either spe-
cies. This result is in agreement with previous compari-
sions of the rat and opossum behavior (Wesierska et al.
2003). Altogether, these data confirm our previous results
showing that opossums preferentially use the high-risk
exploration strategy while rats mostly rely on the defen-
sive behavior (Beardslee et al. 1989, Kalynchuk et al.
2004). Opossums showed also higher within-species
variability of the distance traveled during the session.

Groups of HA and LA opossums and rats were
selected on the basis of the total path length and behav-
ioral differences between the selected groups were
evaluated. Comparison of the HA and LA groups of
opossums and rats showed that in each species these
selected groups differed on another principle: level of
anxiety (avoiding center of the field, freezing, thigmot-
axy) in Wistar rats and level of locomotor activity
(speed of movement, activity in the center, total path
length) in the opossums which may reflect the level of
general arousal (Geyer et al. 1986). Therefore we con-
clude that depending on the investigated species,
results of the open field test may depend on different
factors.

Comparing distribution of activity in the OF we
observed striking differences between rats and opos-
sums. Even in the extended, 2-hour long test rats evi-
dently avoided the central part of the open field and
spent a lot of time in corners. Opossums (both HA and
LA groups) did not avoid the center of the OF and trav-
elled across all parts of the field with equal frequency,
also resting in corners. It is also significant, that LA
opossums moved slower than HA opossums, while the
reverse was true as concerning rats. We conclude, that
rats differed mainly on the factor of level of fear/anxi-
ety, while opossums have a unique way of exploring of
new environment, dependent on the level of arousal,
and not anxiety, that is different from all tested species
of rodents (Geyer et al. 1986, Miyakawa et al. 1996,
van Gaalen and Steckler 2000, Salome et al. 2004) and
also from shrews (our unpublished data). Further
investigations on various species as well as investiga-
tion of the mechanisms of this behavior in opossums
are needed to elucidate the basis of these differences.

Exposition to a novel environment elicits specific
emotional reactions that differ in the selected HA and
LA groups of rats (Meyza et al. 2009). It seems par-
ticularly interesting to examine whether these behav-
ioral difference between rats and opossums depend on
differences in their dopaminergic and serotonergic
emphasize that the basis of locomotor diversity of ani-
mals are differences in the dopaminergic and seroton-
ergic systems of the brain. It is noteworthy that HA
rats have higher basic dopaminergic activity in the
nucleus accumbens (Piazza et al. 1991, Hooks et al.
1992). This difference in dopaminergic activity
between HA and LA rats appears to be dependent in
part on the differences in regulatory inputs to the mes-
encephalic dopaminergic neurons (Lucas et al. 1998).
Hooks and others (1994) have show decreased D2 and
increased D1 receptors binding in the nucleus accum-
bens of the HA rats, in comparison to the LA rats.
However, there are no data yet on the organization of
these systems in the opossum.

One important result of these experiments is docu-
mentation of striking behavioral heterogeneity of the
laboratory opossums and lower heterogeneity of
Wistar rats. We found that levels of spontaneous and
exploratory locomotor activity of the individual rats
or opossums taken from the same colony may differ
by more than an order of magnitude. In spite of the
larger tested group, Wistar rats showed smaller dif-
fences between the most active and least active
animal than the investigated group of opossums. This
indicates that these natural phenotypic differences are
at least partially based on genetic differences.
Laboratory opossums are derived from only seven
wild individuals and kept as an outbred line for over
forty years without any directional selection
(VandeBerg et al. 1997). Yet, their laboratory line is
still very variable, as concerning behavioral characteristics (Wesierska and Turlejski 2000, Wesierska et al. 2003).

It is not known how many founders were used to establish the albino rat line, but there were certainly only a few animals that established the Wistar albino line that is laboratory-bred for over one hundred years. During that time they were (unwillingly) selected for reduced level of aggression and general tameness, but not for reduced level of fear (Pisula et al. 2012). On the other hand, epigenetic influences, especially during development, result in phenotypic differentiation even among genetically identical individuals, so a part of both opossum and Wistar rat variability might have a non-genetic cause.

This genotypic and phenotypic variability results in remarkably large dispersal of various behavioral features of the laboratory animals that weigh on results of various experiments. Naturally active and non-active individuals may differ in their metabolic rate and other physiological conditions, pain sensitivity, reaction to psychotropic drugs etc. (Lish 1969, Thiel et al 1999, Kazlauckas et al. 2005). One way of dealing with this problem might be increasing the group size. This approach has the advantage of closer modeling the natural, heterogeneous populations and allowing for prediction of the effects of applying a drug in such population. Another approach is to select a behaviorally more homogeneous group out of the available population on the basis of results of a test. This approach allows for elimination of various uncontrolled factors, like different levels of spontaneous locomotor activity interfering with the experimentally introduced factor, for example the level of physical training. Such behaviorally selected groups would allow for reduction of the number of animals used for experiment without loosing significance of results.

CONCLUSIONS

Our data show that in the long-time open field test opossums show much higher locomotor activity than Wistar rats and do not avoid the center of the apparatus. Natural variability of the level of activity is higher in the opossums than rats. Low-active rats move faster and avoid center more than highly active rats, while the reverse is true about opossums. Therefore, the low level of activity in rats depends on the increased freezing and fear, while in the opossum it does not correlate with fear, but with general level of activity.

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