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Research Article

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Self Recognition and Social Sense in Rodents: T-maze With a Mirror

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ABSTRACT
Mirrors are very relevant tools for our human identities: from those built with lead in the fifteenth century to the frontal camera of the mobile, these gadgets have been very useful to let us know who, how or what we are. However, it might be possible not all species on the planet see the same as we do. In this research we try to understand what the rodents see in a mirror and, contrary to what was already published, we found mirrors are not aversive stimuli for mice. As an assumption, we could say they see at the beginning something similar to a friend mate in the distance, then after approaching, they could see a bright wall with a sense of profundity. But they do not see themselves. Likewise, as the gorillas on the top of the leafy grove in the arboreality of their common environment, they do not see the image of the mirror as themselves, because their perception is aimed to see other stimuli to survive. Social sense of rodents could have their main value for their own identity and survival: they might not see themselves in the reflex, which could require other brain structures. Sense of social beings *vs* individual beings as different stages in the evolutionary steps of the mammalian the brain is discussed.

Keywords: T-Maze, Laterality, Self-Recognition, Social-Recognition, Mirror, Egocentric Navigation

Introduction

Being able to navigate in an external environment is a crucial ability to survive for all the species. It is so vital to subsistence that it has also conserved in phyla as basic as insects: ants, bees, etc. Navigation also is very present in avians, fish, bats and all terrestrial mammals. These skills have evolved to the extent that some animals are able to navigate in three dimensions rather than two: birds, bats, fish and marine mammals [1].

There are several types of navigations, depending on the keys used to get an orientation: the external and distal cues are used for the allocentric navigation, which involves the hippocampus, entorhinal cortex and surrounding structures. The internal, proximal and intra-organism cues (feedback of self-movement, sense of distance, kinesthetic perception or speed and proprioception), are used for the egocentric navigation and the brain areas related are the dorsal striatum and connected structures. This later navigation can operate in darkness without visual cues and it is used in species with poor sense of view [1,2].

The T-maze might be a test for the egocentric navigation and spatial learning, being a good way to check the working memory and brain areas related to laterality. It also is a practical tool to detect partial dysfunctions in the hippocampus [3]. Consists on a capital T with a main corridor and two smaller arms, with something different in each of them. The animal choice per one arm or the other are recorded, as well as the recalling of their choices in posterior tests. The rodents are showing their free will in the election of two levels or categories, 0-1, because no aversive stimuli are present in this maze or they are smaller and more tolerable than in other mazes (water in the Morris maze). The arms of the T- maze use to be closed or with little light, then the mice felt more comfortable and hidden than in an open field test.

Memory could be defined as the “persistence of the previously acquired information” and together with synaptic plasticity and learning, depends on the changes in brain metabolisms such as postsynaptically glutamate receptor mGluR5 [3,4]. Memory and learning are very related, because “learning is the process by which we acquire knowledge about the world and memory is the process by which that knowledge of the world is encoded, stored and later retrieved” [5]. In the hippocampus some

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histological changes were described for glutamate receptors in the CA1, CA2/3 and dentate gyrus after six months of T-maze training, although these changes were subtle and more related to membrane levels of affinity in the transduction at the intra-cytoplasmic level [6].

Mirror is an ancient object whose form and use has changed over the centuries. The first ones were made of glass and lead, silver or gold, so they were expensive and scarce, only aimed to be in the richest and privileged houses. Until the end of the seventeenth century mirrors became more affordable and began to be a necessity for everyone to perceive their own image, although it never completely lost its magical sense or warnings about vanity (Narcissus story or Perseus *vs* medusa) [7]. In humans the body schema or self image is not innate, but acquired gradually: children become increasingly aware of their own body or self image at the age of 2 or 3 years old. Piaget insisted on the importance of the others for the individual identity, pointing out the relevance of interacting with others to build their own body schema at a very early age [2].

The mirror self recognition test (MSR) has been used to check the own consciousness in several animal species such as upper apes, dogs, and in a large range of species over the past decades, including Asian elephants (*Elephas maximus*), marmots (*Marmota flaviventris*), chimpanzees (*Pan troglodytes*), corvids (*Pica pica*), dolphins (*Tursiops truncatus*), and ants (*Formicidae*) [8]. Dolphins demonstrated their capacity to recognize images in a mirror for motor imitation of conspecific others and even humans, supporting the idea dolphins have an advanced self awareness and own action sense [9]. However only a few species with bigger brains are capable of self recognition in the mirror test, specifically apes species, killer (*Orcinus orca*), false killer (*Pseudorca crassidens*) whales and Asian elephants. Gorillas present inconclusive data concerning the MSR test, compared to those obtained from chimpanzees or orangutans. A recent meta-analysis search into scientific literature showed the differences in methodology and its rigors were not relevant biases that could justify the lack of consistency for gorillas results in the MSR. It was suggested that the natural environment of apes, i. e. arboreality, might have an effect on their own self perception [10].

Other species, such as rodents, seem to behave socially toward the image of a mate``conspecific" in a mirror, thus the mirror it is not clear if it was considered as an aversive stimuli [8,11]. Mirrors are used for some pet avian species to enrich and improve their visual stimulation of isolated housed birds, to alleviate the negative effects (stress, anxiety) of isolation housing. Mirrors in rodents have, however, not this use, because it has been suggested mirrors are slightly aversive to individually housed mice, specially during feeding [12]. It was found mice rarely interacted directly with the mirror while, on the contrary, they were seen to rear against, push behind, or 'wrestle' with the mirror. Some mice occasionally slept close to and directly in front of the mirror. These data therefore indicate that in contrast to some other species, mirrors are probably not a suitable environmental enrichment for individually housed laboratory mice because of their supposed aversive meaning [11,12].

In previous and more recent research it was found the mice C57BL/6 do prefer to stay in the middle of a cubicle only when the walls were covered with mirrors and they could see "something like mates" around, otherwise, without mirrors, they rather hide in the corners of the cubicle, because they found the open environment very stressful [13]. Nevertheless, we cannot know whether they can see other mates in the mirrors or a sense of depth or distance. Mirror might allow mice to recognize other selves of their own species, especially in the distance because of their poor sense of viewing. Mice lemurs are very good in nocturnal vision and they can see quite well. In a mirror test they were shown to respond with curiosity to a mirror. However, whether they could choose between their own images *vs* the company or a real mate, they chose and distinguished the alive creature [8].

Our observational experiment with a mirror wanted to check whether the mice see their own image, the image of a self creature or just a sense of profundity. For that goal we chose to test a few well identifiable animals that had different sizes and contrasting different fur colors (dark, coffee, cinamon, white with dissimilar sizes). Our hypothesis would be that the domestic *Mus Musculus* mice could choose more often the arm of the mirror because it is not an aversive stimuli and in the distance, they might see another friend mate to reunite and to reduce their anxiety to the new experience or a sense of profundity and continuity of the path. But once the animal researches and inspects the mirror, seeing it is only a bright wall with nobody or no path behind, habituation occurs and their posterior arms choices would be indistinct.

Method

We used to analyze and observe the mice behavior one T-Maze with close arms, which required egocentric skills of navigation and demands less stressful brain metabolites to solve, because it is completely safe. Smell residues or previously odor were removed each time with dissolved chlorine solution. For that purpose, two domestic mice rodents *Mus Musculus* (one male, one female) were obtained by acquisition in an official pet store. They mated on February the 14th and subsequent offspring of that couple were used for these experiments. A total of 6 male domestic *Mus Musculus*, an adult group (N = 3) of 7 months old (born 16th March) and a younger group (N = 3) of 4 month old (born 9th and 12th June) were tested with a T- test of two options, having a mirror on the left arm of the T (figure 1). Previously to the test and to avoid any unsureness of their identities, the rodents were "formally presented" to the video camera during the first seconds of recording. While all the animals were tested in each trial and before returning to the home cage, they rested one by one in an intermediate transit cage to restore their anxiety levels [3].

Two sessions were performed, with a distance of 8 days in between. Each session was made with 3 consecutive trials at the same time each day (around 7pm) and a recall trial exactly than the other ones, done on day 5 (total 4 days of recordings), leaving one day in between without testing. The mirror was always in both sessions on the left side, but the difference between sessions where the place of the exit of the maze was in the left side for the first session and in the right side in the second session. Considering escaping from the test is a reward for rodents, we changed the position of this option to neutralize the possible recompense effect of escaping.

The data from the video recording were obtained through the vision of movements of the animals that were clearly distinguishable for the opening carved in the top surface of the cardboard tube (figure 1). The apparatus was elevated 80 cm above the ground. During the first stage (choice run), once the entrance door was opened and closed behind, the animal was allowed to freely choose between the two arms of the Tmaze: the one with the mirror at the end of the left arm or the one with no mirror. Six animals at a time were carried into the T-maze for the experimental behavioral test and each performance and choices of the six mice were recorded for each trial in two sessions. Two dependent variables were reported: time to complete the maze (latency from the entrance to the exit) in seconds and the first choice made (left or right arms) in units (1-0). Specific behaviors, habituation and other events during the test were also recorded.

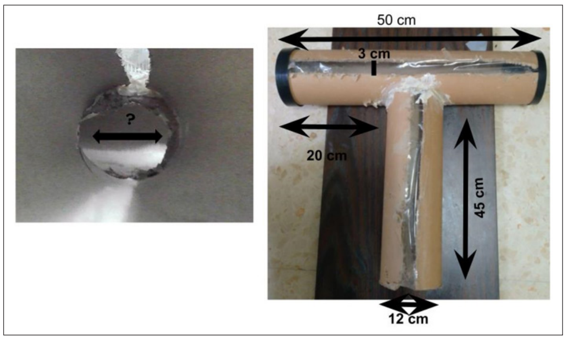

Figure 1: Picture on the left is displaying the view of the mice when entering into the maze inside the main corridor of the T-Maze. The picture on the right depicts measurements and the characteristics of the T-Maze tests: it was made from cardboard tubes, with a top opening to allow observation and registration.

For proper cognitive and motor stimulation (proprioception and kinesthesia) and individual development, these experimental domestic *Mus Musculus* were usually housed with interconnected cardboard cylinders, rotating wheels (vertical and diagonal), creative colorful pipe tube tunnels, made with plastic or wood, with directions in their connections inside the cage and bridging different enclosures. Also with outside and inside spacious nests, several height levels to roam around in each cage, elastic or fixed aerial horizontal ladders made from popsicle sticks and ladders made from bamboo sticks [14,15]. Mice of pet store *Mus Musculus*, known as home Mus Musculus or domestic *Mus Musculus*. have an unknown genetic background, but they are usually sold to become pets or to be food for captive reptiles, when they are certainly good mammalian specimens for obtaining scientific behavioral and experimental data to provide information for the Scientific community.

All mice were group housed per ages and sexes at 25ºC, under standard 12 h day/night cycle, in comfortable cages provided with beds of finely ground Spanish pine sawdust, fresh hay, straw and rodent litter. All the cages were cleaned, changed and dewormed using antiparasitic spray, sepiolite and diatomite litters at least once every week. The food provided was carefully chosen in a healthy variety following veterinary instructions for little rodent necessities: natural seeds without additives, lettuce, nuts (pumpkin seeds, black and white/black sunflower seeds, wheat, walnuts, peanuts in shell, oatmeals, pistachios etc.), pasta (raw macaroni), toasted bread crumbs or bread peaks, fresh and dried proteins [dried or healthy alive tenebrio molitor coleoptera and dried larvae of hermetia illucens (black soldier fly)] [14,15].

All experiments were approved by local authorities and were in accordance with the guidelines for animal experiments of the EU (Directive 2010/63/EU). We also strictly follow the general protocols established for mice by the American Institutional Animal Care & Use Committee (IACUC) concerning the Policy on Investigation Noncompliance and Animal Welfare and the Environmental Enrichment for Animals Standard Operating Procedures (SOP).

Results

Our results added some new insights and ideas, to keep researching this line of reasoning on how the difference between one's own and social identity are considered but with the limited sense of view in these rodents.

First we found there were no differences between age groups in any variable. It was proved there was not an aversive consideration toward the mirror, because they mostly chose the arm with the mirror, no matter where the exit was located (figure 2).

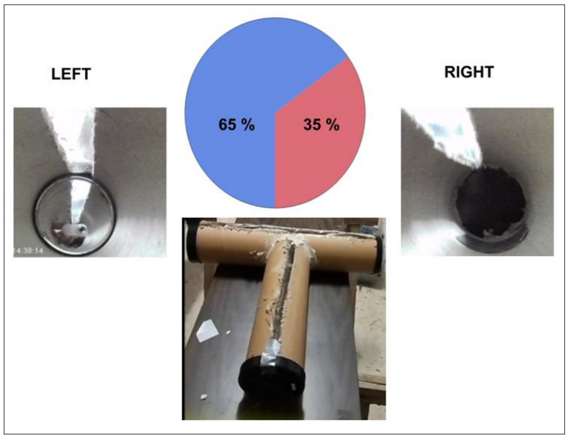

Figure 2: Picture showing the percentage of animals mostly choosing the left arm with the mirror *vs* the right arm with a closed door (65% *vs* 35%). It is noticeable that the animals see at the end of the corridor the mirror with an image of a rodent at the end. In this picture the image is a camera, but readers might have a better idea of what the mice are choosing.

The mirror at the end seems to be the more attractive option for rodents, in both cases, when the exit of the maze was done from the left side or from the right side of the T-Maze test. The way out the door was made with the help of a hollow foam rubber tube, where the animal would climb onto and then it was possible to take out and put it in an intermediate rest cage. That foam rubber transporter tube was replacing the standard door of the maze when the animal finished the exploration of the two arms and then returned to the main corridor. The mirror left choice of the T was majority elected in both cases of the exit gate (figure 3 and 4).

However, doing a more careful reading, we could see how, in some cases, animals chose always the same option, for every trial, and almost in all sessions: subject #1 and #6 choose always the left mirror option in two sessions, while, under the exactly same circumstances, subject # 4 choose almost always, except once on day 3 session 2, the right non mirror option (figure 4). These observations might be suggesting some sort of laterality or side preference in rodents, making in these *Mice Musculi* and their interhemispheric connection a matter of interesting study.

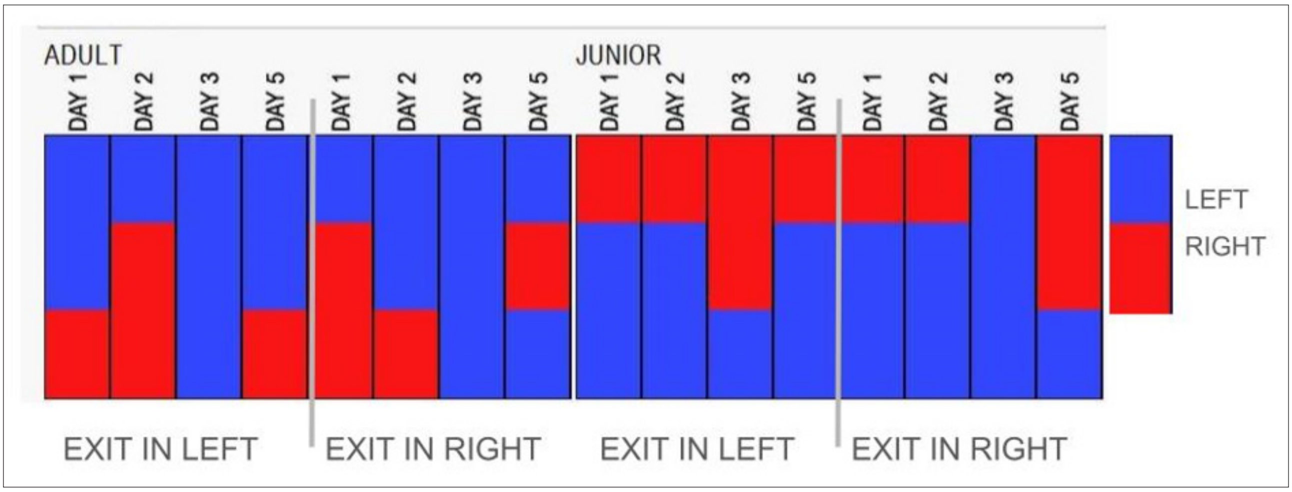

Figure 3: Colorful graph showing the main presence of the mirror left choice (blue color) compared with the right arm with no mirror (red color). The mirror was mostly chosen by both groups, adults and juniors and in two sessions, when the exit was in the left and in the right.

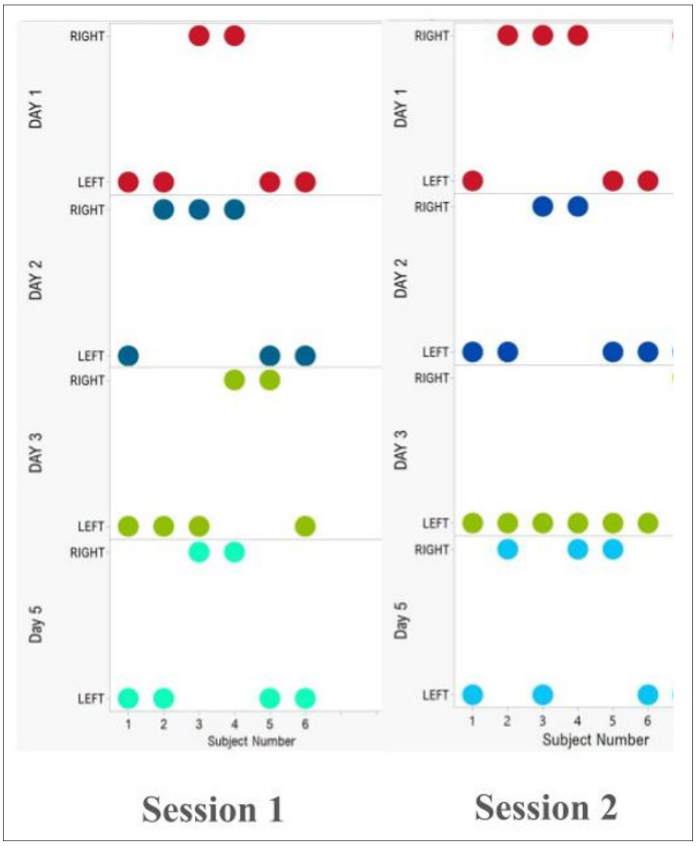

Figure 4: Graph depicting each choice of every experimental subject through the two sessions. Subjects # 1, 4 and 6 showed a systematic direction of choice. Although mostly all animals explored both sides, they seemed to have a route preference.

It is remarkable to mention one of the experimental subject (#1), suffered a vestibular syndrome while doing this experiment, performing the last trial on day 5 of the second session under the effects of this sickness. His affliction occurred on the left inner ear, the place where his head was bowed and consisted on a total lack of balance (walking sideways), loss of equilibrium and proprioception (when he was grabbed by the tail and while he was in the air, he began to make spasmodic and incessant turns towards the side of the injury, showing a deprivation of stability) and likely dizziness.

Another dependent variable was the time spent inside the maze. As a sign of habituation to the task, they showed longer and longer times inside the maze, in a range from less than one minute till almost 5 minutes (figure 5). During this long *tempo* they were in the interior of the maze, some mice spent time in the mirror arm, close to their own images, grooming themselves and finding comfort, a behavior that would be incompatible with the consideration of the mirror as an aversive stimuli.

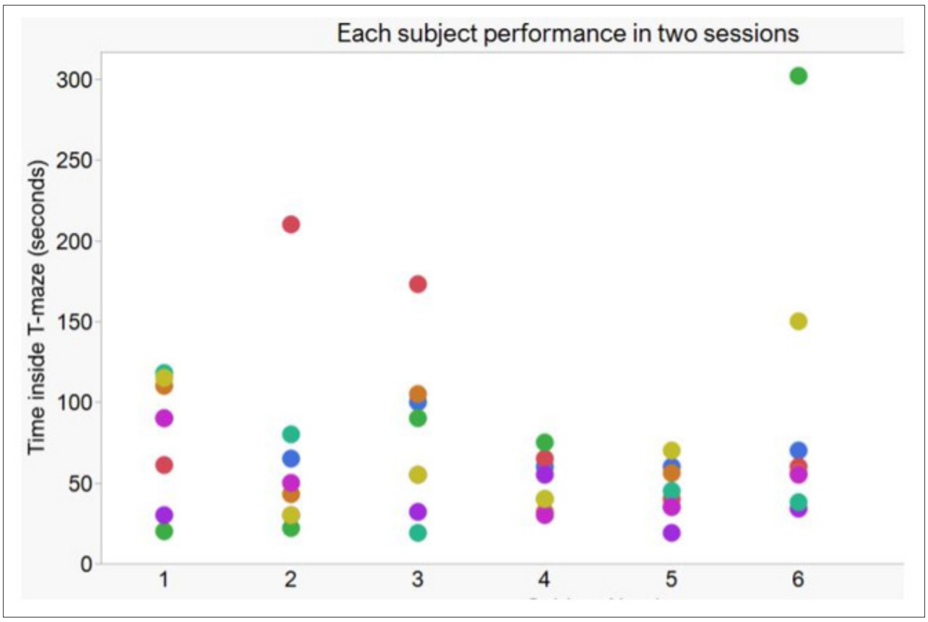

Figure 5: Graph showing the time inside the T-Maze in seconds per each experimental subject. Subjects 1 to 3 belonged to the Junior group, while subjects 4-6 were members of the adult group. It is noticeable how some of them in some trials lasted almost 5 minutes while others went out in less than a minute.

The learning process is noticeable in both groups for the first session, when they perform the task in a shorter time each trial (figure 6) and they show significances in the male adults group between last trial (purple bars) and the previous first trials [Trial 1 *vs* Trial 5: F (1, 5) = -4.5214; p = 0.0031; Trial 2 *vs* Trial 5: F (1, 5) = -2.29; p = 0.0353]. In the second session, habituation to the environment and the mirror stimuli (figure 6) are visible, on how the time spent for last trial 5 was statistically significantly bigger than in the last trial of session 1 and in both groups adults and young: [session 1 *vs* session 2 last trials and both groups: F (1, 6) = 2.10; p = 0. 0449] (figure 6).

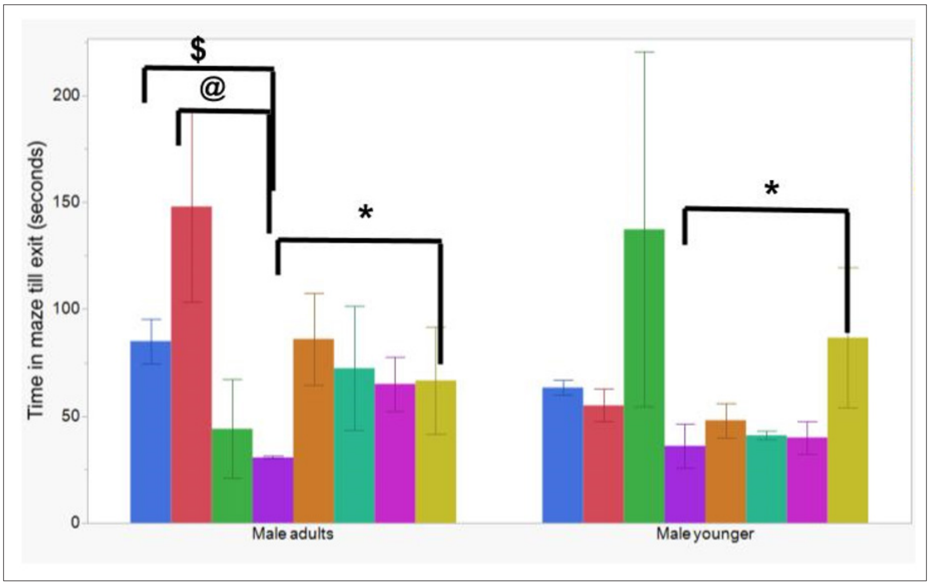

Figure 6: Graph displaying in each group (male adult and male younger) the total time inside the T-maze, till the entrance door closed behind them to they went out. The first four bars in each group belong to the first session, with the exit on the left arm. The last four bars in each group are showing time for the second session and the exit on the right arm. Statistical significances were found: Trial 1 and 2 *vs* Trial 5: $ = p = 0.0031; @ = p = 0.0353; Last trial session 1 *vs* Last trial session 2: * = p = 0.0449;

Discussion

Navigation skills are well preserved among species, because they mark the course of action and direction of routes. They are used to be aimed for getting resources, such as food or finding other locations with conspecifics. What creatures see in the mirror might be dependending on what their brains are prepared to see for reaching those goals of finding required resources together with the environment they have to deal with. Previous studies showed the mice react to the mirror rearing against or pushing behind, perhaps because they see a profundity to walk over on, not necessarily an aversive stimuli to fight against. We found some of them used to do grooming behavior close to the mirror. That could only be possible if the animals do not see a threat in the mirror. Guessing how they perceive, we would say they first see first a conspecific mate, then a bright wall, then nothing of interest. Most probably they do not jump toward self recognition, because their brains are not built for that objective, since they are mainly social creatures where the community is all the meaning of their existence and individualism only a cause of coldness, anxiety and stress.

Laterality in mice was also visible in this research. Some of the mice systematically chose the same arm every time. Although, that is difficult to establish because mice do not perform specific activities with one arm or the other. Their brain neuroanatomy, however, sometimes shows differences between hemispheres in areas such as the hypothalamus and the bed nucleus of the stria terminalis. This asymmetry between hemispheres in the brain may be mediated by a common ontogenetic mechanism for human beings and other species [16,17].The vestibular syndrome mouse is fortunately completely cured after a few drops inside the damaged ear with a cotton swab of ivermectin and insecticide pyrethrins derived from Chrysanthemum. His head is completely in position, although his balance and equilibrium required longer time for a full recovery. Now he looks exactly like his mates.

Conclusions

Phylogenetically speaking, self identity might have been built layer upon layer in the brain and it might be very related to the environmental and social demands each species have to deal with. Navigation skills for ants, mice or gorillas are relevant, but also their ability to identify and be part of a bigger group to successfully stay alive. With T-maze test results of the mice´s laterality preferences and egocentric navigation skills are shown. Metabolic activity of brain glutamate receptors in the hypothalamus, hippocampus (Dentate Gyrus) and Dorsal striatum (caudate nucleus and the putamen) could be measured and analysed together with these behavioral studies to a better understanding of our mammalian brain functions.

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