

Incredible Apodemus Sylvaticus: T-Maze New Definition

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The two rodent strains, *Apodemus sylvaticus* and *Mus musculus domesticus*, are related, but genetic similarity sometimes provides no more resemblance than body shape or dietary preferences: closer analysis reveals entirely different and difficult-to-quantity behaviors between these two murids. With brains of different sizes and weights, these two sub-species have specialized to survive in different habitats, and their behaviors reveal how this specialization has occurred. In this article, we review the response of *Apodemus sylvaticus* and *Mus musculus* to a two-dimensional T-maze, in which the main standard and predictably domesticated variable to measure would be the choice of the left arm with a mirror at the end or the right arm of the maze. With the wild *Apodemus* species, another variable suddenly appeared that needed to be measured: to survive, the wild rodent attempts to anticipate its captor's (researcher's) strategy in order to do the opposite and escape. A research experiment with new behavioral parameters to measure in a new 3D definition of the T-maze that would become a battle of brains in the middle of a natural hunt. The contrast between *Apodemus* (active gripping, climbing, escape attempts) and *Mus musculus* (passive falling) resulted to be striking and is fully described. These results point to how new rodent species and the new psychological era require new behavioral metrics, which could involve an implementation of techniques used in some branches of modern neuroscience: behavioral neuroscience, animal cognition, and comparative psychology.

KeywordsEscaping Behavior, Hunting Behavior, T Maze, Climbing behavior, Arousal, *Mus Musculus*, *Apodemus Sylvaticus*.**Introduction**

The *Apodemus sylvaticus*, (AS) also known as wood mouse, is a sylvester long tailed rodent from the family of *Muridae*. Their phenotype is quite different from the laboratory or domestic mice *Mus Musculus* (MM), although their size is very similar. *Apodemus Sylvaticus* (Figure 1) with very large tail and awake dark eyes, bigger head, quite autonomous front limbs, surprising agility to jump and speed of movements, have shown an excellent performance in the Morris Water Maze when tested, having a completely different style in the performance [1]. It is remarkable that the AS is able to control a cognitive map of 10.000 cm² [2,3], subsequently their brains need to be different from the lab or domestic mice, although some anatomy and connections might seem to be quite similar. It is a challenge for Neuroscience and Neuroanatomy to find out where AS differs in their brain compared

with the MM.

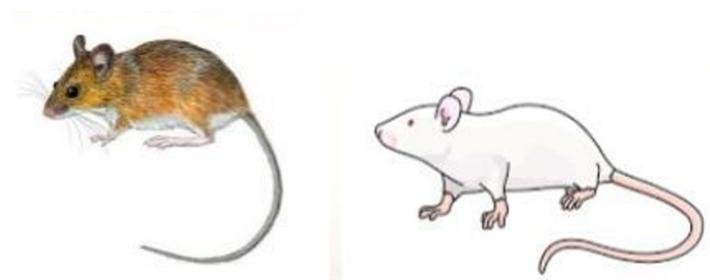


Figure 1: Picture depicted the two rodent strains compared in this study, which are familiarly related but they are notoriously different. In the left picture, the undomesticated *Apodemus Sylvaticus*, with very large tail and awake dark eyes, bigger head, quite autonomous front limbs, surprising agility to jump and speed of movements. In the picture on the right is the domestic *Mus Musculus*, albino mouse with red delicate eyes, very curious about their immediate reality, who are much more appreciative, collaborative and cooperative to produce numbers and graphs for neuroscientists.

It has been proved that the wild wood mouse (*Apodemus sylvaticus*) plays a crucial role in maintaining the health and balance of various European and Mediterranean ecosystems, where they naturally develop their environmental ecosystem [4,5] (Figure 2). For instance they help in forest regeneration by seed dispersal and transport of tree and shrub seeds. They also contribute to improving the soil quality by building burrows and tunnels which open ways to the water and helps for substrate infiltration, those are essential for plant growth and solid aeration. Furthermore, their natural droppings act as a natural fertilizer. They are also very important links in the food chain, being predated by birds (owls, barn owls, and falcons), mammals (foxes, badgers, martens, weasels) and reptiles (snake species). *Apodemus* feeds on harmful invertebrates and insects, helping to regulate their populations in agricultural and forest environments. Moreover they are used as an environmental sentinel to detect signs of pollution or changes in ecosystem quality, because their appearance and abundance might be a direct indicator of a healthy habitat with biodiversity. They are quick seekers, being able to travel per night a total average of 320 meters in woodland against 1201 meters in sand dunes. They are classified as Least Concern on The IUCN Red List because the species is widespread and very common.

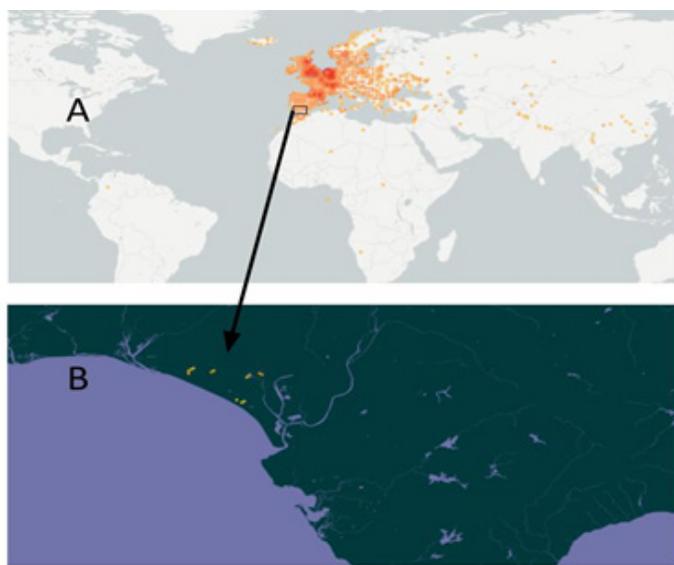


Figure 2: Pictures depicted the *Apodemus Sylvaticus* distribution in Europe (A) and, specifically, in southern Spain (B) (Donana). The species appears to be purely native to Europe, well anchored in the origin of the longest-lasting human civilizations. In South Mediterranean and Atlantic areas, i. e. Donana Natural Park, there are systematic recorders of their appearance [4,5]. The few recorded occurrence in South America might be caused to a different although physically similar rat species, the American pygmy rice rat, *Oligoryzomys longicaudatus*, also known as the long-tailed colilargo or long-tailed pygmy rice rat that is a species of rodent in the genus *Oligoryzomys* of the family Cricetidae, different from Muridae. The Asian occurrence might also be due to other families. Data maps of occurrences were obtained from GBIF.org.

Method

We used to analyze and observe the mice behavior with T-Maze in close arms, which required egocentric skills of navigation and demands less stressful brain metabolites to solve compared with

the Morris maze, because in this T-shaped labyrinth there are no stressful stimuli like deep water. Smell residues or previously odor were removed each time with dissolved chlorine solution. For behavioral research purposes, six male rodents (N = 6) (three domestic *Mus Musculus* and three wild *Apodemus Sylvaticus*), were compared in three different trials with a temporal distance of one week. Three sessions per animal were performed; the visual stimuli of the mirror was on the left side. An internal camera inside the T-Maze was recording the first option to choose by the animal (right or left) and all the behavioral displays inside the labyrinth. Moreover from an external video recording we obtained more behavioral data through the vision of the whole scenario with researchers, helper cats supervising the proper execution of the experiment and experimental rodent movements inside the maze (Figure 3).



Figure 3: Pictures showing the two camera visions of the experiment: one general camera (upper picture) and an inside camera for recording the arm chosen (lower picture).

The T- Maze 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 T-Maze: the one with the mirror at the end of the left arm or the one with no mirror at the end of the right arm. Specific behaviors, habituation and other events during the test also happened.

The method for removing the animal after the stipulated recording time was different in this experiment. Normally, a human hand is placed inside the maze and the animal is removed after being caught. Alternatively, the animal can be removed by entering a

movable section of the maze that is then taken to the animal's usual cage or an intermediate area without touching it [6]. However these options were not advisable in this experiment, as opening the maze for touching the *Apodemus* might be a risk of escaping. We opted to invert the maze from the arm where the animal was located, so that it would fall back into its cage without touching it due to the fall of its own weight. In this way, we transformed the T-shaped maze into a three-dimensional maze, and new and surprising behaviors arose.

All mice were housed at 25°C, 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 nutritional instructions for little rodent necessities: natural seeds without additives, lettuce, nuts (pumpkin seeds, black and white/black sunflower seeds, wheat, walnuts, peanuts in shell, peanut butter, oatmeals, etc.), pasta (raw macaroni), toasted bread crumbs or bread peaks (Spanish *regañas* and *picos*), fresh and dried proteins [dried *tenebrio molitor* coleoptera and dried larvae of *hermetia illucens* (black soldier fly)]. All experiments were approved by local authorities and were in accordance with the guidelines for animal experiments of the EU (Directive 2010/63/EU). The specimens of the wild *Apodemus* species were released into the natural field safe and sound once the three-trial registration period had elapsed. 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

The *Apodemus Sylvaticus* mean choices were: 1- Left, 2- Right, 3- Left. The *Mus Musculus* mean choices were : 1- Left, 2- Left, 3- Right. Therefore, a slight tendency to choose the side of the mirror was detected. Being the percentage of choice left (67%) and right (33 %) (Figure 4).

However, the unexpected surprise of this study, serendipity, was the behavior of the *Apodemus* when we attempted to extract it from the maze. Time and again, we tried to return the *Apodemus* to its original cage from the maze's arms through a tipping over due to the fall of its own weight. But that simple movement was so difficult because the *Apodemus* clung to the walls with its tiny claws, grabbed hold and climbed up the arm to a safe place, avoiding falling again and again (Figure 5). On several occasions, after locating the animal at a specific point and tilting the maze arm over the cage, the *Apodemus* did not fall and hid again. As if trying to hunt the rodent anew, it kept escaping again and again while the cats supervising the experiment watched my clumsy hunting strategy with boredom. All three *Apodemus* exhibited this behavior, and the last one, taking advantage of my tiredness and exhaustion, managed to escape outside and forever by falling into a gap between the maze and the cage.

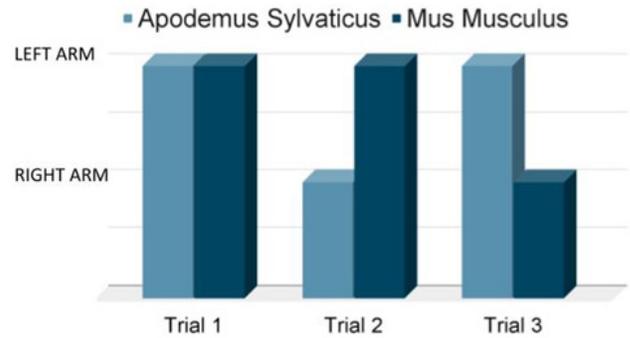


Figure 4: Graph showing the mean of two groups of rodent strains in each trial. Both strains did the same thing (twice to the left and once to the right) and chose the same first option (left side, with the mirror).



Figure 5: This image shows how the *Apodemus Sylvaticus* clung to the walls of the maze to avoid falling. At the end of the tube, which would have been positioned vertically, the open door is visible with the original cage at the end.

On the contrary, the three domestic mice (*Mus Musculus*), when overturned from the maze onto their original cage, instantly fell like dead weights into the cage and, after a few seconds of being stunned, resumed moving and behaving normally. This behavioral difference was so striking that it justifies the elaboration of this research paper.

Discussion

T-maze or the variant Y-maze is a simple forked passage used in

psychological experiments to test animal cognition experiments. It provides the rodent a straightforward choice (right or left arm) and in repeated trials it gives information about memory (reference and working memory) and spatial learning function through applying various discriminative stimuli (reward). The classical maze started in the early 20th century, the decision made by the rodent can be a cause of a natural preference.

This pioneering research highlights the significant behavioral and even metabolic differences between captive and wild animals offering a new perspective for behavioral experiments in rodents. Given that these animals have contributed many, if not all, of the psychological concepts (classical conditioning, operant conditioning, positive reinforcement, punishment, negative reinforcement, etc.) still used today in humans education and other animal species (training dogs, cats, horses, etc.), this new finding on behavioral differences in captivity should not be overlooked.

However, in the 21st century, many other important concerns and questions have arisen and this maze, being still useful, might include some other parameters to be measured according to the time change. For example, currently there is much greater awareness of animal welfare and a higher respect for laboratory animals and their lives. Therefore, in this study we included the novel variable of releasing the specimens into their natural environments after “asking them” about their performance in the T-maze. In this way, there is a minimal impact on its ecosystem or the food chain where they belong.

While both rodent strains exhibited a mild preference for the mirror arm in the T-maze (left arm), the study’s most significant contribution is the unexpected emergence of escape, climbing, and anticipatory behaviors uniquely displayed by *Apodemus sylvaticus*. T-shaped maze became a three-dimensional maze and new and surprising behaviors arose. The vertical rotation of the maze to evacuate the animal after the experiment introduced a new behavioral element: solving another three-dimensional maze. The reinterpretation of the T-maze as a 3D escape and hunting context is original and thought-provoking. This maze, made of cardboard, allowed the rodent to grip the walls showing unexpected escape, climbing, and anticipatory behaviors that were absent in the *Mus Musculus*. This reconceptualization of the T-maze significantly broadens its experimental relevance and opens new avenues for behavioral measurement in wild rodent species such as the European *Apodemus sylvaticus*, new operational definition of behavioral variables could be included for measurement and comparison, for instance: 1) number of failed attempts to tip the maze over, 2) time spent gripping the vertical wall before climbing, 3) the arm of the maze from which it finally fell into the cage, etc.

With new species to study, it is necessary to consider and analyze new behaviors. This observation convincingly demonstrates how wild species actively interpret and counteract human intervention, highlighting cognitive flexibility, arousal, and survival-driven problem solving.

The specific location of this species, *Apodemus Sylvaticus*, in Europe offers an interesting point of reference for future ecological research on the distribution and biodiversity of fauna. Similar species belonging to other families are mentioned on other continents, and new research would be required to define how they are similar or how they differ in their brains and behaviors, besides their teeth (*Muridae* vs. *Cricetidae*).

These results point to how new rodent species and the new psychological era require new behavioral metrics, which could involve an implementation of techniques used in some branches of modern neuroscience: behavioral neuroscience, animal cognition, and comparative psychology.

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