Alternative handling techniques for mice

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Introduction

When laboratory mice are picked up, it has an effect on their stress- and anxiety-related responses. The way an animal is picked up can also have significant effects on its behaviour. It is therefore of great importance that animal handling in (behavioural-)research is standardised, in this way all animals are handled similarly and are accustomed to the procedure.
There are numerous tools for measuring different kinds of behaviour in laboratory animals. Even when we limit ourselves to mice, there are countless behavioural tests available (1). Still, most behavioural set-ups have one thing in common: the mouse must be placed in the set-up at the beginning of the test, and must be removed afterwards (with a few exceptions, for example a set-up in which the animal's task is to find its home cage, or when measurements are taken in the home cage). Both placing the animal in and removing it from the set-up can cause a stress-reaction, in both the animal and the person handling it.
This applies in particular to one behavioural set-up, from which it can be troublesome to extricate the mouse: the so-called Elevated Plus Maze. The set-up consists of two open and two closed arms, and is set on top of a raised platform. Mice will almost always try to escape the researcher's hand, particularly in the enclosed environment of the closed arms. However, there is a way to remove the animal from the set-up that is relatively simple and also reduces stress: using a tube as home-cage enrichment. After the test is finished, this tube can be held adjacent to the set-up so that the animal is drawn to its own scent and enters the tube, after which the tube with the animal can be transported to the home cage. This results in a less stressful handling method for the animal.

Behavioural research and the Elevated Plus Maze (EPM)

The Elevated Maze is a commonly used set-up for measuring anxiety-related behaviour in rodents. Its variants include the Elevated Zero Maze, Elevated X-shaped Maze, Elevated Plus Maze and Elevated T-Maze. Out of these mazes the Elevated Plus Maze, abbreviated to EPM, is the most frequently used (2). The EPM set-up consists of a plus shape (+) with two closed and two open arms, situated approximately 80-100 cm above the ground (fig.1, panel A). The walls of the closed arms can be transparent or opaque. The principle of the EPM is based on »

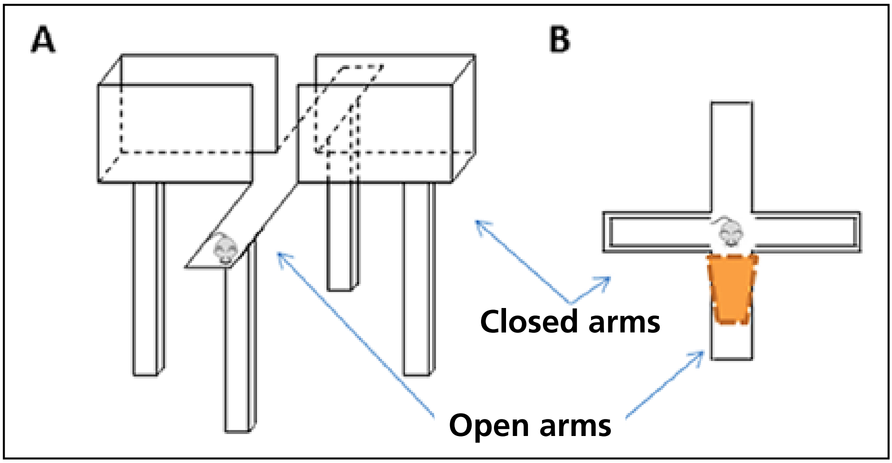

Figure 1: A. Schematic drawing of the Elevated Plus Maze; a commonly-used test for anxiety-related behaviour in mice.
B. Top view with an orange plastic tube on the open arms of the EPM.

the observation that mice avoid high, open and unprotected areas (3, 4). Quantification of the avoidance of these areas is seen as a measure of anxiety behaviour. Other behaviours such as locomotion and exploration can also be scored.

Despite the fact that the EPM is a validated test and is widely used in research on anxiety behaviour, it often gives inconsistent results between different research groups (5, 6). When two identical experiments were conducted at two different locations, in particular exploratory behaviour in the EPM was found to be location-dependent – and thus dependent on the conditions of the location, such as other caretakers (7). This makes the reproducibility of behavioural research very difficult. On top of this can the choice of the mouse strain be affecting the results (8).

Today great efforts are being made to standardise and harmonise experiments. The greatest possible consistency in factors such as the choice of strain/substrain, intestinal microbiota, the lighting schedule in the housing units, the use of cage enrichment, regulating the temperature and humidity of the animal rooms, etc., is expected to make the studies as identical as possible. However, divergent results are still occurring between research groups. One factor that can affect the results of an experiment, in particular anxiety-related studies, is the way the animals are handled.

As stated in the introduction, handling a mouse can generate a considerable stress reaction (9). There is a need for a handling method that minimises the stress reaction and ensures that the innate behaviour of the animal can be measured.

Mouse handling techniques

Research has shown that this so-called 'handling stress' has an influence on both an animal's behaviour and physiology (10). How the animals are handled is therefore very important for both behavioural researchers and researchers interested in, for example, the animal's metabolism, in particular pharmacological or physiological processes. Given that there are often unaccountable variations in studies from different laboratories, it is first of all important that the animals are handled in the same way (standardisation and harmonisation), and second of all it is important to minimize handling stress.

The most common method of picking up a mouse is to grab the animal by the base of the tail. The mouse is then carried (supported on e.g. the forearm) to a new cage (when the cages are changed) or to a behavioural set-up. In 2010 Jane Hurst and Rebecca West wrote that both the use of tunnels and an open hand resulted in a reduced anxiety response in mice, compared with picking them up by the base of the tail (9). The open-hand method entails 'scooping' the mouse from the cage with two hands and allowing it to walk freely on the open hands (a short 'cup

*Figure 2.
Top view of the EPM with the researcher removing the mouse (indicated with red arrow) from the closed arm.*

handling' video can be seen at: https://www.nc3rs.org.uk/handling-and-restraint). Although the mouse's initial reaction will be to jump away, can mice easily be trained in a few sessions to voluntarily climb onto the researcher's hand, and can thus be transported. The downside of this method is the training period; training requires time and researchers often want to test the animals in a 'naïve' state (so that an animal's innate characteristics can be observed without the influence of previous experiences or training). The other option of handling using a tunnel does not have these disadvantages, since it requires no training. The principle behind this method is that the tunnel is part of the enrichment of the home cage and that the animals respond to its familiar scent (a video on 'tunnel handling' can be seen at: https://www.nc3rs.org.uk/handling-and-restraint).

As indicated above, removing the animals from the EPM set-up can be troublesome, since the mouse can easily run to the opposing closed arm (figure 2). Around the time a study by Gouveia and Hurst was published in 2013 (10), we found a similar solution for removing the animals from the EPM. When the animals' cage included a plastic tube as cage enrichment, this tube could be used to remove the animals from the set-up. When the tube was set on the open arm of the EPM (fig.1, panel B), the mice were found to be drawn to the tube relatively quickly and even walked into it (fig. 3), which enabled the mice to be transported with the tube back to the home cage. This method firstly obviated the need to grab the animal by the tail, and secondly, eliminated the stress of the researcher chasing the animal around in order to pick it up.

While it shared our observation that the animal could be transported back to the home cage more easily after an EPM experiment, the study by Gouveia and Hurst (10) went a step further. They studied the effect of various handling methods on anxiety behaviour in the EPM (10). Mice were picked up and transported by the base of the tail, using a tunnel from the home cage or using an unfamiliar tunnel. This study showed that animals transported with both types of tunnels showed less anxiety behaviour than those picked up by the base of the tail. In addition, it became clear that there was also a difference between a tunnel used as cage enrichment and a tunnel exclusively intended for handling (and thus used for many different animals). A number of mice handled with a tunnel from their home cage showed even less anxiety behaviour than »

Figure 3.
Using the plastic tube from the home cage to remove the mouse (indicated with red arrow) from the EPM by placing the tube on the open arm. The animal can then be transported back to its home cage.

Figure 4.
While most mice tend to avoid human contact, there are always exceptions. This mouse (indicated with red arrow) follows the researcher's hand on the open arm, after which it is easy to pick up.

Photos figure 2-4, Anne-Marie Baars

those with the communal tunnel. These researchers recommend housing the animals with a tunnel in the home cage and also using it for handling (10). There are always exceptions that prove the rule, which we also found in our study, since most mice tend to avoid contact with humans, there were also some who came to the researcher of their own accord (personality and character vary between mice) and could easily be picked up this way (figure 4).

Cage enrichment

Besides the function of the plastic tube as a transportation method to a behavioural set-up, has the plastic tube in the home cage another important function, namely that of cage enrichment. Research has shown that mice housed without cage enrichment have impaired brain development, and that they show more stereotypical and anxiety-related behaviour(11). It is therefore to the advantage of both the animal and the researcher to use cage enrichment, since the research results become more reliable when the animals' housing is improved.

Conclusions

The use of a tunnel/tube as both cage enrichment and as a method for transporting mice to and from a behavioural set-up is recommended. This ensures both an improvement of their home environment and a reduction of handling stress. Moreover, this can result in less variance in the studies and is an easy way to remove a mouse from a behavioural set-up.

This article is dedicated to the memory of Prof. dr. Frauke Ohl.

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