

Key considerations for conducting aggressive behavior tests using ARM-II

- 1) The location for conducting ARM-II experiments is inside the animal housing room.

As mentioned earlier, ARM-II should be installed inside the animal housing room, and the experiments should be conducted within that room. Moving the mice to another room just before measuring aggressive behavior can make them feel anxious and subject them to acute stress. Since ARM-II is a device designed to detect the mental state of mice, the introduction of acute stress can affect the experimental data. Due to the high precision of ARM-II in measuring aggressive behavior, even seemingly minor factors can have a significant impact on the results.

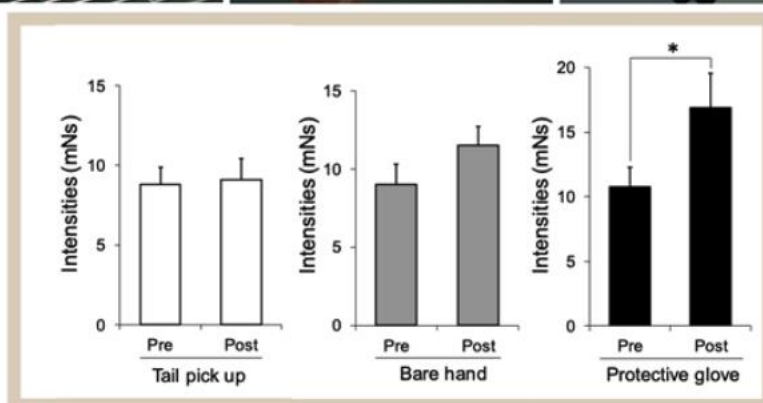
- 2) Building a Trusting Relationship Between the Experimenter and the Mice is Crucial

It is essential to establish trust between the experimenter and the mice. If the experimenter becomes a source of stress for the mice, accurate experiments cannot be conducted. Experimenters should take care of the mice themselves rather than delegating animal care to others. When transferring mice to a cleaned cage, it's recommended to pick up the mice by their tails. This action is similar to handling the mice when placing them in ARM-II's animal chamber, and it doesn't cause anxiety (acute stress) in the mice during the experiment. Experimenters should spend as much time as possible inside the animal housing room, share the space with the mice, and build a trusting relationship with them. The better the relationship between the experimenter and the mice, the more accurate the experimental data will be.

Despite being less hygienic, it is recommended to handle mice with bare hands without wearing gloves. For mice, the smell of a familiar experimenter is comforting. Wearing gloves while handling mice may sometimes enhance aggressive behavior. The photo and graph below show experiments involving intraperitoneal saline injections conducted by an experimenter who has built a good relationship with the mice. When the experimenter used gloves to restrain the mice, post-injection aggressive behavior significantly increased.

Mice dislike the smell of natural predators, but the scent of an experimenter's pet cats or dogs is not stressful. If the mice regularly smell the experimenter's scent, the smells of their pets become part of the experimenter's scent.

Changes in aggression levels before and after intraperitoneal injection of physiological saline.



Left : The mouse was restrained on a wire net, and a needle was inserted into the peritoneal cavity from below. No increase in aggression was observed (n=10).

Middle: The experimenter restrained the mouse by grasping the back of its neck with bare hands and injecting intraperitoneally while the mouse was on its back. Aggressive behavior increased, but the difference was not statistically significant (n=10).

Right: The experimenter wore gloves and similarly restrained the mouse on its back for injection. A clear increase in aggressive behavior was observed after the injection (n=10).

3) Access by outsiders to the mouse housing room should be prohibited on the day of the experiment.

When individuals who are not accustomed to mice enter the animal housing room, the mice become wary and agitated. If such a situation occurs during an experiment measuring aggressive behavior, it can lead to the intensification of the mice's aggressive behaviors, potentially affecting experimental data. The impact of outsiders entering the mouse housing room may persist for several hours. It is recommended to cancel experiments conducted on the day when outsiders have entered the mouse housing room and reschedule them for another day. It is recommended to cancel experiments scheduled for that day when outsiders have entered the mouse housing room and reschedule them for another day.

4) Please pay attention to the noise around the laboratory and to any odors infiltrating into the mouse housing room.

Mouse models for mental illnesses have a propensity to become agitated over minor disturbances. Therefore, during aggressive behavior measurement experiments, if unusual noises are heard, mice may sensitively react to them, potentially intensifying their aggressive behavior. When odors from other animals such as rats infiltrate the laboratory, mice become more cautious, resulting in changes in their aggressive behavior. Even subtle events that might go unnoticed by humans can cause stress for mental illnesses model mice. If you encounter any abnormal data in your experiments, it is essential to investigate the underlying cause.

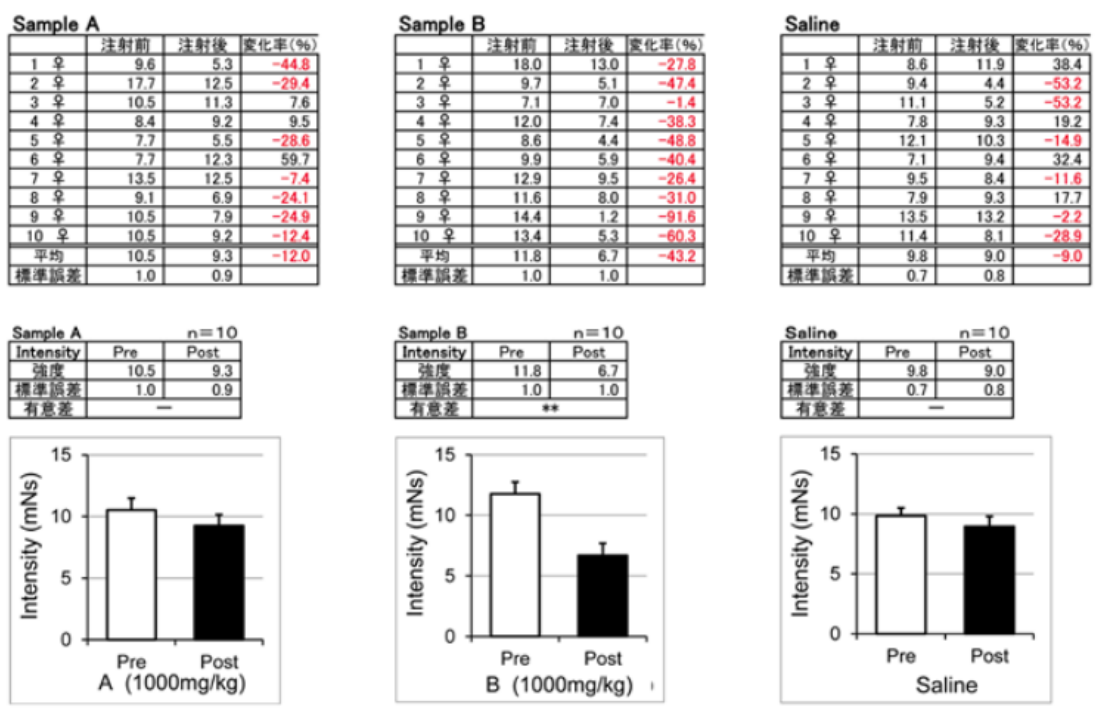
In animal research facilities, various experimental animals are often housed, and it is important to investigate the potential for odors from these animals to infiltrate the mouse housing room. Rooms where odors from different species of animals, such as rats, may infiltrate are not suitable for use as mouse housing rooms. Housing mice in a room where the odor of rats occasionally infiltrates can lead to the manifestation of stress symptoms in many mice, rendering them unusable as normal research animals.

5) Aggressive behavior data should be evaluated on a group basis.

The mood of psychiatric disease model mice is unstable, with some days being good and others where they are feeling down. When measuring aggressive behavior measurement using ARM-II daily in such animals, the numerical values fluctuate from day to day. For example, aggression might be 15 mNs two days ago, 20 mNs yesterday, and 10 mNs today, which can lead to questions like, "What are we even measuring here?" Since the mood of individual animals can vary from day to day, this variation is considered correct. Therefore, the importance of focusing on individual animals is relatively low.

However, in experimental setups where aggression is evaluated on a group level, there can be individuals with heightened and lowered moods that offset each other, making it easier to observe changes in the group as a whole. For instance, in a pharmacological efficacy experiment with antipsychotic drugs, aggression changes in a group of 10 experimental subjects are compared with a group of 10 control subjects using the average values before and after drug administration. If the drug is effective, aggression is suppressed in all individuals in the drug-administered group, leading to a significant difference before and after administration. In the control group, aggression might be enhanced in some individuals and reduced in others after administration, resulting in no significant difference before and after vehicle administration.

Substances A & B: Intraperitoneal Administration Experiment



Please refer to the data table and graph above. This is the verification data for two candidate substances (A & B) for a "calming supplement" requested by a certain research institution. The experiments were conducted using stress disorder model mice.

Sample B shows a significant difference before and after administration, suggesting a calming effect. However, there is no effect observed in Sample A and the control group, which received saline solution. When examining the data table for Sample A and saline solution, you will notice that some individuals exhibited an increase or decrease in values by more than 50% after administration compared to before. Surprisingly, this is a characteristic feature of the psychiatric symptoms in the model animals for mental illnesses. Some individuals become agitated after the injection, while others become more depressed, resulting in no significant difference in the average values for the ten cases before and after the injection.

In contrast, Sample B shows a suppression of aggressive behavior in all ten individuals. ARM-II is highly sensitive, so if the administered substance has a calming effect, it can detect it with high precision.

6) Breeding of the control group (normal mice) used in stress experiments

When conducting experiments to investigate the psychological effects of a specific stress on mice, normal animals without stress symptoms are used in the control group. However, when we measure aggressive behavior of mice using ARM-II, we often detect stress symptoms, even in mice believed to be normal. In reality, "raising mice in a completely stress-free environment" is not easy. It may be difficult for researchers to accurately understand what causes stress symptoms to develop in mice.

For example, researchers do not know the breeding conditions of the mice purchased from animal suppliers. One Japanese supplier reportedly allows mother mice to nurse all their offspring. Since mice typically give birth to ten offspring or more at once, there are not enough nipples for all of them. Naturally, some individuals develop faster than others. Mice with delayed development will inevitably exhibit stress symptoms due to inadequate breastfeeding. When purchasing mice from suppliers, it is important to be aware that mice with stress symptoms may be included.

For these reasons, researchers conducting stress studies are recommended to breed animals themselves. We breed animals within the laboratory, with strict management to ensure that no stress is imposed. Various measures, such as controlling room temperature/humidity, noise, odors, and restricting entry by outsiders, are in place to reduce stress. It is recommended to rear the offspring of the control group in groups of 5 to 8 individuals. Having more than 9 individuals may lead to stress symptoms due to inadequate breastfeeding, while having 2 to 3 individuals will impose stress similar to individual housing (Valzelli, 1969, Aggressive behavior, 70-76). In stress experiments, bonding with mice may also be effective (Gentsch et al., 1988, *Physiol. Behav.* 43, 12-16).

ARM II efficiently detects the emergence of even subtle stress symptoms, making it easy to identify mice raised under stress. In stress experiments, before entering the actual experiment, the intensity and frequency of the mice's aggressive behavior are measured to select the appropriate mice for experimental use. Subsequently, the normal mice are divided into two groups,

with half of them exposed to specific stress, while the other half continues to be reared as usual. ARM II excels at screening mouse aggression.

7) Replacement and Cleaning of Animal Chambers

In principle, please use one animal chamber per mouse. However, it may not always be necessary to replace the chamber with a new one, unless the chamber is soiled with feces and urine. Used chambers can be adequately cleaned with a mild detergent. Mice cohabiting in the same animal room do not exhibit increased vigilance towards the scent of chambers used by other mice.