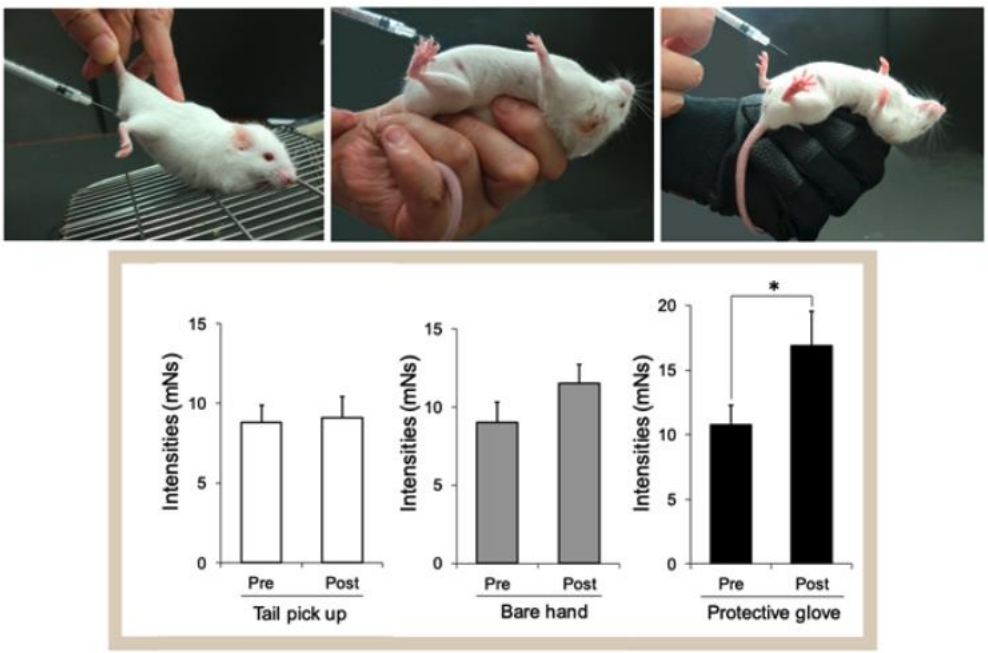
Key considerations for conducting aggressive behavior tests using ARM-II

1) Experimenter should make the mice remember your scent.

As mentioned earlier, if the experimenter themselves become a source of stress for the mice, it's impossible to derive accurate experimental data. Since mice identify their owners through scent, experimenters need to make sure the mice recognize their scent and accept them as caregivers. To achieve this, it's recommended for experimenters to handle the mice without gloves so that the mice can recognize their scent. It's known that when experimenters handle mice with gloves, it enhances aggressive behavior. The photos and graphs below depict an intraperitoneal injection experiment performed by an experimenter who has built a good rapport with the mice. When the experimenter wore gloves to restrain the mice, there was a significant increase in post-injection aggressive behavior.

While mice dislike the scent of predators, such as predatory animals, the scent of cats and dogs that experimenters may have at home doesn't cause stress. If experimenters smell like their own scent every day, the scent of dogs and cats seems to be recognized as part of the experimenter's scent.

Comparing aggression levels before and after intraperitoneal injection of physiological saline solution.



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).

2) In experiments, the entry of outsiders may reinforce aggressive behavior in mice.

Mice tend to be wary of unfamiliar individuals, so the presence of outsiders during experiments may lead to an escalation in aggressive behavior. If such a situation arises and it is determined that it affects the data, it is recommended to reschedule the experiment for another day.

3) 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.

4) 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

Sample A

	注射前	注射後	変化率(%)
1 ♀	9.6	5.3	-44.8
2 ♀	17.7	12.5	-29.4
3 ♀	10.5	11.3	7.6
4 ♀	8.4	9.2	9.5
5 ♀	7.7	5.5	-28.6
6 ♀	7.7	12.3	59.7
7 ♀	13.5	12.5	-7.4
8 ♀	9.1	6.9	-24.1
9 ♀	10.5	7.9	-24.9
10 ♀	10.5	9.2	-12.4
平均	10.5	9.3	-12.0
標準誤差	1.0	0.9	

Sample B

	注射前	注射後	変化率(%)
1 ♀	18.0	13.0	-27.8
2 ♀	9.7	5.1	-47.4
3 ♀	7.1	7.0	-1.4
4 ♀	12.0	7.4	-38.3
5 ♀	8.6	4.4	-48.8
6 ♀	9.9	5.9	-40.4
7 ♀	12.9	9.5	-26.4
8 ♀	11.6	8.0	-31.0
9 ♀	14.4	1.2	-91.6
10 ♀	13.4	5.3	-60.3
平均	11.8	6.7	-43.2
標準誤差	1.0	1.0	

Saline

	注射前	注射後	変化率(%)
1 ♀	8.6	11.9	38.4
2 ♀	9.4	4.4	-53.2
3 ♀	11.1	5.2	-53.2
4 ♀	7.8	9.3	19.2
5 ♀	12.1	10.3	-14.9
6 ♀	7.1	9.4	32.4
7 ♀	9.5	8.4	-11.6
8 ♀	7.9	9.3	17.7
9 ♀	13.5	13.2	-2.2
10 ♀	11.4	8.1	-28.9
平均	9.8	9.0	-9.0
標準誤差	0.7	0.8	

Sample A n=10

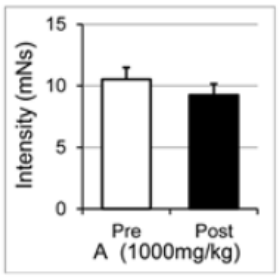
Intensity	Pre	Post
強度	10.5	9.3
標準誤差	1.0	0.9
有意差	—	

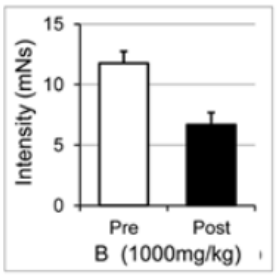
Sample B n=10

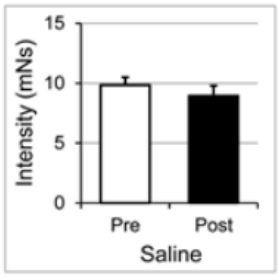
Intensity	Pre	Post
強度	11.8	6.7
標準誤差	1.0	1.0
有意差	**	

Saline n=10

Intensity	Pre	Post
強度	9.8	9.0
標準誤差	0.7	0.8
有意差	—	







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.

5) 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.

6) 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.