

Experimental Procedures for Aggressive Behavior Measuring

1) ARM-II quantifies the intensity of aggressive behavior Measurement theory of emotional aggression

Currently, the predominant method for assessing aggressive behaviors is the Resident-Intruder test. This test measures aggressive behaviors that appears as a part of male animals' "sexual behavior." In this test, researchers record the aggression displayed by resident males towards intruder males who enter their home cages using video recordings. Subsequently, the intensity of the animals' aggression is evaluated by the researcher. In contrast, ARM II is a "device for exploring the mental state" that measures aggressive behaviors expressed by experimental animals (mice) due to "irritation and anger" . [\(Please watch the video below featured in the Japanese version, with English subtitles enabled.\)](#)



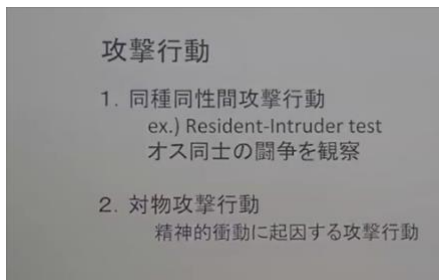
The Resident-Intruder test is a test of aggression in male animals' sexual behavior. On the other hand, the ARM-II test is a quantitative test of emotional aggression in mice.



Video Course for Graduate Students:

Quantitative Measurement of Aggressive Behavior Towards Inanimate Objects,

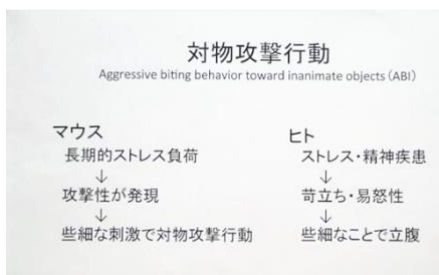
Kagoshima University Graduate School of Medical and Dental Sciences, Satoshi Kuchiiwa, Ph.D.



Observing Aggressive Behavior

1. Intraspecific Same-Sex Aggression
Male Territorial Fights.

2. Observing Object-Directed Aggression
Aggressive Behavior Resulting from Psychological Symptoms.



In mouse models of mental illness, aggression may manifest, and they may exhibit aggressive behavior in response to minor events. Similarly, in some patients with mental disorders, there can be an increase in irritability over minor matters, leading to raised voices and acts of violence. Mice displaying emotional aggression closely resemble these patients.

Testing with ARM-II consists of two sessions. First, a 'provocation session' is conducted, followed by a 'measurement session.' During the provocation session, the hind legs of the mice are lightly pushed up with two thick metal rods to induce irritation (anger) in the mice. The movement of the metal rods is relatively slow, so it does not cause pain, and normal mice do not pay much attention to it. However, in the case of psychiatric disorder model mice, they find these minor stimuli bothersome. When repeated multiple times, they cannot stay still and vigorously kick the rods away with their hind legs. After repeating this 30 times (5 minutes), the mice's anger reaches its peak, and in the subsequent 'measurement session,' they fiercely bite one of the two rods rising under their chin. The rods are positioned at 15mm intervals and stop on either side of the chin, so they do not forcefully push up the mouse's head. Normal animals are indifferent to the rods rising from below, but psychiatric disorder model mice cannot tolerate them and bite them. In stress model mice, a strong

correlation has been demonstrated between the 'duration of stress exposure' and 'biting intensity,' suggesting that the stronger the mental illness, the more vigorously they bite. ([Kuchiiwa & Kuchiiwa, 2014, J. Neurosci. Meth., 228: 27-34](#))

ARM II mechanically and automatically measures aggressive biting behavior, thus eliminating the subjective judgment of the experimenter. ARM II tests are not influenced by the female sexual cycle, allowing experiments to be conducted with female mice at any time. ARM II excels at detecting gender differences in emotional aggression. While there have been reports of gender-specific effects of psychotropic drugs, detecting gender differences in drug efficacy using ARM II is straightforward. ([Please watch the videos below featured in the Japanese version.](#))



Provocation session for normal mice



Measurement session for normal mice



Provocation session for mental disorder



Measurement session for mental disorder

2) How to Use Aggressive behavior measurement system (ARM- II)

① Installation and Configuration of ARM- II

ARM-II experiments should be conducted within the living space of the mice. Transporting the mice from the animal housing room to another room before the experiment can cause unnecessary stress to the mice, so please install ARM- II in the animal housing room. ARM- II is configured with the stimulus rod rising at a speed of 100mm/s and stopping for 1 second at a height of approximately 10 mm above the floor. Furthermore, within one session, this action is programmed to be repeated 30 times at 10-second intervals over a duration of 5 minutes. These settings have been determined based on extensive experimental data collected over several years. It is essential for researchers worldwide to conduct experiments under uniform conditions, so unless there are specific reasons to do otherwise, please carry out the experiments with these settings as they are.



② Mouse Housing and Bedding Change

Experimenters should enter and exit the mouse housing room frequently, personally care for the mice, and spend extended periods of time in the housing room. This allows the mice to memorize the experimenter's scent and helps establish a mutual relationship where the mice do not feel anxious when touched by the experimenter. During bedding changes, mice should be gently picked up by the base of their tails with bare hands and transferred to a new, clean cage. This action is similar to the process of introducing animals to the ARM II animal chamber.

Inside the mouse housing cage, place a cylinder of the same size as the animal chamber. This allows the mice to enter and exit the cylinder, using it as a plaything.

③ Introduction of Mice into the Animal Chamber



Before starting the experiment, mice are introduced into the animal chamber of ARM-Ⅱ. Gently grasp the base of the mouse's tail with your bare hand, lift it, place the mouse on your palm, and position its nose at the entrance of the chamber. The mouse will then enter the chamber on its own. If the mouse does not enter the

chamber voluntarily, it should not be forcibly placed inside. Subjecting the mouse to stress before the experiment can affect the experimental data. If the mouse becomes agitated when placing it in the animal chamber, it is advisable to cancel the experiment for that day.

(An explanation of the method for introducing mice into the animal chamber can be found in the second half of the video below. [Page 8](#))

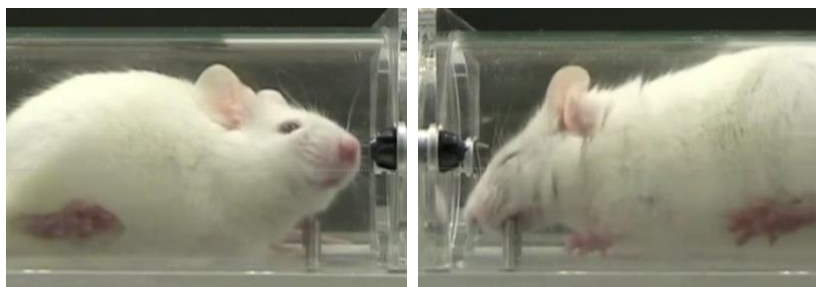
④ Implementation of Provocation Session

Upon entering the animal chamber, the mouse engages in exploratory behavior for a while, but after a while, it settles down. Please wait for a few minutes until the mouse has settled. Once the mouse is calm, please begin the 'provocation session.' The experimenter grips the handle of the ARM II's positioning device and manually moves the stimulator rods to the position of the mouse's hind paw sole. Upon pressing the start button of ARM II, two stimulator rods rise and press against the sole or abdomen of the mouse. The stimulator rods stop in the raised position for one second and then descend, concluding the first provocation stimulus. Nine seconds later, the two stimulator rods rise again. The experimenter observes the position of the animal's hind limbs and positions the two stimulator rods at the location. In the 'provocation session,' this action is repeated 30 times (for five minutes). If the mouse is irritable, it will show signs of

agitation and vigorously kick the two stimulator rods. Conversely, a normal animal will ignore the contact with the stimulator rods."

⑤ Implementation of the 'Measurement Session'

After the 'provocation session' is completed, please begin the 'measurement session' without delay. The procedure for conducting the 'measurement session' is the same as the 'provocation session,' but the position at which the stimulus rods are raised is adjusted to the level of the mouse's eye position. Pressing the start button of ARM II causes two stimulus rods to rise and stop on either side of the mouse's jaw. If the mouse is looking upward, the two stimulus rods will not make contact with the mouse's head. If the mouse is looking downward, the two stimulus rods will rise on both sides of the mouse's jaw. If the mouse is facing either left or right, one of the stimulus rods will push up the mouse's jaw. In case the mouse exhibits 'irritability,' it will bite one of the stimulus rods almost every time. In contrast, normal mice will ignore the movement of the stimulus rods. In mice with mild irritability symptoms, although they may bite the stimulus rods frequently, the biting force is not significantly strong. Therefore, mouse models with mild irritability tend to show a higher frequency of biting behavior expression rather than an increase in biting intensity."



⑥ Assessment of the Presence or Absence of Biting Behavior

During each trial of the 'measurement session,' the experimenter should observe the mouse's behavior. When the experimenter judges that the mouse has bitten the stimulus rod, press the 'response button'. The criteria for determining the occurrence of biting behavior include the following three conditions: (1) When the experimenter visually perceives that the mouse has bitten the rod. (2) When the graph displayed on the screen clearly exhibits the

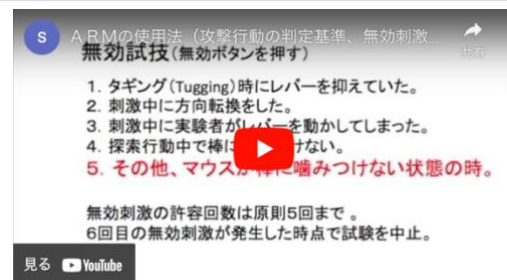
characteristic waveform of biting behavior. (3) When the display shows a value of 3 mNs (millinewton-second) or higher. It is difficult for the human eye to accurately judge the presence of mouse biting behavior. Even if it appears that the mouse has bitten the stimulus rod, it is often not the case. The reason for using 3 mNs as the threshold value is that the load sensor detects the movement when the mouse merely touches the stimulus rods. It is known that a value of 3 mNs or higher will not be displayed unless the mouse actually bites. Note that 3 mNs is the standard when using mice weighing 30 grams or more. When using smaller mice, it may be necessary to reduce the threshold value."

⑦ Criteria for Invalid Trial Determination

The experimenter needs to judge whether each trial was conducted correctly. In cases like the following, the 'error button' should be pressed, and the trial should be treated as invalid. When the invalid button is pressed, ARM II repeats the trial again. The higher the number of invalid trials, the greater the potential impact on the measurement results. It is advisable to discontinue the experiment when there are six invalid trials within one session. The experiment using that animal will be rescheduled for another day. The following circumstances are considered as invalid trials:

1. When the mouse tugs while the experimenter is holding the moving lever.
2. When the mouse changes direction as the stimulus rods start to rise and cannot bite the stimulus rods.
3. When the mouse is biting the stimulus rod, at the same time, the experimenter accidentally moves the moving lever.
4. When the mouse begins exploratory behavior during the experiment and becomes indifferent to the movement of the stimulus rod.
5. When the stimulus rod rises in a posture where the mouse cannot bite, such as when the mouse is lying on its back.

(Please watch the video on the right featured in the Japanese version, with English subtitles enabled.)



攻撃行動計測システム

Aggression Response Meter

ARM

室町機械株式会社



This video provides an explanation of the ARM-001, which was sold by the Muromachi Kikai Co Ltd. The operation of ARM-II follows the same principles as ARM-001, so please watch this video until a new one is available.

対物攻撃行動の計測

1. 強度 (mNs)
2. 頻度＝攻撃回数/session

攻撃行動の判定基準

1. 目視下に攻撃行動を確認
2. 攻撃行動特有の波形出現
3. 攻撃強度・・・3mNs以上

条件を満たす攻撃行動の回数を記録

Measurement of aggressive behavior

1. Intensity of aggressive biting
2. Biting frequency=biting action/session

Criteria for Assessing aggressive behavior

1. Biting behavior was visually confirmed.
2. A typical waveform of biting has appeared.
3. The biting intensity was 3 mNs or higher.

Record the number of biting behavior that meet criteria

No.	積分値(mNs)	反応あり	無効データ
23	6.66	0	0
24	6.17	0	0
25	57.26	0	0
26	6.55	0	0
27	5.45	1	0
28	5.79	1	0
29	13.25	1	0

When you press the 'response button', the number '1' is recorded in the 'response detected' field.

無効試技(無効ボタンを押す)

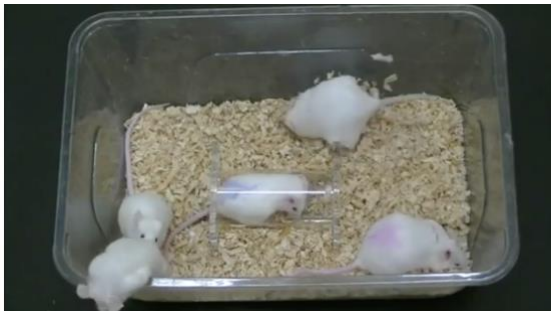
1. タグging (Tugging)時にレバーを抑えていた。
2. 刺激中に方向転換をした。
3. 刺激中に実験者がレバーを動かしてしまった。
4. 探索行動中で棒に噛みつかない。
5. その他、マウスが棒に噛みつかない状態の時。

無効刺激の許容回数は原則5回まで。
6回目の無効刺激が発生した時点で試験を中止。

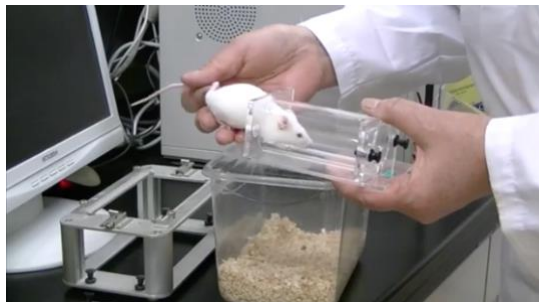
Invalid trials (Press the 'Error Button')

1. When mouse tugged, the experimenter was holding the lever.
2. During the trial, the mouse changed direction.
3. During the trial, the experimenter accidentally moved the lever.
4. The mouse is in exploratory behavior and indifferent to the stick.
5. When the mouse is in a posture where it cannot initiate biting behavior towards the sticks.

It is permissible to allow up to five invalid trials, but if there are more, the experiment should be discontinued.



When a tube of the same size as the ARM-II animal chamber is placed inside the mouse's cage, the mouse will enter and play with it. By doing this, during the experiment, the mouse will enter the chamber on its own without feeling anxious.



When placing the mouse into the animal chamber, the experimenter gently grasps the base of the mouse's tail, lifts it, and places it on the palm of their hand. Then, by bringing the mouse's face close to the entrance of the chamber, the mouse voluntarily enters the chamber.



Since mice may urinate during the experiment, urinary pads are necessary. Urinary pads are created by folding highly absorbent paper multiple times and punching two holes at 15mm intervals. This is completed by inserting them onto the two Stimulus Rods of the ARM-II.