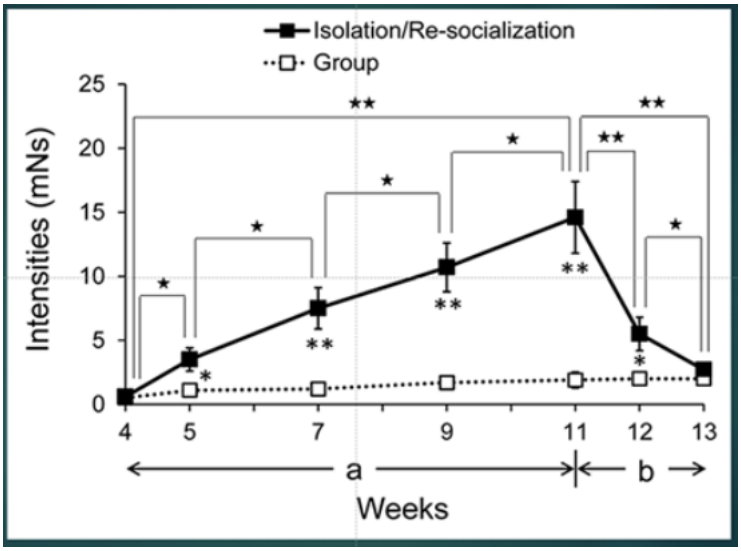
What ARM-Ⅱ can do

1) Quantitative Measurement of Emotional Aggressive Behavior

ARM-II allows for the quantitative measurement of the intensity and frequency of aggressive behavior towards inanimate objects (object-directed aggressive behavior) in mice, assessing the mental state of mice with explosive anger caused by pathological irritability. In other words, it brings out the frustration and anger within the minds of mice with psychiatric disorder as aggressive behavior towards inanimate objects and uses it as an indicator to evaluate the severity of the mice's mental symptoms.

The graph below tracks changes in object-directed aggressive behavior over seven weeks after transitioning from group housing to individual housing (isolation) at four weeks of age. Furthermore, it examines changes in aggressive behavior for two weeks after returning to group housing. Under individual housing conditions, the mice experience loneliness stress, and their stress symptoms progressively worsen. Stress symptoms are detected within one week of individual housing, and there is a clear linear progression of symptoms during the stress exposure period. However, when the mice return to group housing after more than seven weeks of isolation, they return to a normal state within two weeks. (n=13)



2) Screening Mouse Aggressive Behavior

Even among psychiatric disorder model mice created under the same conditions, there are individual differences in the expression of psychiatric symptoms. For example, even when exposed to the same level of stress, the intensity of stress symptom manifestation varies among mice. In tests examining the effects of psychotropic drugs, variations in symptoms among individuals can result in differences in drug response, making drug efficacy evaluation unclear. Therefore, it is effective to conduct experiments with animals exhibiting roughly the same level of symptoms, and thus, screening the symptoms of the animals becomes necessary.

ARM-Ⅱ is capable of accurately and efficiently screening the mental symptoms of animals. ARM-Ⅱ can easily quantify the intensity and frequency of aggression in psychiatric disorder model mice. Since the time required for evaluating a single mouse is within 15 minutes, screening dozens of mice in a day is feasible, allowing the selection of animals for experiments. By pre-selecting mice with similar levels of psychiatric symptoms, clear results can be obtained.

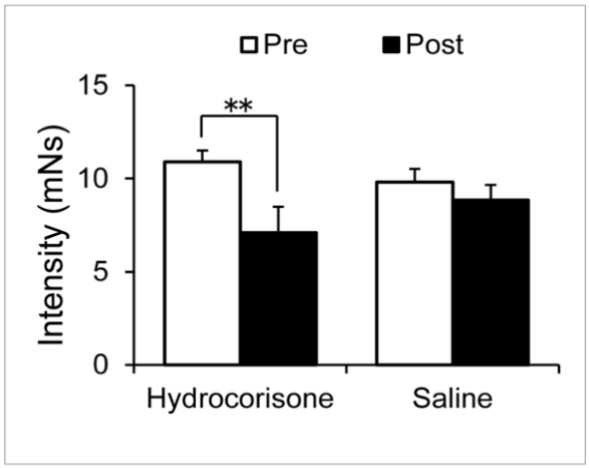
Animal screening with ARM-Ⅱ is not limited to aggression tests but is highly effective in evaluating psychiatric symptoms in other behavioral experiments as well, such as elevated plus-maze tests, open-field tests, light-dark box tests, social interaction tests, and more. We typically select around 20 mice with roughly the same level of irritability (averaging around 10 mNs), allocating half to the experimental group and half to the control group. In many experiments we have conducted so far, clear results have been obtained with this number of animals.

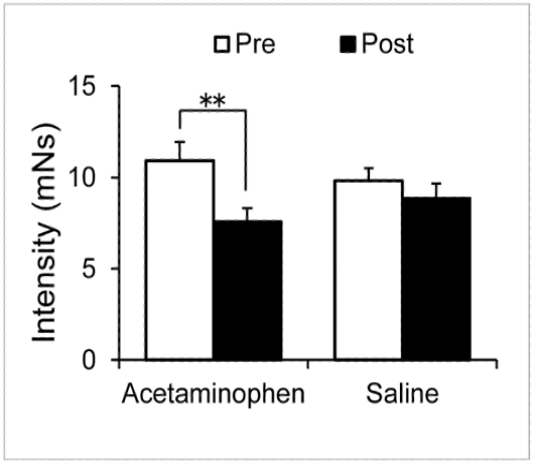
3) ARM-Ⅱ is possible to detect subtle changes in aggressive behavior (mental symptoms).

When using psychiatric disorder model mice and wanting to investigate changes in aggressive behavior after administering a certain drug or applying specific stress, other methods (such as the Resident-Intruder test) often fail to

detect significant changes in aggressive behavior unless they are relatively large. In contrast, ARM-Ⅱ can detect even slight changes in animal behavior, allowing for a significant reduction in the experimental duration. For instance, as shown in the graph above, ARM-Ⅱ was able to detect aggressive behavior in mice subjected to one week of isolation-induced stress. In comparison, the Resident-Intruder test requires a minimum of three weeks of isolation to confirm the expression of aggression (Yen et al., 1959: Arch. Int. Pharmacodyn. 123:179-185), highlighting the high sensitivity of ARM-Ⅱ. It is capable of detecting subtle changes in mental symptoms that may remain undetectable by other methods.

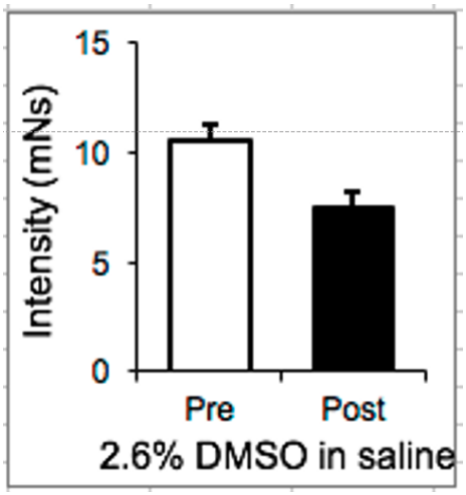
We reported at the Japan Pharmacological Society that there is an inhibitory effect on aggressive behavior in substances such as analgesics and anti-inflammatories (e.g., NSAIDs, glucocorticoids, acetaminophen), olive oil, and DMSO. Our data presented at the conference were met with skepticism from other researchers, as detecting the inhibitory effects of these substances on aggressive behavior is impossible using methods other than the ARM II object-directed aggressive behavior test. Without using ARM II, it would be hard to even notice their existence. However, with the increasing number of researchers using ARM II, it will become a widely recognized fact in the near future that anti-inflammatory and analgesic agents have a mild sedative effect.





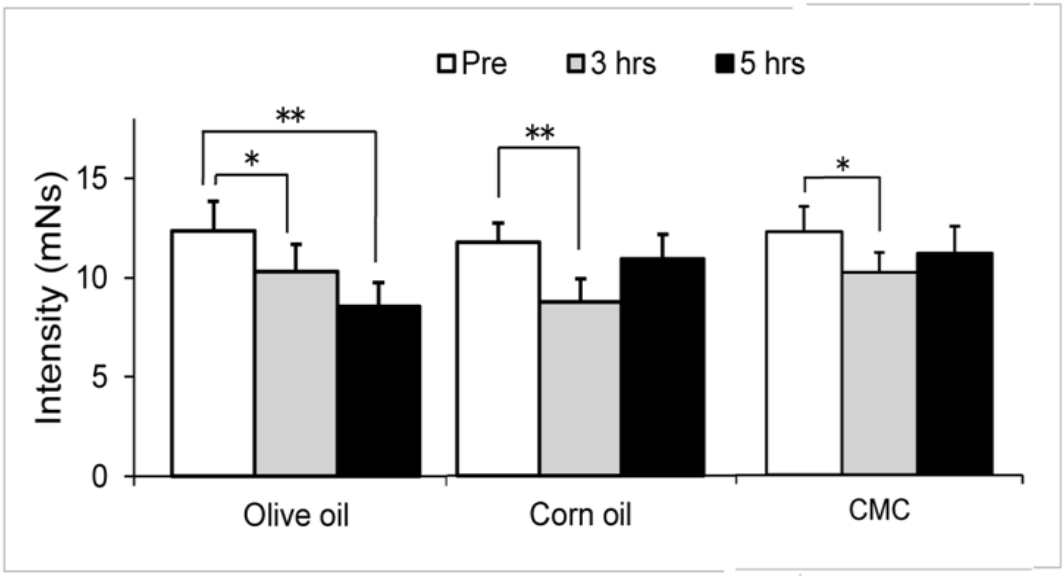
The figure below indicates a significant reduction in aggressive biting behavior following intraperitoneal administration of DMSO (dimethyl sulfoxide) in the ARM-II object-directed aggressive behavior test. DMSO is known to have an effect on blocking the conduction of C-fibers that transmit pain perception (Evans et al., 1993, Neurosci. Lett., 150: 145-148). Therefore, it can be inferred

that the analgesic effect of DMSO resulted in a sedative effect on the mice. DMSO is commonly used as a solvent for drugs. In our experiments using the ARM II for aggressive biting tests, we experienced disruption in the experimental animals' aggressive behavior when a sedative substance was dissolved in DMSO. We have reservations about using DMSO as a solvent in experiments related to psychotropic drug efficacy testing.



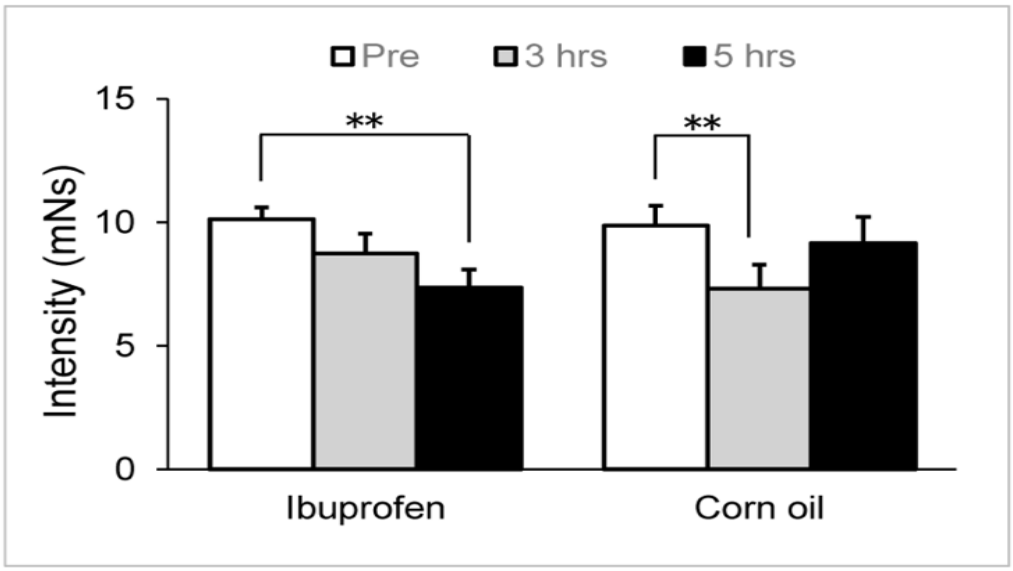
The figure below represents an experiment conducted to verify the sedative effect of olive oil. In the control group, we used corn oil or CMC (carboxymethyl cellulose), which have similar viscosity to olive oil. We orally administered an amount sufficient to satiate the mice and measured the intensity of object-directed aggressive behavior using ARM II at 3 hours and 5 hours after administration. As shown in the figure, at 3 hours after administration, all substances exhibited suppression of object-directed aggressive behavior. While corn oil and CMC returned to their original levels after 5 hours, olive oil continued to suppress aggression even at 5 hours. Since aggression suppression appeared 3 hours after gastric administration of any substance, this phenomenon can be inferred to be caused by parasympathetic nerve stimulation due to gastric wall compression (satiety effect). The loss of the aggression-inhibiting effect of corn oil and CMC after 5 hours can be attributed to the movement of these substances from the stomach to the intestine.

The continued aggression suppression effect of olive oil even after 5 hours suggests that it contains substances with sedative properties. Olive oil is known to contain a substance (oleocanthal) structurally similar to ibuprofen, which has anti-inflammatory and analgesic effects (Beauchamp et al., 2005, Nature, 437: 45-46). Therefore, the continued sedative effect after 5 hours is indicative of the action of oleocanthal.



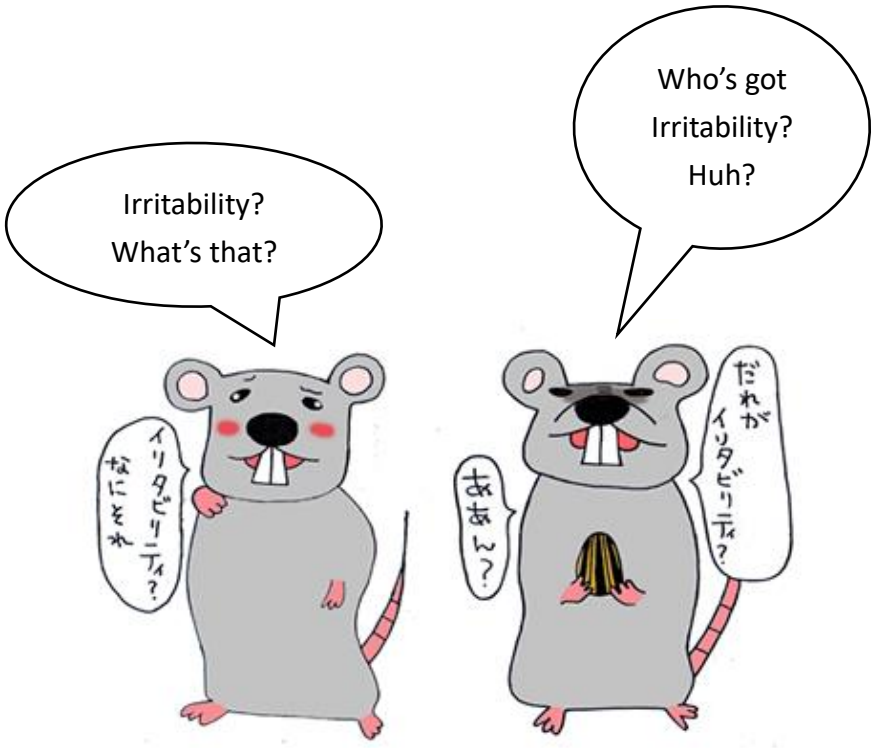
The figure below is an experiment conducted to confirm this. When ibuprofen was dissolved in corn oil and orally administered, similar to olive oil, aggression behavior suppression continued even after 5 hours.

While olive oil is used as a solvent for drugs, caution is necessary when using it as a solvent for psychotropic drugs.

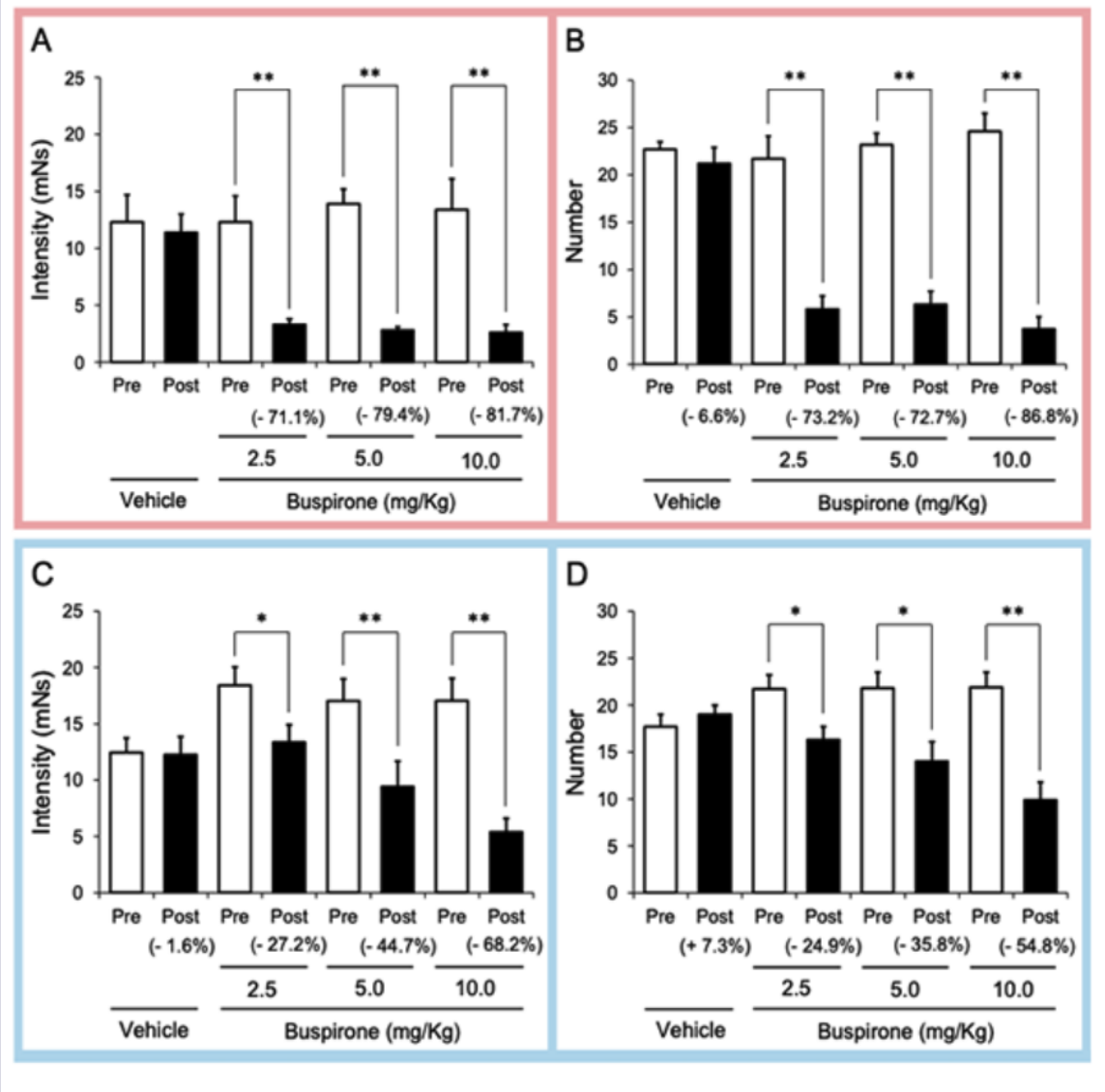


4) Detecting Gender Differences in Mental Activity

It goes without saying that emotional aggressive behavior is expressed in both males and females. Researchers familiar with the Resident-Intruder Test may be surprised when they hear about measuring aggression in female animals. Still, anger stemming from mental frustration is common in both humans and animals, regardless of gender. Patients with pathological irritability, regardless of gender, tend to lash out at those around them when their symptoms of irritability intensify. Likewise, in psychiatric disease model mice, when they become irritable, they may bite anything, be it an experimenter's finger or a stimulus stick of the ARM-Ⅱ when they become irritable. While it is believed that there may be clear gender differences in such mental states, trying to quantify and graphically represent them is not easy. However, when comparing male and female aggression using ARM II, it is easy to detect clear gender differences. While there have been few studies examining gender differences in aggressive behavior, using ARM II makes these differences so evident that it is predicted that there will be an increase in research taking gender into consideration.



Analgesic agents suppress an aggressive behavior



The graph above investigates gender differences in the effects of the psychotropic drug (Buspirone) on aggressive biting behavior. The upper pink section represents female mice, while the lower blue section represents male mice. Aggression behavior intensity (A, C) and aggression behavior frequency (B, D) were compared before and 30 minutes after administration. In females, aggression behavior was almost completely suppressed with a dose of 2.5 mg/kg, but in males, even a dose of 10 mg/kg did not completely suppress agonistic behavior. (Note: The criterion for aggression behavior intensity is 3 mNs or higher.)

5) Long-Term Tracking of Changes in Aggressive Behavior

The Aggressive Response Meter II (ARM-II) allows for long-term tracking of changes in aggressive behavior. The aggressive response test conducted with the ARM-II itself has been demonstrated to induce very minimal stress in normal animals (Kuchiiwa & Kuchiiwa, 2014, J. Neurosci. Meth., 228: 27-34). However, it is indeed stressful for mice used as models for psychiatric diseases. In general, when chronic and repetitive stress is administered, stress symptoms tend to worsen. Nevertheless, the ARM II-based aggressive response test does not exacerbate stress symptoms even when the same animal is used repeatedly (Kuchiiwa & Kuchiiwa, 2014, J. Neurosci. Meth., 228: 27-34). This is believed to be due to the ARM II's ability to detect "reflexive behaviors" in response to unpleasant stimuli. This characteristic of the ARM II makes it well-suited for conducting long-term investigations to track the effects of drug administration or stress.

By the way, in the ARM II test, the rising time of the stimulus rod is set at one second, as it is designed to detect only reflexive behaviors that occur immediately after the stimulus rod rises. Aggressive behaviors initiated after a delay in response to the stimulus rod are considered non-reflexive, and the ARM II excludes them from analysis.