Calculating aggressive behavior toward inanimate object using a machine (Aggressive Behavior Measurement system: ARM-Ⅱ)

With the development of a psychotropic drug, we assess how aggression of the laboratory animal changes after giving it the new drug, surveying the details and assessing the effect of said drug on the brain. Generally, male laboratory animals are made to fight each other and it is analyzed for aggression (Resident Intruder Test). But the results of this method may vary based on the relationship between the animals, as well as bias of the observer. Furthermore, since male hormones are required for aggression to be exhibited, the inability to use female laboratory animals is a disadvantage, and since we know the effect of the psychotropic drug differs greatly between the two genders, performing the aggressive behavioral test on male laboratory animals alone is an issue. With the development of this psychotropic drug, the appropriate thing to do would be to introduce an aggressive behavioral test that is not influenced by male hormones.

What do you mean?

Am I getting annoyed ?

A mental disorder model mouse that reacts hypersensitively to being touched.

A normal mouse does not respond when touched with a rod.

Our research group has developed a tool to measure aggressive behavior towards inanimate objects (Patent number 4858996 Aggression Response Meter: ARM). This tool focuses on the mouse's aversion to objects that contact it's body, and the increase in aggression to said objects in mice that have stress disorder and depression-like diseases. Normal animals don't react much

when poked with a stick, but those with mental illnesses cannot tolerate it and tries to eliminate it by biting it aggressively. The aggressive behavior that are exhibited in these animals with mental illnesses is known as aggressive biting behavior towards inanimate objects. As these mental symptoms get stronger, their aggression towards objects becomes stronger. Therefore, if we were to measure the force at which the animals bite the stick, we can evaluate how heavy the mental symptoms in said animals are, and since it is a mechanical measurement, there is the advantage of subjectivity and bias not affecting the results and lowers the differences in data based on individual researchers' bias. The tool we have invented was technology transferred to Muromachi Kikai Co. Ltd. and is being manufactured (Aggression Response Meter: ARM).

Analysis of Aggressiveness in Psychiatric model mice

When young mice that have ingested dioxin via placenta/breast milk reach puberty, an irritated behavior becomes prominent. Since said state matched with the behavior of model animals with depression, our research group inferred that there is a possibility of depression being caused by dioxins. We thought to diagnose those symptoms quantitatively, but until then a behavioral research method to measure this irritation did not exist.

Patients who are abnormally more irritable react strongly to trivial things and tend to explosively switch from a calm mental state exposing discomfort or anger. An abnormal increase in irritability is not only a dioxin disorder but rather stress disorder, depression, bipolar disorder, drug-induced mood disorder, schizophrenia, personality disorder, dementia, pervasive development disorder, ADHD, disruptive behavioral disorder, and such, which can be seen on a daily basis on many patients of mental diseases and development disabilities. They may also appear as a side effect to psychotropic medicines and drugs. Furthermore, they may also appear when interrupting the use of these drugs.

Similar to patients with mental disorders, various model mice or rats with mental illness are also exposed to abnormal behavior due to irritability. For that reason, they generally have a cumbersome nature to an experimenter. For example, if you were to grab a model animal with a mental illness by hand, the animal aggressively runs around their home cage, and at times even biting your fingers. If you persistently touch the heads of animals with a stick, they bite it and try to eliminate it (aggressive behavior towards inanimate objects). (Please refer to videos 1 and 2.) Since the behavior of these model animals with mental illnesses cannot be observed in normal laboratory animals, it can be thought that they suffer from brain diseases or types of symptoms associated with brain disorders. Aggressive behavior towards inanimate objects is the characteristic unusual behavior in animals that have abnormal irritability symptoms. We believe that it is necessary to establish technology to quantitatively evaluate the behavior in animals when we think that various mental illnesses and brain disorders are accompanied by abnormal irritability.

A part of model animals with mental illnesses is that they are bred by loading chronic stress on them. Long term isolated breeding models (breeding one per cage for a long period of time) are

a typical example of this, and are widely used for evaluation tests and such for psychotropic drugs (since mice are animals that live in groups, putting them in isolation accumulates stress and symptoms stress and symptoms of depression appear). Our research group keeps mice in isolation for long periods of time to develop symptoms of stress in them, and using aggressive behavior as an index we conduct an evaluation test on their irritability. We also investigate the change in reaction after administering the psychotropic drug, and verify the validity of whether quantitative evaluation of aggression uses their response behaviors as indices.

In this research, in order to measure expression of touch escape behavior and aggressive biting behavior towards inanimate objects, we developed a research tool (Aggression Response Meter: ARM) and examined the change in both behaviors. After beginning isolated breeding, the intensities of touch escape and aggressive biting behaviors gradually increased, and conversely, when the animals were returned to group breeding the intensities for both responses decreased remarkably. Not to mention, when anxiolytic drugs are administered to the irritated animal of isolated breeding with strong touch escape or aggressive biting behaviors, both decreased significantly. Through these experiments, we found that along with stress load, both touch escape and aggressive biting behaviors increase, and that administration of anxiolytic drugs both weaken said behaviors. This research revealed to us that by measuring touch escape and aggressive biting behaviors, we can accurately assess symptoms of mental illnesses in animals.

Photic Sneeze Reflex

The phenomenon of photic sneeze reflex is the occurrence of reflexive sneezing triggered by exposure to light stimuli. It happens at the moment when one experiences brightness, such as when moving from indoors to the outdoor sunshine or when direct sunlight enters the eyes. The photic sneeze reflex occurs only while perceiving brightness, so the number of sneezes is typically one or two, and it does not happen repeatedly.

The photic sneeze reflex is a physiological (normal) reflex that exists only in some individuals, not everyone. In our survey study conducted with a sample of 749 individuals, it was found that approximately 25% of Japanese people exhibit this reflex (Medicine and Biology, 1992, Vol. 125, No. 6, pp. 215-219). However, within this group, there are individuals who rarely experience the reflex or had it frequently in their youth but not anymore. Therefore, it is estimated that only about half of those with the photic sneeze reflex experience it regularly. The photic sneeze reflex is expressed within families, suggesting that it is likely transmitted to descendants through dominant inheritance. Even among individuals with the photic sneeze reflex, there is a significant individual variation in the light intensity that induces sneezing. Some people only experience the reflex with intense light, such as direct sunlight, while others may sneeze with weak light, like

indoor lighting. Regarding the mechanism behind this phenomenon, our research group speculates as follows.

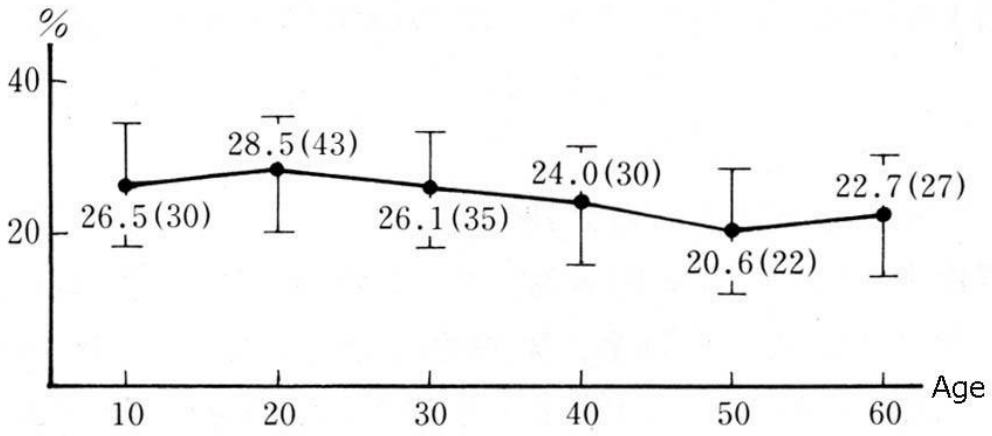
Sneezing can occur not only when foreign substances enter the nasal cavity but also in response to signals from the brain. For some reason, when a group of nerve cells called the salivary nucleus in the medulla oblongata becomes excited, parasympathetic nerve fibers originating from it reach the pterygopalatine ganglion (a group of nerve cells located in the pterygopalatine fossa deep behind the upper jaw) through the facial nerve. Furthermore, nerve excitation is transmitted to the nasal mucosa, leading to nasal mucus secretion and blood vessel dilation. As a result, a tingling sensation occurs in the nasal mucosa, which is the cause of the sneezing reflex.

Our research group anatomically demonstrated that the pterygopalatine ganglion is not only controlled by the facial nerve but also by the oculomotor nerve (The Journal of Comparative Neurology, vol. 300: p301-308, 1990). Intense light entering the eyes is transmitted to the pretectal olivary nucleus in the midbrain and further relayed to the Edinger-Westphal nucleus (EW nucleus). The parasympathetic nerves originating from the EW nucleus are conveyed through the ciliary ganglion located behind the eyeball to the pupillary constrictor muscle, causing the pupillary light reflex (contraction of the pupils in response to bright light). We discovered that a portion of the nerves originating from the EW nucleus reaches the nasal mucosa control cell region of the pterygopalatine ganglion. It can be inferred that the EW nucleus, while inducing the pupillary light reflex, simultaneously stimulates the nasal mucosa control cell group in the pterygopalatine ganglion, leading to vasodilation and nasal mucus secretion.

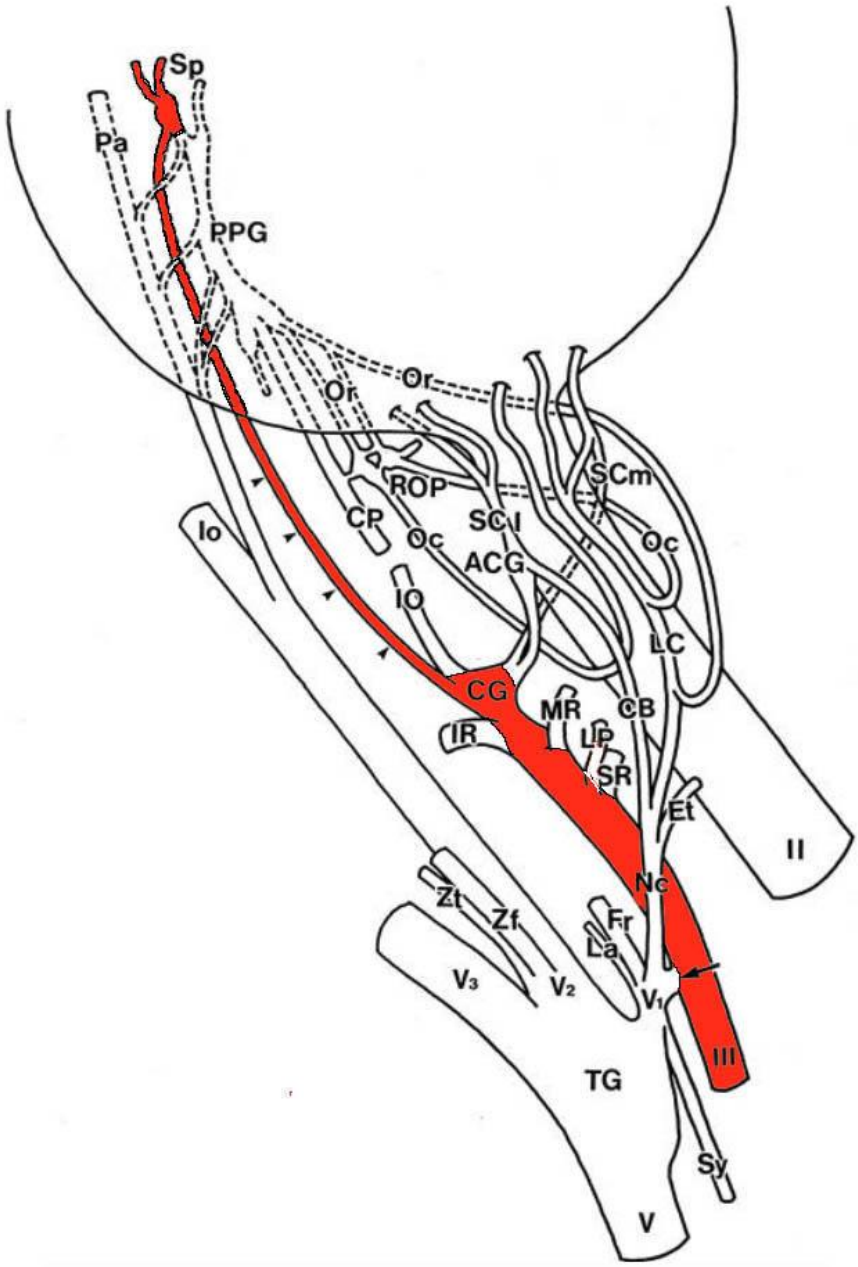
Incidentally, the facial nerve is part of the neural system associated with emotions. The facial nerve is responsible for creating facial expressions, shedding tears, and causing facial flushing in response to emotional movements. Intense emotional states can also lead to nasal mucus secretion and vasodilation, so although rare, some individuals may experience sneezing when indulging in sexual fantasies. (Bhutta and Maxwell, 2008)

Photic Sneeze Reflex Ownership Rate by Gender

Gender	Number of survey participants	Number of Owner	Number of Non-owner	Ownership rate (%)
Female	358	88	270	24.6
Male	391	99	292	25.3
Total	749	187	562	25.0



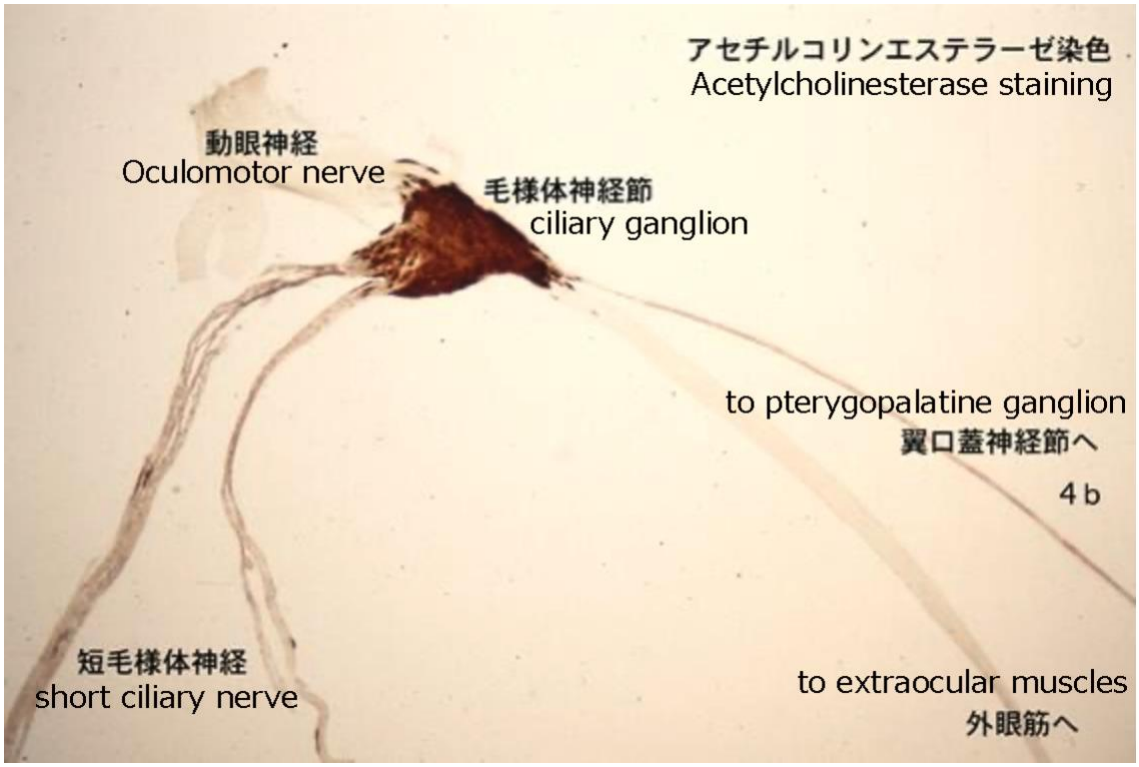
Photic Sneeze Reflex Ownership Rate by Age Group



Neural Network between the cranial base and the orbit
(Indicating the connection of the oculomotor nerve and
the pterygopalatine ganglion)

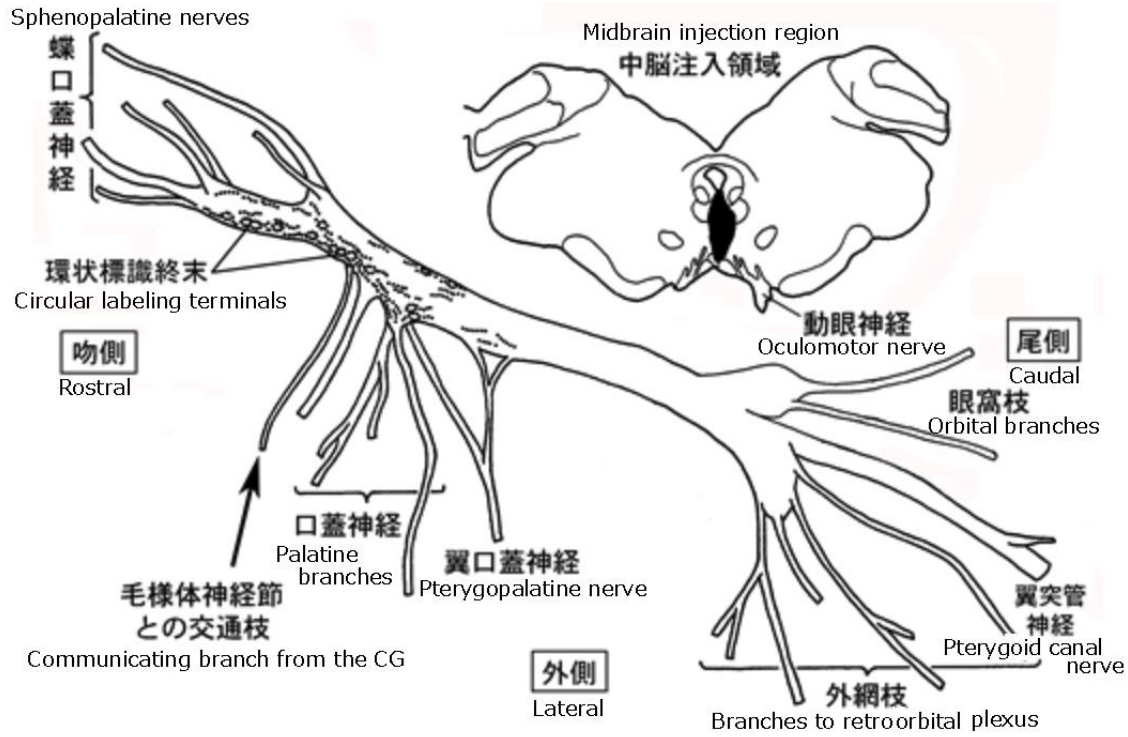
The parasympathetic preganglionic fibers originating from the Edinger-Westphal nucleus (EW nucleus) enter the orbit along the oculomotor nerve (III) and reach the ciliary ganglion (CG). A nerve that pass through the ciliary ganglion (indicated by four arrowheads) then travel to reach the rostral part of the pterygopalatine ganglion (PPG). From this region of the ganglion, the sphenopalatine nerve (Sp) emerges, distributing to the nasal mucosa and palate. II, optic nerve;

V, trigeminal nerve; ACG, accessory ciliary ganglion; ROP, posterior orbital nerve and ganglion. Or and Oc represent vasomotor nerves projecting to the eyeball from the pterygopalatine ganglion or posterior orbital ganglion. Arrow indicate communicating branches between the trigeminal nerve and the ciliary nerve (including parasympathetic preganglionic fibers reaching the ciliary ganglion via the trigeminal nerve); CB represents the trigeminal root entering the ciliary ganglion.



Microscopic photographs of acetylcholinesterase staining (AChE staining) specimens of the oculomotor nerve. In the nerves traveling from the ciliary ganglion to the pterygopalatine ganglion (4b), both AChE-positive and AChE-negative fibers are present. The preganglionic fibers of the parasympathetic nervous system are AChE-negative, while the postganglionic fibers are AChE-positive. Therefore, AChE-negative fibers traveling from the ciliary ganglion (CG) to the pterygopalatine ganglion (PPG) may originate from the Edinger-Westphal (EW) nucleus, serving parasympathetic preganglionic fibers to nasal mucosa control cells located in the rostral part of the PPG. On the other hand, AChE-positive fibers are postganglionic fibers of the CG cells, and they may pass through the PPG, distributing to the nasal mucosa via the sphenopalatine nerve

(Sp). These findings strongly suggest the potential involvement of the EW nucleus in the regulation of blood flow and nasal secretion in nasal mucosa.



Midbrain sections and pterygopalatine ganglion sections in an experiment involving microinjections of a tracer substance into the region containing the Edinger-Westphal (EW) nucleus in cats: illustrating the injection site of the tracer substance and showing the distribution of anterograde labeling.

The injection site covers the Edinger-Westphal nucleus (EW nucleus). Anterograde labeling, arranged in a circular pattern around large postganglionic neurons in the rostral part of the pterygopalatine ganglion (PPG), was prominently observed. This suggests the presence of anterograde projections from the EW nucleus to the rostral part of the PPG, where labeling appeared is known to autonomically control the nasal mucosa through the sphenopalatine nerve (Sp). Large cells of the PPG are known to control exocrine glands (quoted from "Why do we sneeze when we see the light?" Clinical Neuroscience, Vol. 33, 2015).