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Cori Bargmann's Lecture Part 1:
Genes, The Brain and Behavior

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1. Keywords and Terms

Brain disorders, neuroscience, neuroethology, G protein coupled receptors (GPCR), olfaction, *C. elegans*, ODR-10, circadian rhythm, narcolepsy, cataplexy, hypocretin, social behaviors, oxytocin and vasopressin

2. Lecture Notes

For Part 1: Your Introductory Lecture

Why do we think we can understand brain disorders by studying genes, and why do we use model organisms to understand gene functions?

Familial risk of psychiatric illness is much higher in identical twins than in the general population or even between siblings. This serves as a basic foundation of the

hypothesis that genetic, heritable elements could be a major contributor to brain disorders. Phenylketonuria (PKU), the first genetically defined brain disorder, leads to mental retardation and movement disorders. PKU patients have mutations affecting a single gene, which produces the enzyme phenylalanine hydroxylase, which converts L-phenylalanine to L-tyrosine. In the presence of the mutated enzyme, toxic chemicals accumulate and neuronal growth deteriorates. With the understanding of this altered metabolic state in PKU, we can limit phenylalanine in the diet of PKU patients, and therefore limit the production of the toxic chemicals. By extension, we hope that understanding disease genetics will greatly facilitate improved treatments for other brain disorders.

Humans share most of their genes with other organisms. In fact, almost half of our genes are present even in invertebrate, and only about 1% of our genes are unique to humans. Brain regions and functionality are similarly conserved from human to animals, extending as far as sharks and mice. Using molecular biology tools to study genes in simple model organisms may provide insight into the role of the same genes in the human brain and behavior.

What is neuroethology?

Neuroethology is the study of animal behavior and the underlying neurological mechanisms that are common and conserved among species in evolution. For example, Niko Tinbergen's work on stickleback fish established the idea that specific external stimuli can induce stereotypical behavioral responses like courtship, food searching, and aggression. Konrad Lorenz's work on imprinting in geese suggested that the nervous system has an internal drive to elicit certain behaviors that only can be established at certain developmental stages. Studies of social behaviors of honeybees by Karl von Frisch demonstrated that social structure is widespread among animals.

Classical neuroethology has helped to construct a general framework of behaviors – animals receive environmental stimuli through perception, or through internal state and motivation drive (with or without modification from internal states like memory), to make decisions and then produce behaviors. Modern neurobiology is using newly emerged genetic and molecular biology tools to study questions from classical neuroethology, and to understand how animals use genes, neurons, and brain regions to perform a series of actions.

GPCRs – an important class of molecules regulating animal behaviors

G-protein coupled receptors (GPCR) are a large class of transmembrane proteins that detect stimuli outside the cell and propagate signals inside the cell. These biochemical signals then impact cellular function; especially when the cells are neurons, the ligand can impact behavior. Stimuli that are recognized by GPCRs include external cues such as odors and light, as well as internal cues such as hormones, neurotransmitters, and neuropeptides. Therefore, GPCRs initiate sensory processes like vision and olfaction, and also mediate signaling between neurons, as well as between neural system and other tissues.

Chemical sensation – a receptor expressed in sensory neurons encodes behavioral responses

C. elegans is a valuable animal model for studying the nervous system and behaviors. Every neuron in *C. elegans* is known, and therefore can be studied in details. For example, ODR-10, a G-protein coupled odor receptor, is expressed in the *C. elegans* sensory neuron AWA where it detects the attractant odor diacetyl. Detection of the odor activates AWA, then a decision-making network, and followed by motor neurons that drive the attractive behavior toward diacetyl. In a typical chemotaxis assay, wild type *C. elegans* will approach and accumulate at the diacetyl spot, and mutant animals without ODR-10 will ignore the odor and scatter on the plate.

When ODR-10 is deleted from AWA neuron by a genetic mutant, then introduced into another neuron with a genetically engineered transgene, we observe a new behavior.

Expressing ODR-10 in AWB, a neuron that normally senses repellent, gives rise to the reprogrammed animals that avoid diacetyl instead of approaching it. The combination of a genetic mutant and transgenic manipulations demonstrated that in *C. elegans*, sensory neurons encode behavioral responses, i.e. the sensory neuron AWA drives attraction while the AWB sensory neuron drives repulsion.

Similar principles may apply in other animals, including mammals. Most mammals favor sweet food and avoid bitter tastes, as an evolutionarily conserved strategy to acquire nutrients and avoid toxins. This innate preference is hard-wired in the mouse brain in a fashion that reminds us of AWA and AWB in *C. elegans*. An artificial receptor called RASSL responds to a synthetic chemical that does not exist in nature. When RASSL is

expressed in the cells in the taste bud that sense sweet food (TIR taste cells), mice will drink water flavored with the synthetic ligand, but normal mice won't. When the same RASSL ligand is expressed in T2R taste cells that sense bitter taste, mice reject the synthetic chemical. This experiment shows that animals from *C. elegans* worms to mammals may use the fixed sensory neurons and anatomical pathways to perform innate behaviors such as avoiding dangerous food and favoring nutritious food.

Using fruit flies to understand sleep, an internally generated behavior

Sleep is conserved throughout the animal kingdom. Like humans, the fruit fly Drosophila has sleep and waking, and an activity pattern that maintains a 24-hour rhythm even in constant darkness. This internal activity rhythm is defined as a circadian rhythm; an internal pacemaker that drives sleep and wake cycles. Benzer and colleagues pioneered the genetic analyses of circadian rhythms by searching for fly mutants with abnormal sleep patterns. Intriguingly, they found different mutants in a single gene, per, can cause either a circadian cycle longer than 24 hours or a cycle shorter than 24 hours, or a completely random circadian cycle. These results suggest that per may play a key role in determining sleep cycles.

Indeed, additional studies have shown that the intracellular circadian clock of flies and many other animals is controlled by a negative feedback loop composed of per and several other genes that regulate transcription on a 24-hour cycle. The basic 24 hour cycle can be simplified down to two genes, per and clock, as though many others are involved. At night, a transcription factor clock gene turns on the expression of the per gene. At the transition to day, per expression has risen to a threshold and the PER protein starts entering the nucleus. Per is a transcriptional repressor that inactivates the clock gene, interrupting the expression of clock downstream target genes that include per itself. Thus over the day per expression falls, resulting in a reactivation of the clock gene by the time night arrives again. The principle of circadian rhythm regulation by an oscillation pattern of transcriptional regulations involving per and clock, is conserved from flies to humans.

Hypocretin – a class of molecules that affects sleep and sleep disorders in mammals

In humans, specific brain areas that express circadian genes such as per and clock, including the hypothalamus and suprachiasmatic nucleus (SCN), function as a master

clock for the entire body. Studying a rare sleep disorder narcolepsy-cataplexy has elucidated the link between the brain regions and behavior. In narcolepsy-cataplexy, the internally generated sleep state invades the waking state. Patients have trouble staying awake during the day, in addition they can sometimes have dream-like hallucinations when they are awake, or lose muscle control suddenly as if they are about to fall asleep.

Narcolepsy-cataplexy is very rare in humans. There is a similar, very rare disease in some dogs. Genetic studies of dogs and mice with narcolepsy led to the discovery of neuropeptide called hypocretin, and its receptor that couples the circadian clock to behavior. Dogs with mutation in the hypocretin receptor have narcolepsy, and mice or human that carry this mutation in hypocretin peptide also have a similar disorder. In the human brain, ~2000 neurons (out of billions) that make hypocretin in the hypothalamus connect to neurons in the circadian rhythm master clock to link that clock to sleep-wake cycle. Secreted hypocretin can act on many brain regions, showing how one brain area releases a neuropeptide to regulate behavior. Most human patients with narcolepsy-cataplexy have lost the neurons that produce the hypocretin peptide through autoimmune destruction.

Social behaviors are rapidly evolved and can be distinct in two closely related species

Social behaviors are widespread among different species, and yet highly variable within and between species. Social behaviors can differ depending on social context and the environment. They often differ in young animals and adults. They also can be distinct in closely related species. For example, rodents called meadow voles are mostly solitary, non-territorial, and polygamous; while closely related rodents called prairie voles are colonial, territorial, and monogamous, forming strong pair bonds that can last their entire life. What has caused these two closely related voles to evolve such drastic differences in social strategies? Clues have come from studying two similar neuropeptides called oxytocin and vasopressin. These neuropeptides are important in mammalian reproductive physiology and behavior. Insel and Young discovered that both prairie voles and meadow voles possess the neuropeptides oxytocin and vasopressin, and their receptors. However, the ways that these molecules are expressed in the brains are distinct in different species. Monogamous prairie voles express the vasopressin receptor V1R in areas that are involved with reward and the formation of positive associations, leading to pair bonding, while polygamous voles express V1R in a region of brain that does not form rewarding memories and thus does not promote pair bonding. Although brain anatomy and genetic elements evolve slowly, changes in gene expression of “old” genes like two vasopressin receptors can impact

behaviors and allow the rapid generation of new behaviors with conserved repertoires of genes and neurons.

3. Recommended Reading

The study of instinct, Niko Tinbergen, Oxford University 1991

Beyond the connectome: how neuromodulators shape neural circuits. Bioessays, 2012

Seven-Transmembrane Proteins as Odorant and Chemosensory Receptors, Science, 1999

Odr10 and T1R reviews: Prasad and Reed, Chemosensation: molecular mechanisms in worms and mammals, Trends in Genetics, 1999

Sleep, per/clock review:

Life's 24-hour clock: molecular control of circadian rhythms in animal cells. Young, Trends Biochem Sci. 2000

Neuropeptide Transmission in Brain Circuits, Neuron, 2012

Oxytocin, vasopressin and pair bonding: implications for autism, Hammock and Young, Philos Trans R Soc Lond B Biol Sci. 2006

4. Review Questions

1. What is the disease PKU and how can PKU be treated by diet?
2. Dr. Bargmann describes a family of molecules that transmits extracellular signals into intracellular biochemical processes. What is this family? What kinds of external and internal stimuli does it detect?

3. How do sensory receptor proteins and the neurons that express them act together to generate behavior? What determines whether a taste is appealing or aversive in a worm or a mouse?
4. What is a circadian clock, and what happens when circadian clock genes are disrupted?
5. Review how clock and per genes function in flies to regulate circadian rhythm. Draw both per and clock gene expression levels over a 24-hour cycle in a wild-type fruit fly.
6. What is narcolepsy?
7. How does hypocretin play a role in the disease state of human narcolepsy?
8. Review the differences in social behavior between the two closely related species, the meadow vole and the prairie vole. How do their vasopressin and vasopressin receptor expression patterns differ in the brain?
9. What are the three major mechanisms by which a neuron communicates with other neuron(s)?

5. Answers to Review Questions

1. Phenylketonuria (PKU), the first genetically defined brain disorder, leads to mental retardation and movement disorders. PKU patients have mutations on a single gene, phenylalanine hydroxylase, which converts L-phenylalanine to L-tyrosine. In the presence of this mutated protein, toxic chemicals accumulate and neuronal growth deteriorates. With the understanding of this altered metabolic state in PKU, we can limit the phenylalanine in the diet of PKU patients, and therefore limit the production of the toxic chemical.
2. G-protein coupled receptors (GPCR) are a large class of transmembrane proteins that detect stimuli outside the cell and propagate signals inside the cell. These biochemical signals then impact behavior. The extracellular stimuli include external cues such as chemical odors and environmental light, as well as internal cues as hormones, neurotransmitters, and neuropeptides. GPCRs not only provide the sensation of smell from external stimuli like food, but also give rise to sensations such as reward signaling from internal stimuli like the neurotransmitter octopamine.

3. The innate preference is hard-wired in the neural circuitry to drive attraction or repulsion toward a certain stimulus. For example, worms without the diacetyl receptor odr-10 will ignore the odor and scatter on the plate. The GPCR protein ODR-10 is normally expressed in AWA, an attractant sensing neuron, to drive chemotaxis toward the odor diacetyl. If ODR-10 is expressed only in AWB, a repellent sensing neuron, these reprogrammed animals respond inappropriately to avoid diacetyl.

4. The internal and entrainable rhythm of sleep-wake oscillations is defined as a circadian rhythm -- an internal pace maker directly driving sleep and wake cycles. Mutants with circadian rhythms disrupted may have abnormal sleep patterns such as random sleep-awake cycles, or longer or shorter cycle lengths than 24 hours.

5. The intracellular circadian clock is controlled by a negative feedback loop composed of at least two main genes, per and clock, which regulate the expression patterns of other molecules. At night, the transcription factor clock turns on expression of per. At the transition to day, per reaches a peak threshold and starts entering the nucleus where it inhibits the clock gene. This in turn causes a decrease in per expression resulting in a reactivation of the clock gene by the time night arrives again.

6. Narcolepsy is a human sleep disorder in which the internally generated sleep state invades the wake state. Patients will have hallucinations when they are awake, and will lose muscle control suddenly as if they are about to fall asleep.

7. Human patients with narcolepsy or catalepsy have lost the neurons that produce the hypocretin peptide through autoimmune destruction.

8. Both meadow voles and prairie voles possess the neuropeptides oxytocin (OT) and vasopressin (VP), and their receptors. However, the ways that these molecules are expressed in the brains are extremely distinct. Monogamous prairie voles express the vasopressin receptor V1R in areas that are involved with reward and the formation of positive associations leading to pair bonding, while polygamous voles express V1R in a region of brain that does not form rewarding memories and thus does not promote pair bonding.

9. Synaptic transmission, gap junction, and neuropeptide/peptide receptor interactions.

6. Discussion Questions

1. What have we learned from neuroethological studies of behavior? Please provide a few examples.
2. How do GPCRs sense the stimuli outside the cellular membrane and produce signals into the cell?
3. How can modern genetics and molecular biology help us to revisit neuroethology questions? In other words, what kind of experiments can we perform with these modern tools, to dissect the mechanisms of animal behaviors?
4. *C. elegans* uses the sensory neuron ASH to detect noxious stimuli, such as high osmolarity, and which elicit reversals and a rapid escape response. Wild type *C. elegans* does not respond to capsaicin because it has no capsaicin receptor. How would you expect a worm to respond to capsaicin, if we use transgenic technologies to install a rat capsaicin receptor on the ASH cell, and why?
5. Anatomy doesn't change quickly, but behavior can change rapidly in sister species. In the example of voles and vasopressin receptor, what precise genetic element do you think has been altered during evolution to enable their distinct behavioral difference?
6. Meadow voles and prairie voles are two closely related species with distinct behaviors. Using modern genetics, we can change V1R expression in meadow voles by expressing fragments of prairie vole DNA encoding V1R. What change of behaviors would you expect to see in this transgenic meadow vole?

7. Answers to Discussion Questions

1. Neuroethologists have demonstrated that animal behaviors are influenced by both internal states and external cues. For example, Konrad Lorenz’s work on imprinting in geese suggested that the nervous system has an internal drive to elicit certain behaviors that only can be established at certain developmental stages.
2. G-protein coupled receptors (GPCR) are a large class of transmembrane proteins that detect stimuli outside the cell and propagate signals inside the cell. These biochemical signals then impact cellular function; especially when the cells are neurons, the ligand can impact behavior.
3. Modern genetics provides tools to dissect the system and test the hypothesis that was proposed by the neuroethologist. For example, we can use *C. elegans* to

understand how avoidance and attraction behaviors are hardwired in the nervous system by manipulating the diacetyl receptor ODR-10 in AWA and AWB neurons.

4. They will escape capsaicin if the receptor is expressed in ASH, because just as AWA encodes attraction behavior, ASH is hardwired to acute escape behavior.
5. It could be genetic elements that control expression pattern (e.g. the promoter region), protein folding (e.g. 3'UTR), or post-translational modifications (the modification enzymes), etc.
6. You should expect the behaviors of meadow voles to change from being polygamous to monogamous.

8. Explain or Teach These Concepts to a Friend

1. *C. elegans* is a soil nematode species that is innately attracted to many kinds of odors, e.g. diacetyl. Explain to a friend how to engineer a *C. elegans* worm that will avoid diacetyl, and why.
2. Explain to your friend about narcolepsy and how hypocretin molecules affect sleep regulations in mouse, dogs, and humans.
3. Explain to your friend the difference in vasopressin expression of monogamous and polygamous voles, and how neuropeptides and their receptors influence animal behaviors.

9. Research the Literature on Your Own

1. Learn about the signaling pathways and molecular players downstream of GPCR. See Robert Lefkowitz' iBioSeminar
2. Learn about other genetic components contributing to circadian rhythms. See A PER/TIM/DBT interval timer for Drosophila's circadian clock. Saez, Young, et al Cold Spring Harb Symp Quant Biol. 2007