Genes and Genomes: Impact on Medicine and Society

Genes, Genomes, and Medicine
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Cornelia Bargmann, Ph.D., University of California, San Francisco, CA
Genes, Behavior, and the Sense of Smell

Introduction by Andrew R. Marks

Andrew R. Marks: So it's my pleasure to introduce the next speaker, and first I want to apologize for the lack of coffee out there. I announced a coffee break and people were asking where the coffee was.

Dr. Cori Bargmann received her B.S. at the University of Georgia and a Ph.D. from MIT. As a postdoc in Bob Horvitz's lab at MIT, she established the worm C. elegans as a biological system for behavioral analysis of chemosensation. She is currently a professor of anatomy at UCSF and investigator in the Howard Hughes Medical Institute. She identified the first olfactory receptor for a specific odor, and used the C. elegans system to achieve seminal discoveries matching the sense of smell to behavioral responses. Today she will tell us about her remarkable work exploring the links between genes and behavior. Cori, welcome to Columbia.

Genes and the Brain

Cornelia Bargmann: Thank you for that introduction, and am I audible? No. I'm on? Okay, thank you. And thank you very much for inviting me for this celebration of Columbia's 250th anniversary.

The human brain is a wonder of nature. It's the seat of the mind, the seat of our perceptions, emotions, thoughts, memories, and feelings. The human brain is also a biological organ, and like other biological organs, it's built by genes, which are the tools of biology. The object of the brain is to generate behavior, and at some level that means that genes affect the behavior of animals and of humans.
Now to say that genes affect behavior is not to say that genes control behavior; no serious person believes that in any way, but no serious person denies either the fact that genes have a role in generating behavior. William James, the father of psychology, in the nineteenth century had already written that one clear implication of Darwin's theory of natural selection is that the behaviors of animals would also be selected for in ways that were appropriate for their survival. But going from that to understanding how that occurs is a different question.

Now it's been appreciated for a long time, throughout the history of human genetics, that genes can affect the brain. In fact, one of the first genetic diseases identified in humans was a neurological disease, phenylketonuria, identified in 1934. And this disease is associated with behavioral issues, late social skills, mental retardation, and various movement disorders, and it's related in a straightforward way to an inborn error of metabolism, a defect in handling the amino acid phenylalanine, one of the eight essential amino acids in the human diet.

Now phenylketonuria, this disease, remains a paradigm for human genetics in a whole set of ways and helps to illustrate—this is the pointer?—great—and helps to illustrate principles of thinking about how genes affect behavior in ways that remain to this day. First of all, the gene does not act directly on the behavior but rather through a set of intermediate steps. The gene is interpreted in the context of a cell. In the particular context of this disease the mutation phenylalanine hydroxylase leads to smaller and fewer neurons. This in turn leads to altered function of the brain and brain circuits, and these altered circuits lead up to a person who has altered behavior. And so understanding genetic influences involves understanding all of these steps along the way in the generation of the behavior.

Now the success of genetics as a tool to find this gene also led to a success in terms of the ultimate health and well-being of the patients affected with this disease. As a disease of amino-acid metabolism, PKU can be controlled by a low-protein diet, and a low-protein diet remains the treatment of choice for this disease to this day. And the success of genetic approaches has also been quite prominent in studying all kinds of neurological diseases, neurological diseases being distinguished from psychological diseases or psychiatric diseases by the fact that neurological diseases result in something you can see. And the major neurological diseases all consist, at least in part, of diseases that have genetic origins or genetic components.

**Genes and Human Behavior**

So to what extent do genes affect behavior, however, as opposed to the structures of the brains, and the viabilities of nerve cells? And what is the evidence that genes are involved in behavior, specifically human behavior? Probably the strongest evidence for a genetic component to human behavior...
results from studies of identical twins who are essentially genetically identical at all loci, and a comparison of the traits of these twins with other siblings or first-degree relatives, who share only half of their genetic material. Now twins are raised in identical environments, but much of the power of this approach has been the ability to extend it to twins that are reared in different environments, identical twins reared apart, exemplified here in the eyes of the entertainment industry, to illustrate that twins reared apart might share only some and not all of their characteristics. So in this case both twins grow up to be highly paid movie stars, but only one of them is the governor-elect of the state of California.

And in fact for many different kinds of behavioral and psychiatric traits, it turns out that there's a substantial but by no means overwhelming genetic component, that about 40 percent of the sorts of quantifiable behavioral assays, personality indices, psychiatric diseases, can be ascribed to genetic causes. And this has been very notably the work of many groups, but particularly Tom Bouchard and his colleagues at the University of Minnesota.

So this problem is interesting not only as sort of an intellectual problem, but in terms of an enormous amount of human suffering, human disease, disability, lost work, economic indices. Depending on exactly how you count, seven of the top ten reasons that people take time off from work because of disability are directly related to psychiatric disease, including depression, drug and alcohol abuse, obsessive-compulsive disorder, and schizophrenia. So understanding something about the genetic bases of these diseases is important, and in fact the genetic risk for these diseases is quite substantial. If you look at the genetic risk associated with either an identical twin or a sibling for these common psychiatric diseases, it's comparable or higher to the genetic risk associated with type 2 diabetes, which is clearly recognized to have at least a partial genetic origin, as Eric Lander just told you. So these genetic diseases, however, are still waiting for their PPAR-γ, the specific molecular information that would lead you to be able to design some sort of an intervention to help the individual.

So to know that these diseases have a heritable component is at some level to know nothing. It doesn't give you any information that you can use to understand more about the underpinnings of the disease, and it doesn't give you any information that you can use to intervene in a useful way. And so what we'd like to understand about genetic variation and behavior is how many genes are involved, what kinds of molecules and pathways are involved in generating behaviors, what kinds of changes distinguish one kind of individual from another, and how do those changes affect behavior. And somehow this information is imbedded within the 30,000 genes of the human genome. But we don't really know where to look.

This is a pie chart from one of the papers about the human genomes dividing genes up by category of function, and I draw your attention here to the fact that the largest piece of this pie, about 41 percent of all genes, fall in a category
called molecular function unknown. So despite what we do know about human genes, there's a great deal that we do not know. And the tools that have been valuable for reaching and understanding here are really tools that were conceptualized for the first time for animals by Thomas Hunt Morgan and his colleagues working at Columbia University, which was the idea that simple animals would represent tools that could be used to understand general laws, initially of chromosomes and heredity, later of developmental biology, and ultimately of behavior. And Morgan himself was interested in the question of behavior, although he did not address it in his own career.

And we now have a quantitative sense of what Morgan appreciated qualitatively, which is that most human genes are shared with other organisms, that the number of genes that are specific for humans alone is going to be extremely small, and even the genes that are specific for vertebrates represent only twenty-some percent of all the genes in the human genome, that the overwhelming majority of genes are shared by all animals, and many even shared by unicellular organisms, and that these much simpler and genetically tractable organisms would represent a place to create the power to understand these problems.

**Circadian-Rhythm Genes**

Now Morgan ultimately moved to Cal Tech and the genetic analysis of behavior really travels in pretty much of a straight line to Cal Tech through the work of Seymour Benzer and his colleagues, who used Morgan's experimental organism, the fruit fly *Drosophila melanogaster* to make the first inroads into understanding the genetic basis of behavior. And the behaviors that they chose to study are behaviors that have circadian rhythms, patterns of behaviors that fruit flies exhibit over long periods of time. So flies are diurnal, they buzz around and do their feeding and mating and carrying on during the day, and during nights they have low activity levels. These chits here represent some sort of activity, flying around of a fly. Many animals have circadian rhythms. The interesting and remarkable thing about circadian rhythm is that they are not directly controlled by the environment, but are in fact internally generated by the animal's nervous system. So, for example, if you take an animal and shift it to constant darkness or constant light in the absence of any information about the day, the animal will continue on a twenty-four-hour cycle involving high levels of activity punctuated by low levels of activity.

And Konopka and Benzer in 1971 reported the first genetic analysis of this complex, long-range, innate behavior by identifying mutations in which this periodicity of the animals when shifted to an environment without cues was disrupted, and in particular they identified mutants that had short-day rhythms, as short as 18 or 19 hours, long-day mutants that might be as long as 28 hours instead of 24, and mutants that were completely lacking in circadian rhythms. And remarkably all three of these classes of mutations could be isolated in a single gene, implicating this gene as having a central function in the generation
of these regular behavioral rhythms. And the name of that gene is \textit{per}, and molecular studies and further genetic studies initiated, starting from that particular starting point, has led to a molecular understanding of a network that generates behavioral rhythms in the fly.

And that network consists of a gene regulatory network that consists of genes, including \textit{per} and several other related genes, which regulate each other's synthesis positively and negatively in such a way that the levels of these gene products rise and fall with a regular twenty-four-hour oscillation. And the names of these genes are \textit{per}, \textit{tim}, \textit{timeless}, \textit{clk}, and \textit{bmal}. The result of their positive and negative regulations is that these gene products oscillate on different cycles, and therefore different ones dominate at different times of the day. And just to sort of simplify what that entails, a gene called \textit{clk} is active through most of the day, and the genes \textit{per} and \textit{timeless} are active through most of the night. And so the alternating activities of these two gene sets account for the differences in activities and many behaviors that insects exhibit.

Now the power of this approach was evident to Joe Takahashi and his colleagues at Northwestern University in the late 1980s and early 1990s. They wondered if you could take the same kind of single gene-forward genetic-mutagenesis approach to comparable behaviors in more complex animals, such as rodents. And indeed Joe in a genetic screen identified a mouse mutant which in the same kinds of activity logs when shifted to constant darkness has ever-increasing day lengths, in other words, had a long circadian rhythm, and which could in addition, when homozygous, be shifted to a completely arrhythmic pattern. So mice, like flies, have endogenous circadian rhythms. They're the opposite of flies in that they run around at night and sleep during the day, but these kinds of activity monitors look quite similar between vertebrates and invertebrates, and the mutations look remarkably similar in terms of the kinds of effects that they have.

The mutations are also remarkably similar in terms of the molecules that they affect. And so the central circadian oscillator of mouse circadian rhythm is essentially identical, with a few modifications, a few duplications and a few bells and whistles, to the central circadian oscillator that accounts for fly circadian rhythms. And so it's the same network of oscillating genes, in fact the \textit{clk} gene, one of these genes, that was first identified in the mouse, later in the fly. The others were first identified in the fly, later in the mouse. One is able to move seamlessly between these experimental organisms, really validating Thomas Hunt Morgan's idea that you would be able to study genetic processes in simple animals to understand more complex animals. And the same ideas of these oscillating gene products that are dominant at different times of the day explains the behavior of the mouse.
Genes and Human Sleep Disorders

The final example and the completion of this idea of genetic conservation and the role of genes and behavior is indicated by a rare group of human patients who suffer from something called advanced sleep-phase syndrome, and these patients can be shown in normal conditions to have very unusual behaviors compared to most individuals in our culture. They wake up at about three o'clock in the morning when left to themselves and have an enormous amount of difficulty staying awake past six o'clock at night. And they never go to any good parties, they never get into any of the cool clubs, they're absolutely wretched and they complain to their doctors about the effect on your social life of essentially not having the free evenings, which are when in our culture you have most of your social interactions. And sort of in order to characterize these further, a set of ASPS patients volunteered to be placed in conditions where they had no external information about day and light cycles, so no television, no clocks, no radios, and their activities were monitored. And when these patients were monitored over a period of days the shift in their activity cycles, toward the left side, indicates that they had shortened circadian rhythms, that their clocks actually run fast, on the order of twenty hours instead of twenty-four hours. So they're constantly pushing themselves to try to stay on a schedule which in fact wants to shift earlier and earlier every day. And this behavior pattern is virtually indistinguishable from rodents or flies that have short circadian rhythms, where you have shifting patterns that move to earlier and earlier periods of time.

Louis Ptacek and Ying-Hui Fu at the University of Utah identified a mutation associated with this advanced sleep phase syndrome in these patients and discovered that this mutation is precisely a mutation in the human ortholog of the fly \textit{per} gene; that is, the identical gene that affects circadian rhythm in the fly is responsible for this behavioral difficulty in humans. And in fact it's found in an exact residue which is known to regulate the cycling and the turnover, and therefore the rate of oscillations of these gene products. So the conclusion of this is that it's possible to understand a fairly complex long-range behavior in terms of molecules that are conserved through evolution that organize whole groups of behaviors, in this case transcriptional molecules.

And I have to say one of the reasons that this was such a powerful model was that sleep is a fabulous behavior, so one of the difficulties for understanding behavior is the difficulty of scoring it. You know when organisms are asleep, you know when they're awake. They don't have to tell you, they don't have to write it down on a form. And as a result sleep genetics is something that of the behavioral systems we probably know more about than other kinds of behavioral disorders.

So a second example of that is the syndrome of narcolepsy / cataplexy. This is a very unusual disorder, it's quite rare, it's observed in human patients, and it represents a fragmentation of the separation between sleeping and waking...
behavior, such that behaviors appropriate for sleep creep into everyday waking life. These patients are sleepy—they fall asleep early, they enter REM sleep prematurely, and can even enter REM sleep while they're awake and therefore have hallucinations. They can have a loss of muscle control under excitement and a paralytic response, and that's the cataplexy response. Again, paralysis is appropriate for sleep but not for waking states. And this is a human disease which has been mapped to susceptibility locus, for this has been mapped by linkage mapping, but at some level this was a failure of genetics. And the reason it was a failure of genetics is that the linkage mapping identified a particular genotype at the HLA, the major histocompatibility locus, as the source of narcolepsy / cataplexy risk. And so on the one hand that tells you something about what gene is responsible, but on the other hand it tells you nothing, because what it tells you is that there's something, probably some sort of immune disorder, probably some sort of autoimmune destruction of something that leads to this sleep disorder and this curious behavioral fragmentation. But it doesn't tell you how to go about finding out what exactly has been lost, and how exactly these different manifestations are coupled to each other.

**The Mechanism Behind Narcolepsy / Cataplexy**

So the same group that worked on the human genetic linkage, Emmanuel Mignot's lab at Stanford had a backup plan in case that didn't work out, and their backup plan involved a genetic analysis of the dog. This dog is Prancer, is a Doberman Pinscher, and Prancer is lying on the ground in a state of cataplexy, because Prancer, along with a few but not most Doberman Pinschers, has a genetic syndrome indistinguishable from narcolepsy / cataplexy syndromes in humans. So this has been observed several times in several different dog breeds. It's not something that every animal in the breed will have, just a few individuals have it, but since these are animals that you can breed with one another you can do genetic mapping, and you can try and find out what genes are involved in this disorder, and ask if they're related in some way to the genes involved in the human disorder.

And so the Mignot lab working in dogs, and actually in a parallel study at the Yanagisawa lab working in mice, identified a particular signaling mechanism as essential in the generation of this narcolepsy / cataplexy syndrome. And that mutation was in a G-protein-coupled receptor called the hypocretin-2 receptor, also known as the orexin receptor, whereas the mouse disease was in hypocretin orexin itself, the ligand, the small peptide ligand for this receptor. So these are molecules that are expressed in nerve cells. It was known that they were expressed in the brain, it was not known what they did; in fact, they were somewhat misnamed with the idea that they might have something to do with feeding, whereas the genetic results suggest that they have something to do with the sleep-waking regulation.
Now neuropeptides represent a very interesting kind of molecule to think about, genes that might be binding together groups of different responses in the form of coherent behaviors. So you can contrast neuropeptides with classical neurotransmitters in the brain. Neurons signal to one another using both classical transmitters and neuropeptides. Classical neurotransmitters generate rapid, precise, local transfers of information involved in rapid, precise processes like perception and thought. Neuropeptides can be released from the same neurons, but they work in a very different way. They act over much longer time scales of seconds to hours, rather than milliseconds. They can act over a distance, many different groups of neurons or over circuits. There are many different neuropeptides and many different neuropeptide receptors, dozens or hundreds, and they're all expressed in very specific patterns and specific groups of neurons. And they're slow action in their ability to act over a distance. It's quite interesting in terms of thinking about how you would bind together a set of different behaviors in regulating, for example, the transition between sleep and waking.

Further insight into this came from asking where orexin was expressed in the brain. It turns out that in the human brain there are about only 2,000 neurons out of the billion or so neurons of the human brain that express orexin, this peptide. They're localized in the hypothalamus, which is involved in regulating many instinctive behaviors, hunger, thirst, sexual behaviors. And this particular set of neurons, once they were characterized, could be looked at carefully, and they were found to project to many different regions of the brain that were already known to be involved in sleep and arousal. So there many parts of the brain that were known to be regulating sleep and waking, but what wasn't appreciated until the genetic study of narcolepsy was that these parts of the brain were essentially bound together by a master control region in the hypothalamus that expressed a particular neuropeptide that communicated with all of them, and gave them the potential to coordinate with one another.

So this gives an insight into how the brain works that was not understood prior to the genetic insight. It also enables an insight for going back and understanding the autoimmune basis of the human disease, because the human patients who suffer from narcolepsy have a specific loss of the hypocretin-containing neurons in the hypothalamus, associated with the gliosis. Compared to normal individuals it suggests that they're undergoing some kind of an immune destruction. So the use of genetics in multiple organisms was actually powerful for understanding something that at one genetic level in the human was already understood, but which required the other organisms to go back and understand it at the level of the brain and the circuit, these intermediate levels of going from a gene to a behavior.

**Species-Specific Social Behaviors**

What other kinds of behaviors are interesting to study, what other kinds of behaviors are interesting to think of from a genetic basis? Social behaviors
represent behaviors that are innate to species and shared by all individuals within a species often. All species of animals have to be able to recognize other individuals of the same species, for example, so that they mate with the right individuals, but how elaborate their social structures are in addition to this varies enormously between different species. Social behavior has different advantages and disadvantages, and it's evolved and been lost many times independently during evolution. Because of things like game theory, there's times that it's advantageous to cooperate, there's times that it's advantageous to go it alone, and different species will exploit these different strategies to different extents. So on one extreme you have the elaborate social constructs of humans or of social insects like ants or bees. At other extremes you have animals that really only get together to mate, but in all cases these responses are innate, they involve recognition, and they're relatively fast evolving. So one example of the fast evolution is that species that are quite closely related can exhibit different social strategies. Among the big cats, tigers and lions are closely enough related genetically that they can mate and have offspring, but tigers have a solitary life pattern—in Brooklyn—and lions spend their entire lives in a social group.

So what kind of genetic changes might occur in the mouse species or individuals to be different from one another? You can actually break this problem down further to think about differences between individuals within a species. And one slightly artificial but intuitive example of differences in social behavior is represented by the behavior of different breeds of dogs. So dogs have been bred by humans for particular traits to work with humans, and many of these traits are quite specifically and explicitly behavioral traits. So, for example, golden retrievers were bred by English hunt clubs to be able to work during hunting with any number of the hunt clubs, and so these dogs are relatively easy to get along with, easy to work with, don't have strong preferences for particular individuals, they basically have an extroverted sort of character. By contrast, German shepherds and other shepherd dogs have been bred specifically to work with one individual, and in particular to work with that individual to protect the sheep from thieves or from other predators, and so they tend to encounter new individuals with some degree of skepticism and mistrust, and instead form very strong bonds with a single individual, so strong actually that German shepherds are no longer used as seeing-eye dogs because they form such a strong bond with their initial trainer that they have difficulty reforming a bond with another individual.

So these are traits that you can expect to be reliable among golden retrievers and among German shepherds, but we have no idea what the genetic basis would be that would lead to their differences in social strategies. And so we'd like to understand that and take this idea of Morgan's of working with a simple animal to see if we can use genetic tools to figure out what kinds of molecules or pathways could be involved in this sort of behavior pattern. And the simple organism that we work with is the soil nematode *C. elegans*, a millimeter long, which feeds on soil bacteria. As Mike Levine pointed out to you it doesn't have particularly fascinating developmental patterns, but I would argue that it has
fabulous behaviors and—at least as good as flies buzzing around. A little sibling rivalry here.

Social Behaviors in *C. elegans* Strains

And different isolates of *C. elegans* have been isolated for many different places in the world and grown in the laboratory. And this is the standard laboratory isolate that Sydney Brenner introduced to the world. It's a British strain called N2, and if you place it on a lawn of bacteria in the laboratory it observes a characteristic feeding pattern in which the animals pay no attention to each other whatsoever. And this we call a solitary feeding pattern.

Now by contrast, other isolates of *C. elegans* from different places, such as this strain, RC301, isolated by Randy Cassada in Germany, exhibited a different feeding pattern in which animals gather into groups of dozens or hundreds of animals. So there are bacteria throughout this field, but the *C. elegans* will not feed on the bacteria until they have first gathered into a feeding group, and only then do they exploit the bacterial source. And these traits are reliably different from one another, and they’ve both been observed many times. So about a third of all strains are solitary, and these include strains isolated from England and from various places in the U.S. About two-thirds of all strains exhibit the social feeding pattern, and these include strains from continental Europe, Australia, Hawaii, as well as the continental U.S. And these two different kinds of nematodes appear to be able to coexist with one another. Both a solitary and a social worm were isolated from the same compost pile in Pasadena, California, implying that these two different strategies are both under some sort of positive selection which provide advantages to the animal under different circumstances.

So the power of this experimental organism is the simplicity of its lifestyle and the rapidity of its generation time. These animals grow up in about three days, and you can use genetics to understand what the basis is of the genetic difference between these true breeding variations.

And what Mario de Bono found when he was studying this is that a single gene, the neuropeptide receptor *npr-1*, can explain the difference between social and solitary strains. So this gene, like the hypocretin-2 receptor implicated in narcolepsy, is a neuropeptide receptor, a molecule expressed on neurons that responds to peptide ligands. *C. elegans* has about a hundred neuropeptides and about a hundred neuropeptide receptors, again a complex network of these molecules that are expressed on neurons in specific patterns. And what Mario found was that this molecule was reliably different between the social and the solitary strains, that the solitary strains invariably, in five out of five cases, has a valine, a particular residue, implicated in the strength and specificity of G-protein coupling, whereas the social strains invariably, in twelve out of twelve cases, had a phenylalanine residue at that same location. And this was true regardless of
the original site of isolation of the animal, there was a perfect correlation of these amino acid residues.

And Mario has gone to show that these residues result in functional changes in the receptors that are detectable in their G-protein-mediated responses to their neuropeptide ligands. So different ligands, the solitary isoform represents a high level of activity of this receptor that enables it to respond strongly to its ligands and to respond to a broader variety of ligands. The phenylalanine or social version represents a low level of activity of this receptor in which a higher level of ligand is required to stimulate this activity. So the activity of this gene is to stimulate solitary behavior when expressed at high levels, or at high levels of activity.

So what's the argument that this gene is not only correlated with but central to these behavior patterns? In fact, that argument is based on genetic manipulations that you can do in either the solitary or the social backgrounds that indicate it functionally in these different outcomes. So the particular 215 valine allele is necessary for solitary behavior. If the npr-1 gene is inactivated and it is the only gene inactivated in a solitary strain on animal, these animals are fully converted to social feeding behavior, which is actually a complex constellation of behaviors that include social feeding and several other responses to the environment.

Conversely, this allele is also sufficient for solitary behavior in an otherwise social background. So if we take different social isolates from Europe or Australia or the United States and introduce into them a transgene which contains only this gene in the allele that corresponds to the solitary version, these strains are immediately and completely converted to the solitary feeding pattern. So based on the arguments of genetics, this represents a necessary and sufficient argument that indicates that this gene accounts for the difference between these two strains.

**Brain Circuits in *C. elegans* Social Behavior**

So what I've just told you is that when we grow these animals in the standard lab environment, the level of activity of this gene, whether it's low or high, is sufficient to determine the behavior of the animal. So a low level of the activity of the gene corresponds to social feeding, a high level to solitary feeding. But having said this, at some level again we've said nothing. We've said that there's a gene and we've said that there's a behavioral output, but we've missed the stages of translation in between, the understanding of the neurons and the understanding of the circuits that will enable us to see what it is that these behaviors truly represent.

And so David Tobin and Jesse Gray, two graduate students in the lab, have tried to understand that, in particular using this gene and other genes as tools to go
into *C. elegans* and dissect the circuits in the same way that the orexin gene was done, to dissect the narcolepsy and sleep and waking circuits in the human brain. And what they discovered was a set of different sensory neurons and sensory inputs were actually essential for generating these different kinds of behavior. And in fact, those sensory inputs could be modified, at this point we can modify them at will, in ways that lead us to believe that we understand something about the genetic nature of this behavior.

So if we move animals from a standard lab environment to a high-stress environment—and there are many different ways of doing this that involve food deprivation or various kinds of environmental stressers—the animals exhibit social feeding. And in fact in a high-stress environment, animals, regardless of their phenotype at this neuropeptide locus, will exhibit a social feeding pattern.

Conversely, if the lower the level of stress in the animals' environment—and again there are various kinds of sensory manipulations that we use based on our understanding of this sensory circuit, and the identification of the olfactory neurons and other sensory neurons involved in it—we can make animals solitary feeders, regardless of the genotype at the npr-1 locus. So this experiment, or this series of experiments, tells us some things rather generally about the way that genes interact with the environment to generate behavior, that first of all the genes do not determine that ability to generate the behavior, because animals at either genotype are capable of exhibiting solitary feeding behavior or social feeding behavior, but rather what the genes are doing are regulating the probability that the behavior will be generated in a very specific set of environmental conditions. And second, they give us some sort of an idea, which we have been able to explore further using these exact environmental conditions, about what the nature of the behavior is that we're looking at. And that is in fact that the social behavior that we're studying is a behavior that is induced by stress. So social behavior, based on evolutionary theory, can carry a variety of advantages to an animal: it can carry advantages for feeding, it can carry advantages for defense against predators or toxins, it can carry advantages for mating and reproduction. But social behavior does come at a cost: you have to share your food with other individuals, and you're more susceptible, for example, to infectious diseases which occur at high density. And so the decision whether to engage in a social behavior will be regulated by other kinds of stimuli that tell the animal whether it's appropriate. And *C. elegans* at high stress levels, the animals engage exclusively in social feeding; in low stress levels, they're more exploratory and engage exclusively in solitary feeding. And what we believe is that the genetic polymorphism in fact represents a polymorphism in the response to moderate stress levels, because it turns out that our laboratory conditions are actually a little bit unnaturally and moderately stressful to the animal, and that in that particular environment the animals that have the social genotype are cautious and assume that the moderate stress is stressful enough that they respond as though it was a high-stress environment, whereas the animals that
have the higher activity or solitary version of the gene are bolder and they exhibit the behavior appropriate for a low-stress environment.

And this kind of a behavioral axis between cautious and bold is one of the behavioral axes that you see varies between individuals in almost all species. There's almost always a range of behavior that can include these two extremes, because they'll be valuable depending in a very online way in the particular environmental conditions. And so you see, for example, in subspecies of fish in different lakes if there are predators within the lake, very rapidly you start to select for cautious fish, and if there are no predators in the lake, very rapidly you start to select for bold fish. But you can imagine that the introduction of a predator would immediately switch these things back. So this is a kind of behavioral axis that would toggle back and forth easily in evolution.

So one of the reasons that we find this result engaging is that we think that perhaps there are ways of thinking about social behaviors and genes involved in the behaviors that might extend to multiple systems. Work of Tom Insel and his colleagues has studied a different level of social behavior, and that is the evolutionary components of social behavior, in particular the differences of social behaviors in mammals. Now all mammals are social because mammals have to raise their young and therefore interact with other individuals, at least partly, in their life; however, there are many different social strategies in mammals. About 3 percent of all mammalian species are monogamous, whereas the other species tend to be polygamous or what's called promiscuous, mating freely and not maintaining long attachments. And sometimes very closely related species will show these differences. So voles, which are closely related to rodents, very similar individuals can exhibit monogamous, strongly pair-bonded forms, or polygamous, promiscuous individuals that only get together to mate, and in fact the mothers don't even stay around the pups very long. And Insel and his colleagues have over the years implicated a set of neuropeptides strongly in the variation and the creation of these different behaviors.

So in this case it's a different neuropeptide receptor, in this case the vasopressin V1 neuropeptide receptor. And the activity of this receptor is essential for the monogamous behavior of male monogamous voles, blocking its activity blocks their appropriate behavior, and moreover the expression of this receptor in the brain is very different between the monogamous and the polygamous species of vole.

So this expression pattern, this molecule that belongs to the same set of molecules, and the antagonist experiments provide evidence that these molecules are actually engaged in social behaviors. A transgene taken from a monogamous vole and introduced into a mouse which has more of a polygamous lifestyle appears to stimulate affiliative behavior in mice, suggesting that, as in the case of the neuropeptide receptor system that we studied in worms, this neuropeptide receptor may actually be able to modify behaviors and bind
together groups of behaviors in a way that correspond to the affiliative behaviors of rodents.

Research on Human Psychiatric Diseases

I'd like to close by talking a little bit about genes and complexity, and returning to human disease and the importance of genetic systems in understanding how diseases arise. And I would like to start with some of the things that we do know about human psychiatric disease.

Probably the strongest influence on thinking over the past twenty years or so about the fact that psychiatric has a strong biological component has come from the discovery of effective drugs that in many cases, somewhere between 20 and 50 percent, can actually treat major affective disorder, particularly depressive disorder and anxiety disorder. And these are the so-called SSRIs or antidepressants, known by their names of Zoloft, Prozac, Paxil. And the role of these molecules is to increase the effective levels of the neurotransmitter serotonin. Serotonin is a small molecule that sort of acts somewhere between a classical neurotransmitter and fast neurotransmitter and a neuropeptide neurotransmitter in terms of the fact that it can act on slow-ish time scales and it can act across distances. The involvement of the SSRIs in affective disorders like depression and anxiety has really provided evidence and very strong thinking in people's minds that in some way serotonin is involved in these affective disorders.

Now the exact molecular target of these drugs is known, and it's worth saying that these targets, that this whole link was discovered completely by accident based on side effects of medications used for cardiovascular patients, and that if we understood more about these diseases it would be quite possible that we would understand more about ways of designing effective interventions for patients.

But although this molecule is known, this molecule has been sequenced in many, many patients, the link between the serotonin reuptake transmitter, the molecule affected by Prozac and this family of molecules, and the genetics of human affective disorder was extremely weak and discouraging. And this just gives a sense of how difficult it is to do human genetics, because this link is now starting to be made but it required an enterprise of immense proportions, the so-called Dunedin Multidisciplinary Health and Development Study. And what this represents is a study done in New Zealand initiated by Silva, carried on by Poulton and colleagues which has been going on for thirty years, where a thousand children born in the same hospital in New Zealand in 1972 have been tracked, starting from the age of 3 to most recently the age of 26, and they've been monitored very closely for all kinds of health outcomes, for environmental influences, for behaviors, and also for genotypes. And using this very large cohort and doing the kind of effort that it takes to understand human genetics and
human behavior it's actually been possible to demonstrate not only that the serotonin reuptake transmitter, the target of Prozac, is involved in depression and depressive disorders, but that it's involved in a very specific way that involves an interaction with an environment.

There are two major genotypes at this locus, and this represents truly natural variation; about half of all individuals have one genotype or the other in the Caucasian populations. And if you look at the risk of a depressive event, depending on the genotype, both genotypes actually have plenty of depression. These individuals between the ages of 21 and 26, about 17 percent had one depressive episode, so this represents about two hundred events in these thousands patients. So the gene itself is not instructive. Both kinds of people got depressed, and that fits the lack of correlation observed in many studies.

But what's remarkable is what's seen when looking at the S-genotype when actually tracking these individuals for what was happening in their life over this period of time. And it turns out that individuals that have an S-genotype when confronted with multiple traumatic and stressful events, such as poor health, the loss of a job, the death of a family member, a divorce—if they had three or more events of this sort, their risk of affective disorder shot up in a way that those of L-genotype did not. And in fact if you work through these numbers, it appears that about 25 percent of depressive disorders within this cohort can be explained specifically by the interaction between the S-genotype and a series of environmental insults. And the depth and intensity of this study is wonderful because it really lets you exclude many trivial explanations for this result. For example, you can ask what's cause and what's effect by looking at depression between the ages of 18 and 21, and then depression at later ages, and figuring out when the events happened and when the depression came, so you can figure that out.

So I think is a very powerful and very helpful insight into the real basis of human disease, again, the importance of neurotransmitter system in behavioral disorders. But at another level we know almost nothing, and there's a lot that we still don't understand about serotonin means in disease, like where, when, and how it acts. And so for this I think the importance of the animal models and of genetic systems for understanding behavior and psychiatry is as great now, or even greater, than it was. And I want to close by just mentioning the work of René Hen and his colleagues, to end at Columbia University as I began, in this study.

René's work on serotonin and particularly on anxiety-related disorders in the mouse has helped to localize, for example, the brain regions and circuits that appear to be affected by serotonin, including the hippocampus in the cortex, in generating affective disorder like syndromes in the mouse. And the timing of these events, which is fascinating—at least one receptor appears to act very soon after the mouse is born—for its adult behavior, its adult level of anxiety. It
reflects the activity of this gene very early. So this represents a place where the classical ideas of psychiatry, of formative early childhood experiences, and the ideas of genetics may come together. And there's even hints from René and others about the mechanisms of some of these pathways, that they may act, for example, by stimulating neurogenesis of neurons.

And when I come back for Columbia's 300th anniversary I hope that there will be answers to many of these kinds of questions as well that will emerge from these studies. Of course there are many genes still left to go, and having one insight into depression leaves much of human psychiatric disease unknown. There's more of the brain to know about, there will be much to learn about how genes and the environment and the brain and the environment interact. It's increasingly clear that experience alters gene expression, just as the fat in your diet changes the expression of LDL receptors in your liver, the experiences in your life change the expression of significant genes in the brain and its biological properties thereafter. And ultimately we can hope to understand more than disease, but rather how genes make us what we are, and how we in turn transcend them to become what we can become.

Thank you.