Brain and Mind

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Scents and Sensibility: Towards a Molecular Logic of Perception

Introduction by Gerald Fischbach

Gerald Fischbach: Richard Axel is a University professor at Columbia and was, seems to me, born and educated at Columbia, has been an extraordinary influence not just in neuroscience but I think in American biomedical science. He had a career in molecular biology—and still does—that has enormous influence over the way we think about genes and gene expression, and it is the infusion of new talent—from molecular science, from physics, from chemistry—into neuroscience that has invigorated this field. If you really think about some of the advances and some of the people speaking today, you will see that people are joining this field from very different walks, I think because of the challenges that the brain presents, both from the molecular point of view and from the more integrative behavioral point of view.

Richard had a career in medicine, and to illustrate how creative and innovative he is, when he got his degree in medicine, they promised they would give him the degree only on the condition that he never touched a live patient. So he switched fields, and he'll tell you the rest of that story. But he switched fields to our great, great benefit. Richard has won many, many awards. A few years ago his laboratory made a startling discovery, one of the most revolutionary discoveries of our time: that there is a gene pool, a very large gene pool, perhaps the largest pool of genes in the genome, that encode receptors in the nose that detect odors and olfaction. This was a triumph that is really hard to describe in terms of the impact on the field of sensory biology. But as he will describe, it promises to go far beyond that to the point of really trying to understand how we deal with sensory information, how we perceive it, and what the meaning of this is for the functioning human organism.

Perceptions are Internal Constructs

Richard Axel: Thank you, Gerry. As Gerry pointed out, indeed, I was afforded an MD with the promise that I never, ever practice medicine on live patients. And I kept my promise and returned to Columbia in the pathology department here where I did a year of pathology and afterward the chair of pathology offered board

certification if I promised never, ever to practice medicine on dead patients. And indeed this led to my current endeavors, and for this and for many other things I owe this university an enormous debt of gratitude. I love this place.

This is not a nose. It is a portrayal by the Belgian surrealist René Magritte of his own brain's representation of the external world. It is a vignette of image and reality locked in mutual consolation. The sense of slippage between image and object is a source of creativity persistent in art brought to its culmination by the surrealists. The problem as to how the brain represents the external world is not only at the center of modern art, but it is at the very core of philosophy, psychology, and neuroscience. All organisms have evolved a mechanism to recognize sensory information in the environment and transmit this information to the brain where it then must be processed to create an internal representation, a map of the external world. And the existence of a map in the brain immediately implies that different species—and at the extreme even different individuals within a species—will represent the world in different ways. Indeed studies on the evolution of sensory perception reveal the important fact that each species lives in its own unique sensory world of which other species may be partially or totally unaware.

Bats, for example, extract remarkably detailed information about the position, velocity, and size of objects in their surroundings from biosonar, or echo location. Some fish use conceptually very similar modalities that involve electromagnetic waves to navigate and detect prey. Snakes, here you go, boas, pythons, pit vipers shown here, maintain highly sensitive infrared imaging systems that target prey in the absence of visual information. These snakes have evolved a highly specialized sense apparatus, the pit organ, which detects heat from emitted infrared irradiations. These sixth, seventh, and eighth senses, which humans do not possess, illustrate quite clearly that each species perceives but a meager image of the richness of the outside world. The brain functions, then, not by recording an exact image but by creating its own selective picture. Our perceptions are not direct recordings of the world around us; rather, they are constructed internally according to innate rules. Colors, tones, tastes, smells are active constructs created by our brain out of sensory experience. They do not exist, I argue, outside of the brain. That which an organism can perceive is determined by the unique allotment of neurons with which it is genetically endowed, and we are therefore trapped in the representation of the world made possible by our genes.

But how can genes provide insight into the astonishing problem of how the brain represents the outside world? The brain, after all, consists solely of a collection of excitable neurons. And how is it that the rich array of mechanical, optical, and chemical properties that define touch, hearing, vision, smell, taste can be represented by bits of electrical activity that can essentially only vary in two parameters, time and space?

Odor Recognition and Discrimination

Now we are interested in how it is that olfactory information is represented in the brain. In humans smell is often viewed as an aesthetic sense, as a sense capable of eliciting enduring thoughts and memories. But smell is a primal sense. For most organisms it is that sense that affords them the ability to detect food, predators, and mates, and evolutionarily it is the most primitive sense. What we wish to determine is how brain space represents chemical structure, how the brain knows what the nose is smelling.

Let's consider this in the context of the anatomy of the nose. The olfactory sensory neurons reside within a sheet in the posterior recess of the nose. The neurons themselves are very simple structures: they're bipolar. Shown schematically, here the individual neurons send a process, a dendrite, out to the surface of the nose in contact with the external environment, and it is on these processes that reside the receptor molecules capable of interacting with the universe of odors. The energy of binding of odors to these receptors is transduced into electrical activity, which then travels down a second process, the axon, which actually courses through the skull and synapses, communicates, in the first relay station of the brain, the olfactory bulb. And so we have a direct connection between the outside world and the brain by a single neuron.

Now as reductionists, we have reduced the problem of determining how the brain knows what the nose is smelling into two problems. The first problem is, How do we recognize this vast array of molecular structures that are defined as odors? Humans, for example, are thought to be able to recognize tens of thousands of discrete odors, and second, conceptually more difficult, is the problem of discrimination; that is, how do we tell, how do we discriminate, among this vast repertoire of odorous molecules?

The first problem, the problem of recognition, was at least solved at a superficial level by a fellow in the laboratory, Linda Buck. Linda Buck isolated the genes encoding the receptor molecules that bind odors on the tip of olfactory sensory neurons. And the identification of these genes provided significant insight into the problem of recognition. These genes appear to operate in a manner that's quite distinct from the receptor genes that operate in other sensory systems. So in the eye, for example—in this mélange of Magritte again—in the eye we are capable of detecting several hundred hues by only three photo receptors encoded by three genes that have an overlapping specificity for different wavelengths of light. In the case of taste, we have only about thirty genes. But in the case of olfaction, there are a thousand genes encoding receptors. The implication, then, is that the vast diversity of molecular structures that define[s] the universe, the repertoire of odorous molecules in our environment, cannot be accommodated, cannot be recognized by a small number of promiscuous genes, but rather we have a very large number of, so to speak, chaste genes in the chromosome. And this principle of a large number of genes accommodating odors threads through virtually all

eukaryotic species such that the simple worm has as many genes as does man. And so a genome which maximally may have within it 30,000 genes contains 3 to 5 percent of its genes in the form of odorant receptors.

Now the identification of odorant receptor genes, then, provides us a solution to the recognition problem. We recognize this diversity of molecules that are defined as odors by virtue of having in our genome a vast array of genes encoding odorant receptors. But the identification of the odorant-receptor genes provided us with a surprising insight in the more complex question as to how it is that the brain knows what the nose is smelling. For we could now translate this question into molecular terms, we could logically ask, How is it that the brain knows which receptors have been activated by a given odorant? For if we could come up with a cogent model by which the brain knew which of the thousand receptors were activated, we would have the beginnings of a discriminatory model.

The problem was simplified even further by an observation which demonstrated that within the sensory epithelium of the nose, each of the individual 10 million sensory cells, for example, mate only a single receptor gene. This allowed us to reduce the problem even further, for the problem of the brain discerning which receptors had been activated could now be reduced to a problem of the brain discerning which neurons had been activated. And by analogy with other sensory systems, we could perhaps argue that the brain could determine which neurons had been activated by segregated defined neurons in space, by creating a map in the brain.

A Cortical Map of Sensory Information

Now it's been known for over a hundred years that the segregation of sensory modalities and submodalities is a basic principle of cortical organization in man. What we see here is that each of the individual sensory modalities projects to cortex in a discrete region. Not only do the individual sensory modalities project within a discrete region of cortex, but within a modality region, the somatosensory region, or the auditory region, there exists an internal map, and that spatial order serves to define both the position of a sensory stimulus in space and the quality of a sensory stimulus. If we return to the bat, for example, to the bat's auditory cortex, what we observe is that one important quality of auditory information, frequency, is linearly mapped along the bat's auditory cortex such that low-frequency sounds are on the right and high-frequency sounds are on the left. And so the brain uses the position of a signal to determine the quality of a sound bit. Importantly this map, this spatial map, is not proportional. You see within the center of this map a region encompassing about a third of the auditory cortex, which is finely tuned to sounds around 60 kilohertz. Sixty kilohertz is precisely the sound of the bat's echo, so this disproportion in the allocation of space is uniquely designed over evolution to meet the specific ecologic and evolutionary needs of a specific organism.

This disproportion is also evident in the somatosensory cortex where . . . I cannot see this slide, can you? But in somatosensory cortex, what we observe is that there is a disproportionate representation of the body surface of the hand and face at the expense of the trunk and limbs. But most importantly the somatosensory cortex illustrates a second principle, and that is that this sensory map is not static, it changes with experience. So if one looks at the region of the sensory map in the brain of a violinist, his fifth digit on the left hand, which is used for fingering, occupies three times as much brain space as does the fifth digit in the normal population. And so in these sensory systems, we have a spatial map that meets both the evolutionary, ecologic, and experiential needs of the organism.

Now in the olfactory system the brain does not map in a traditional way the position of an olfactory stimulus in space. And relieved of this requirement, we asked whether the brain uses space to map the quality of an olfactory stimulus. And the answer is very clear: indeed it does. In these sorts of experiments, performed in the laboratory by Peter Mombaerts and Fan Wang, what we did was to genetically engineer, to alter a mouse such that all of the neurons that make a given odorant receptor and are therefore responsive to a given odor are turned blue. And they're turned blue both in the sensory sheet shown here—this is the internus of the mouse nose—but also along their projections as they course through the skull into the brain. And what we observe is that in the sensory epithelium, neurons that make a single one of the thousand receptors are randomly distributed, but order is restored in the brain. The processes of these neurons all project back and converge on a fixed point in the first relay station of the brain.

Importantly, that point is fixed, it's invariant, in all individuals in a species, and that point differs for all the thousand receptors expressed in the nose of an individual within that species. The consequence of this is indeed that there is an anatomic map. And it follows, then, that individual odors will activate a subset of receptors, which in turn will activate a subset of points in brain space such that the quality of an odor may be defined by unique spatial patterns of activity in the brain.

Linking Sense to Activity

But is that anatomic map . . . for after all I've shown you, is an anatomic map . . . is this anatomic map functional? Does an odor indeed elicit precise patterns of activity, and do these patterns of activity have any consequence for the behavior of an organism? To address these points, we turned our interest to an analysis of the representation of olfactory information in an insect brain. Insects are capable of rather complex olfactory-driven behavior that's mediated by a brain which is five orders of magnitude simpler numerically than the brain of a mammal. This is a mammal, and this is the insect *Drosophila melanogaster*, and what we observe is the nose of the insect is the antenna, and, remarkably, upon analyzing the genes and circuitry of the fly nose, we observe that despite the 600 million years of evolution that separate these two species, the basic principles of anatomy and

functional organization of the peripheral olfactory system appear to be shared between these two organisms.

Here we see the nose of a fly, and we're lighting up cells that are expressed in a single neuron. And, indeed, what we observe is this very principle of the existence of multiple genes such that only one of the multiple genes is expressed in a single sensory neuron is preserved in the fly, and, moreover, all of the neurons that make a given odorant receptor each project back to a fixed point in the fly brain. So now we're looking at the fly brain, and this is the functional and anatomic equivalent of the olfactory bulb that I described to you in the mammalian brain. And so despite the 600 million years of evolution that separate the two species, the two species appear to have evolved—in this instance, independently—the same basic solution to the organization of the peripheral olfactory system, suggesting that this solution is in fact one of a relatively few that solve this essential and rather complex problem.

And this organization indeed suggests that different odors in the fly will activate different loci—these are known as glomeruli—such that banana might activate one combination of loci which is overlapping but nonidentical, and the quality of an odor would be encoded by spatial patterns of activity. But is this anatomic map functional? And importantly, are there behavioral consequences to the activation of a discrete set of loci in the brain? To address this problem, we made use of the genetic facility of the fly, and in a series of experiments performed by Jing [Wang] and Allan Wong in the laboratory, what we were able to do is to express within all fly olfactory neurons a reporter protein, which fluoresced upon elevations in calcium in the neuron. And calcium is an indicator of electrical activity, and this allowed us to perform imaging experiments on the fly brain to actually examine the patterns of brain activity in response to odors in real time and actually look at what is happening in the fly brain in a two-photon microscope over real time with a sensitivity and a spatial resolution that is perhaps a thousand times greater than the sorts of human-brain-imaging experiments.

And so what Jing and Allan Wong were able to do was to develop a fly-brain population which allows this sort of imaging at the submicron level in an isolated brain preparation that functions for several hours under a microscope. And this is what it looks like, looking now at the antennal lobe of a fly in response to two odors, caproic acid, which you will see in red, and pyridine, which you will see in green. And indeed, what we see is that pyridine activates these two loci in the brain whereas caproate activates these two loci in the brain. So indeed there is a functional representation of the anatomic map in the fly brain such that different odors elicit different patterns of activity. The anatomic map is functional, but it is of behavioral consequence?

In a series of experiments that we recently performed with Seymour Benzer and David Anderson at Caltech, we began to study the observation that flies are averse—they are repulsed—by what is known as an alarm pheromone, an alarm

substance, that is emitted by stressed flies. We were able to demonstrate that a major component of the alarm substance is CO_2. And we were able to demonstrate that, indeed, CO_2 elicits upon imaging a very simple pattern of activity in the brain. It activates one specific locus, one specific glomerulus here, and through genetic chicanery what we were able to do is essentially inhibit activity in this locus and ask, If we inhibit activity within this locus what is the consequence to behavior? And the flies no longer show an aversive response to CO_2. The inhibition of activity in all other regions of this antennal lobe have no effect upon the response to CO_2. So indeed odors elicit distinct patterns of activity in the brain, and these spatial patterns are of behavioral consequence.

The Binding Problem

What I have clearly shown thus far is that different odors elicit different patterns of activity, and that these patterns of activity can be read out as specific behaviors. The implication is that the nervous system, in the case of olfaction, dissects and deconstructs the odor into its structural components such that a given odor is represented by multiple spatially invariant loci of activation. So in this model, apple may activate these three loci, [and] banana these three overlapping but nonidentical loci. And I can look down at this map of activity and with accuracy I can discern what it is that the fly is smelling. But I've accomplished this with my eyes and my brain. How does the fly do it? The fly has no eyes in his brain, so what or who in the fly brain is actually looking down upon this map and reconstructing this deconstructed image into a meaningful percept? This is a simple form of the binding problem, how bits of electrical activity are bound into a meaningful percept. And inherent in the binding problem is a related problem, the parsing problem. Consider these two odors. Each of these odors elicits an overlapping but nonidentical pattern of activity. If I expose an organism to a mix of two odors, that organism is often able to discriminate the two individual odors within the mix. But the pattern of activity that we observe in the mix now consists of five loci. How is it that the organism is able to segregate, to parse, these three points of activity and identify it as apple, and these three points of activity and identify it as banana, rather than looking down and seeing a new pattern of five bits of activity? This is the parsing problem.

Superimposed on these conundra is the added complexity that the ultimate percept not only reflects sensory input but brain context, experience, expectation, and even emotion.

Now the binding problem in its simplest form as I've described it is one that is shared by all sensory systems. Elegant early work by Hubel and Wiesel and independently by Semir Zeki and in talks tomorrow have shown us that in the visual system, to obtain knowledge of what it is seeing, the brain does not merely passively represent images reflected on the retina; rather, it must actively deconstruct and reconstruct the visual world. A visual world is deconstructed first in the retina and ultimately by parallel processing pathways that report the distinct

components of a visual image, thus color is represented in a region of the cortex known as V4, whereas cells in V5, as we'll hear tomorrow, are responsive to motion, and an adjoining region is responsive to form. V3 and V5 are indifferent to the color of a stimulus, and lesions on the color area V4 allow an individual to see an image with clarity, but only in shades of gray.

This segregation in the visual system immediately poses a problem not dissimilar to what I've described in the simpler olfactory system. It's a problem of reconstruction or binding. How is the spatial map read? How are bits of electrical activity integrated to allow for meaningful recognition of a sensory image? I've already argued that sensory input, the bottom-up process, is incomplete, that it often results in a meager and selective image of physical reality. The image is completed by the brain in a top-down process that brings experience and expectation to the binding process. And if this is true, then perception is, as originally suggested by Richard Gregory, only a hypothesis, a best guess that only asymptotically approaches reality. Our assumptions are based in part on input and in part from the brain's stored record, a record nourished by our visual experience.

Consider this particularly clear example of binding. Some of us will bind well and see immediately, and others will require more time to see this Dalmatian sniffing the ground in front of a tree. Once having seen, bound, and parsed this image, binding on second view will be instantaneous and you will never forget it. Experience shapes the way you integrate incoming sensory information.

Now if perception is really just an assumption, a best guess, then you can get it wrong. The Kaniza triangle is a classic example of what I call illusory binding. This is an image that consists of three black Pacmen, but this is not at all what you see. What you see, what you are focusing on, is an equilateral triangle whose sides should be quite clear to you. But these sides to not exist. Your brain is trying to use its preconceived notions of the visual world and makes what is not. This is an illusion.

Finally if perception is indeed a hypothesis it can be challenged, as Zeki has pointed out, and this is precisely what René Magritte did. Magritte defies the common sense of our brain deliberately and with great success. The painting *Carte Blanche* is confounding. It goes against everything the brain has ever seen, learned, or stored in its memory. We can have no preconceived notion here because the brain has no representation of this bizarre scene. It is an act of the imagination that fascinates precisely because we cannot find a solution. It is a classic example of nonbinding.

Let me then return to the biology of the binding problem. How is the deconstructed map in the olfactory system reconstructed? One disturbingly seductive model argues that the combination of signals from the antennal lobe might be brought together to report to a locus in high brain, so this combination of signals might connect to a single locus in high brain that would then provide us with a refined

olfactory image, the notion of a jasmine cell in olfaction analogous to a grandmother cell in vision. Indeed recent data in our laboratory suggest that the next level of olfactory processing does precisely this, that active loci in the antennal lobe, which are insular and segregated and have minimal communication, actually project to high brain to form a second map. But this second map is of a different character. The termini of neurons from the antennal lobe are no longer insular or segregated, but now interdigitate, and this sort of dispersive interdigitation affords the opportunity for integration, so it allows for the communication of these bits of activity in high brain and perhaps for their reading.

So these observations leave us closer to a solution, but we're left with a higher-order problem. For there is not likely to be a single master area to which all signals ultimately report in any sensory system. Moreover if there were, who would look at it, who would read its spatial image? Rather, the sensory representation, I would argue, is likely to be distributive, and how this distributive ensemble is read and ultimately elicits appropriate behavioral or cognitive responses is what Vernon Mountcastle has described as "the big in-between, the ghost in the machine." And it would be presumptuous of me, a geneticist, to try and approach this old and complex problem of the ghost in the machine. Who reads the image, who listens to the music? And so I will leave this task to the ghost busters, to Bill Newsome, John Searle, Christof Koch, who will address the problem of consciousness tomorrow.

Thank you.

Question and Answer

I'm happy to take questions.

[Question inaudible.]

The question is there are some odorants that have powerful effects on the organism. Have I examined those odors and how they map? Indeed it's true that there are a set of odors within each species that elicit innate and strong responses. And so pheromones in most mammals below primates, for instance, elicit an innate mating response. And indeed we have looked at pheromones, as you might imagine. Pheromones activate a set of neurons in a different nose, what I call the erotic nose, the vomeronasal organ, which projects to a different part of the brain. The main nose that detects the universe of odors projects to what you might consider to be cognitive brain, whereas the pheromones are detected by a nose that projects to amygdala, the emotive brain.