

Brain Gas **Jay Hosler**

Consider the possibility that any man could, if he were so inclined, be the sculptor of his own brain.

-Santiago Ramon y Cajal

The fact that learning changes the brain in some fundamental way is something many of us take for granted. But, in doing so, we fail to consider the wondrous nature of the event. Our experiences can lead to substantial changes in the physical, chemical and electrical architecture of our brains. These changes may give us the ability to memorize our favorite poem, sight-read a song we have never heard or remember the smell of our grandparents' home. By restructuring our brains, we construct memories that can change our behavior and persist throughout our entire lives.

My interests lie in how memories of odors are established. Odors play a fundamental role in the lives of most animals in directing reproductive behavior, guiding the search for food and communicating with friends and rivals. But despite odors' pivotal role, olfaction may well be the most mysterious and hard to describe of our senses. In her book *A Natural History of the Senses*, Diane Ackerman points out the difficulty we have in describing

Bookend Seminar Presentation, October 10, 2001

smells. While we can identify colors in very specific terms (“that apple is red”), we tend to describe odors in terms of something else (“that smells fruity”) or in terms of how they make us feel (“that smells disgusting”). Perhaps it is difficult because smell is our most ancient sense. Unlike visual information that gets processed in our large and wrinkly cerebral cortex, the first stop for olfactory information is an ancient part of our brain called limbic system. The limbic system is the seat of drives and emotions that we often find equally difficult to articulate. The fact that many sensory biologists consider olfaction to be the first sensory system animals developed underscores the evolutionary significance of learning about smells. As we shall see, a strong evolutionary need to smell and remember odors has led to some interesting processing similarities among very different species. The research in my lab focuses on three different facets of odor memory formation: 1) how learning about odors changes the way animals behave, 2) the brain chemicals involved in learning odors and 3) how the brain’s electrical signals are modified once a new odor has been learned. Of these, the easiest to examine are behavioral changes.

We use classical conditioning to teach bees specific odors. Ivan Pavlov was a physiologist studying digestion when he developed classical conditioning to collect saliva from his dogs. The protocol was simple. Pavlov rang a bell and then gave his dogs some food. Under these conditions, his dogs started to associate the bell with food and started salivating in response to the bell alone. The dogs had learned to *associate* the bell with food and now *anticipated* food when the bell was rung. In our research, we have replaced the bell with a puff of odor and the dog’s food with sugar water (as a proxy for nectar). This pairing is very effective for bees and they usually learn to associate the odor with sugar water after only a few exposures. By manipulating how and when odors are presented and rewarded, we can learn a great deal about how and what honey bees learn about those odors.

So, why study the way bees learn odors? What is the relevance of olfactory memory formation in insects? There are a number of answers to this question. The first is that it interests me. This is a critical, often unstated, element of the scientific (or, for that matter, academic) process. Curiosity about a system drives creativity in research. Olfaction in honey bees is also interesting from an evolu-

tionary standpoint. Insects constitute over 75% of all animal species. They are far and away the most successful multicellular organisms on the planet, and understanding how they do anything will contribute to our understanding of their success. From an economic standpoint, honey bees are the primary pollinators of many crops and honey bees' ability to pollinate depends heavily on their sense of smell.

But perhaps the most interesting reason to study olfaction in honey bees is the striking similarities we see between the neural wiring in their brains and in ours. All animals that smell have a specific region of their brains that process incoming olfactory information. In most vertebrates (animals with backbones like us) odors are processed in the olfactory bulb, a little patch of neural tissue right above the nose. In honey bees, the brain region that processes smells is called the antennal lobe and it sits just inside the head at the base of the antenna (a good location since the antenna is a bee's nose). Information about smell travels unmolested through the antenna down long nerve cells called olfactory receptor neurons. When olfactory receptor neurons leave the antenna, they plug into the antennal lobe and things start getting complicated. The antennal lobe is a Gordian Knot of three types of neurons: 1) receptor neurons from the antenna, 2) local interneurons in the antennal lobe and 3) and projection neurons, which carry information from the antennal lobe to other parts of the brain. When stimulated, these three types of nerves begin a complicated conversation, the result of which is the filtering of the incoming olfactory information. This filtering is critical for a honey bee's ability to use olfactory information. The world a bee encounters is awash in olfactory noise. To make associations that are meaningful, the bee needs a filter to separate important smells from not-so-important smells. The wiring of the nerves in the antennal lobe makes this filtering possible. What is truly remarkable about this circuitry is that it is nearly identical to the wiring in our own olfactory bulbs!

The similarities, however, do not end with circuitry, and that brings us to the second facet of research in our lab: the brain chemicals involved in learning odors. Neurons use a chemical language to communicate with each other. The chemicals they use are called neurotransmitters and many of the same neurotransmitters are

found in the antennal lobe and olfactory bulb. Neurotransmitters are released from one neuron, diffuse across the space between cells (called a synapse) and activate a second neuron by interacting with a receptor in the second cell's outer membrane. While this is the traditional way in which neurotransmitters work, I would like to focus on a very interesting non-conformist. At least one neurotransmitter in both mammals and insects is a gas.

Nitric oxide is a free-radical gas produced when the enzyme nitric oxide synthase (NOS, for short) converts the amino acid arginine into citrulline. Unlike a traditional neurotransmitter, nitric oxide does not move unidirectionally from one cell to the next. Once produced, it spreads in an expanding sphere to all cells in the immediate vicinity. As a gas, it can pass like a wraith through cell walls and once inside, it activates a chemical called guanylyl cyclase, which then activates the cell. The first to suggest that nitric oxide gas might be playing a physiological role in the body was Ferid Murad in 1977. In the 25 years since then, nitric oxide has been found to be critical for the regulation of systems controlling blood pressure, the heart, infections, shock, the lungs, the brain and cancer. Viagra, one of the most commercially important drugs in recent memory, targets a nitric oxide system. Clearly, this gas is an important player in physiological systems. Nitric oxide caught our attention because it is found in high quantities in the honey bee's antennal lobes, suggesting to us that it might play a role in the processing of olfactory information. In fact, a neurotransmitter that could exert regional effects on several cells at once would be very useful in an information filter. We hypothesized that if NO was important for memory formation, then removing NO during learning would alter memory formation relative to a control group. But how do you remove gas from the brain of a bee?

As stated above, NO is produced through the action of an enzyme called nitric oxide synthase (NOS). If NOS is turned off before classical conditioning, the production of NO is turned off, effectively removing NO from the brain (for a short time at least) during learning. We targeted NOS with an inhibitory chemical called LNAME. To apply LNAME to the bees' brains, we surgically exposed their antennal lobes and injected them with 3 microliters of drug treatment. We used several treatment groups in this experiment, but I will focus on three in particular. In the control group,

we treated antennal lobes with an innocuous saline solution. In the LNAME experimental group, we treated the antennal lobes with the NOS inhibiting chemical LNAME. A comparison of the responses from these two groups would indicate whether preventing NO production has any effect on memory formation. Finally, we treated a third group with a cocktail containing LNAME and a second drug called SNAP. SNAP is a chemical that donates NO to a system. In effect, this last treatment knocks out the native (or endogenous) NO and replaces it with foreign (or exogenous) NO. In theory, if NO was playing a role in memory formation, then this last group should show responses similar to those treated with saline, since NO (albeit exogenous NO) will be in the system.

We treated bees with saline, LNAME or LNAME/SNAP prior to training them. There was no difference among the bees in all three groups in their ability to form an association between odor and sugar water. The differences arose in the specificity of the odor memories they formed during training. Saline treated bees could remember the odors to which they were trained and did not mistakenly respond when exposed to different odors. However, bees who had their endogenous NO blocked by LNAME responded equally well to conditioned and different odors. Bees in the LNAME/SNAP group retained some of their ability to discriminate between conditioned odor and different odors.

These results support our initial hypothesis that NO plays an important role in setting up specific odor representations in the antennal lobe. The bees treated with LNAME learned something about the odors to which they were conditioned but without NO present during the process their memories are fuzzy. They made more mistakes than did the saline controls when asked to recall the odor to which they were trained. Interestingly enough, Keith Kendrick obtained comparable results when doing similar experiments with sheep. These studies suggest a functional analogy between NO release in the insect antennal lobe and the vertebrate olfactory bulb that complements the strong structural similarities the two structures share. In other words, our olfactory bulb is wired like a bee's antennal lobe and they appear to work in much the same way.

These similarities further suggest some very interesting things about the evolution of odor processing regions in a brain. All liv-

ing things share a common ancestor at some point on the evolutionary family tree. For humans and insects, that shared ancestor lived hundreds of millions of years ago. Did that ancestor carry some genetic program for wiring a nose that humans share with insects? It may be possible. The homeobox gene *pax-6* directs eye development in insects and humans (and many other multicellular animals) although they develop radically different eyes. Such a gene has not yet been found for noses. The other possibility is that in the evolutionary history of bees and humans, both lines have found the best solution to extracting the most information they can from their olfactory world. Regardless of how we got here, the study of how insects process odors yields valuable insight into how humans do it and how sensory information is processed in general.