

*Conversations with six leading neuroscientists
on timely topics in brain research*



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What's New in Neurogenesis



FRED H. GAGE, Ph.D.

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Q: Your 1998 Nature Medicine article reporting that new neurons are regularly born in the adult human hippocampus helped overturn a long-held central tenet of neuroscience—and set off a wave of follow-on research that is just beginning to crest. What has surprised you most in the ensuing research?

A: I admit being surprised at the flood of articles, and by the sheer number of individuals who have gone into the field. One reason for that is because the tools that are available now for investigating neurogenesis are repeatable in the laboratory, and better techniques are being developed all the time. The repeatability of the findings and the consensus that developed quickly in the field have been encouraging.

I remember presenting our findings on adult neurogenesis at the Society for Neuroscience annual meeting 10 years ago, when there were only about 10 posters on the subject. There was a lot of skepticism about it, and a lot of debate going on. Contrast that with the 2006 meeting, where there were aisles and aisles of posters on neurogenesis. Investigators are now looking at this from many different directions, not just as a phenomenon itself but also at its role in epilepsy, stroke, spinal cord injury, etc. And there are reports of neurogenesis occurring in multiple brain areas, outside of the hippocampus and the olfactory bulb where much of the work has focused. The controversy remains in some domains, but the core observations about neurogenesis are solid.

Q: You've recently developed a complex computer simulation of hippocampal neurocircuitry to track the developmental progression of newly born neurons. Describe what your aims are and why a computational model is the best approach.

A: It has become clear over the last 10 years that neurogenesis is a process, not a single event. It encompasses a series of phenomena in the adult brain, one of which is self-renewal or maintenance of the stem cells within the niche in the brain where these cells originate. But neurogenesis as a process also involves the migration of those stem cells from the niche; the initiation of differentiation into various types; polarization, in which dendrites form and axons extend out from the cell body in a polar manner; and connectivity, which is how the inputs from, say, interneurons in the entorhinal cortex or inhibitory neurons in the hilus make contact with these new cells. The process as a whole takes time—up to a month or more from the cells' emergence to their integration into the system. Over the course of their maturation, the cells change their pattern of activity, and there is a transient period of hyperexcitability during which the cells are particularly sensitive to input signals. Finally, there is the question of what role these cells play in the normal functioning of the intact hippocampus and dentate.

For each of these steps, there is a plethora of new data being generated. So, in trying to decide what the next most important questions were and what experiments to do next, we wanted to take into consideration as much of the existing information as possible. Our goal was not so much to model the hippocampal system as to describe the current state of knowledge about neurogenesis mathematically.

Because adult neurogenesis is a newly recognized phenomenon, it doesn't easily fit within the current understanding of hippocampal function, in terms of physiology, anatomy, or behavior. In order to better understand how it may fit in, we need to put together as much information as we

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can about what is actually happening throughout the process of neurogenesis relative to the existing circuitry. From there, we can propose theories that can be tested to see whether we are right or wrong.

occurs at a second time point. The current thinking about the dentate gyrus is that part of its role is to keep these two events separate—what we call sparsification. By looking at a model encompassing all of the existing data on what these cells

are hypothesized to do in the context of the existing neurocircuitry, we can deduce that they seem to be linking these two independent events.

When you remember a past experience—let's say a summer vacation—you may pick up a very specific memory of say, a dinner you had and the people who were there at the table. As you draw that memory up, it will at the same time open up other memories of things that happened at the same time but were not directly related to that dinner and the specific events that occurred at it. Our brains have a way of linking things together that occurred generally close in time; we hypothesize that perpetually generating new cells is a mechanism by which the brain accomplishes this.

While we may mostly think of memory associations in terms of

very short time frames—seconds or minutes—this is more of an extended linkage related to the time course in which these cells remain in the process of integration into the circuitry.

Q: How are these new understandings contributing to an evolving view of hippocampal function and plasticity?

A: In the theory that we're currently working with, the general idea is that these new cells provide an added level of plasticity, of dynamic action within a hard-wired circuit that is pretty dynamic to begin with. This is really another level of plasticity beyond what we see in existing hippocampal neurons, which are known to

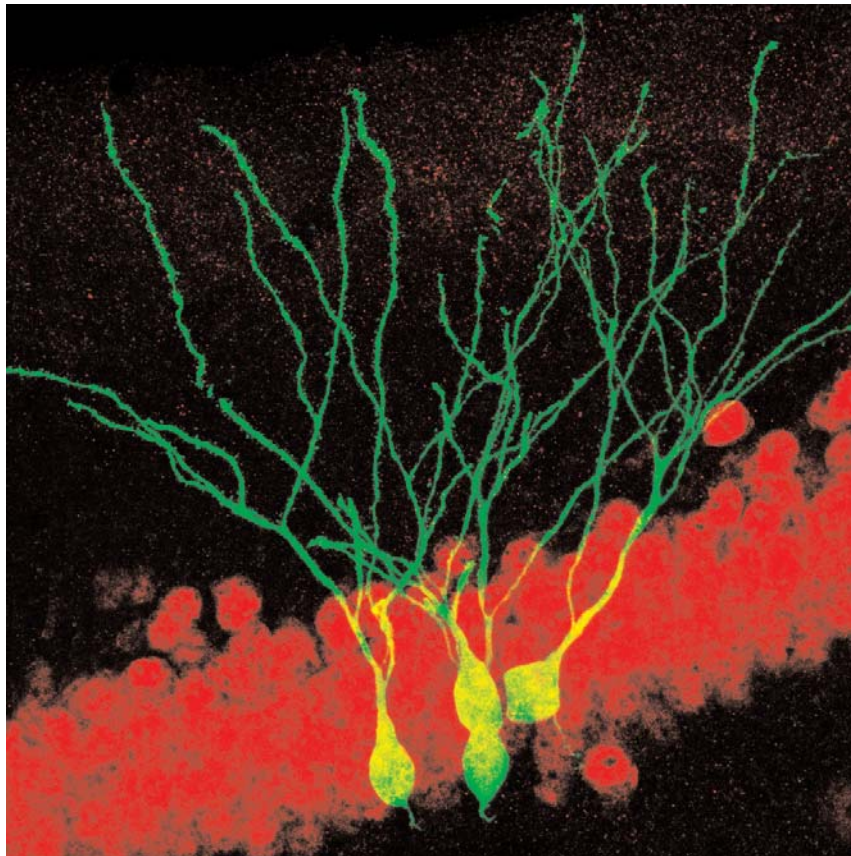


IMAGE COURTESY OF CHUNMEI ZHAO, GAGE LAB, SALK INSTITUTE

Newborn neurons in the adult mouse hippocampus

Q: Where are you with that research right now?

A: The modeling has led my colleagues Brad Aimone, Janet Wiles, and me to generate certain hypotheses about the function of newly born cells. We recently published a completely theoretical article (in *Nature Neuroscience*, June 2006) on a potential role that the newly born hippocampal granule cells could play in information processing and memory, specifically in what we're calling "time-coding" of events.

Our theory is that the new neurons link existing events. So, you have an event that occurs at a certain time, then you have another event, which

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change synaptic affinity in response to activity. With neurogenesis, new synapses and whole new neurons are actually being added into the circuitry. So it is the level of plasticity that is important.

Originally, the hippocampus may have been thought of as a structure involved in learning and memory, but it's now clear that it's less involved in long-term memory. So the earlier view was that it would be unreasonable to have newborn cells in

“What's driving the excitement and the theories is the acquisition of basic knowledge...about the cells' maturation.”

a circuitry that involves long-term memories because that might disrupt existing memories. That is less of a concern now because the current conceptualization of the hippocampus is that it is involved more in the formation of memories as opposed to memory storage.

Answering questions related to the function of these cells is important, and we will have a much better handle on this over the course of time. I suspect many of these questions will be resolved in the next five years. What's driving the excitement and the theories is the acquisition of basic knowledge about the sequence of events underlying the cells' maturation. This is a great example of how basic biological research drives applications and drives the ability to extrapolate about what is going on.

Q: What would you say is the focal point of neurogenesis research right now?

A: There are a few things. One is understanding the cellular/molecular events that constitute the progression from an adult stem cell *in vivo* to an

integrated functioning neuron. For many of us, that's enough—just to understand that process of fate/maturation in an adult context. A separate set of questions is: why does this area of the brain allow this to happen, when it doesn't really happen anywhere else? What's so important about this part of the hippocampus?

The third layer is applying this to understanding diseases. In many disease states it looks like there are changes occurring in the rate and the function of these cells relative to the normal progression: either they're not developing as much, as in aging and depression, or they're developing too much, as in epilepsy and stroke. For some of these diseases, this is actually the first time there has been an anatomical locus to pay attention to. This is particularly true in the affect disorders. This area of the brain (the hippocampus) is known to be involved in depression, schizophrenia, and others, but now there is evidence of dynamic changes in neurogenesis occurring in correlation with changes in disease states.

Q: What does all of this mean to the average person? Is there a “neurogenic” lifestyle that will help us ramp up the volume of new neurons in our brain?

A: Based on the experimental evidence that currently exists, there are several things that one could conclude. Physical exercise, environmental complexity, and specific types of learning are three conditions that have been robustly shown to increase neurogenesis. On the other side of that, both acute and chronic stress decrease neurogenesis, so the implication is that decreasing the pathogenic properties of stress should have less of a detrimental effect on neurogenesis.

Given those observations, my guess is that it's probably a pretty good idea to globally decrease stress, increase physical activity and environmental enrichment, and seek to continually acquire new information within your environment—to continue to be stimulated, in other words. I don't think that's going out on a limb. ■

Toward a New Approach to Depression



PAUL GREENGARD, Ph.D.

Professor & Head
Laboratory of Molecular
and Cellular Neuroscience
The Rockefeller University

Q: Your laboratory recently discovered a protein, dubbed P11, that you believe may be a key to understanding and treating depression better. What is the significance of the P11 finding?

A: P11 is important because it controls the localization of a very important class of serotonin receptors (the 5-HT_{1B} receptors). Within nerve cells, P11 recruits the 5-HT_{1B} receptors from the interior of the cells, where they are not functional, to the membrane on the cell surface, where they become functional and interact with serotonin molecules released by other cells. P11 is required for this movement.

To put this in context, there are currently three general classes of antidepressant drugs: SSRIs, tricyclics, and MAO inhibitors. All three cause an increase in serotonergic signaling, though they do it in different ways: the SSRIs block serotonin reuptake; the tricyclics block serotonin and norepinephrine reuptake; and the MAO inhibitors block serotonin and norepinephrine breakdown. The common short-term effect of all three classes of drugs is to raise the level of serotonin in the presence of a fixed number of serotonin receptors.

What P11 does is increase the level of receptors in the presence of a fixed amount of serotonin. Theoretically, increasing the level of serotonin receptors should produce an antidepressant effect because there would be more receptors to detect the serotonin. In fact, we found that if we knocked out P11 in mice, the animals behaved in a depressed manner, and if we over-expressed P11, the animals behaved as if they had been given an antidepressant.

We also found that, with extended use, antidepressants raise the level of P11 in the brains of experimental animals. And both in animal models of depression and in human post-mortem brain tissue, we found that depressed subjects had lower levels of P11 than non-depressed controls. To the best of my knowledge, this is the first example where there is a very good correlation between the level of a protein and state of depression, suggesting that P11 may be a key determinant in whether or not we are depressed. So this is a rather exciting starting point for trying to understand the biology of P11 and its relation to depression.

Q: Where are you now with research on P11?

A: Based on our work to date, we can make several conclusions. We know that antidepressants raise the level of P11; that P11 recruits serotonin receptors to the cell membrane; and that depressed animals and people have lower levels of P11 than normals. We also know that if you lower P11 levels, animals get depressed, and if you raise P11, the depression is relieved. One of the projects we're working on now is to try to understand the mechanisms by which antidepressants raise the level of P11. It's possible that this may be the key to how antidepressants are working, a theory that is not incompatible with other theories of how antidepressants exert their effects, such as the recognized effect these drugs have on neurogenesis.

We also want to understand how P11 recruits serotonin receptors to the membrane, and how this increase in serotonin receptors at the membrane leads to the observed antidepressant behavior. These are the more urgent questions we're trying to address.

Beyond these central questions, we want to know if P11 levels in the blood can be used as a biomarker for depression. We're also looking at other members of the large family of so-called S100 proteins, to which P11 belongs. Since there are many different types of serotonin receptors, we're now asking which of the S100 proteins

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interact with which serotonin receptors, to see if these phenomena we've observed could have broader significance. We don't know the answers yet; we're just setting up the methodology to do these studies now.

Q: Does this suggest that P11 might be used as a new form of antidepressant?

A: Because P11 is a protein, it couldn't be taken orally—it wouldn't be in the form of a pill like Prozac. Conceivably, P11 could be harnessed to treat depression using gene therapy approaches aimed at raising its level of expression. We're doing some studies in collaboration with Michael Kaplitt at Cornell to see whether we can use RNA interference technology to knock down or raise the level of P11 in specific brain regions suspected to be important in depression, and then see what happens behaviorally in experimental animals. Theoretically, this work could prove useful. The more traditional approach (which we are also doing) is to identify the mechanisms by which current antidepressant drugs raise the level of P11 and develop drugs that do that more effectively.

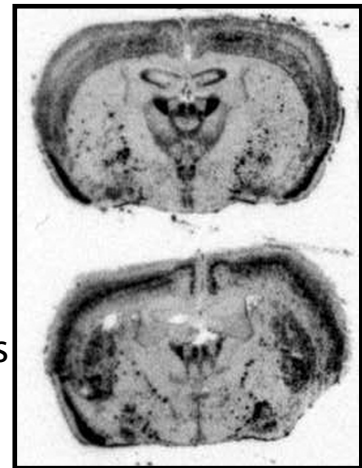
Q: Why do we need another antidepressant?

A: About one-third of patients who are severely depressed don't respond to any antidepressant. Among the two-thirds who do respond, many often suffer from side effects, which are sometimes severe. Current antidepressants take two to three weeks or more to have an effect, which is a very worrisome situation in severely depressed people because there may be a suicide risk. P11 would be a totally new approach, one that conceivably could provide benefits to a population of patients who either don't respond to antidepressants now on the market or who could respond with fewer side effects.

Q: You were awarded the Nobel Prize in Physiology or Medicine in 2000 for your work on post-synaptic pathways in the dopamine system, and last year you turned 81. What drives you in your research these days?

A: What continues to drive me is my excitement about understanding the brain, which is greater than ever; I feel there's so much more that's exciting now. It's a very stimulating environment because there are so many excellent people, including talented younger people, coming into the field, and there's so much more information coming out all the time. It would be impossible not to be excited about all the progress we've made in neuroscience.

Helpless



Non-helpless

IMAGE COURTESY OF PER SVENNINGSSON, KAROLINSKA INSTITUTE, STOCKHOLM, AND PAUL GREENGARD

Brain tissue slices from mice with a condition that models human depression ("Helpless," top image) show a dramatic reduction in P11 protein levels (dark areas) compared to control mice who show no signs of depression ("Non-helpless," bottom).

Q: What would you like to see occur in your lifetime in brain science?

A: It would be nice to understand the locus of depression in the brain. What are the abnormalities in the neural circuitry that lead to depression? There is some progress being made in this area. In Alzheimer's disease, it would be nice to know the difference between vulnerable and non-vulnerable neurons. For all of the diseases involving the dopamine system, it would be very nice to understand why dopaminergic neurons in the substantia nigra degenerate to a much greater extent than those in the ventral tegmental area, and what the adaptations are in the cells in the striatum that are the target of those dopaminergic neurons.

It would also be interesting to learn more about the causes of schizophrenia; we really know so very little. There's good evidence that it is a devel-

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opmental disorder, but the actual cause is still unclear. The genetic studies have provided some exciting leads, but not very many. How is the dopamine and glutamate signaling circuitry involved in producing the schizophrenic state? A lot of these problems are approachable now, whereas they weren't 10 years ago.

“The field has advanced much faster than I could have imagined. That’s largely due to the revolution in molecular biology and the development of a lot of very powerful tools.”

Q: What surprises you most about science today?

A. One thing that surprises me is the progress that has taken place in the computer revolution, particularly the increased speed of calculation and the ubiquitous application of new methods of calculation. These things are advancing far faster than those of us not in that field expected. Right now, computational models are being used to digest the vast amounts of data being generated by modern molecular biological techniques. That has had a profound impact on research, especially in genomic and genetic studies.

I think these tools will become increasingly important to understanding the brain. As we learn more about all the genes that are expressed in all the different types of cells in the brain, and about the myriad connections that any given nerve cell has with others in the brain directly or indirectly, there is going to be an increasing role for computer modeling.

Q: Did you ever imagine you'd be able to do these sorts of things when you started out in neuroscience?

A: The field has advanced much faster than I could have imagined. That's largely due to the revolution in molecular biology and the development of a lot of very powerful tools. For example it is now possible to change the level of a given gene in any given cell type at any stage of development. You can now remove or over-express all sorts of genes to study their effects on biochemical, physiological and behavioral properties of animals modified in that way. It is now possible to change the level of a protein in a single cell type at a certain time, to create inducible, conditional knockouts. We have new electrophysiological techniques that enable one to record from single cells, including from genetically modified cells or cells from genetically modified whole animals. There are also advances in imaging that make it possible to visualize, in real time, how cells change in live animals under various experimental conditions.

These advances have all greatly informed research, and make it possible to test hypotheses in ways that were never feasible before. Because all of these techniques are available, there is also more and more collaboration among laboratories. So I'm extremely optimistic about the future of the field.

Q: How important is it that scientists “sell” science to the public?

A: I think the more that scientists can communicate the excitement of our field to the lay public, the more support the lay public will want to see the scientists given by the government. We're moving closer and closer to major advances in many different areas of medicine, and I have the sense that this is also true for every field of science. The more we can communicate this work to the lay public, the more likely they will be to urge their Congressional representatives to support these areas of research. ■

When Stress Messes Up Memory



STANFORD NEWS SERVICE

ROBERT SAPOLSKY, Ph.D.

Professor of Biological Sciences,
Neurology, & Neurological Science
Stanford University

Q: Much of your work focuses on the effects of stress on the brain and, in particular, memory processing. How does emotional stress impact what we remember and what we forget?

A: Naturally, in a complex way. The sound bite about the subject is that “Stresses messes up memory.” The more complicated picture is built around a) how severe and prolonged the stressor is, and b) what kind of memory we’re talking about. Let’s start with conventional memory—conscious declarative facts that can be retrieved, as in “I am called a mammal” or “I have a dentist appointment next Tuesday.”

When a stressor is relatively mild and transient, the formation of new memories is improved. So too, although to a lesser extent, is the retrieval of old memories. What’s that about? Well, what is mild and transient stress? It’s what we would call “stimulation.” However, when stress is more severe and prolonged, consolidation and retrieval of declarative memory is impaired.

The impairments are focused in a key area of the brain involved in this type of cognition, the hippocampus. While mild transient stress increases the metabolic rate and excitability of the hippocampus, the more severe scenario involves lower metabolism, less excitability, atrophy, and even death of neurons. A key class of stress hormones secreted by the adrenal gland, called glucocorticoids, mediates much of these effects.

There is a second type of memory that is also relevant, which we call implicit traumatic memory. Examples are a memory of an earthquake’s

vibration, or the accent of the person who did the unspeakable thing to you. This type of implicit, non-conscious memory formation and retrieval is centered in the amygdala, and glucocorticoids do something VERY different there. Major traumatic stress causes enhancement of those implicit memories and, as a result of the elevated glucocorticoid levels, increased excitability and growth of neurons in the amygdala. Thus, a major traumatic stressor can facilitate the implicit memory of an event at the same time that it can impair the explicit components.

Q: From a practical perspective, if we really want to be sure we remember something, do we need to learn it in an emotional setting, or otherwise put ourselves under stress?

A: As outlined above, you’d want to do it with the RIGHT kind of stress, namely the circumstance we call stimulation: mild and transient activation of the stress response.

Q: You’ve spent a lot of time studying baboons on the Serengeti. What can baboons teach humans about stress?

A: There are several things that are relevant. For example, we have learned that in a stable dominance hierarchy, low-ranking animals have the most indices of stress-related disease. In contrast, in an unstable, rapidly shifting hierarchy, those traits are seen in high-ranking animals.

These general rules can be greatly modulated by individual personality factors. Among those of the same social rank, individual males are likely to have more indices of stress-related disease if they have trouble recognizing a neutral interaction with a rival as not being threatening, if they exert little social control, and if they have little participation in affiliative behaviors (e.g., grooming) with other animals.

Taken to another level, these individual-specific factors can be modulated by community-wide factors. In one troop of baboons, for example,

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low-ranking animals may be groomed a lot more, on average, than in another troop, and this will impact their physiology accordingly—that is, by making them less susceptible to stress-related disease.



IMAGE COURTESY OF LISA SHARE.

Robert Sapolsky with one of the wild baboons that he has studied for more than three decades on the East African Serengeti.

Q: A recent report from your lab shows that a specific type of gene therapy improves memory in stressed rats. What are the clinical implications of this work, and how long before we might see clinical applications based on it?

A: Both unfortunately and fortunately, things are still far from the clinic. There are a lot of “plumbing” aspects that need to be solved in the gene therapy field, by which I mean that the major challenges are related to how best to deliver genes to neurons, as opposed to what gene to deliver. On one hand, this is unfortunate because gene therapy could theoretically be immensely helpful. At the same time, this is fortunate, because a lot of bioethical work will need to be figured out when/if this should be done. For example, should this be used for someone who, greatly rushed and

stressed, is trying to figure out how best to save someone in an Emergency Room? Should this be used for someone who, greatly rushed and stressed, is trying to figure out how to most efficiently ethnically cleanse a village? These are ethical quandaries that society has yet to grapple with.

Q: What gets you most excited about your research?

A: One thing is the business of doing something that might actually help someone, and in the way that biomedical science does best: you could be flailing in the wilderness, and nothing is working, but then if, against all odds, you hit the jackpot of discovering something important, you may wind up helping lots of people as a result. That aspect of research always reminds me of a story: two people are standing on the edge of a fast-moving river. They spot someone being washed down the river. The first of the two people on the bank dives in to save the person; the second one just watches. More time passes, and another person is carried down the river. Again, the first person leaps in, while the second watches. This happens a third, then a fourth time. Finally, the first person yells at the second, “Why don’t you help?” And the second answers, “I am helping. I’m trying to figure out why people keep falling into this river.” The first person is a physician, and the second is a biomedical scientist; it’s that latter way of doing good that appeals to me enormously.

Q: What are the next steps?

A: For our work concerning how stress and stress hormones (glucocorticoids) can have adverse effects in the nervous system, we hope to understand in detail a very counterintuitive finding that we and others in the field have been uncovering—namely, that these hormones, renowned for their anti-inflammatory effects, can occasionally be pro-inflammatory in the injured nervous system. For our gene therapy work, our goal is to help move the field toward more clinical trials by further refining what genes would be most logical to deliver to the injured nervous system in various circumstances. ■

Sorting Out Memories and Emotion



JOSEPH LEDOUX, Ph.D.

University Professor
Henry and Lucy Moses
Professor of Science
Center for Neural Science
New York University

Q: You often make the distinction between “memories of emotions” and “emotional memories.” What is the difference?

A: We remember life’s important moments especially well. Emotional experiences, whether good or bad, leave strong traces in the brain. It was once thought that there was a single memory system in the brain. Now, however, we know that memories are formed in a variety of systems that can be roughly divided into two broad categories: systems that support conscious memory (i.e., explicit memory systems), and systems that store information unconsciously (i.e., implicit memory systems). Memories about emotional situations are often stored in both kinds of systems.

Much of our understanding of the neural systems’ underlying implicit emotional memory has come from studies utilizing Pavlovian fear conditioning as a behavioral paradigm. This work has implicated the amygdala in the formation and storage of emotional memories. That these memories are implicit is illustrated by the fact that people can be conditioned to respond to stimuli that they are not conscious of. Moreover, damage to the amygdala interferes with the ability of humans to be conditioned in this way. Such people have conscious explicit memories of being conditioned (i.e., they have memories about the emotional situation), but do not have the implicit emotional memories that allow the stimulus to elicit emotional responses.

Q: How is the amygdala involved in emotional memory?

A: In the case of implicit memory the information is learned and stored in the system that processes the relevant stimulus information and produces the learned response. This differs from explicit memory, which involves a system (the medial temporal lobe system) that has no obligatory responses associated with it. The amygdala is hard-wired by genetics to respond to certain kinds of stimuli that have traditionally been dangerous to our species. When such stimuli are encountered, behavioral, autonomic nervous system, and hormonal responses are expressed that help the organism cope with the danger. In such situations, stimuli that are associated with the danger, and thus predictive of future dangers, acquire the capacity to elicit emotional responses. Thus, amygdala is involved in emotional memory because it is involved in emotional processing, and it is involved in emotional processing because of its wiring to sensory and motor systems.

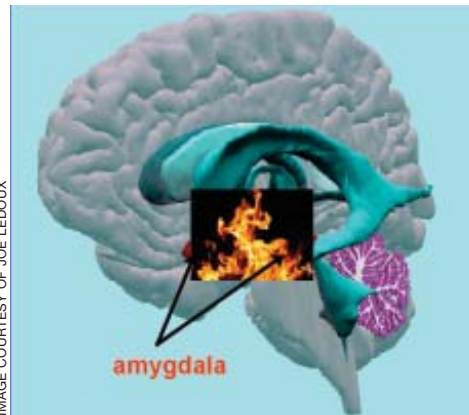


IMAGE COURTESY OF JOE LEDOUX

**YOUR
BRAIN
ON FEAR**

The amygdala is hard-wired to respond to stimuli that have traditionally been a threat to survival.

Q: You’ve suggested that the long-held concept of a “limbic system” that governs emotions is misguided. What has led you to this conclusion? Is there an “emotion circuit” in the brain?

A: The limbic system was proposed as an all-purpose solution to the problem of how the brain makes emotions. Several concepts were key to the theory. First, the limbic system is involved in emotion and not cognition. Second, the hippocampus is the centerpiece of the limbic system, and hence the emotional brain, because it integrates the internal and external environment. Third, the

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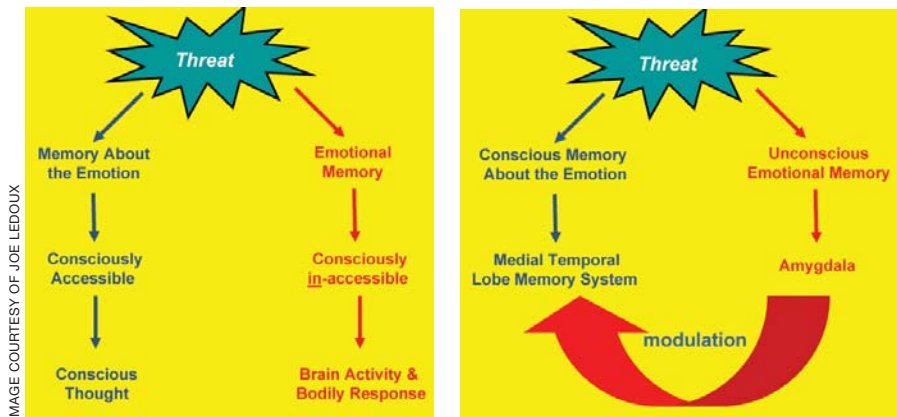
limbic system exists anatomically. Each of these ideas has been called into question. Soon after the limbic system theory was proposed, it was shown that the hippocampus, the system's purported centerpiece, was involved in cognitive memory. As it turns out, the hippocampus does not play a key role in emotion. This weakened the first two concepts about the limbic system. Regarding the third point, it has been very difficult to come up with criteria that allow one to say which brain areas belong to the limbic system and which do not.

More than any other region, the amygdala has been consistently implicated in emotion. Because

The limbic system was brilliant in its time, especially as a psychological theory about differences between emotional and cognitive evolution. The brain part was not quite right. But Paul MacLean, who originated the concept, should be praised rather than criticized. We know a lot more now than was available at the time, and he did a heck of a job with what was available.

Q: Beyond “academics,” why should the public care about research on emotional memories? What are the clinical implications of this kind of work?

A: There is both an upside and a downside to the fact that emotional states make memories stronger. The upside is that we remember our emotional experiences to a greater extent than non-emotional ones. The downside is that we remember our emotional experiences to a greater extent than non-emotional ones. By understanding how emotional memories, and memories about emotions, are formed and stored, we hope to be



Emotional memories and memories about emotions are processed differently by the brain.

the amygdala was part of the limbic system, evidence implicating the amygdala in emotion has been viewed as vindication of the limbic system concept. This constitutes faulty reasoning.

One thing we need to guard against is replacing the limbic system concept with the amygdala concept of emotion. Much of what we know about the amygdala has come from studies of fear, and moreover, of a limited kind of simple fear. We need much more research on emotions and their neural basis before we can assign a particular brain region or circuit as the “emotion system.” My preference is to talk about the “limbic fore-brain”—regions related to the medial cortex—without referring to these as a functional system. Functional systems have to be discovered through research. Broad theories like the limbic system are probably detrimental because they imply that we know the answers.

in a better position to help relieve suffering in people who live with traumatic memories that intrude upon daily life. Recent studies in rats suggest that it is possible to weaken emotional memories by giving certain drugs during the retrieval of the memory. This has implications not only for traumatic memory but also for the implicit memories that sustain addiction.

Q: What gets you most excited about your research? What are the next steps?

A: The most exciting thing about my research is the young people I work with. I am constantly being challenged and amazed by their creativity and insights. My next steps are really steps we take together. It's hard to say where we are going. I don't run the lab with a master plan. I kind of let today let us know what to do tomorrow. ■

Why Just Saying No Is Not Enough



MICHAEL KUHAR, Ph.D.
Candler Professor and Chief
Division of Neuroscience
Yerkes National Primate Research
Center of Emory University

Q: Why is the idea that drug addicts can “just say no” to drugs misguided?

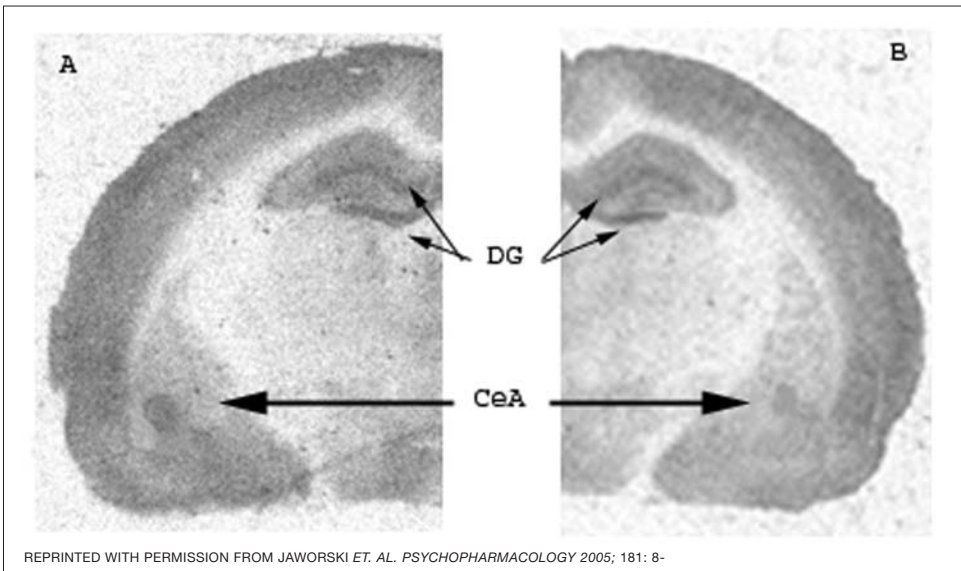
A: A current view in the field of drug abuse research is that the action of addictive drugs on the brain is partly to impair judgment and cognitive function. We are all aware how strong ordinary habits can be and how difficult some are to break. Becoming dependent on drugs is not only like a habit but like a habit in which other brain functions are impaired. This makes it more difficult to stop taking drugs. The evidence that drugs change the brain is abundant and really cannot be argued against. These brain changes are the basis of drug dependence and cognitive impairments. Sometimes, the reaction to drugs or even to things associated with drugs (so-called drug cues such as a crack house or white powder) are so strongly conditioned that it becomes some-

what unconscious. In this case, the craving for drugs cannot be stopped simply by saying, “go away”; treatment is necessary to reduce these automatic responses.

In summary, drugs impair our ability to just say no.

Q: For years, you have studied the effects of CART (or cocaine- and amphetamine-regulated transcription factor), a genetic switch by which cocaine and other psychostimulants disrupt dopamine signaling. What are the clinical implications of your discovery about CART, and where do we stand as far as applying this finding to the development of therapeutics for drug addiction?

A: We started working on CART shortly after its discovery when there were only a few papers in the literature; now there are hundreds. CART is a peptide found in many places in the brain, including drug reward/reinforcement areas. We found that injecting CART into these areas has little or no effect by itself, but co-injecting CART and cocaine results in an attenuation of the effects of cocaine. This attenuation suggests that drugs that mimic CART (agonists) could be used to blunt the effects of psychostimulants and thus be useful medications to treat drug addiction. The fact that



Animals exposed to perinatal stress have a greater tendency to self-administer alcohol, and this behavior is related to the density of brain receptors for the neurotransmitter GABA. Figure shows differences in GABA receptors depending on the kind of stress animals were exposed to perinatally.

DG=dentate gyrus

CeA=central nucleus of the amygdala

Why Just Saying No Is Not Enough

they don't totally block the cocaine effect is likely to be an advantage, since complete blockers are not always accepted by addicts. Recently, we have identified a CART receptor, a molecule that binds to CART to initiate a signaling cascade. These new understandings about the underlying mechanisms by which CART acts to blunt cocaine's effects make it possible to develop small-molecule medications that would be testable in classical clinical trials. While the receptor hasn't yet been cloned—

“It is known that early life stresses in humans predispose them to many problems later in life, including various neuropsychiatric disorders.”

a key step to designing agonists—the binding and signaling assays we used to discover the receptor are adequate for screening potential pharmaceutical compounds. This is a very interesting and potentially clinically useful area of research.

Q: You have found that separating rat pups from their mothers early in life increases the pups' vulnerability to addiction. What is your current thinking in terms of the neural mechanisms underlying this increased vulnerability, and how does it apply to humans?

A: This also is a very interesting field. The work in our lab and elsewhere shows that separating rat pups from dams (moms) daily, for varying periods of 15 or 180 minutes during the first two weeks of life, changes the vulnerability of the pups to addiction when they are adults. It appears that perinatal treatments can actually change an

animal's vulnerability to take drugs for the rest of its life. This is remarkable!

How does it happen? The answer is not fully known, but data suggest that the dams behave differently after the separations, which influences the pup brains. It also appears that stress is linked to this response because the stress-response axis is changed in some of the separated pups. A combination of stress to the offspring and the mother's responses seems to be involved in producing lifelong changes in the offspring.

It is known that early life stresses in humans predispose them to many problems later in life, including various neuropsychiatric disorders. This maternal separation model with rats is likely to be helpful in testing various ideas about treating humans who have a history of perinatal stressors.

Q: What are the next steps in your research?

A: In the CART project, we want to work with the receptor and set up a drug-screening protocol to identify possible medications. This is a reasonable and achievable goal. We also want to unravel how CART produces the cocaine-blunting effects in brain areas such as the nucleus accumbens. If we understand the mechanisms, then maybe we can use that knowledge to manipulate the effects in novel and helpful ways.

In the maternal separation project, we hope to determine the changes in the dopamine system that cause the changes in vulnerability to drugs. We want to clarify the mechanisms for this, in general. We also want to see what can reverse the increased vulnerability: Will anti-depressants do it? Will an enriched rearing environment do it? Findings in the field suggest that the answer to these questions is yes, and we want to extend that work. This is a very exciting area with important implications for humans. ■

Addiction and the Prefrontal Cortex



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Q: What is I-RISA and how does it apply to the study of addiction?

A: Together with Dr. Nora D. Volkow, we have recently emphasized that human drug addiction can be characterized by Impaired Response Inhibition and Salience Attribution (I-RISA), where the motivation to procure drugs overpowers the drive to attain most other non-drug-related goals. In this model, we mapped the core clinical symptoms in drug addiction, including craving, or “drug wanting” to the brain mechanisms that underlie the ability to control behavior, especially in an emotionally salient (e.g., drug-related) context. In this model we postulated that drug-addicted individuals attribute excessive salience (i.e., importance, relevance) to the drug and drug-related cues. At the same time, insufficient salience is attributed to non-drug-related reinforcers, stimuli such as food or social relationships that increase the probability of a subsequent behavior. We further hypothesized that this change in salience attribution, which is modulated by prefrontal cortical brain regions, would be predictive of impaired control of behavior (impulsivity).

This I-RISA model advances the notion that drug addiction cannot be fully understood without looking beyond the “pleasure principle,” the classical brain-reward circuit. The circuit encompasses subcortical regions such as the ventral tegmental area, where dopamine, a neurotransmitter critically implicated in drug self-reinforcement, is manufactured, and the nucleus accumbens, where dopamine is released. We also emphasized the

importance of cortical brain regions within this reward circuit, particularly the prefrontal cortex (PFC), which includes the orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC).

These PFC regions are involved in higher-order cognition (e.g., decision making) and emotion (regulation). In our I-RISA model we have specifically implicated these PFC regions in determining the salience of a given reinforcer. Knowing this, it may be possible to increase the salience of a non-drug reinforcer (e.g., the prospect of being employed) to buffer a strong emotional response to a drug-related reinforcer. These notions are consistent with a modern view of dopamine function that advances its role beyond reward to salience and novelty processing.

Q: What have you learned about how drug-addicted individuals respond to non-drug rewards?

A: In our translational research we use a combination of cognitive tests, self-report questionnaires, and brain-recording tools. For example, we record study participants’ behavioral responses on reaction-time tasks. We ask subjects how they think/feel about certain stimuli by using subjective rating scales. Finally, we image subjects’ brain structures and functions while they are performing these tasks, using functional magnetic resonance imaging (fMRI), positron emission tomography (PET), or event-related potential (ERP) recordings.

Preliminary results from our laboratory using this multimodal research approach¹ indicate differences in responses to non-drug rewards as a function of addiction. We found that when drug-addicted individuals think about a hypothetical situation during which they are “under the influence,” the importance of a drug reward exceeds

¹ I want to acknowledge the contribution to our studies of many talented and dedicated researchers, including Nelly Alia-Klein, Dardo Tomasi, Patricia Woicik, and Thomas Maloney from the Neuropsychimaging group and also Frank Telang, Gene-Jack Wang, Joanna Fowler, Chris Wong, and numerous others from the PET group.

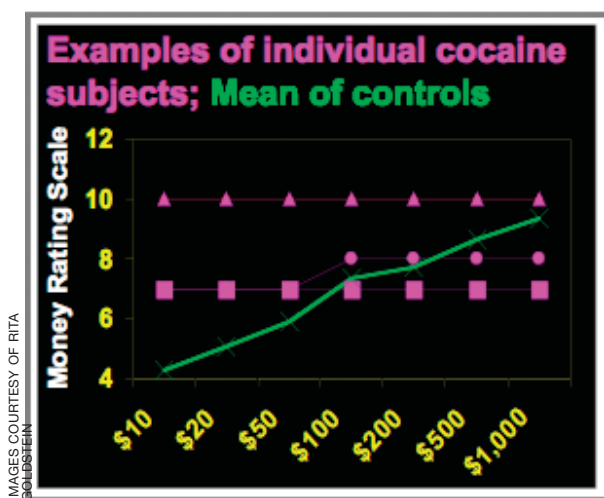
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that of other primary reinforcers, such as food. Using a self-report instrument we've recently developed, we also asked subjects to rank their feelings of "wanting" vs. "liking" the drug while they were thinking about this hypothetical situation. Overall, cocaine-addicted individuals—but not healthy controls—ranked "wanting" a drug higher than "liking" a drug. Furthermore, the addicted subjects with the highest "wanting" over "liking" rankings had both a higher frequency of recent drug use and greater reactivity to drug cues. This supports the fact that they indeed may be craving the drug (or at least unable to ignore it) even when the drug is no longer pleasurable.

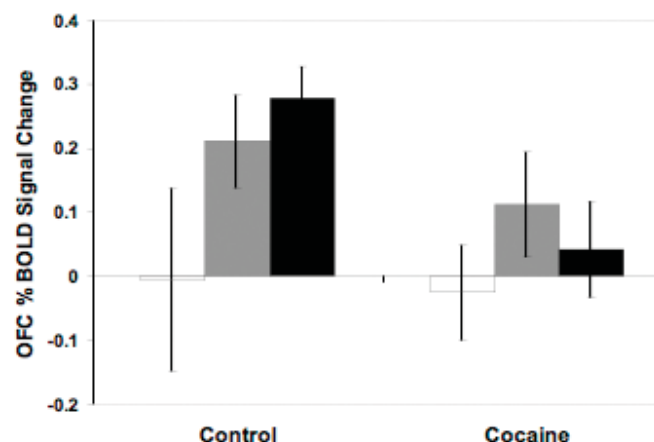
Our results further suggest a compromise in the ability to process the relative value of secondary non-drug-related rewards (e.g., money). In a group of 16 individuals with cocaine-use disorders, nine (56%) subjects demonstrated decreased sensitivity to differences between levels of abstract monetary rewards. When asked to rate seven monetary amounts (\$10, \$20, \$50, \$100, \$200, \$500, \$1000) on a scale of 0 (not at all valuable) to 10 (most valuable), these subjects rated \$10 to be equally valuable to \$1000; all amounts received a rating of 10. Only 2 of 13 (15%) control subjects demonstrated this flattened sensitivity to

monetary reward, a statistically significant group difference (Figure 1A). In the drug-addicted subjects, the PFC response (as measured with fMRI) accounted for 85% of the variability in this compromised sensitivity to monetary reward. In particular, this compromise in subjective sensitivity to relative monetary reward was paralleled by the OFC response to money: while OFC activity monotonically increased in the healthy control subjects, its activity was reduced and not linear in the cocaine subjects (Figure 1B). Importantly, we did not ask our study volunteers to *choose* between \$10 and \$1000; instead we asked them about the *subjective* value of these amounts.

This result makes sense if one thinks about the desire to use drugs in drug addiction: even very small amounts of money can bring an individual closer to this goal. It still remains to be determined whether this compromised sensitivity to gradients in reward predicts choice behavior—whether it will predict more severe drug use symptomatology, for example. This may indeed be the case because if the relative context of reward is compromised, the addicted individual may be more amenable to making disadvantageous decisions such as trading something of high personal value for the opportunity to get high.



(A) 9/16 (56%) individuals with cocaine use disorders but only 2/13 (15%) controls had compromised subjective sensitivity to the value of different gradations in abstract monetary reward (\$10=\$1,000).



(B) The orbitofrontal cortex (OFC) responds in monotonically positive fashion to monetary reward (white=neutral, gray=low, black=high) in healthy control subjects (N=13) but not in individuals with cocaine use disorders (N=16).

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Q: How does this relate to the propensity for drug relapse?

A: Our results also point to a disrupted perception of inner motivational drives (or the inability to translate perception into action), which could contribute to impairments in self-control in the drug-addicted individuals. Thus, while healthy control subjects were able to modify behavior based on the perceived relative value of a reward, drug-addicted individuals were not able to do so. This impairment may represent not only a compromise in perceiving the value of a reward, but also in utilizing this knowledge to modify behavior.

“ Consistent with the compulsive and chronically relapsing nature of drug addiction, our findings may help explain why efforts to control addiction through reinforcement can be compromised. ”

These results suggest an underlying cognitive-emotional mechanism in drug-related situations: when the value of a drug stimulus is higher than all other available rewards, which are perceived as equally less important than the drug, the ability to use non-drug reinforcement to control drug-taking behavior would necessarily be compromised. This would predispose an individual to relapse and drug use. Indeed, preliminary studies from other laboratories suggest that, in initially abstinent drug-addicted subjects, stronger drug cue- or stress-induced brain activations in the PFC during the early abstinence period are predictive of earlier or more severe relapse.

Q: What is your current thinking regarding the neural mechanisms underlying this flattened sensitivity to non-drug rewards?

A: Individuals with lesions to certain regions of their PFC, including the OFC, have difficulties in modifying behavior appropriately in response to altered reinforcement situations in their environment. Similarly, drug-addicted individuals also have PFC structural changes (e.g., reduced volumes), OFC and ACC functional changes (e.g., increased response when craving), and parallel behavioral changes (e.g., increased impulsivity). These findings led us to ask what role the OFC and ACC play in the drug-addicted individual's ability to modify behavior based on the salience and value of a given reinforcer.

We think that drug addiction may be better understood as a disorder of neural regulation. Here's why: even though the OFC and ACC are not sufficiently engaged in the processing of non-drug-related rewards, they are activated—in addicted individuals but not controls—in response to drug-related cues (e.g., words/pictures/videos of drug taking or pharmacologically similar drugs). Indeed, our preliminary fMRI results suggest that a possible communication breakdown between PFC sub-regions (OFC and dorsolateral PFC) may underlie the disrupted perception of motivational drive and the impaired control of behavior that characterized the drug-addicted individuals in our study.

Q: What does this work suggest in terms of clinical implications for treating drug addiction?

A: Consistent with the compulsive and chronically relapsing nature of drug addiction, our findings may help explain why efforts to control addiction through reinforcement can be compromised. It is possible that instead, efforts should be focused on devising new training and skill-development strategies and on supervised pharmacological interventions, all with the goal of decreasing the reinforcing effects of the drug, enhancing the relative value attributed to non-drug-related rewards, and increasing control of behavior. Together, these

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approaches may enhance the ability to control drug-taking behavior even in situations when the desire for the drug exceeds that for other rewards.

More research is required to delineate the optimal treatment approaches. As we acquire basic knowledge about addiction-related brain circuits and their interaction with environmental variables, dual approaches pairing behavioral interventions with medications will likely offer new and effective treatments for drug addiction and its associated neurobiological changes. For example, one could conceive of interventions designed to “exercise” brain circuits using specific cognitive and behavioral therapies to remediate and strengthen the circuits affected by chronic drug use, analogous to some interventions currently used for reading disabilities and traumatic brain injury. Such dual interventions that specifically activate and strengthen circuits involved in inhibitory control and salience attribution (e.g., the PFC) may increase successful abstinence from drug taking.

Q: What are the next steps in your research?

A: In our next project we target the I-RISA model using a newly developed fMRI task, the drug Stroop task, to directly test the effect of salient cues on inhibitory control in cocaine-addicted individuals. While participants perform this fMRI task, we plan to administer a pharmacological challenge (methylphenidate, or Ritalin) that increases extracellular dopamine and enhances the striatal-PFC activity that marks an event as salient. We will test whether response to this pharmacological salience enhancement predicts clinical outcome at follow-up. Results of this study may be helpful in devising intervention strategies to counteract the overwhelming salience that drugs of abuse have on addicted individuals, with the goal of minimizing relapse. This study will also allow us to directly probe the dopaminergic circuit in human subjects addicted to cocaine, to be accomplished for the first time with pharmacological fMRI.

In addition, we plan to test the predictive utility of our self-report instruments *vis-à-vis* choice behavior. We want to know if drug-addicted individuals

who report wanting drugs more than liking drugs and who show flattened sensitivity to non-drug-related rewards would choose drugs over other salient reinforcers (e.g., money) more frequently and/or despite severe consequences.

Lastly, our interest in personality traits (e.g., a tendency to avoid harm vs. approach risk) has led us to examine genetic vulnerabilities in drug-

“ Results of this study may be helpful in devising intervention strategies to counteract the overwhelming salience that drugs of abuse have on addicted individuals.... ”

addicted individuals. Can we associate heightened I-RISA risk with a modified genotype? If so, what are the gene candidates most related to the underlying neurocognitive I-RISA mechanisms? A better understanding of the interactions between genes, environment, and neurobiology may offer new targets for the development of pharmacological and non-pharmacological interventions.

Such future studies could help elucidate the following questions: did the neurocognitive impairments develop secondary to drug abuse and addiction, or were they a predisposing factor? The answer probably lies between these two possibilities and varies among individuals. Most importantly, can we identify susceptible individuals before addiction develops, thus preventing the onset of this vicious cycle? And, can we offer the intense treatment needed to individuals at highest risk for the most severe forms of addiction, reducing the high morbidity and mortality associated with this chronic disease? ■