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STUDIES PINPOINT SPECIFIC BRAIN AREAS AND MECHANISMS ASSOCIATED WITH DEPRESSION AND ANXIETY

Scientists investigate promising new target areas for treatment

SAN DIEGO — Research released today reveals new mechanisms and areas of the brain associated with anxiety and depression, presenting possible targets to understand and treat these debilitating mental illnesses. The findings were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

More than 350 million people worldwide suffer from clinical depression and between 5 and 25 percent of adults suffer from generalized anxiety, according to the World Health Organization. The resulting emotional and financial costs to people, families, and society are significant. Further, antidepressants are not always effective and often cause severe side effects.

Today's new findings show that:

- A molecule in the immune system may contribute to depression, suggesting a potential biomarker for the disease (Georgia Hodes, PhD, abstract 542.1, see attached summary).
- Decreasing a chemical signal in the amygdala, a brain area associated with emotional processing, produces antidepressant-like effects in mice (Yann Mineur, PhD, abstract 504, see attached summary).
- MicroRNAs, tiny molecules that alter gene expression, correlate with how mice respond to socially stressful situations that cause depressive-like behavior. The findings may help determine why some people are more likely to suffer from depression than others (Karen Scott, PhD, abstract 731.2, see attached summary).

Other recent findings discussed show that:

- A pathway between two brain regions, the amygdala and the hippocampus, plays a significant role in anxiety. Shutting down this connection can decrease anxiety-like behavior in mice (Ada Felix-Ortiz, MS, presentation 393.01, see attached speaker summary).
- Aversive experiences can change how humans, particularly those with anxiety disorders, perceive stimuli. After a severe negative incident, patients with anxiety disorders over-generalize the experience and have increased emotional responses to subsequent similar situations (Rony Paz, PhD, presentation 295.05, see attached speaker summary).

"Today's findings represent our rapidly growing understanding of the individual molecules and brain circuits that may contribute to depression and anxiety," said press conference moderator Lisa Monteggia, PhD, of the University of Texas Southwestern Medical Center, an expert on mechanisms of antidepressant action. "These exciting discoveries represent the potential for significant changes in how we diagnose and treat these illnesses that touch millions."

This research was supported by national funding agencies such as the National Institutes of Health, as well as private and philanthropic organizations. Find more information about depression and anxiety at *BrainFacts.org*.

Related Neuroscience 2013 Presentation:

Symposium: **The Emotion Triad: The Role of Interactions Between the Amygdala, Hippocampus, and Medial Prefrontal Cortex in Mood and Anxiety**
Monday, Nov. 11, 8:30—11 a.m., Room 6A

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Abstract 542.1 Summary

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Rethinking the Role of the Immune System in Depression

A molecule produced in white blood cells may predict depression risk

When treating debilitating mental disorders, researchers look not only to the brain, but also to the body for answers. A new study in mice shows that levels of the inflammatory cytokine IL-6, a molecule that is produced and secreted by white blood cells of the immune system, can be used to predict how animals might react to social stress. The findings were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Social stress is one of the most significant contributors to depression in humans, yet some individuals experience no adverse effects, while others are vulnerable. Understanding the differences could drastically affect how depression is treated.

"This study changes the way we understand the relationship between our immune system and stress responses that can lead to depression. We now know that a specific reaction in the immune system actually predates, predicts, and can shape how we'll respond to stress," said lead author Georgia Hodes, PhD, of the Ichan School of Medicine. "This may represent a legitimate biomarker for depression and could represent a new chapter in the effort to accurately diagnose and better treat mood disorders."

The research showed that levels of IL-6 not only predict whether mice are susceptible to the effects of social stress, but that artificially increasing or decreasing the molecule can change how an animal responds.

To investigate the role of this molecule in depression, the researchers used social defeat stress, a test that puts a smaller mouse in the same cage with a larger, more aggressive strain of mouse. The smaller mouse is quickly defeated, and long term exposure to this defeat produces depression-like behavior, such as social avoidance. But not all mice are susceptible; some are less sensitive and will continue to interact with other animals in spite of the social defeat. IL-6 may make the difference.

Researchers found that higher levels of IL-6 released by stimulation of the white blood cells before the defeat predicted depression-like behavior, while lower levels predicted stress-resistance. They also showed that reducing IL-6 in the body made mice immune to social stress. Conversely, increasing IL-6 with a bone marrow transplant from a stress-susceptible mouse had the opposite effect, provoking depression-like behavior. The results suggest that measuring stimulated IL-6, a chemical easily found in the blood, could serve as a bio-marker for stress sensitivity. Furthermore, treatments to reduce IL-6 in the body may be effective in treating depression and related disorders.

Research was supported with funds from the National Institute of Mental Health, the Johnson & Johnson International Mental Health Research Organization, and the Brain & Behavior Research Foundation.

Scientific Presentation: Tuesday, Nov. 12, 9–10 a.m., Halls B–H.

542.1, Individual differences in peripheral inflammatory signaling controls susceptibility to social defeat stress
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TECHNICAL ABSTRACT: Interleukin-6 (IL-6) is increased in the blood of subjects with depression and may reflect hyperactivity of the peripheral immune system. We utilized repeated social defeat stress (RSDS), an animal model of depression, to examine individual differences in the peripheral immune response to stress. After exposure to RSDS some animals termed susceptible show a spectrum of depression-like behavior, whereas resilient animals behave more akin to controls. Susceptible mice exhibit heightened IL-6 levels following their first defeat, which remains elevated 48 hours after the last defeat. To examine if IL-6 levels contribute to individual differences in stress sensitivity, peripheral mononuclear cells (PBMCs) were isolated before mice were exposed to RSDS. Cells were stimulated with lipopolysaccharide (LPS) in vitro to examine IL-6 release. PBMCs from animals that later developed a susceptible phenotype had an

exaggerated release of IL-6 in response to LPS stimulation. IL-6 release negatively correlated with social interaction ratio scores. Furthermore, mice that became susceptible to RSDS had more circulating PBMCs than mice that displayed a resilient phenotype. The number of isolated PBMCs also negatively correlated with the individual animals' social interaction ratio score. To test the functional relevance of what appears to be a heightened peripheral IL-6 response to stress, we blocked susceptibility to RSDS by systemically injecting an antibody that neutralized IL-6 in the periphery. Additionally, IL-6 knockout mice also displayed resilient behavior. We then ablated the peripheral immune system of C57BL/6J mice and replaced it with bone marrow from IL-6 knockout mice. Bone marrow transplantation from an IL-6 knockout mouse promoted resiliency to RSDS. Furthermore, bone marrow transplants from susceptible mice induced social avoidance following a sub-threshold microdefeat. Together these studies indicate that individual differences in the inflammatory response to stress underlie the development of depression-like behavior in the social defeat model.

Abstract 504.01 Summary

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To Raise Mood, Researchers Look To Brain Protein

Animal study points to acetylcholine receptors as possible new drug targets for depression

Decreasing a specific protein in the amygdala, an area of the brain involved in mood, creates antidepressant-like effects and reduces anxiety in mice. The findings, presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health, may help identify new molecular drug targets

"Our data provide a new mechanism and location in the brain that can be used to study depression," said lead author Yann Mineur, PhD, an Associate Research Scientist at Yale University School of Medicine. "These findings could lead to new tools to understand and diagnose depression, and might be the key to creating more effective antidepressants."

Previous studies show that drugs that block specific acetylcholine receptors (β2 nAChRs) can have antidepressant properties. To zero in on the role of this interaction, researchers developed mice with localized reduction of β2 nAChRs expression and measured responses to social defeat stress tests.

Mineur and his colleagues found that this change in the mouse amygdala appeared to protect against depression and anxiety in mice. The findings could guide researchers to a better understanding of the molecular mechanisms of depression and assist in the development of new drugs to treat mood disorders.

Research was supported with funds from National Institute of Mental Health.

Scientific Presentation: Tuesday, Nov. 12, 8–11 a.m., Room 33C.

504.01, A role for site specific β2-subunit containing nicotinic receptors in the control of stress and mood
Y. S. MINEUR, G. M. FOTE, S. A. BLAKEMAN, S. ZHOU, M. R. PICCIOTTO; Psychiatry, Yale Univ. Sch. Med., NEW HAVEN, CT

TECHNICAL ABSTRACT: It has been shown that increased cholinergic tone can lead to symptoms of depression in human subjects. In line with these clinical findings, we have found that blockade of acetycholine (ACh) degradation in the hippocampus can decrease stress resilience and increase and anxiety- and depression-like behavior in mice. Both animal and human studies have suggested that compounds which interact with nicotinic acetylcholine receptors (nAChRs) can have antidepressant-like properties. These compounds can also decrease c-fos expression in the basolateral amygdala. We therefore investigated the circuitry underlying the stress-regulating effects of nicotinic compounds and acetylcholine. We used AAV-shRNAs to down-regulate β2 subunit-containing (β2*) nAChRs in the amygdala, the prefrontal cortex, and the hippocampus, three structures involved in the regulation of depression-like behaviors in mice. At baseline, β2 nAChR down-regulation in the amygdala induced robust antidepressant-like effects in several mouse models of antidepressant efficacy, and also led to an increase in stress-resilience in the social defeat paradigm. Further, the antidepressant-like effects observed following systemic injection of mecamylamine were significantly blunted by knockdown of β2 nAChRs in the amygdala. When a similar strategy was applied in the prefrontal cortex (PFC), the results suggested a state-dependent effect on depression-like behaviors that did not explain the pharmacological effects of nicotinic modulation. Finally when β2 nAChRs were knocked down in the hippocampus, there was no change in depression-like behavior or stress resilience in the social defeat test. These results suggest that decreasing nicotinic signaling through β2* nAChRs in the amygdala is antidepressant-like, likely due to the high level of tonic ACh input to this structure at baseline. Conversely, the effects of nicotinic neurotransmission in the PFC are complex and may depend on the level of stress-induced ACh release during the test paradigm. Interestingly, while increasing ACh levels in the hippocampus can induce stress and anxiety-like behavior, blocking β2* nAChRs did not change depression-like behavior on its own, suggesting that cholinergic signaling through hippocampal β2* nAChRs at baseline does not modulate these behaviors. The current results suggest that changes in β2* nAChR activity in the amygdala are important for mood regulation and that stress-induced ACh release may have effects on mood through other receptor subtypes.

Abstract 731.2 Summary

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Small Molecules Could Be Big News for Depression Risk

New research shows that microRNAs affect response to stress and risk of depression-like symptoms in mice

Researchers have uncovered a potential mechanism determining how mice respond to stress and whether they develop depression-like symptoms as a result. The findings were presented at Neuroscience 2013, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

"These studies may help us to better understand why some individuals are more likely than others to experience mood disorders and depression," said Karen Scott, PhD, a researcher in the laboratory of John F. Cryan at University College Cork and lead author on the study. "Understanding what makes different individuals protected against or sensitive to mood disorders after severe life stress could open new avenues to prevent the development of depression."

Clinical research in mice has shown that not everyone responds to stress in the same way. Severe social stress can contribute to the development of anxiety and depression in some, while others appear to be less sensitive to the effects. This new research suggests that microRNAs, small molecule sequences that influence gene expression, may play a previously unrecognized role in how animals respond to severe social pressures.

To determine the role of microRNAs in depression, Scott and her colleagues looked at two different mouse models exposed to social defeat stress, a common model used to mimic human mood disorders. To induce social defeat stress, a mouse is introduced into the cage of a larger, more aggressive mouse. The smaller interloper is quickly defeated, and then placed behind a partition, where it is still able to sense the aggressor. After 10 days of exposure to these defeats, one mouse model type showed social avoidance of other mice, while the other type was resilient to the stress of social defeat.

The researchers then looked at several microRNAs associated with the development of depression, focusing on the hippocampus, an area of the brain associated with learning, memory, and emotional processing. They found a specific microRNA, miR-16, was elevated in stressed mice, while another, miR-34c, was associated with protection from the effects of social stress. The researchers will next try to determine how each microRNA is involved in stress sensitivity or protection, in the hopes of understanding how individuals might be predisposed to mood disorders following stress.

Research was supported with funds from Health Research Board (Ireland).

Scientific Presentation: Wednesday, Nov. 13, 11 a.m.–12 p.m., Halls B–H.

731.2, Susceptibility and resilience to repeated social defeat stress is associated with altered expression of hippocampal microRNAs

***K. A. SCOTT**[1], R. M. O'CONNOR[1], H. M. SAVIGNAC[3], G. M. MOLONEY[1], T. G. DINAN[2], J. F. CRYAN[1]; [1]Anat. and Neurosci., [2]Psychiatry, Univ. Col. Cork, Cork, Ireland; [3]Psychiatry, Univ. of Oxford, Oxford, United Kingdom

TECHNICAL ABSTRACT: Social stress is a particularly salient stressor and is often used to model symptoms of human mood disorders in laboratory animals. As in humans, stress susceptibility varies greatly amongst individuals and likely reflects multiple gene-environment interactions. The effects of social stress can differ according to stressor type or quality, as well as the strain of laboratory animal used in such studies. We have shown that male BALB/c mice exposed to a specific social defeat stress regimen (10 days of one defeat per day) exhibit avoidance during social interaction tests whereas C57BL/6J mice are resistant to this effect. The hypothalamic-pituitary-adrenal (HPA) axis of BALB/c mice also appears to be more sensitive to social stress, as control and socially defeated BALB/c males manifest elevated plasma corticosterone following social interaction tests, an effect not evident in C57BL/6J males (Savignac et al., 2011). Understanding the molecular basis for this susceptibility and resilience may give insight into the development of novel therapeutic strategies for stress-related disorders. There has been an increasing emphasis on the role of microRNAs in brain function and behavior. MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs that affect gene expression, typically by targeting mRNAs for degradation or by preventing their translation to protein. In the current study we have assessed the expression of different miRNAs associated with cognitive function and stress adaptation in these 2 mouse strains. miR-16, which has been implicated in the development of depression and is altered by successful antidepressant treatment, was significantly elevated within the hippocampus of socially defeated BALB/c mice ($P<0.05$), whereas miR-16 expression was unaffected in socially defeated C57BL/6J mice. Conversely, C57BL/6J exhibited significantly higher levels of hippocampal miR-34c than BALB/c mice, regardless of stress treatment ($P<0.05$). This supports literature reporting stress-protective effects of hippocampal miR-34c overexpression. Hippocampal miR-134 expression was unaffected by stress or strain in our current studies. The expression of these miRNAs within other corticolimbic structures, the effects that they have on gene and protein expression and how their manipulation may contribute to the etiology of mood and anxiety

disorders are currently under investigation. Our preliminary findings suggest that miRNAs play an important role in differential stress susceptibility. Understanding their role may in turn inform novel and more efficacious treatments for stress-related disorders such as depression and anxiety.

Speaker Summary (393.01)

Speaker: Ada Felix-Ortiz, MS (617) 324-8135 / (617)515-3974
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Optogenetic Manipulations of Amygdala Projections to the Ventral Hippocampus Modulate Anxiety-Like Behaviors

Monday, Nov. 11, 8–9 a.m., Halls B–H

Anxiety disorders represent one of the most common class of mental illnesses experienced worldwide, with a lifetime prevalence of 16.6 percent. Several areas of the brain are key actors in the production of anxiety. Using human brain imaging, neurochemical and pharmacological techniques, scientists have discovered that two regions, the amygdala and the hippocampus play significant roles in most anxiety disorders.

The amygdala is a deep brain structure thought to be critical for emotional processing, acting as a liaison between the parts of the brain that process incoming sensory signals and the parts that interpret these signals. The amygdala can alert the rest of the brain that a threat is present and trigger an anxiogenic response. The hippocampus has been known to play a role in the memory formation of threatening events. Furthermore, studies in humans and rodents have shown that the hippocampus appears to be smaller after exposure to chronic stress. Although previous studies established correlated activity between these areas during anxiogenic conditions, the causal relationship between the amygdala and the hippocampus was unknown, until now.

To test whether the pathway from the amygdala to the ventral hippocampus is sufficient to control anxiety-like behavior in mice, we used an optogenetic approach where neuronal firing can be controlled by pulses of light in the brain of freely-moving rodents. To test for anxiety-like behavior we used three well-validated anxiety tasks: the elevated plus maze, the open field, and the novelty suppressed feeding test. In the wild, mice have evolved to prefer enclosed spaces where they are less vulnerable to environmental threats. In the case of elevated plus maze, the rodent will expend more time on the closed arm when they feel more anxious and more time on the open arm when they feel less anxious.

Our novel results, soon to be published in Neuron, show that silencing amygdala to ventral hippocampus projections significantly decreased anxiety in the elevated plus maze, as shown by increased open arm exploration and in the open field by increased center exploration. In contrast, activation of the same projection significantly increased anxiety-like behaviors, as shown in the elevated plus maze by decreased time in the open arms and in the open field by decreased center exploration. Furthermore, in a different group of animals, hungry mice were subjected to the novelty suppressed feeding test. On this test, latency to feed on a familiar food pellet in the center of a novel anxiogenic arena was measured. Activating amygdala to ventral hippocampus projections increased the latency to approach the food pellet, indicating an increase in anxiety.

In summary, our research indicates that the amygdala-to-ventral hippocampal projection can control anxiety-like behaviors in a bidirectional manner. Furthermore, our research provides a better understanding of the interaction between the amygdala and other downstream brain regions, like the ventral hippocampus, by using optogenetic technique.

By learning more about how the brain creates anxiety and by studying how brain structures interact with each other, researchers may be able to devise more specific treatments for anxiety disorders that are more effective with fewer side effects.

Research was supported with funds from the JPB Foundation, Picower Institute Innovation Funds, The Whitehall Foundation, and the Picower Institute for Learning and Memory and the Department of Brain and Cognitive Sciences at MIT.

Speaker Summary (295.05)

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From Adaptive Learning To Anxiety In Primate Networks

Symposium: The Emotion Triad: The Role of Interactions Between the Amygdala, Hippocampus, and Medial Prefrontal Cortex in Mood and Anxiety

Monday, Nov. 11, 8:30–11 a.m., Room 6A

Our findings suggest that aversive experiences can induce changes in early perceptual mechanisms, and these in turn can lead to anxiety disorders in extreme cases or affect choice behavior in daily life scenarios.

We tested the effect of aversive learning on sensory discrimination and found that subjects that underwent conditioning between a neutral auditory stimuli to an aversive reinforcer, showed decreased sensitivity (i.e. worse discrimination) around the conditioned stimulus. In other words, they could not discriminate between the conditioned tone and a similar tone, although they could do so before the conditioning.

We show that this is a robust phenomenon that applies for many reinforcer modalities (olfactory, auditory, and visual) and also for secondary reinforcers (monetary loss). We also show that it affects later on choice behavior, even at the cost of more losses.

We suggest that anxiety patients might have an even more decreased sensory sensitivity after learning, which suggests that traumatic experiences lead to less discrimination, and hence to overgeneralization of the aversive experience. Therefore, they would respond emotionally to stimuli that are somewhat similar to the original traumatic experience, although other people would not perceive it as similar “enough” to evoke a response.

We delineate the underlying brain mechanisms using fMRI in anxiety patients and controls, and record electrophysiologically in an animal model to unveil the network architecture that underlies it. We show an involvement of the amygdala, the prefrontal cortex, and the primary auditory cortex.

Our findings highlight a model in which perception plays a major role in overgeneralization after emotional experiences, and points to the underlying mechanisms.

Research was supported with funds from the European Research Council, the Israel Science Foundation, and the Minerva Foundation.