development takes place. It has been widely presumed that the cell of origin for medulloblastomas is the granule cell precursor, which undergoes rapid proliferation during this stage[8]. Consistent with this idea, mutations that activate the Sonic hedgehog pathway have been identified in human medulloblastomas[9]. A mouse in which mutation of the Patched receptor leads to constitutive activation of Sonic hedgehog pathway also develops medulloblastoma[10], providing additional evidence that this pathway is involved in cerebellar progenitor cell transformation. Many important effectors of hedgehog's mitogenic function have been identified, including transcription factors such as N-myc[11] and Gli family members[12]. These proteins are upregulated in the 'desmoplastic' variety of medulloblastoma in humans[13] and are expressed in the medulloblastomas of the Patched mutant mice[9,10], providing additional evidence that activation of the Sonic hedgehog pathway in granule cell precursors can cause medulloblastoma formation. Small-molecule compounds specifically targeting the hedgehog pathway hold promise for treating medulloblastomas associated with hedgehog pathway activation[14,15].

However, as Lee and colleagues discuss, the so-called 'classic' medulloblastomas are not associated with hedgehog pathway activation and are genetically and pathologically distinct from the hedgehog-dependent, desmoplastic medulloblastomas[8,13]. An intriguing possibility raised by the study of Lee *et al.* is that the prominin-1 positive cerebellar stem cells constitute an alternative cell of origin for medulloblastoma. If so, the evidence that cerebellar stem cells proliferate in response to bFGF stimulation rather than to stimulation with Shh suggests that compounds blocking the bFGF pathway or its downstream effectors could be therapeutic in classic medulloblastomas. Such targeted therapy would be preferable to current medulloblastoma treatments, which do not distinguish the therapies for these two tumor subtypes, and carry potential physical, developmental and psychological side effects.

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Blue genes: wiring the brain for depression

Stephan Hamann

How do genes act in the brain to influence susceptibility to mental illness? An imaging study suggests that healthy carriers of a gene variant associated with depression risk have decreased brain volume and neural coupling in affective circuitry involved in depression.

The genetic test result comes back: your baby carries a high-risk gene for depression and anxiety. What does this portend for his future? How will this gene affect his developing brain and temperament, and what can we do to protect him from developing depression? The idea that genes affect risk for mental illness is widely accepted, but exactly how genes influence the brain to increase vulnerability is a mystery that has only recently begun to unfold. A new study[1] in this issue uses multiple brain imaging techniques to show that carrying a high-risk variant of the serotonin transporter gene profoundly affects both anatomy and function in a key emotion circuit. This work has broad implications for understanding how genetic vulnerability to depression is manifested in the brain's response to emotional stimuli.

Serotonin is an important modulator of emotional behavior, and considerable evidence links serotonergic dysfunction to depression. Serotonin also shapes the brain's development, including that of the limbic system's affective circuitry[2]. Given these links, genes regulating serotonin have become prime suspects in tracking down genetic factors in the development of depression. A variation in the promoter region of the serotonin transporter gene (5-HTTLPR) has attracted particular interest. The short (*s*) allele is associated with reduced serotonin availability compared to the long (*l*) allele, and individuals who carry at least one *s* allele (*s/s* or *s/l*) have increased anxiety-related traits and risk for depression[3].

Depressed patients have decreased brain volume in the subgenual part of the anterior cingulate, together with abnormal activity in a key affective circuit involving the anterior cingulate and amygdala[4]. However, it has been unclear whether these abnormalities precede the development of depression or are caused by the depressed state. A straightforward approach to this question is to study healthy individuals with respect to their genetic risk for depression to see whether brain anatomy and function in those at higher risk resembles that of the depressed brain, in effect identifying neural markers that signal vulnerability to depression. The new work is one of the largest published imaging genomics studies to date, with 94 subjects. In this study, Pezawas *et al.*[1] used a three-pronged imaging approach, hypothesizing that healthy individuals who carry the high-risk 5-HTTLPR *s* allele would show depression-like changes in brain structure and function in the cingulate-amygdala circuit. They used structural MRI to examine gray matter volume, functional MRI to look at responses during a task with emotional stimuli previously shown to elicit greater amygdala activation in *s* allele carriers[5], and analysis of the functional responses to determine the degree of functional coupling in cingulate-amygdala circuitry.

The primary results strongly supported the conclusion that depression-like changes in anatomy and function are indeed present in healthy carriers of the high-risk *s* allele, constituting neural markers for vulnerability to depression. Carriers of the *s* allele had more than a 25% reduction in gray matter volume in the perigenual anterior cingulate and an approximately 15% reduction in the amygdala (**Fig. 1**). Notably, the greatest decrease was in the same region where reduced volume

Stephan Hamann is in the Department of Psychology, Emory University, 532 North Kilgo Circle, Atlanta, Georgia 30322, USA.
e-mail: shamann@emory.edu

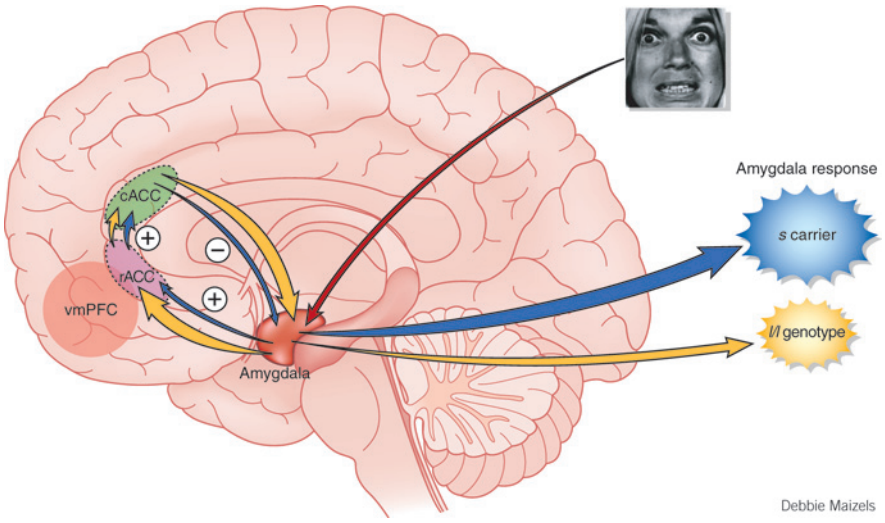

Figure 1 Differences in processing of emotional stimuli between *s* allele carriers (blue arrows) and homozygous *l* allele carriers (yellow arrows). Negative emotional stimuli are evaluated by the amygdala (red arrow) after preliminary analysis in the ventral visual pathway (not shown). Carriers of the *s* allele have markedly reduced positive functional coupling between the rostral anterior cingulate (rACC; purple oval) and the amygdala, which results in a net decrease in inhibitory feedback from the caudal anterior cingulate (cACC; green oval), via connections between rACC and cACC (short upward arrows). Brain volume was also substantially reduced in *s* allele carriers in the rACC and, to a lesser extent, the cACC and amygdala. The consequence of these genotype-based alterations is an emotional hyper-responsivity to negative affective stimuli in *s* allele carriers (large blue cloud) compared with individuals lacking this allele (small yellow cloud), which may be related to an increased risk of developing depression. As found in a previous study[7], functional coupling between the vmPFC (pink circle) and the amygdala was also increased in *s* allele carriers.

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has been observed in depression[6], the rostral anterior cingulate (rACC), supporting the link to depression. This is the first evidence that healthy *s* allele carriers show significant volume reductions in brain regions implicated in depression, and the magnitude of these effects is striking. In line with the idea that these regions constitute an affective circuit, the magnitude of volume decreases in the cingulate and amygdala were highly correlated within individual subjects.

The authors next examined whether genotype also affected functional coupling between the amygdala and perigenual cingulate regions, where *s* allele carriers showed decreased gray matter volume. Across all subjects, analysis of moment-to-moment fluctuations in fMRI activity during a task with fearful and angry faces showed that bilateral amygdala activity correlated positively with rACC activity and negatively with caudal anterior cingulate (cACC) activity. Activity in rACC and cACC was positively correlated as well. These correlations suggested a basic affective circuit involving a positive connection between the amygdala and rACC, a positive connection between rACC and cACC and, finally, a negative feedback connection from the cACC to the amygdala. Consistent with the authors' prediction that *s* allele carriers would show alterations in this functional circuit, the high-risk allele was associated with decreased positive coupling between the amygdala and rACC (**Fig. 1**) and decreased negative coupling or feedback between the cACC and amygdala, which may lead to elevated amygdala responses to emotional stimuli in *s* allele carriers[5]. Subjects' scores on a personality test assessing anxiety-related traits associated with depression correlated strongly with dynamic coupling between the amygdala and rACC, but not with functional and anatomic measures in individual areas, supporting the link between the imaging results and depression susceptibility and highlighting the importance of examining dynamic functional interactions.

A recent study[7] also looked at 5-HTTLPR genotype and affective processing with fMRI but, intriguingly, found increased rather than decreased functional connectivity between the amygdala and an area near the rACC, the ventromedial prefrontal cortex (vmPFC), (**Fig. 1**). Pezawas *et al.* reanalyzed their own data and found qualitatively similar results. In part because the vmPFC may lack significant direct connections to the amygdala, the authors of the current study propose that the rACC, which has abundant direct connections from the amygdala, is part of a primary affective circuit and that the vmPFC has a more indirect, compensatory role. Statistical techniques such as dynamic causal modeling, which go beyond simple temporal correlations to explore directional, modulatory and hemispheric asymmetry effects, will help clarify the wiring of this extended affective circuit. Interestingly, Heinz *et al.*[7], using emotional scenes as stimuli, found that positively valenced emotional pictures also elicited amygdala activation, consistent with a general role for the amygdala in both positive and negative emotion[8], but only responses to negative pictures showed 5-HTTLPR genotype effects. This suggests that the nature of the experimental emotional stimuli can influence whether genotype effects will be detected. Future studies using multiple stimuli selected to evoke different types of affective responses may clarify what aspects of emotion are modulated by genotype and may lead to more sensitive probes of gene effects.

Environmental factors are thought to be critical in determining whether individuals with genetic vulnerability will eventually develop depression. Though somewhat controversial, recent studies suggest that carriers of the high-risk serotonin transporter allele may never develop depression unless they are exposed to stressful and traumatic events, particularly in early life[9]. Even with such exposure, although the serotonin transporter gene clearly influences affective responses, it is only one of an unknown number of genes that may potentially contribute to mental illness susceptibility. Important remaining questions are whether healthy *s* allele carriers who show stronger anatomical and functional markers will develop depression at a higher rate than those who do not show these markers, and how stressful life experiences factor into this process. The answers will require longitudinal, prospective studies following individuals for several years. Another line of questions concerns how early in life the anatomical and functional alterations associated with 5-HTTLPR variation develop. Altered serotonin levels can affect early brain development in animal models, suggesting that by birth the effects of this genetic variation may already be present[2]. Determining the developmental mechanisms that underlie the structural and functional changes observed here in humans is an important direction for further study.

Surprisingly, most people of European descent carry at least one high-risk allele, and thus along with the baby in our original example, are at higher risk for depression[3]. Why should a genetic tendency toward the blues be

relatively common? As has often been noted, the function of genes is not to cause disease, and surely, the short 5-HTTLPR allele does not exist for the purpose of causing depression. Instead, it seems to tune and prune affective circuits so as to heighten responses to negative emotional stimuli, a consequence that could easily prove adaptive in threatening environments. Conversely, carriers of the low-risk *l* variant have attenuated amygdala responses[5,7] to negative emotional scenes or faces signaling threat. Always looking on the bright side of life is fine for warding off depression, but a blunted response to aversive stimuli carries its own risks.

The current study demonstrates the power of imaging genomics to uncover alterations in brain structure and function related to genetic risk for psychiatric disorders. Combining the strengths of functional imaging with genomics holds considerable promise in identifying the neural underpinnings of human individuality and understanding how this variability interacts with the environment to produce both adaptive emotional behavior and disease states.

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Shaking up sleep research

Joan C Hendricks

Sleep deprivation causes all too familiar behavioral impairments and increased need for sleep. A new Drosophila mutant with alterations in the *Shaker* potassium channel sleeps less than normal but does not show the usual effects of sleep deprivation.

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Did you sleep well last night? Are you sleepy now? Most of us probably know and envy someone who annoyingly claims to need only a few hours of daily sleep. For many people though, the inability to fall asleep when we want to or the feeling of sleepiness at inopportune times are familiar sensations. In a recent issue of *Nature*[1], Cirelli and colleagues report the identification of a remarkable Drosophila mutant that not only sleeps less but also to some degree is immune to the effects of sleep deprivation (albeit at the price of a shortened life span). This discovery makes an important contribution to our understanding of the remarkably elusive questions of how and why we sleep.

In both mice[2] and humans[3], potassium channels are implicated in sleep, but studies in *Drosophila melanogaster* provide the opportunity for a better quality of proof. Sleep-like behavior in Drosophila[4,5] was documented just a few months before the fly genome was published. Hopes were immediately raised that flies could help discover the genetic basis for sleep[6], despite differences between fly sleep and 'true' sleep (most notably, the apparent absence of sleep stages analogous to REM and non-REM). These initial hopes were soon fulfilled by identification of conserved genes[7,8] and the demonstration that sleep loss—caused by mutant genes[9,10], environmental stimulation[10] or drugs[11]—is lethal for flies. In the current study, Cirelli and collaborators identified a mutant that slept very little but appeared normal during waking, which they named *minisleep* (*mns*). Did these flies need less sleep? Probably not, as the authors also found that *mns* and other short-sleeping flies with mutations in the same gene had shortened lives.

To identify their mutant, the authors took full advantage of the efficiency and low cost of breeding Drosophila, using random mutagenesis, unbiased screening of 9,000 lines of mutant flies, large numbers for statistical analyses and mature genetic tools. The ethyl methane sulfonate (EMS) mutagenesis that led to *mns* produces point mutations that are notoriously difficult to map. However, the authors noted that the mutants shook when exposed to ether anesthesia. As a perfect example of chance favoring the prepared mind, the authors recognized this phenotype and focused their attention on the highly conserved *Shaker* gene, which encodes a voltage-gated potassium channel heavily expressed in the CNS. They soon discovered that *mns* flies carried a point mutation in the α subunit of the *Shaker* channel.

However, when the authors examined other existing, independently generated lines of flies with *Shaker* mutations, they discovered that only one null mutant showed short sleep. The authors cleverly considered the possibility that accumulated modifiers might have altered the phenotype and allowed the mutants to show normal sleep. That is, to compensate for a maladaptive mutation, altered forms of genes that counteracted the *mns* mutation would have provided a selective advantage to the population over successive generations. To test this possibility, the authors bred the null *Shaker* mutants that did not show short sleep to unrelated lines of flies that had not been under the same sort of selective pressure (using well-established Drosophila tricks to preserve the *mns* mutation). The resulting offspring displayed not only the short-sleep phenotype but also the short-lived phenotype observed in *mns*. This tendency to accumulate modifiers that prolong both sleep and life further solidifies the evidence that sleep serves an important biological purpose across evolution.

There are several interesting implications of the study for understanding sleepiness and sleep need. The *mns* flies had reduced baseline sleep but were normally responsive and active. Wild-type flies sleep more deeply after sleep deprivation, which the investigators measured by their ability to sleep during complex mechanical noxious heat stimulation. The *mns* flies responded to deprivation by increasing their total sleep appropriately, but they did not show the changes in sleep intensity. Normal fly sleep is also more consolidated following deprivation, meaning that they sleep for longer continuous periods. However, even after experimentally imposed all-nighters, *mns* flies continued to break up their sleep with arousals, fragmenting sleep into relatively short bouts, and they woke up readily when stimulated—they were even hyper-responsive. One might reason that *mns* flies either have a deficit in deep, consolidated sleep, or that they need to sleep less because

Joan C. Hendricks is at the Department of Clinical Studies, Philadelphia School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.
e-mail: jch@vet.upenn.edu