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STUDIES IN GENETICALLY MODIFIED MICE SUGGEST NOVEL MECHANISMS OF MOOD REGULATION

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The field of biological psychiatry has been hindered by the paucity of animal models of depression and bipolar disorder that are reliable across laboratories, assess constructs with relevance to core symptomatology of these disorders, and include inducing conditions that are based on solid theoretical rationales about the etiologies of these disorders. Two papers published in this issue of *Biological Psychiatry* used molecular genetic techniques in rodents to reveal previously unidentified mechanisms involved in the neurobiological regulation of mood and affect, moving beyond the traditional targets of current pharmacotherapies. These papers suggest roles for γ -aminobutyric acid (GABA) A receptor and Circadian Locomotor Output Cycles Kaput (CLOCK) transcription factor in depression and bipolar disorder. Since malfunctions in such regulatory mechanisms lead to affective symptoms of depression and mania, these findings provide new insights regarding the etiology of mood dysregulation and its potential treatment.

Role of the $\gamma 2$ subunit of the γ -aminobutyric acid (GABA) A receptor in mood regulation suggests a new integrative hypothesis about the role of GABA and its receptors in depression

Shen and colleagues showed that female mice, that were heterozygous for the deletion of the $\gamma 2$ subunit of the GABA_A receptor, exhibit depression- and anxiety-like behaviors when tested in the novelty suppressed feeding (NSFT), forced swim (FST), tail suspension (TST), and sucrose consumption (SCT) tests [1]. These GABA_A receptor deficient mice also exhibit dysfunction of hypothalamic-pituitary axis (HPA) activity, as reflected in elevated baseline corticosterone levels, independent of whether the genetic lesion was introduced during embryogenesis or delayed until adolescence. Nevertheless, the corticosterone response to a stressor did not differ between heterozygous and wildtype mice. The authors conclude that because of the mixed anxiety/depression like phenotype of these mice, these mice appear to be a model of melancholic depression.

Interestingly, all abnormalities seen in the $\gamma 2$ -deficient mice, including the higher baseline corticosterone levels, were reversed by chronic, but not subchronic, treatment with the tricyclic antidepressant and norepinephrine reuptake inhibitor desipramine, while only the anxiety-like behavior in the NSFT was reversed by chronic treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine. It should be noted, however, that some of the

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reported effects of desipramine and fluoxetine are not clear reversals or lack of reversals. Specifically, desipramine increased both sucrose consumption and the sucrose/water consumption ratio, a more sensitive measure of anhedonia than sucrose consumption (especially since the heterozygous mice exhibited significantly decreased consumption of both water and sucrose), in both wildtype and $\gamma 2^{+/-}$ mice in the SCT. In the case of fluoxetine, the latency to immobility in wildtype mice in the FST was decreased by fluoxetine (opposite to the typical effect of fluoxetine in this test) to the levels seen in the $\gamma 2^{+/-}$ mice. Thus, the effects of desipramine in the SCT may reflect a non-specific increase in fluid consumption and not a specific anti-anhedonic effect, while the effects of fluoxetine in the FST are difficult to interpret due to the atypical effects of fluoxetine on FST in the Shen study.

Much of the strength of the conclusions derived from this work is that several different tests of depression- and anxiety-like behaviors were used, as well as two antidepressant treatments that differ in their selectivity for norepinephrine versus serotonin transporters. As exemplified above, each one of the behavioral tests used has limitations, and one may question whether some measures provided by these tests, such as the FST, actually reflect a construct with relevance to depression or anxiety, or whether these measures are only reflective of antidepressant/anxiolytic activity [2]. Indeed, most of the inconsistencies and unexplainable differences in findings in both the Shen and the Mukherjee (discussed below) studies were derived from the FST. In both reports, increased immobility was interpreted as depression-like behavior when in fact the FST was originally designed only as a test of tricyclic antidepressant activity (it later had to be modified to reflect the effects of SSRIs), and therefore this test does not necessarily reflect depression-like behavior [2]. Nevertheless, the consistency of the $\gamma 2^{+/-}$ mouse phenotype across tests provides converging evidence that these mice exhibit depression/anxiety-like behaviors and HPA dysfunctions that are reversible by chronic treatment with desipramine in most cases.

An interesting aspect of this work relates to the demonstrated interaction between GABA_A receptor function and HPA activity. The authors assessed (i) behavioral responses and corticosterone levels during different times in development, and (ii) in both global $\gamma 2^{+/-}$ mouse and mice that had dysfunctional GABA_A receptors only in the telencephalon, and not in the hypothalamus. Accordingly, the authors concluded that (i) the GABA_A deficit leading to elevated corticosterone levels was extrahypothalamic; and (ii) the elevated corticosterone levels alone are insufficient to induce the behavioral changes observed in $\gamma 2^{+/-}$ mice. The latter conclusion is also supported by the finding that fluoxetine reversed the elevated baseline corticosterone levels but did not reverse most of the behavioral changes seen in these mice.

In the context of the role of GABA neurotransmission in depression, the findings by Shen and colleagues provide new evidence potentially explaining some of the apparent inconsistencies between human and animal data about the role of GABA in depression. Measures of GABA activity indicate that there is decreased GABAergic neurotransmission in humans suffering from depression [3]. In apparent contradiction to these findings, investigations of various genetically altered mice that have GABA_B receptor deficits in the FST, the learned helplessness, and the chronic mild stress procedures, as well as the use of pharmacological antagonists at GABA_B receptors in the same tests, suggest that antagonist actions at GABA_B receptors may have antidepressant properties [for review, 4]. By identifying the possibility that dysfunctional GABA_A receptors contribute to depression/anxiety-like behaviors, the work of Shen and colleagues may help to reconcile this conundrum. Thus, all currently available findings suggest the following parsimonious, albeit speculative, hypothesis about the role of GABA in depression. Depressive and anxiety symptoms, that are often comorbid in depressed patients, may be mediated by decreased

transmission through the GABA_A receptor engendered by lowered synaptic GABA levels (human findings) and/or dysfunction of GABA_A receptors [1]. This abnormality may be remedied by antagonist actions at GABA_B receptors, some subtypes of which are found presynaptically as both heteroreceptors primarily on glutamate, serotonin, and dopamine terminals, and as autoreceptors regulating GABA transmission. That is, through trans-synaptic actions or antagonist actions at GABA_B receptors regulating GABA transmission, GABA_B receptor antagonists may increase GABA release to act at postsynaptic GABA_A receptors.

***Clock* gene expression in the ventral tegmental area (VTA) regulates mood and results in bipolar-like behaviors**

Mukherjee and colleagues [5] examined how a knock down of the expression of *Clock* gene in the ventral tegmental area (VTA) of mice, through the use of RNA interference (RNAi) technology, altered behavioral functions relevant to mania and electrophysiological activity of VTA dopamine neurons. Mice with *Clock* knock-down exhibited hyperactivity in a novel environment, abnormal circadian locomotor rhythms reflected in wheel running activity, increased risk-taking behavior in the elevated plus maze, the light/dark box, and the open field, decreased depression-like behavior in the FST and the learned helplessness procedure, as well as increased VTA dopamine neuron activity. Most of these behaviors and the increased dopamine neuron activity were also seen previously in mice that had a constitutive mutation in the *Clock* gene (*Clock*Δ9), except that these mice showed decreased depression-like immobility in the FST (opposite to that seen in the RNAi-treated mice). Thus, these two different genetic techniques to modify the function of the *Clock* transcription factor lead to some similar and some different forms of mood dysregulation. Most notably, these disparate genetic methods lead to comparable increases in mania-like motor activity, which appear to mimic findings of hyperactive responses to a novel environment in acutely ill manic patients [6]. While both the global and constitutive disruption of *Clock* and the VTA-specific reduction of *Clock* expression in adulthood lead to shortened circadian cycles, hyperactivity, and reduced anxiety-like behavior, only the latter increases putative measures related to depression (learned helplessness) and antidepressant activity (FST). The increased electrophysiological activity of VTA dopamine neurons that project to forebrain structures, such as the nucleus accumbens, the frontal cortex, and the amygdala, may explain, at least partially, the behavioral phenotype of mice with dysfunctional *Clock* gene expression. For example, increased dopamine release in the nucleus accumbens is likely to mediate the hyperlocomotion in a novel environment. Increased dopamine release in the amygdala may underlie increased risk-taking behavior due to decreased fear of aversive situations (e.g., bright light). Finally, increased dopamine release in the frontal cortex may modulate the hyperdopaminergic activity in other areas through negative feedback loops.

Because *Clock* is a transcription factor, it regulates the expression of many genes. Using microarray analysis, the expression of several genes already implicated in mood regulation, such as cholinergic receptors, ionotropic and metabotropic glutamate receptors, the glycine receptor, glutamate receptor interacting protein 2, and trophic factors, were shown to be altered after *Clock* knock-down in the VTA. In the context of the Shen and colleagues study discussed above, it is interesting to note that an increase in the expression of the α4 and α2 subunits of the GABA_A receptor was observed in the VTA of the *Clock* knock-down mice. Although Mukherjee and colleagues interpreted these increases in GABA_A receptor subunit expression as potential compensatory reactions to counteract the increased activity of dopamine neurons in the VTA, the data by Shen and colleagues suggest an intriguing alternative interpretation. That is, deficits in GABA_A receptor function may be associated

with increased depression- and anxiety-like behaviors, while increased GABA_A receptor function may be associated with mania-like behaviors.

Summary and Conclusions

Two multidisciplinary studies in mice provide novel insights into the neurobiology of mood regulation. GABA_A receptor deficits result in depression- and anxiety-like behavioral phenotypes, concomitant with increased HPA activity, similar to the symptomatology of melancholic depressed patients. These new data enable the integration of previous findings in the literature that appeared contradictory and suggest a new hypothesis that there is decreased synaptic GABA levels and dysfunctional GABA_A receptors in depression whose effects may be reversible by antagonist actions at GABA_B autoreceptors that will restore GABA synaptic levels and GABA_A neurotransmission. By contrast, dysfunction of the *Clock* gene globally or in the VTA resulted in behaviors characterizing mania, such as increased activity, dysfunctional circadian locomotor activity, and increased risk-taking. Interestingly, knock-down of the *Clock* gene expression in the VTA was also associated with increased GABA_A receptor subunit expression, possibly leading to increased GABA_A transmission. Alterations in *Clock* gene function within the VTA lead to changes in the expression and function of several gene products that may also be involved in mood regulation, in addition to the GABA, norepinephrine, and dopamine systems implicated with the results of these studies. These new findings, taken together with results from the literature, contribute to a broader understanding of brain mechanisms regulating mood that is altered in several psychiatric disorders, and most notably depression and bipolar disorder.

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