Priority Communication

Biological Psychiatry

Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles

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ABSTRACT

BACKGROUND: Examining transcriptional regulation by antidepressants in key neural circuits implicated in depression and understanding the relation to transcriptional mechanisms of susceptibility and natural resilience may help in the search for new therapeutic agents. Given the heterogeneity of treatment response in human populations, examining both treatment response and nonresponse is critical.

METHODS: We compared the effects of a conventional monoamine-based tricyclic antidepressant, imipramine, and a rapidly acting, non–monoamine-based antidepressant, ketamine, in mice subjected to chronic social defeat stress, a validated depression model, and used RNA sequencing to analyze transcriptional profiles associated with susceptibility, resilience, and antidepressant response and nonresponse in the prefrontal cortex (PFC), nucleus accumbens, hippocampus, and amygdala.

RESULTS: We identified similar numbers of responders and nonresponders after ketamine or imipramine treatment. Ketamine induced more expression changes in the hippocampus; imipramine induced more expression changes in the nucleus accumbens and amygdala. Transcriptional profiles in treatment responders were most similar in the PFC. Nonresponse reflected both the lack of response-associated gene expression changes and unique gene regulation. In responders, both drugs reversed susceptibility-associated transcriptional changes and induced resilience-associated transcription in the PFC.

CONCLUSIONS: We generated a uniquely large resource of gene expression data in four interconnected limbic brain regions implicated in depression and its treatment with imipramine or ketamine. Our analyses highlight the PFC as a key site of common transcriptional regulation by antidepressant drugs and in both reversing susceptibility– and inducing resilience–associated molecular adaptations. In addition, we found region-specific effects of each drug, suggesting both common and unique effects of imipramine versus ketamine.

Keywords: Depression, Imipramine, Ketamine, Resilience, RNA-seq, Susceptibility

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Depression is a complex and heterogeneous disorder and a leading cause of disability worldwide, yet existing pharmacotherapies have limited efficacy (1). Virtually all drugs used to treat depression today target the same basic mechanisms identified more than 60 years ago, inducing full remission in fewer than 50% of affected individuals (2). Earlier treatments, such as tricyclic antidepressants (e.g., imipramine), target multiple neurotransmitter systems. Specifically, imipramine inhibits reuptake of serotonin and norepinephrine (thought to mediate its therapeutic actions) and influences numerous monoaminergic and cholinergic receptors. More recently developed antidepressants have greater selectivity at inhibiting serotonin and/or norepinephrine transporters but have roughly the same intrinsic efficacy as older tricyclic medications. Moreover, the therapeutic actions of both tricyclics and more selective reuptake inhibitors require weeks or months of treatment. Although the initial target of these drugs is known, the slowly developing drug-induced adaptations that mediate antidepressant outcomes remain unknown (3,4). There is a great unmet need to develop more effective and more rapidly acting treatments for depression, ideally guided by an improved understanding of the pathophysiology of the syndrome.

Several groups have shown that ketamine, a dissociative anesthetic, induces rapid antidepressant effects in approximately 50% of patients who are resistant to available tricyclic and

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reuptake inhibitor antidepressants (5,6). Although ketamine's mechanism of action as a noncompetitive *N*-methyl-D-aspartate glutamate receptor antagonist has been studied with regard to its anesthetic and recreational use at high doses, the functional and molecular underpinnings of ketamine's antidepressant action at lower doses are a matter of ongoing study, with several attractive models of altered synaptic and structural changes proposed (7–9). Unbiased genomewide transcriptional profiling may shed new light on the molecular mechanisms targeted by both established and experimental pharmacotherapies, thereby facilitating the development of novel antidepressant treatments.

A key challenge in understanding the mechanism of action of existing pharmacotherapies for depression is to identify the brain regions in which antidepressant treatments exert their effects. Neuroimaging studies of depressed patients, and findings in animal models, show that depression is a circuit-level disorder in which several functionally interconnected brain regions are affected (10–13). One involved circuit is the highly studied corticomesolimbic reward system consisting of several limbic brain regions, including the nucleus accumbens (NAC), prefrontal cortex (PFC), hippocampus (HIP), and amygdala (AMY). The NAC integrates information from glutamatergic inputs from the PFC, AMY, and HIP, among other regions (14). Structural, functional, and transcriptional changes in each of these brain regions have been reported in both rodent depression models and depressed humans (12,15–25). Thus, examining how antidepressant drugs regulate transcriptional profiles in these functionally interconnected brain regions may offer important mechanistic insights into their therapeutic actions.

In studying the mechanism of action of antidepressant drugs, it is important to address both the individual receiving the treatment and the heterogeneity of treatment response. Antidepressants do not elevate mood in nondepressed individuals, suggesting that unique responses may occur in depressed patients. Likewise, analyzing drug-induced transcriptional changes in both responders and nonresponders may be particularly informative in distinguishing drug-induced therapeutic changes from off-target effects. A key question is whether the lack of response reflects simply the lack of drug-induced therapeutic changes or induction of aberrant transcriptional programs that mask antidepressant actions.

Here, we compared imipramine and ketamine action in mice subjected to chronic social defeat stress (CSDS), an ethologically validated model of depression and social stress-related disorders (26,27). Chronic, but not acute, administration of imipramine or other standard antidepressants has been shown to reverse a range of behavioral abnormalities in roughly 60% of mice (26,28). Recently, single doses of ketamine were shown to induce roughly equivalent treatment responses (29). We used RNA-sequencing (RNA-seq) to characterize transcriptomic responses genomewide to either chronic imipramine or acute ketamine within the limbic circuitry noted above: NAC, PFC, HIP, and AMY. Our findings demonstrate fundamental differences in the molecular and brain region targets of these two medications in responders and nonresponders, results that have important implications for antidepressant drug discovery efforts.

METHODS AND MATERIALS

More information is available in the Supplement.

CSDS, Behavioral Testing, and Drug Treatment

An established CSDS protocol was used to induce depressive-like behaviors in mice (26,27). C57BL/6J mice were subjected to 10 daily, 5-minute defeats by a novel CD1 aggressor, and social avoidance behavior was assessed in a two-stage social interaction (SI) test 24 hours after the final defeat. In the first 2.5-minute test (no target), the experimental mouse was allowed to freely explore an arena containing a plexiglass and wire mesh enclosure centered against one wall of the arena. In the second 2.5-minute test (target), the experimental mouse was returned to the arena with a novel CD1 mouse enclosed in the plexiglass wire mesh cage. Time spent in the interaction zone (IZ) surrounding the enclosure was measured. Resilient mice spent more time in the IZ in target than no target, and total time in the IZ in target was >60 seconds. Susceptible mice spent less time in the IZ with target than with no target, and total time in the IZ in target was <60 seconds.

Susceptible mice were treated with either saline, ketamine, or imipramine. Twenty-four hours after the final injection, mice were subjected to a second SI test (SI2). Mice were defined as responders to imipramine or ketamine treatment if they spent more time in the IZ in target after antidepressant treatment and had an increase of >20 seconds in the IZ in target from SI1 to SI2. Mice were defined as nonresponders if they spent less time in the IZ in target after treatment or had an increase of <10 seconds in the IZ in target from SI1 to SI2. Saline-treated resilient and susceptible animals were included in transcriptome-wide analyses if they continued to meet the SI1 criteria in SI2. All control animals were included in downstream analysis.

RNA Isolation, Library Preparation, and RNA Sequencing

Mice were killed 2 days after SI2, and NAC, PFC, HIP, and AMY tissues were rapidly dissected and frozen on dry ice. Tissue from two mice were pooled for each sample for three to five biological replicates for each brain region and phenotype. RNA isolation, quantitative real-time polymerase chain reaction, and data analyses were performed as described (12). Libraries were prepared using the TruSeq RNA Sample Prep Kit version 2 protocol (Illumina, San Diego, CA) and sequenced with 50 base pair paired-end reads (Supplement).

Statistical and Bioinformatic Data Analysis

Differential Expression Analyses. Pairwise differential expression comparisons were performed using Voom Limma (30) and a nominal significance threshold of fold change >1.3 and $p < .05$ (Supplement).

Enrichment Analyses. Enrichment between gene lists was analyzed using the GeneOverlap R package (www.bioconductor.org/packages/release/bioc/html/GeneOverlap.html).