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Persistence of Drug Memories: Melting Transcriptomes

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One of the defining characteristics of drug dependence is relapse after a period of abstinence. Cue-induced drug craving and relapse risk not only persist during abstinence, but increase over time, a phenomenon termed incubation (1). This progressive increase in risk factors likely requires enduring “memory-like” changes in synaptic plasticity, but there has been little exploration of the underlying molecular mechanisms. It is assumed that this remodeling of the brain is initiated by changes in gene expression (2). The artwork by Salvador Dali titled *The Disintegration of the Persistence of Memory* offers a symbolic illustration of how extrinsic factors shape the transcriptome over time (<https://www.wikiart.org/en/salvador-dali/the-disintegration-of-the-persistence-of-memory>). Viewed from this perspective, the bricks in the painting evoke the assembly of RNA sequencing reads into a transcriptome in a surrealist landscape that implies a relationship to memories and time, while the water simulates the depth of changes by drugs of abuse. These persistent influences produce a transcriptome melting pot.

For addiction research, in-depth transcriptome analysis entails study of multiple brain regions comprising the addiction neurocircuitry, as well as the temporal patterns of drug intake, withdrawal, and relapse. The work by Deena Walker and colleagues (3) provides a transcriptome-wide analysis of the molecular changes induced by cocaine self-administration as a function of withdrawal time and brain region, and specifically tests the short- and long-term withdrawal effects on cocaine self-administration. This analysis takes advantage of a rich dataset and signifies a bioinformatics tour-de-force.

Another strength of the study is that neuroadaptive changes were examined in response to a history of cocaine self-administration rather than passive drug administration (investigator administered). This is an important component given the critical role of cognitive processes in behaviors associated with addiction (4). The authors also used a creative approach to complex phenotypic data reduction by studying multiple parameters of operant responding for cocaine self-administration to create a composite addiction index for each subject, which was then associated with specific patterns of transcriptional regulation. The goal of transcriptome profiling is to identify candidate genes and pathways that have eluded traditional methods of measuring one or a few genes or biological functions. For example, Walker and colleagues reported regulation of immune-related genes, such as *Lcn2* and *Map4k2*, during prolonged drug abstinence that suggests neuroimmune candidates for

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functional validation. Another innovative approach in the data analysis was to examine unidirectional changes in gene expression in multiple brain regions across the addiction circuitry, with the assumption that these genes signify top candidates for identifying common upstream regulators of transcription. This is based on the hypothesis that overlapping expression changes in the brain may be key sites for therapeutic intervention. Their analysis identified the well-characterized addiction regulator CREB as a proof-of-principle example, as well as a number of nuclear receptors that have received limited attention for addiction-related disorders (3). These include peroxisome proliferator-activated receptors for which there are therapeutic agents that have shown some efficacy in preclinical addiction models (5), suggesting that this approach may aid in the discovery of new molecular targets.

Walker et al. (2018) examined transcriptome profiling after both short (24-hour) and long (30-day) abstinence from cocaine self-administration together with time-matched cocaine and saline treatments in the long abstinence group (3). The cocaine treatment would be expected to increase relapse-like behaviors, but no operant behavior was studied (levers were retracted) during withdrawal. The marked individual differences in operant responding during acquisition of cocaine self-administration were key to implementation of the addiction index, and thus measurement of responses during abstinence/extinction would be informative. Further analysis of gene expression changes during the two time points is also warranted, as another study from this group noted a 10-fold increase in differentially expressed genes in the central amygdala and orbitofrontal cortex after methamphetamine in long compared with short abstinence groups (6). Although this does not appear to be the case in the current study, more discussion of time-dependent and brain regional differences would have been of interest.

Here, as is common to a number of RNA-seq studies, the authors used PCR to validate the changes in expression, once again showing that the two platforms give similar results. We believe it is important to point out that RNA-seq methodology is sufficiently established and that future studies may now abandon the drain of resources from using an arguably less reliable approach to 'confirm' findings. A much more appropriate use of resources would be to confirm the importance of expression changes at a functional or behavioral level.

As noted by the authors, this dataset will be a great resource for data mining and thus it will be important to make this available to the public. Future analyses could further examine the cell-type specificity of the transcriptional changes induced by cocaine self-administration. Although a cell-type analysis was mentioned in a supplemental table, this was not thoroughly explored. Mapping the transcriptional changes found in this study at a cellular level, and leveraging other single cell RNA datasets (7), will broaden our understanding of drug-induced transcriptional changes in addiction. Another potentially exciting application of these data is to use computational approaches that predict drug treatments from transcriptome changes (8). This was recently applied using a genetic mouse model of high alcohol consumption to predict novel drugs that reduced alcohol intake (9). The explosion of transcriptome profiling in addiction biology, and all of neuroscience, raises the question of how this information can inform diagnosis and treatment. Neuroscientists should pay close attention to the cancer field which has demonstrated some success in using transcriptomics

to computationally select unexpected therapeutic compounds (10). As addiction researchers merge an increasing number of refined RNA datasets, we can hope to visualize and then target the transcriptional landscape at different times in the addiction process.

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