Biobehavioral Therapies: 
Complex Systems of Behavior, Brain, Epigenetics

Ted Hoppe’s message to the Snowflake Community, 29 March 2013 4:51 PM EST

The neurofeedback presentations we’ve been hearing at the conference for the last two years, as well as the “Ware K” system we heard about this year, prompted great discussion but also left many questions unanswered. It would be worth following up on those discussions here. My question about these methods of treatment centers around the need for biological evidence to support the claims. For example, there are claims that neurofeedback is an effective treatment for addiction and depression, both of which create epigenetic modifications. See Eric Nestler’s research: [Scientific American, December 2011, pp. 76-83.]

What Changes Exactly? Even a single dose of cocaine can alter the epigenetic landscape of genes in the nucleus accumbens, a part of the reward center. In the absence of drugs (a), methyl marks predominate, keeping the affected chromatin tightly wound and its genes quiet. Cocaine causes acetyl groups to predominate and chromatin to loosen (b). Then many genes encoding proteins involved in the pleasurable response to the drug become active.

Is there a need for research that would demonstrate a “resetting” of the “markers” to justify the neurofeedback’s treatment claims?

In the absence of such research what exactly is neurofeedback, or the “Ware K” treatment, accomplishing that can be measure biologically?

Ted

Fred’s Fantasia #2: Some perspective on those issues.

Ted has raised the issue of the importance of biological substrates of neurofeedback and biobehavioral therapies. Several perspectives are implied by this inquiry.

1. **Is the treatment effective?** Answer: I cannot be sure that it is or isn’t at this point due to my own unfamiliarity with the topic. But I suspect that the literature is not yet entirely conclusive on this point. The kinds of controlled research that is required is difficult to accomplish.

2. **If so, is that sufficient reason for employing or not employing it?** Not necessarily until other issues are resolved. See #5 infra.

3. **If it is effective, and even if information on brain processes are not required to justify its use, would not it be interesting to search for neural correlates?** Of course. After all this line of research started in the septum of rats (Olds,)

4. **For any biological correlates involved, is there any compelling evidence that other treatment modalities, such as yoga exercises, and the body work of Mark Filippi,**
George Muhs, Ken Ware, and others that yield similar results, work through the same neural and epigenetic channels as the biofeedback? I don’t know, but the question has been raised by others, and would certainly be worth pursuing. Ken’s work has some answers with physiological and brain wave measurements.

5. Fifth, Martin Gardiner raised the issue of could there be the possibility of deleterious side effects of biofeedback? This is certainly a possibility in all therapy, medical, pharmacological, psychological, and environmental, such as provided by his example of Jayne’s Primal Scream. Nestler’s discussion of addiction might be a precaution to make sure other reward systems might be addictive, or at least involve epigenetics.

6. Can we realistically demand that all the biological foundational research be more complete before continuing the clinical practice? I suspect not. The system is too complex to get all the answers quickly. Certainly scientific curiosity would demand it, and for me, that, rather than clinical result are of primary interest. Whether behavioral alone, or a mix of behavioral and biological evidence would satisfy Martin’s objection is not clear.

7. Why is epigenetics important? Roulette Wm. Smith has been regaling us over the past several years with the importance of junk dna and epigenetic networks for bodily, behavioral, and social systems. The early work with electron microscopy showing chromosomal activations (Porter, Palade, De Robertis, the 1950’s, I forget exactly whom), along with Waddington’s (1957) suggestion of an epigenetic landscape (see Roulette and Maze & Nestler (both 1912), seem to have reached new heights of experimental and theoretical sophistication. This works seems to suggest that the brain-epigenetic system seems to be an integral system embedded within its genetic-biological-behavioral-environmental system.


Nestler:

‘The environment can influence gene activity by regulating the behavior of epigenetic writers and erasers—and thus the tagging, and restructuring, of chromatin. Sometimes the tags persist for just a short time, say, to allow a nerve cell to respond rapidly to intense stimulation by producing a sustained wave of neurotransmitter release. Often the tags stay put for months or years—or even for the life of the organism: strengthening or weakening the neural connections involved in laying down memories, for example. (p. 80.)

“The addition and removal of acetyl and methyl groups—and other marks—can thus help the brain to respond and adapt to environmental challenges and experience. My lab and others are now finding in animal studies, however, that these beneficial epigenetic processes can go awry in conditions such as addiction and depression, where alteration of the normal array of modifications may serve to activate cravings, induce feelings of defeat or otherwise predispose an animal to a lifetime of maladaptive behavior. Examination of human brain tissue, retrieved post-mortem, suggests that the same may be true in people. (p. 80.)
“Because the brain’s reward center reacts to such a wide variety of stimuli—including food and
sex—manipulating the activity of neurons in this center can fundamentally alter the way an ani-
mal behaves. (p. 81.)

“Looking more closely at the mice’s DNA, we saw changes in epigenetic modification across
some 2,000 genes in the brain’s reward center. For 1,200 of these genes, we measured an
increase in a particular epigenetic mark—a form of histone methylation that represses gene
activity. So it seems that depression may shut down genes important to activating the part of the
brain that allows an animal to feel good, creating a sort of “molecular scar.” Many of these
stress-induced changes, we found, could be reversed by treating the mice for one month with
imipramine, a widely prescribed antidepressant. Similar epigenetic changes have been detected
in human brain samples obtained from individuals who were depressed at their time of death.
(p. 81.)

“Although these results are promising, the inhibitors currently on the market are not likely to be
useful for combating mental illness. The acetyl erasers—histone deacetylases—regulate
epigenetic markings in cells throughout the brain and all over the body, so drugs that disable
them indiscriminately have serious side effects and can be toxic. One alternative would be to
generate medicines able to selectively inhibit the forms of histone deacetylases that are enriched
in areas of the brain most affected in specific psychiatric conditions—the reward center, for
example. Another option would be to identify novel proteins involved in epigenetic modification
in the brain. In the end, though, the most fruitful approach might be to determine which genes
are the subjects of epigenetic modification in depression or addiction: the genes for specific
neurotransmitter receptors or signaling proteins, say, that are involved in neural activation. We
can then focus our efforts on designing drugs that target the activity of those particular genes—or
the protein products of the genes—directly.” (pp. 82-83.)

Maze & Nestler:

Abstract

“Drug-induced alterations in gene expression throughout the reward circuitry of the brain are
likely components of the persistence of the drug-addicted state. Recent studies examining the
molecular mechanisms controlling drug-induced transcriptional, behavioral and synaptic
plasticity have indicated a direct role for chromatin remodeling in the regulation and stability of
drug-mediated neuronal gene programs, and the subsequent promulgation of addictive
behaviors. In this review, we discuss recent advances in our understanding of chromatin
phenomena—that is, epigenetics, by one definition—that contribute to drug addiction, with the
hope that such mechanistic insights may aid in the development of novel therapeutics for future
treatments of addiction.

Epigenetics:’ a definition

“Interactions between genes and the environment that result in specific biological phenotypes
are one way in which the term “epigenetic” is used. In 1957, the idea of an “epigenetic
landscape” was first described by Conrad Waddington, a developmental biologist who was
interested in explaining how identical genotypes could result in such a wide variety of phenotypic variation throughout the process of development\textsuperscript{5}. Later, this concept would evolve to include the additional suggestion that “potentially heritable changes in gene expression [occur] that do not involve changes in DNA sequence”\textsuperscript{6-8}.

“While many questions remain, mechanistic insight into Waddington’s landscape has taken a clear molecular form with documented evidence indicating that environmental signals can be transduced to promote stable alterations in chromatin structure. These changes occur via a number of multisubunit complexes that act to regulate DNA's access to the transcriptional machinery, thus activating or repressing specific gene programs\textsuperscript{11}. Such events ultimately result in the promulgation of specific gene expression patterns in response to distinct environmental cues through a combination of chromatin remodeling activity, enzymatic modification of DNA and histones, and nucleosomal histone subunit exchange\textsuperscript{11-13}. The regulatory control exerted by mechanisms of chromatin remodeling, as well as the potential stability of such modifications, make epigenetic regulation a prime candidate for mediating some of the persistent alterations in transcriptional and neural plasticity that are thought to underlie aspects of drug addiction.

**“Epigenetic mechanisms**

At its most basic level, chromatin functions to ensure the proper organization, storage and readout of genetic information with remarkable spatial and temporal precision during processes of cellular differentiation and organismal development\textsuperscript{10}. The nucleosome core particle exists as the fundamental repeating unit of chromatin, each composed of 147 bp of DNA organized in approximately two superhelical turns around an octameric core of histone proteins (two copies each of H2A, H2B, H3 and H4, or variants of these proteins) (\textbf{Figure 1A})\textsuperscript{14}. Structural variations of the nucleosome particle can be introduced via a number of tightly regulated mechanisms, including post-translational covalent modifications of histones, histone variant deposition, and chromatin remodeling complexes that promote altered levels of chromatin compaction, resulting in more “open” (euchromatic) versus “closed” (heterochromatic) transcriptional configurations. Such configurations typically reflect more “active” versus “inactive” states of gene expression, respectively\textsuperscript{11,13}. For example, histone modifications that weaken or disrupt histone:DNA contacts, such as histone acetylation, correlate with transcriptionally active states, whereas modifications that increase histone:DNA contacts, such as histone methylation at certain basic amino acid residues, promote transcriptional repression\textsuperscript{13}. Combinations of numerous post-translational modifications occurring on N-terminal histone tails, including phosphorylation, methylation, sumoylation, and others, have been demonstrated to affect condensation of chromatin and to result in altered levels of gene expression in cells (\textbf{Figure 1B})\textsuperscript{15}. Likewise, direct methylation of cytosine bases within DNA controls the activity of the affected genes.
Figure 1
Post-translational modifications of histones regulate gene expression. Shown in panel (A) is the nucleosome core particle, representing the functional repeating unit of chromatin, composed of 147 bp of DNA wrapped around a core octamer of histone proteins ... [bp = base pairs]

“These events occur through a very dynamic and complex system of enzymatic events that employ various “writer/eraser” proteins to catalyze the addition or removal, respectively, of histone marks at specific substrates. “Effector” proteins with distinct “reader” modules recognize specific histone modifications, or combinations thereof, to promote various downstream events. Ultimately, many different modifications occur at distinct histone residues throughout the genome, and these modifications are summated to determine the transcriptional output of genes into messenger RNAs and proteins as well as numerous non-coding RNAs. One important issue to consider is the apparent stability of certain chromatin modifications. This stability is evident in X-inactivation or genetic imprinting, whereby DNA methylation promotes lifelong gene silencing. Despite such high levels of apparent stability of some epigenetic modifications in vivo, most, if not all, forms of chromatin modifications are potentially reversible, and specific enzymes or mechanisms exist to mediate the addition or removal of associated marks. The in vivo mechanisms that control the transiency versus stability of specific chromatin modifications remain largely unknown.

Epigenetics and drug addiction
“Repeated exposure to numerous drugs of abuse alters gene expression profiles throughout the reward circuitry of the brain. Recent data suggest that repeated exposure to the psychostimulant cocaine promotes alterations in histone acetylation, phosphorylation and methylation levels, as well as DNA methylation levels, in the NAc. It is thus hypothesized that these modifications may be involved in mediating drug-induced behaviors.
“Remarkable progress has been made in documenting distinct regulatory patterns of chromatin modifications and the subsequent activity of transcription factors and their downstream targets in response to administration of several drugs of abuse. However, many important questions still remain regarding the impact and persistence of such events in the development and maintenance of the drug addicted state in both animal models of abuse and in human addicts as well as possible effects of different classes of abused substances on chromatin endpoints.

“Although epigenetic mechanisms represent attractive candidates to explain long-lasting, and potentially even permanent, alterations in neuronal function following chronic drug exposure, it is still unclear as to how long after drug exposure these changes in chromatin structure persist. As mentioned previously, many of the genes known to display altered expression levels following repeated drug administration do not remain elevated or repressed during periods of extended withdrawal. Thus it appears necessary to shift the focus of current research to more thoroughly examine alterations in the transcriptional inducibility of gene targets following drug/cue re-exposure, as well as the epigenetic phenomena underlying such events. Doing so may aid in the identification of novel gene targets, which could prove useful for the development of future drug therapies. Furthermore, given that drug addiction is a highly heritable illness, it will be interesting to further examine whether drug-induced alterations in gene expression at the level of chromatin are likewise transmittable to future generations. This would involve stable, drug-induced epigenetic modifications in germ cells, which persist in offspring and affect addiction vulnerability. Whether such modifications occur remains a subject of intense investigation.

“Another important question will be to ask on which genes, and in which regions of the genome, are drug-induced changes in chromatin structure occurring, as well as how these chromatin remodeling events ultimately affect the activity of genes in response to drug exposure? Although attempts have been made to address these questions through the use of genome-wide promoter analyses (ChIP-chip) and microarray technologies, the advent of next generation massively parallel genomic sequencing techniques (e.g., ChIP-Seq, RNA-Seq, etc.) promise enhanced DNA/RNA sequence-level resolution of chromatin binding events and transcriptional expression patterns. When examined at the level of numerous histone modifications (i.e., comparing combinatorial patterns of various histone marks), these techniques will allow for a stronger characterization of the genomic landscape following drug exposure. They also will permit future investigations into both the effects of drug treatment on gene expression, as well as the impact of chromatin regulation at non-coding genomic sequences (i.e., miRNAs, retrotransposons, enhancer elements, etc.), thus teasing apart another layer of complexity in our understanding of potential molecular mechanisms guiding addictive-like behavioral responses.

“High-throughput genomic analyses of chromatin regulation, as well as targeted approaches, such as those described throughout this review, will further address questions concerning the importance of many other types of chromatin modifications (e.g., sumoylation, nucleosome remodeling and ubiquitination, to name a few) to drug addiction, and will aid in future investigations examining the similarities and/or differences between chromatin-mediated gene regulation in response to various drugs of abuse throughout numerous brain regions implicated in addiction. Such studies will allow for a fuller understanding of the ways in which drug-induced gene expression profiles throughout numerous limbic forebrain regions affect neural connectivity and electrophysiological communication between associated structures.

“Lastly, it is important to consider that many of the brain regions affected by drug of abuse do not exist as homogeneous cell populations, but rather as heterogeneous structures composed of many distinct cell types with different signaling outputs and electrophysiological properties. For example, although the NAc is composed of approximately 95% medium spiny, GABAergic
projection neurons receiving common inputs from several afferent pathways (e.g., glutamatergic, dopaminergic, etc.), these neurons express distinct dopamine receptor subtypes—dopamine receptor 1 (Drd1) and 2 (Drd2), which promote opposing downstream signaling cascades leading to very different transcriptional outcomes and, oftentimes, quite distinct behavioral responses to drugs of abuse. Analogous studies are needed for the several types of interneurons present in the NAc as well as glial cells. Therefore, it will be necessary to further examine drug-induced chromatin modifications in a cell type specific manner to more fully understand the contribution of differential modes of transcriptional plasticity in distinct cell types that may be involved in the development of addictive behaviors.

“In conclusion, although our understanding of the molecular mechanisms underlying drug addiction remains incomplete, the identification of chromatin remodeling as an important mediator of drug-induced transcriptional and behavioral plasticity represents an exciting new area of research with potential therapeutic benefits. The ability to reverse the epigenetic landscape controlling the addicted state offers an approach that may aid in the development of more effective treatments for addiction, not only through direct targeting of aberrant chromatin regulation, but also through the future identification of target genes involved in addiction pathogenesis.”

**FF2 Postscript**

These are but a few passages from their articles, and while we may not have targeted addiction as a topic, I think it important to evaluate just how complex a dynamical system the brain-genetic-epigenetic-environmental system is. These epigenetic changes occur not only to chemical substances, but to ordinary neural, behavioral, and environmental events, and in a very interactive way. Furthermore, they point out that not only NAc but the rest of the limbic reward system is involved, which also means most of the rest of the brain as well.

Alan Bachers has rebutted the challenge of Ted’s demand for more biological investigation, with the citation of EEG and neuroimaging work on neurofeedback, and I mentioned Sterman’s work. He also emphasizes the neurofeedback phenomena as a self-organizing system, operating in Wolfram IV territory (which has now morphed into the more inclusive and definitive realm of the μ-bifurcation, use of that terminology is now compulsory, Abraham, 2013). My own research on recovery of function in the brain verifies this point of view (Abraham et al., 1973). I think this is a stand-off. More research is clearly needed, but meanwhile the nice promise of work such as Jean Alavarez’s positive results with cognitive deficits after breast cancer surgery suggest that this work should proceed.

John Strong, participant a year ago at the Winter Chaos Conference 2012 sums up this discourse nicely: “Thanks Fred, that gives a nicely balanced summary for me. This is quite a passionate area that has potential from snake oil through to a true breakthrough in science (I intend no offence and make or judgment just a statement of the huge range of potential). I have just finished reading *Mind over Mind* by Chris Berdik which explores the placebo effect and the mind’s potential for self-change to actualize a belief including healing. We have a way to go to understand the complete scope of epigenetics.”

**References**


