Translation of Addictions Science into Practice

Edited by:
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PREFACE

From Bench to Bedside: Diffusing Addictions Science into the Real World

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Abstract: Translation of basic to clinical sciences and from clinical sciences to the applied arena is an essential step toward our understanding and successful treatment of substance abuse disorder. Unfortunately, the movement of addictions research along this continuum has been a sluggish one, and the gap between what we know about addictions and what we practice is wide. We know much more than we apply. The ultimate aim of this volume is to provide a forum to close that gap, and speed the process of technology transfer.

Recent research on the genetic, neurochemical, behavioral and cultural underpinnings of addiction has lead to rapid advances in our understanding of addiction as a disease. Scientific progress in basic science and the resulting development of new pharmacological and behavioral therapies are occurring at a faster pace than can be assimilated, not only by clinical researchers, but also by policy makers and practitioners. Translation of science-based addictions knowledge into prevention and treatment is increasingly important.

Currently, there is a wide gap between the development of science-based knowledge of the nature (e.g., as a brain disease) and/or treatment (i.e., pharmacotherapies) of addictions and the application of these ideas and treatments in the real world. This is due to many factors, all of which are outlined and discussed in our final chapter on "Pathways to Innovation in Addiction Practice."

An illustrative example of this research/treatment disparity is the limited uptake of adjunctive pharmacotherapy in the treatment of alcohol dependence. Most alcoholism practitioners have little knowledge of FDA-approved medications

for alcohol dependence (e.g., naltrexone, acamprosate) and, despite evidence of efficacy, many practitioners question their utility in clinical practice (Meza et al., 2001). Evidence-based pharmacotherapy is not in widespread use among alcoholism practitioners (Anton & Swift, 2003; McLellan, 2002) and, even physicians who specialize in addiction medicine prescribe alcoholism medications for less than 15% of their alcoholic patients (Mark et al., 2003).

This gap between addictions research and clinical practice has been the subject of an influential Institute of Medicine (IOM) report (Lamb, Greenlick & McCarty, 1998) that prompted substantial responses from several federal agencies in the USA. Such programs as the Center for Substance Abuse Treatment's (CSAT) Addiction Technology Transfer Centers and its Practice Research Collaboratives, the National Institute on Alcohol Abuse and Alcoholism's (NIAAA) Research-to-Practice Forums and its Researcher-in-Residence Program, and the National Institute on Drug Abuse's (NIDA) Clinical Trials Network have been established to address this problem.

The primary aim of this book is to provide a needed link between advances in addiction science and innovations in clinical practice. Our goal is to stimulate ideas designed to close the gap between bench and bedside, and to do so as rapidly as possible. While the pace of scientific advances in the addictions field is ever-increasing, the process of moving from basic to clinical to applied research is a slow and tedious one. Unless this transfer process is expedited, as Brown (1995, 2000) has aptly noted, clinical innovations may be created only to be interred on the shelves of academia or in the files of remote federal agencies.

While this book is focused primarily on translation, it also encompasses scientific advances that are relevant to dissemination and implementation, and will provide a useful tool for encouraging innovative thinking. In fact, we see the term "translation" as an all-encompassing one that runs the gamut from single-cell neurochemical research with animals (e.g., as in the later chapter by Mulholland and Chandler) to dissemination and implementation of evidence-based treatments into real-world clinical practice (as in the chapter by Sorensen, Hettema, & Chen). Translation is an attempt to move research progress more rapidly along this continuum, so that neurochemical research is smoothly translated into drug development, drug development into initial laboratory testing, laboratory testing into clinical trials, clinical trials into effectiveness studies in real world settings, and finally, studies into implementation research with treatment providers.

Translational efforts to increase the impact of basic or clinical research on the next stage of research development can take many forms. Examples are collaboration among basic and applied scientists, speculation on the future implications of current research work, and research that specifically focuses on a better understanding of ways to progress research more efficiently along the translational continuum. For example, the goal of animal research into single brain cells or

neurotransmitters is to not simply to increase our understanding of addictions for its own sake. The ultimate goal is that this knowledge will lead to more effective prevention and treatment strategies, and to public policies that can address the tragic and worldwide impact of addiction on individuals, their social networks, and their societies.

Accordingly, this book is intended to generate interest in application opportunities emanating from both research and theoretical advances. We invited distinguished and experienced addictions researchers to prepare chapters that summarized recent scientific advances and their immediate or short-term applications. We also asked them to speculate creatively on applied possibilities of the research in the longer term. Speculation by experts in the field is rarely encouraged or even allowed in traditional scientific journals or even in formal presentations at most scientific conferences. While focusing on empirical data and avoiding speculation that goes beyond that data is a basic characteristic of good science, we argue that conjecture, supposition, and educated guesswork certainly have an important place in generating novel ideas that may lead to future breakthroughs. At the very least, these activities may grease the wheels of translation, and speed the bench-to-bedside movement of research. So, while we intend that this book should provide a concise summary of existing research and thinking on key areas of the science and practice of addictions, our ultimate aim is to generate new and exciting ideas for the application of basic research to improve clinical practice and social policy.

The concept for this volume grew out of Addictions, 2004, an international conference on "Crossing Boundaries: Implications of Advances in Basic Sciences for the Management of Addiction." The conference, sponsored by Elsevier, was held in Queensland, Australia, and included noted addictions researchers from around the world. Conference proceedings were published in *Addictive Behaviors* as a Special Issue (Volume 29, Number 7, 2004) as part of the journal's *Annual Review of Addictions Research and Treatment* series. So much enthusiasm and cross-fertilization of ideas transpired at this interdisciplinary conference that the idea of this more comprehensive edited book was born.

Many of the same researchers contributed to *Addictions 2004* and the current book. This volume includes chapters from addictions researchers at various stages on the translational continuum, and with very different research interests. The combination of these disparate sub-specialities and interest areas is intended to provide a cross-fertilization of ideas that goes far beyond the contribution of the individual chapters.

Our hope is that this volume will help to speed the "bench-to-bedside" translational process and to encourage cross-collaborations and cross-fertilization of ideas among basic scientists, clinical researchers, and practitioners. If just one new idea or new application is derived from reading this book, the effort will have been worthwhile.

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SECTION I
GENETICS AND NEUROSCIENCE

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CHAPTER 1

The Interplay between Genotype and Gene Expression in Human Brain: What Can it Teach Us about Alcohol Dependence?

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Abstract: Molecular expression studies of the pharmacological profiles of receptors in key brain regions and cell types delineate alterations in neurotransmission in alcoholic brain. Individual variations in propensity for brain damage and comorbidity modulate these changes. Receptor subunit composition and switching that play central roles in cell killing will define likely paths to neuroprotection. This will guide the development of new precisely targeted ameliorative and preventive drugs.

Abbreviations: AMP, adenosine monophosphate; ATP, adenosine triphosphate; DNA, deoxyribonucleic acid; GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate; mRNA, messenger ribonucleic acid; SFC, superior prefrontal cortex; SNP, single nucleotide polymorphism; WHO, World Health Organization; 5HT, 5-hydroxytryptamine (serotonin)

DRUG AND ALCOHOL ABUSE

The current era has seen a tremendous upsurge in the abuse of psychoactive substances around the world. Despite the public attention lavished on illicit drugs (psychostimulants, heroin, marijuana) and the "war" to contain them, by far the greatest impact on human health and societal well-being is exerted by two licit drugs, tobacco and alcohol, which are highly comorbid in usage. Alcohol consumption has declined slowly in recent years in developed countries, although this may not continue because transnational corporations "aggressively … target young people in advertising and promotion campaigns (WHO, 2004)". Consumption has grown steadily in developing countries, particularly in WHO's Western Pacific region (WHO, 2004). Subjects in the region show patterns of hazardous drinking that exacerbate the harmful effects of alcohol on the brain (WHO, 2004). Understanding the mechanisms that constitute this harm is a major research priority.

On a standardized estimate of disease load, disability-adjusted life years, tobacco gives a value of 4.1%, alcohol 4.0%. In contrast, all illicit drugs combined contribute an average of 0.8% to global disease burden (WHO, 2004). Figures are higher for men and markedly higher in special populations. A comprehensive transnational survey found that although the prevalence of hazardous/harmful drinking varies from country to country, drinking behavior and alcohol-related problems show many common features (Saunders et al., 1993). Harmful drinking in Australia was estimated at ~12% in a recent survey (Simpson et al., 2000).

Alcoholic Brain Damage

Harper and colleagues used quantitative stereometric analyses to establish that alcohol misuse leads to selective brain pathology (Harper & Kril, 1993). Global effects such as generalized brain shrinkage, reduced white-matter volume, and dendritic pruning, may be reversible with abstinence. Superior prefrontal cortex (SFC) is particularly vulnerable, while most cortical regions are much less affected (Kril et al., 1997; Kril & Harper, 1989). Alcoholics differ markedly in their neuropathological presentation, ranging from little or no damage to severe frontal lobe and/or cerebellar atrophy. Common comorbidities of alcohol abuse, including cirrhosis of the liver and the Wernicke–Korsakoff syndrome, are associated with greater brain atrophy (Harper & Kril, 1993). These diseases are more prevalent with high rates of alcohol consumption (Saunders & Latt, 1993), so it appears that the pathology worsens with increasing disease severity.

Discrete Subsets of Neurones may be Selectively Vulnerable

Neurones in specific regions of the brain (cerebellar Purkinje cells and SFC neurones) are selectively damaged (Harper & Kril, 1990, 1993; Kril & Harper, 1989); the alterations in SFC are in line with known changes in cognitive function. Subsets of neurones may be particularly susceptible to ethanol toxicity as a consequence of possessing a specific protein profile (Dodd & Lewohl, 1998; Kril et al., 1997; Kril & Harper, 1989). While neuronal losses are apparent in alcoholics without comorbidity, the severity of damage is greater in alcoholics with concomitant cirrhosis of the liver (Kril & Halliday, 1999). Hence, liver damage may have additive effects on alcohol neurotoxicity. One possibility is that alcoholics with varying degrees of pathology represent stages on a dosage continuum, with more-severe damage correlating with greater lifetime consumption. Alternatively, alcoholics with liver damage may be more susceptible to alcohol neurotoxicity as a result of a genetic predisposition.

Mechanisms Influencing Brain Function

Alcohol abuse leads to cognitive, physiological, and structural changes in several cortical and sub-cortical structures. The cellular and molecular mechanisms that bring this about are poorly understood. Ligand-gated ion channels mediate acute drug intoxication (Weight et al., 1992). In experimental animals, channels for anions (negatively charged ions) that are gated by γ-aminobutyric acid (GABA) and glycine, and channels for cations (positively charged ions) that are gated by glutamate, acetylcholine, 5HT, and ATP, are affected by physiologically relevant concentrations of alcohol. Of these, $GABA_A$ and *N*-methyl-D-aspartate (NMDA) receptors are major cell-membrane targets (Toropainen et al., 1997). Glutamate operates 67–73% of cortical synapses in human brain and is the major excitatory transmitter: the influx of cations it mediates depolarises post-synaptic cells, which have a negative internal polarity in the resting state. In contrast, the influx of anions mediated by GABA (and glycine) hyperpolarizes the post-synaptic cell; GABA is used at 16–25% of cortical synapses, where it is the major inhibitor (Hornung & de Tribolet, 1995). Excitation-inhibition balance is a key determinant of neuronal viability: when tilted toward excessive excitation it is termed *excitotoxicity* (Olney, 1994).

In experimental studies, such a shift may be elicited in several ways. What is not clear is whether such mechanisms occur in human brain *in vivo*, and how the localization of pathology comes about. Plausible options include locally overactive NMDA or underactive $GABA_A$ receptors, and a diminished clearance of glutamate from the synaptic cleft (Dodd, 2002). Receptor and transporter

pharmacology are determined by the expression of many genes; if some are switched off, and others switched on, profound changes in receptor function or transport capacity are brought about. It is thus of interest that alcoholism risk is differentially associated with alleles of several transmission-related genes. The products of these genes (receptors, transporters) are targets for several drugs used to treat alcoholism.

Alcoholism Causes Changes in Transcript mRNA and Protein Expression

Sustained ethanol exposure in animals changes the expression of many genes, including those for mediators of the actions of transmitters, signalling molecules, molecular chaperones, transcription factors, and cytokines (Miles, 1995). Differential display and microarray studies have revealed altered expression of mitochondria- and genome-encoded genes in the brains of alcohol-treated rats and human alcoholics (Chen, Hardy, & Wilce, 1997; Fan et al., 1999; Lewohl et al., 2000). It is not possible to predict which genes are involved from *a priori* neurobiological or pharmacological considerations. Recent advances in microarray technology have enabled the simultaneous analysis of more than 50,000 messenger-RNA (mRNA) transcripts in SFC in chronic alcoholics and controls (Lewohl et al., 2000; Liu et al., 2004; Mayfield et al., 2002). Differentially expressed transcripts fall into functional groups that include metabolism, immune response, cell survival, cell communication, signal transduction, and energy production. Transcripts coding for several synaptic proteins differ in abundance by 40% (1.4 fold) or more between alcoholics and controls, suggesting that synaptic transmission is affected by chronic alcohol use. Pathologically vulnerable and resistant cortical regions show distinctive patterns, such that alcoholics and controls partition completely (Liu et al., 2004).

It is not known whether alcohol has a direct effect on alcohol-responsive genes, or an indirect effect involving many systems. Activation or repression of alcohol-responsive transcription factors could alter the expression of genes that possess the corresponding control elements (Miles, 1995). In contrast, alcohol misuse is known to alter the translation of some proteins without changing mRNA levels. We found marked differences in $GABA_A$ receptor α_3 isoform protein expression between alcoholic and control SFC, but little difference in α_3 mRNA – whereas α_1 isoform protein and mRNA varied in concert (Dodd & Lewohl, 1998). On a gene-by-gene, protein-by-protein basis, it is difficult to discern general patterns. Small changes in low-abundance transcripts can lead to profound changes in cell function. This is often reflected in large variations in protein abundance through altered mRNA half-life brought about by modified activity of post-translational processing enzymes. These proteins are targets for therapeutic intervention.

Neuroadaptive models posit long-lasting changes at the molecular and cellular level to explain compulsive drug use. Differing adaptive responses may account for individual differences in susceptibility to alcohol. Alcohol can be metabolised by mitochondrial oxidation, especially in brain, giving rise to high levels of free radicals, particularly peroxides. Free radicals are highly reactive with protein. While the cell has effective mechanisms for repairing DNA that is damaged by free-radical action, there are no repair mechanisms for damaged proteins. These accumulate within cells and become increasingly defective with time. Adaptive changes that lead to chronicity (such as receptor adaptation) underpin short-term withdrawal, but other factors such as morphological or biochemical remodelling in sensitive cells are important mediators of alcohol-related behaviors in the longer term (Koob, Sanna, & Bloom, 1998). In support of this concept are observations of changes in second messengers and inducible transcription factors, and thence in the expression of downstream genes, in animals following repeated exposure (Koob, Sanna, & Bloom, 1998).

Preliminary Proteomic Studies

We performed a study on samples that had been used for microarray analysis to assess mRNA expression by Lewohl et al. (2000). Samples were SFC pieces taken at autopsy from well-characterized chronic alcoholics and controls matched for age, sex, and cause of death. Poly-drug abusers and cases with comorbid liver cirrhosis or Wernicke encephalopathy were excluded. Tissue pieces were homogenized in water and frozen, and 2-dimensional proteomics was performed on the soluble fraction (Lewohl et al., 2004).

Overall, 182 proteins differed by more than 2-fold between case and control samples. Of these, 144 were less abundant in alcoholics, 33 more abundant, and 8 were new or absent. To date, 63 of these proteins have been identified using MALDI-MS and MS-MS. Four 14-3-3 isoforms (ε, γ, η, ζ) and two members of the synuclein family of proteins (α- and β-synuclein) were identified to be at least 2-fold lower in SFC of alcoholics (see Table 1). A varied group of proteins were up-regulated in alcoholic SFC (Table 2). Comparison of the protein and mRNA data sets revealed several patterns, although both sets had members that did not appear in the other. Some proteins and transcripts had comparable differential expression between alcoholics and controls. However, in several instances the trends were not the same. A number of proteins that showed differential expression in this pilot study of pathologically "simple" alcoholic brain damage also occur in published lists from the proteomic analysis of pathologically "complex" Alzheimer disease (Schonberger et al., 2001; Tsuji et al., 2002). This may provide insights into common pathways of neurodegeneration.

Table 1
Some Examples of Proteins Down-regulated in Alcoholics

Fold change*	Database entry†	Protein name
–14.90	SYUB_HUMAN	β-synuclein
–5.80	L36674	α-synuclein
–3.40	143F_HUMAN	14-3-3 protein η
–2.40	143E_HUMAN	14-3-3 protein ε
–2.40	NM_003406	14-3-3 protein ζ
–2.30	gi12655023	14-3-3 activation protein
–2.10	143Z_HUMAN	14-3-3 protein ζ/δ
–2.10	gi481360	14-3-3 protein γ

*Expression abundance, Alcoholics/Controls.
†Annotation in the SwissProt database. Triplicate protein profiles of control and alcoholic SFC tissue extracts were analyzed for differential display using PDQUEST v7.0 image analysis software (Bio-Rad, Hercules, CA, USA). Images were obtained in triplicate for each sample, for two pH gradients, pH 4–7 and 6–11.

Table 2
Some Examples of Proteins Up-regulated in Alcoholics

Fold change*	Database entry†	Protein name
+5.20	PDX2_HUMAN	Peroxiredoxin 2 (Thioredoxin peroxidase I)
+2.20	FABB_HUMAN	Fatty acid-binding protein, brain
+3.10	AOP2_HUMAN	Antioxidant protein 2
+2.00	KRCB_HUMAN	Creatine kinase, B chain
+6.50	HS7C_HUMAN	Heat shock cognate 71 kDa protein
+1.90	HNT1_HUMAN	Histidine triad nucleotide-binding protein
+2.10	POR1_HUMAN	Voltage-dependent anion-selective channel #1
+3.90	KPY1/2_HUMAN	Pyruvate kinase, M1 or M2 isozyme

*Expression abundance, Alcoholics/Controls.
†Annotation in the SwissProt database.
See Table 1 legend for details.

Genetics of Alcohol Misuse

Individuals vary in their propensity for alcohol misuse through a complex interaction among societal, environmental, and genetic factors. Subjects may be susceptible to drug misuse problems for a variety of reasons, including genetic predisposition, inherent personality traits, and so on. Alcoholics also vary in their likelihood of developing comorbidities such as cirrhosis of the liver. There are gender differences in alcoholism, and the number of female alcoholics is increasing in most countries (Walter et al., 2003). Women are more sensitive to alcohol than men because they have less body water, and hence higher blood alcohol levels after equal alcohol intake (Baraona et al., 2001; Brienza & Stein, 2002; Ely et al., 1999; Hommer et al., 2001; Tapert et al., 2001; Walter et al., 2003).

Linkage and twin-adoption studies are generally not feasible with severe alcohol abusers. Such subjects are often itinerant, and it is rarely possible to obtain clinical histories or DNA from next of kin, so extensive pedigrees often cannot be constructed. Genetic effects can be small, and epistasis and other interactions can confound analysis. A large linkage study of alcoholics under way in the USA (COGA) has produced low LOD scores with broad peaks, in part through difficulties in diagnosis and ascertainment. It has been estimated that these approaches have identified less than half the chromosomal regions containing genes for dependence. Nevertheless, they have suggested number of gene candidates for further study and fine mapping, and they underpin genome-wide scans of informative single nucleotide polymorphisms (SNPs).

Case-control studies of candidate genes are used to search for genetic associations. An adjunct approach is to ask whether genotype partitions the extent of brain damage in alcoholic subjects, and the molecular mechanisms that underpin it. The drawbacks to this approach are that plausible markers must first be found; that the marker often has to be very close to the disease locus for a significant association to be detected; and that population stratification can compromise analyses where cases and controls potentially contain subjects of differing ethnicities (some populations may be over-represented among drug abusers). Differences in allele frequencies between cases and controls can be confounded with differences in population allele frequencies.

Candidate Genes for Alcohol Misuse (for references see Foley et al., 2004)

Drug dependence is linked to mesolimbic/cortical dopamine reward and reinforcement mechanisms. Alcohol activates dopaminergic transmission in experimental animals, and some dopaminergic drugs reduce alcohol intake. Bromocriptine, a D2 agonist, is most effective for treating alcoholics who possess *DRD2* A1 alleles. *DRD2 Taq* I A and B polymorphisms have been associated with alcoholism. The dopamine transporter DAT1 gene (*SLC6A3*) contains a 3′ UTR variable tandem repeat; associations have been found between 9-repeat alleles and alcohol withdrawal symptoms. 5HT reuptake inhibitors have some value in the treatment of alcoholism, but genotyping studies on the 5HT transporter gene (*SLC6A4*) 5HTTLPR polymorphism have been inconsistent. A glutamate transporter EAAT2 gene (*SLC1A2*) polymorphism is associated with risk-taking behavior in alcoholics. Alcohol dependence has been rather unstably associated with variants of the NMDA receptor NR2 subunit gene *GRIN2B* (Schumann et al., 1995, 2003). The NMDA receptor enhancer and $GABA_B$ receptor antagonist acamprosate reduces relapse rates in recovering alcoholics. Ethanol interacts with $GABA_A$ receptors; benzodiazepines, which are $GABA_A$ modulators, are used to treat alcohol withdrawal. The $GABA_A$ subunit gene cluster at 5q33-34 is implicated in alcoholism by studies of gene