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Using autopsy brain tissue to study alcohol-related brain damage in the genomic age

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Abstract

The New South Wales Tissue Resource Centre (NSW TRC) at the University of Sydney, Australia is one of the few human brain banks dedicated to the study of the effects of chronic alcoholism. The bank was affiliated in 1994 as a member of the National Network of Brain Banks and also focuses on schizophrenia and healthy control tissue. Alcohol abuse is a major problem worldwide, manifesting in such conditions as fetal alcohol syndrome, adolescent binge drinking, alcohol dependency and alcoholic neurodegeneration. The latter is also referred to as alcohol-related brain disease (ARBD). The study of postmortem brain tissue is ideally suited to determining the effects of long-term alcohol abuse, but it also makes an important contribution to understanding pathogenesis across the spectrum of alcohol misuse disorders and potentially other neurodegenerative diseases. Tissue from the bank has contributed to 330 peer-reviewed journal articles including 120 related to alcohol research. Using the results of these articles, this review chronicles advances in alcohol-related brain research since 2003, the so-called genomic age. In particular it concentrates on transcriptomic approaches to the pathogenesis of ARBD and builds on earlier reviews of structural changes (Harper et al. *Prog Neuropsychopharmacol Biol Psychiatry* 2003;27:951–61) and proteomics (Matsumoto et al. *Expert Rev Proteomics* 2007;4:539–52).

Keywords

Alcohol-related brain damage; neurodegeneration; autopsy tissue; brain banking

Introduction

Postmortem or autopsy brain tissue is a precious and valuable resource for neuroscience and neurological disease research. The complexity of the human brain combined with the uniqueness of human neuropsychiatric conditions means that studies utilizing postmortem tissue make important contributions to research.

Neurodegeneration is an important sequel of chronic alcoholism that is also referred to as alcohol-related dementia or alcohol-related brain damage (ARBD). More generally, the term ARBD incorporates the changes in brain plasticity that underlies alcohol addiction, tolerance and withdrawal effects. ARBD differs from other neurodegenerative diseases such as Alzheimer's disease in that there is brain atrophy but modest neuronal loss (Kril et al. 1997). Furthermore the recovery of brain function in abstinent alcoholics is unique among these

disorders (Pfefferbaum et al. 1995, Pfefferbaum et al. 2006). Therefore the study of brain tissue from chronic alcoholics may afford opportunities for gaining an understanding of general mechanisms of neuronal dysfunction prior to neuronal loss and offer potential avenues for therapeutic intervention.

The NSW TRC is one of the few human brain banks worldwide dedicated to alcohol-related research (<http://www.nswbrainbanknetwork.org.au/about/NSW-TRC.php>). There have been approximately 120 peer-reviewed papers on alcohol research utilizing tissue from the NSW TRC, since its inception in 1994. In 2003, Harper and colleagues reviewed the studies alcohol-related research carried out using NSW TRC tissue, documenting the structural brain changes seen in chronic alcoholism and associated disorders such as Wernicke-Korsakoff syndrome (WKS) (Harper et al. 2003).

In the same year the human genome project was officially completed, a biomedical milestone that has led to a number of spinoff technologies. Since 2003, these -omic technologies in combination with NSW TRC tissue have been used to prioritize single nucleotide polymorphism (SNP) results from genome-wide association studies (GWAS) on alcohol dependence (Edenberg et al. 2010) and quite extensively in proteomic studies. The majority of proteomic studies were carried out during or before 2007 and have been previously reviewed (Matsumoto et al. 2007). Matsumoto *et al.* described a limited overlap between different brain regions but a common downregulation of the thiamin-dependent enzymes and a likely role of subclinical thiamin deficiency in ARBD.

The predominant -omic methodology utilizing NSW TRC has been whole-genome gene expression or transcriptomic studies. These studies commenced in 2000, and were included in an excellent review on neuroadaptive changes in chronic alcoholism in 2006 (Flatscher-Bader et al. 2006). Given the rapid advances in technology and gene annotation it seems timely to revisit this area. The major aim of this review is to explore how NSW TRC-facilitated research and transcriptomic studies, in particular, have improved our understanding of ARBD. We also take the opportunity to highlight some of the NSW-TRC's own research addressing challenges inherent to using human autopsy brain tissue.

Characterization of donor tissue

NSW TRC brain tissue is largely obtained from the NSW Department of Forensic Medicine (coronial cases) but it also operates a prospective consent program enabling members of the community to donate their brain for neuroscience research after they die. In consented cases a full autopsy precedes the neuropathological examination, an increasingly unusual event in brain banks worldwide but vital in complex phenotypes like chronic alcoholism where co-morbidities and drug use affect brain pathology.

The NSW TRC staff characterize the donated cases clinically including age, gender, body mass index, ethnicity, cause of death, toxicology including blood alcohol level, rapidity of death, agonal events and postmortem interval (PMI). Information is gathered from a variety of sources including standardized questionnaires to attending physicians and next-of-kin and is used to determine a diagnosis according to DSM-IV classification. These sources are also used to estimate mean daily and lifetime alcohol consumption, quantify other lifestyles factors such as smoking and establish medication histories. Mean daily alcohol consumption is classified as light (< 20g/day, and such individuals are considered controls), moderate (20–50g/day), heavy (50–80 g/day) or chronic. The latter individuals have consumed greater than 80 g of alcohol (ethanol) per day for the majority of their adult life (usually >30 years). Where chronic alcoholics do not meet the DSM-IV criteria for alcohol abuse or dependence, they are classified as 'harmful use'. All tissue supplied for research purposes is from

individuals with chronic alcohol use and either DSM-IV criteria. DSM-IV is based largely on psychosocial criteria but NSW TRC clinical information shows that 'dependent' individuals also have higher lifetime alcohol consumption than those fitting the abuse criteria (D. Sheedy et al., unpublished findings). The NSW TRC is currently investigating how these categories, including 'harmful use', relate to ARBD by quantifying the extent of neuropathology.

Exclusion criteria include infectious disease, other neurological diseases, postmortem intervals (PMI) > 60 hours or severe agonal events such as a sustained period of ventilation immediately prior to death. All donated brains undergo a thorough macroscopic and microscopic examination to exclude co-existing pathologies and to examine for pathologies associated with alcoholism such as hepatic encephalopathy or Wernicke-Korsakoff syndrome (Harris et al. 2008a). In addition, the postmortem indices brain pH and RNA Integrity Number (RIN) are measured from frozen cerebellar samples. A comprehensive account of the NSW TRC banking procedure have been previously published (Sheedy et al. 2008).

Alcohol and the Human Brain

Surprisingly, given the well-recognized clinical symptoms of intoxication, the exact mechanism of action of alcohol on the brain remains unknown (Harris et al. 2008b). Alcohol or ethanol is amphipathic being soluble in both lipids and water so it extremely well distributed throughout the entire body. It is presumed that alcohol simultaneously suppresses excitatory and stimulates inhibitory neurotransmission in the central nervous system (CNS). Alcohol appears to act both as a noncompetitive antagonist of excitatory N-methyl-D-aspartate (NMDA) receptors and as a GABA receptor agonist (reviewed by Kumar et al. (Kumar et al. 2009)).

At autopsy, individuals with ARBD have reduced brain weights and their degree of atrophy correlates with lifetime consumption (Harding et al. 1996). Brain shrinkage (atrophy) is regionally specific but mainly occurs in the frontal lobes. ARBD is a neurodegenerative disease but atrophy and is largely due to white matter (WM) loss (Krill et al. 1997). The mild atrophy that does occur in the grey matter (GM) in ARBD results mainly from the retraction of the neuronal dendritic arbor. Neuronal loss is an uncommon feature outside the pyramidal neurons of the prefrontal cortex (PFC) in alcoholics free of liver pathology or thiamin deficiency (Krill et al. 1997). In cytoarchitectural terms, the ARBD brain is surprisingly normal with neuronal loss confined to regions such as the PFC and only detectable by systematic quantification (Krill et al. 1997). This selective impact of ARBD on the PFC is a common theme explored by a number of studies discussed in this review. However, hippocampal atrophy in ARBD is not associated with neuronal loss in either amnesic or non-amnesic individuals (Harding et al. 1997). This point will be discussed further when we consider a role for reduced neurogenesis in ARBD.

Alcohol is known to have wide ranging effects on the brain including damage to cell membranes, blood brain barrier injury and increasing oxidative stress (Haorah et al. 2008), but the major working hypothesis for chronic alcohol intoxication revolves around substantive and concurrent increases in NMDA receptors with decreases in GABA receptors (Kumar et al. 2009). The earliest studies utilizing NSW TRC tissue concentrated on GABA receptor populations. Alcohol is largely seen as a ligand of the GABA_A receptor (GABA_AR), and GABA_AR density was shown to be significantly greater in the PFC of chronic alcoholics versus the motor cortex, an area relatively unaffected by neuronal loss (Dodd et al. 1992). However, as we will see, GABA_AR gene dysregulation has not been a prominent feature of transcriptomic studies.

Alcohol also affects other transmitter systems such as serotonergic and dopaminergic systems that are involved in the predisposition to, and reinforcement of, excessive alcohol consumption, respectively (Heinz and Goldman 2000). In contrast, the endogenous opioid system has been implicated in alcohol addiction as an anti-reward system and is upregulated in response to mesolimbic dopamine activity (Bazov et al. 2013). The majority this research has concentrated on reward effects mediated via μ receptors, but more recently the dynorphin-kappa opioid receptor (KOR) system has been shown to also play an important role in excessive alcohol consumption. For example, the Bakalkin group from Sweden has shown that there is an upregulation of both dynorphin and kappa receptor transcripts in the dorsolateral PFC of alcoholics in the absence of changes in other components of the endogenous opioid system (Bazov et al. 2013). They have also shown that increased methylation of a 3' untranslated region single nucleotide polymorphism (SNP) in the promoter region of the prodynorphin gene (*PDYN*) is involved in alcohol dependence (Taqi et al. 2011). This epigenetic modification is postulated to facilitate transcriptional factor binding and increase *PDYN* transcription. Epigenetic effects are emerging as a major theme of genomic studies of ARBD and will be discussed further below.

RNA quality and transcriptomic studies

A major research theme of the NSW TRC is to improve our knowledge of how tissue quality indices affect downstream molecular studies (Sheedy et al. 2012). It was originally thought that postmortem factors such as postmortem interval (PMI) and handling time at room temperature were the major determinants of RNA quality. However, it is now clear that RNA integrity is more affected by premortem factors (Monoranu et al. 2009, Durrenberger et al. 2010). Low brain pH is a good indicator of a severe and prolonged agonal period and RIN is highly correlated with brain pH (Monoranu et al. 2009).

Brain pH and RIN decrease with the duration of the agonal period and the number of adverse, often hypoxic, events that occur in that period (Durrenberger et al. 2010). Hypoxia is associated with lactic acidosis and cellular damage and it appears that RNA degradation occurs due to the release and activation of acid lysosomal RNases (Barton et al. 1993).

Neurological disorders generally have a lower pH/RIN compared with controls (Monoranu et al. 2009). For example the NSW TRC relies heavily on sudden-death coronial cases for control tissue with myocardial infarction being a common cause of death. Research from the NSW TRC has shown that alcoholics have significantly reduced brain pH and RIN compared with controls (Sheedy et al. 2008, Sheedy et al. 2012).

Previous studies looking at mood disorders utilizing human post mortem tissue have shown expression patterns associated with low pH brain samples can exceed the effects of age, gender and disease (Li et al. 2004). Li et al. suggested that differential gene expression was not solely due to RNA degradation in low pH samples but rather represented a 'coordinated biological response' to terminal stress in the agonal period. We recently carried out a microarray study of white matter from alcoholics and controls and found that low RIN samples were associated with upregulation of genes involved in protein translation (Sutherland *et al.*, unpublished findings). Notwithstanding the common practice of excluding low RIN samples from studies, researchers need to be vigilant for residual RIN effects in their transcriptomic data.

Transcriptomics and ARBD

The earliest transcriptomic studies were carried out using focused cDNAs arrays incorporating genes of interest. As miniaturization technology improved, oligonucleotide or probe-based arrays replaced cDNA platforms and the density of features grew to incorporate

the whole genome or mRNA transcriptome (Sutherland and Kril 2012). In an interdependent relationship, array capacity has continued to grow with our increasing understanding of the complexity of the genome. Alternative splicing, alternative promoter use, anti-sense and non-coding RNA transcription, including microRNAs, is now known to be commonplace at most gene loci. This has not only led to the redefinition of the gene from a single protein coding entity to a 'mosaic' of biological information (Mattick et al. 2010) but a redefinition of the transcriptome. In parallel, the continual improvement in annotation of the human genome has meant that modern arrays are not only more comprehensive, but more accurate than their predecessors.

The pace of change in transcriptomic methodologies means it is useful to consider studies chronologically as some of the findings from earlier studies can be potentially redundant. This pace is about to be ratcheted up another notch with the introduction of next generation sequencing to transcriptomic analysis (RNA-Seq). RNA-Seq represents a disruptive technology for microarrays, as this digital format not only provides greater capacity and accuracy, but it can simultaneously assay all RNA species. Importantly RNA-Seq requires no prior knowledge of the genome for probe design, so hitherto unknown genomic features will be uncovered (Sutherland and Kril 2012). A major challenge will be to adapt our analytical strategies to match this genomic mosaic and move away from the gene-centric approach that dominates current practice (Ponomarev et al. 2012).

The Harris group, based at the University of Texas, Austin, carried out the first microarray study utilizing NSW TRC tissue in 2000 (Lewohl et al. 2000). This study employed a focused array interrogating around 5600 genes, pooled copy DNA (cDNA) samples and targeted the PFC. Their major finding was the down-regulation of myelin genes consistent with WM atrophy being the predominant pathology in ARDB. In particular the authors found that *MAG* (encoding myelin associated glycoprotein) was downregulated while the gene encoding α -synuclein (*SNCA*) was upregulated although the latter result could not be replicated in a subsequent RT-qPCR study (Janeczczek et al. 2012).

The Harris group then extended their studies to compare the PFC with, the motor cortex (Mayfield et al. 2002). Again, pooled cDNA samples were used along with a focused cDNA array. Mayfield *et al.* found that genes were more downregulated in the PFC of alcoholics with the greatest changes in expression in myelin and protein trafficking-related genes. However the direction of gene dysregulation was not necessarily the same as that seen by Lewohl *et al.* with *MAG*, for example, being upregulated here. In a follow up study using individual rather than pooled cDNA samples Liu *et al.* reported "a remarkable downregulation of genes in the ubiquitin-proteasome system in both brain regions" (Liu et al. 2004). While differentially expressed genes involved in myelination were again prominent the genes themselves and directions of change varied from the previous studies. Liu *et al.* noted that, in comparison to cancer gene lists being generated at the same time, their gene expression changes were relatively small. A further study by the Mayfield and Harris team, focused solely on the PFC, noted that only 10% of their 232 annotated genes had been reported previously (Liu et al. 2006). Of these 27 genes, 21 were regulated in the same direction including the down-regulated myelin-related gene, PMP22. Apart from myelin-related genes chronic alcoholism affects genes involved in immune responses, apoptosis, cell adhesion, and ubiquitin-mediated protein degradation. The observations of the Harris and Mayfield team, that few pathways were common to all previous studies and that the magnitude of differences was small were reiterated by the Wilce group from Queensland Australia (Flatscher-Bader et al. 2006).

ARBD also incorporates the changes in brain plasticity responsible for addiction. The nucleus accumbens is the major destination of mesocorticolimbic dopamine innervation, and

is considered the likely origin of dysfunction in many addictive behaviors including alcoholism. The major output for the NA is the ventral pallidum, which projects to the thalamus, and onto the PFC and striatum. The Wilce group compared the PFC and nucleus accumbens (NA) of chronic alcoholics and controls (Flatscher-Bader et al. 2005). In the PFC, Flatscher-Bader *et al.* found similar changes to previous studies but these were quite distinct to the pattern seen in the NA. The latter was characterized by a downregulation of genes encoding key proteins involved in vesicular transport (neurotransmission) and cellular architecture but not myelin production.

ARBD is a complex disorder with varying influences from nutritional deficiency, hepatic encephalopathy and co-abuse with other drugs, including nicotine. Smoking in particular is difficult to pare apart from the direct effects of alcohol, because approximately 80% of alcoholics are heavy smokers. This Wilce group considered the differential impact of smoking and alcohol on four 'alcohol-sensitive' genes in the PFC (Flatscher-Bader and Wilce 2006). Flatscher-Bader *et al.* found that smoking not only affected gene expression in the PFC but also was probably responsible for a previously described alcohol effect on *APOD* (encoding apolipoprotein D) (Lewohl et al. 2000, Mayfield et al. 2002, Flatscher-Bader et al. 2005).

Wilce and colleagues then turned their attention to the origin of the mesocorticolimbic reward pathway, the ventral tegmental area (VTA) (Flatscher-Bader et al. 2008). Using pathway analysis to summarize their list of differentially expressed genes, they showed that synaptic transmission and neuronal plasticity were the most dysregulated processes in the alcoholic VTA. In terms of specific genes, those encoding glutamate transporters were consistently different from controls. As smoking was also known to affect the VTA, Flatscher-Bader and colleagues extended their study using quantitative PCR and western blotting to examine these glutamate transporters in smoking and non-smoking alcoholics. Here alcohol co-abuse attenuated the effects of smoking on *SLC17A6* and *SLC17A7* suggesting nicotine exposure rather than alcohol was the major driver of increased glutaminergic signaling in the VTA.

In 2010 the Wilce group continued their interest in co-abuse of nicotine and alcohol by carrying out a second microarray analysis of the NA, the major destination of the VTA (Flatscher-Bader et al. 2010). The most significant pathway in the NA was "regulation of the actin skeleton". Genes in this pathway were elevated by both alcohol abuse and smoking. These changes were largely confirmed by RT-qPCR study but when the same genes were re-evaluated in the VTA, smoking co-abuse now had a dampening effect. Flatscher-Bader et al. concluded that alcohol's affect on cellular architecture is modulated by nicotine co-abuse in a region-specific manner.

As an aside, Goate and colleagues used NSW TRC tissue to genotype known SNPs in the nicotinic acetylcholine receptor $\alpha 5$ subunit (*CHRNA5*) gene cluster in smokers and non-smokers and quantify mRNA expression in their frontal cortex using RT-qPCR (Wang et al. 2009). They found that a diplotype consisting of a low-expressing variant in combination with a non-risk genotype of a missense variant significantly lowers the risk for both nicotine dependence and lung cancer. Incidentally there were no differences in mRNA levels between alcoholic and non-alcoholic subjects.

Kryger and Wilce also examined the amygdala, a structure that also receives dopaminergic input from the VTA but one that is also tightly regulated by powerful inhibitory circuits from the medial PFC (Kryger and Wilce 2010). The amygdala, along with the PFC, are the two areas known to suffer neuron loss in ARBD (Alvarez et al. 1989). In terms of neurotransmission, Kryger and Wilce reported down-regulation of the excitatory amino acid

transporters GLAST (*SLC1A3*) and GLT-1 (*SLC12*) that had been previously found to be upregulated in the VTA (Flatscher-Bader et al. 2008). Kryger and Wilce suggested that the down-regulation of these transporters would increase glutamate activity and predispose to excitotoxicity. Their findings again reinforce the region-specific response of chronic alcohol intoxication.

It has been known for sometime that that ARBD and particularly brain atrophy are more severe in alcoholics with cirrhosis compared with those without cirrhosis (Kril and Harper 1989). In comparison to their previous findings in non-cirrhotic alcoholics (Liu et al. 2006) the Mayfield group found a two-fold greater number of differentially expressed genes in the PFC of cirrhotic alcoholics (Liu et al. 2007). The upregulated pathways in cirrhotic alcoholics included cell adhesion and mitochondrial function, while apoptosis and cell proliferation pathways were downregulated.

The increased changes in the alcoholics with cirrhosis may reflect the impact of hepatic encephalopathy (HE), although not an obligatory, consequence of liver cirrhosis (Harper and Kril 1989). HE is a pathological diagnosis based on the number of swollen, Alzheimer type II astrocytes per high power field in the basal ganglia and cerebral cortex (Harris et al. 2008a). Recent work by the NSW TRC suggests that up to 20% of alcoholics have pathological evidence of HE (D. Sheedy et al., unpublished findings). One hypothesis for the pathogenesis of HE is that cerebral ammonia toxicity causes astrocyte swelling that triggers oxidative and nitrosative stress of both astrocytes and neurons (Haussinger and Gorg 2010). In particular glutamine synthetase (GS), the enzyme responsible for the formation of glutamine from glutamate and ammonia, is itself targeted by ammonia via protein tyrosine nitration. In 2010 Haussinger and colleagues confirmed the nitration of GS in HE alcoholic cerebral cortex along with its decreased activity and an overall increase in RNA oxidation (Gorg et al. 2010). It appeared that excessive ammonia and radical species combine in a vicious cycle to disturb the glutamate-glutamine cycle between astrocytes and neurons.

HE also results in a lactic acidosis presumably through the inhibition of the tricarboxylic acid cycle enzyme α -ketoglutarate with a consequential decrease in glucose utilization and increased lactate synthesis (Butterworth 2010). As might be expected, alcoholics with cirrhosis and a pathological diagnosis of HE alcoholics have a significantly lower brain pH and RIN than those without HE (Sheedy et al. 2012).

Transcriptomics studies in ARBD had revealed a generalized and subtle effect on biological pathways with myelin-related genes being one of the few pathways in common. Given the prominence of WM atrophy, we wondered whether confining our analysis to WM only might be more informative. Furthermore we were keen to explore the influence of HE on gene expression patterns, so we carried out a microarray study of WM in alcoholics with and without HE (Sutherland *et al.* unpublished findings). For each individual, we assayed, the PFC and motor cortex. Similar to our previous study (Sheedy et al. 2012) the HE alcoholics had a lower mean brain pH and RIN. In a RIN-matched sub-cohort we found few changes in the WM of non-HE alcoholics and no real differences between the prefrontal and motor cortices. Surprisingly, and in contrast to our initial hypothesis there were no differences in myelin-related genes or pathways. Our results remained consistent with the previous studies utilizing whole brain samples where alcohol-related signatures had been relatively subtle. This could reflect the myriad of effects alcohol has in the brain or, as is described immediately below, due to constraints of our analytical approaches.

In contrast the transcriptome of HE-alcoholics was characterized by the downregulation of energy metabolism pathways and the upregulation of interferon responsive genes. The downregulation of energy pathways is consistent with the metabolic acidosis seen in HE, a

feature it shares with hypoxia during the agonal period. The upregulation of genes responding to the pro-inflammatory interferons may be consistent the hypothesis that systemic inflammation combines with ammonia to cause HE (Shawcross and Jalan 2005).

Overall, low RIN, HE and smoking are three confounders that can potentially influence gene expression in ARBD to a greater extent than alcohol itself. This highlights the importance of obtaining tissue with the maximal amount of clinicopathological data so well informed exclusion criteria can be applied. However our experience is that residual effects of these potential confounders should still be explored in post-hoc analyses.

As discussed in the introduction to this section, the genomic age has brought with it a continual improvement in detection reliability and gene annotation on transcriptomic platforms. However these improvements have also shown, and are continuing to reveal, just how complex gene expression is in the human brain. To date most transcriptomic analyses have utilized gene-centric approaches but the Mayfield group have recently suggested that transcriptomic analytical approaches need to adapt to the reality of gene co-expression in order to maximize discovery potential (Ponomarev et al. 2012). Ponomarev *et al.* used a network-based analysis approach to reveal that ARBD was characterized by epigenetic changes to chromatin and global DNA hypomethylation. Alcohol appeared to precipitate the latter by decreasing folate levels and thus *S*-adenosyl methionine (SAM), the substrate used for DNA methylation. Their most unexpected finding was that gene GC content could partially determine the patterns of expression and regulation by chronic alcohol exposure. Ponomarev *et al.* suggested that transcription factors and other DNA-binding proteins that are part of chromatin modification complexes preferentially bind to GC- or AT-rich motifs.

However in contrast to Ponomarev *et al.*, a second study found no differences in methylation patterns in the PFC of alcoholics and neurologically normal controls (Manzardo et al. 2012). Manzardo *et al.* employed the Roche NimbleGen platform that uses methylation-specific immunoprecipitation in combination with hybridization of input DNA to an oligonucleotide tiling array. Notwithstanding the alternative platforms used in the two ARBD studies it is not clear why these studies reached different conclusions.

Along with epigenetics the other major change in transcriptomics has been the increasing awareness of the importance of non-coding RNA species including micro (mi) RNAs. miRNAs bind to the 3' untranslated regions of mature mRNAs and can either suppress their expression or target them for degradation. Their inherent promiscuity means they are capable of achieving extensive combinatorial targeting and the ability to modulate pathways rather than just individual genes. Lewohl and colleagues looked at miRNA in PFC of 27 individual human cases found that 35 miRNAs were upregulated (Lewohl et al. 2011). Furthermore their putative targets coincided with the downregulated mRNAs from cDNA microarray data they had previously obtained (Liu et al. 2006). A pathway analysis showed the most overrepresented pathways were fatty acid and lipid biosynthesis along with ubiquination and cell death. Specific miRNAs included those targeting the myelin-related gene *PLP1*. In a review of this area Nunez and Mayfield discussed the potential roles of miRNAs in ARBD including their modulation of TLR (NF- κ B) signaling and synaptic plasticity and their reciprocal regulation of epigenetic factors (Nunez and Mayfield 2012).

In 2012 Kryger and colleagues reported the upregulation of a long non-coding RNA, called MALAT-1 in the cerebellum, hippocampus and brainstem of alcoholics although not in the prefrontal or motor cortices (Kryger et al. 2012). Kryger *et al.* suggested that increased MALAT-1 expression could account for changes in NMDA receptor subunits seen in chronic alcohols. It is almost certain that many more ARBD-related non-coding RNAs will

be discovered in the near future as next generation sequencing (RNA-Seq) replaces microarrays as the major transcriptomic platform.

Neurogenesis and ARBD

Although not related to genomics per se, the discovery of adult neurogenesis in the human brain in 1998 (Eriksson et al. 1998) coincided with the human genome project and now represents a major research theme in neuroscience and brain diseases. Animal models of both binge and chronic alcohol consumption have shown a decrease in hippocampal (Nixon and Crews 2002) and SVZ (Hansson et al. 2010) neurogenesis and it has been suggested that if such decreases are replicated in humans, then this might explain symptoms such as depression and deficits in learning and memory (Nixon 2006) and smell sensation (hyposmia) (Hansson et al. 2010).

A number of human studies including those using NSW TRC tissue would appear to make this scenario unlikely. Firstly, a hippocampal type memory deficit (encoding/retrieval) is not seen in chronic alcoholics. Rather, there is a working memory deficit that has been attributed to pathology in the dorsolateral PFC (Pfefferbaum et al. 2001). Second there is no correlation between hippocampal atrophy and impaired memory function in chronic alcoholics (Sullivan et al. 1995). Third, there is no loss of hippocampal neurons in either amnesic or non-amnesic alcoholics (Harding et al. 1997). Hippocampal atrophy is largely due to WM loss and is proportional to that seen over the entire brain. Fourth, the few studies that have investigated neurogenesis in the SGZ of normal aged humans suggest that new neurons are extremely sparse (Low et al. 2011). Low et al. have also shown that, in the same individuals, proliferation in the SVZ is 700x greater in comparison.

We investigated whether there were any differences proliferative events in SVZ and the olfactory bulb between chronic alcoholics and neurologically normal controls using the endogenous marker, proliferative cell nuclear antigen (Sutherland et al. 2013). Our findings suggest that chronic alcohol consumption does not affect proliferation in the SVZ or olfactory bulb of adult-born neurons in the human olfactory system. Neurogenic deficits are therefore unlikely to contribute to hyposmia or cognitive deficits in chronic alcoholics.

Summary and future challenges

Postmortem brain tissue is an important and precious resource. It not only provides a research model in its own right but it allows benchmarking of findings from animal and cell culture models. Since the first review of research on chronic alcoholism using NSW TRC-derived tissue (Harper et al. 2003) there has been a burgeoning number of -omic studies on ARBD. The transcriptomic studies in particular have continually shown that the direct effects of alcohol are paradoxically subtle. This is likely to reflect the myriad of effects that alcohol has on the brain, but also the complex pathogenesis of ARBD with potential contributions from clinical or subclinical effects of liver disease, thiamine deficiency and co-abuse of neuroactive substances such as nicotine. A major research challenge for future studies will be to continue to pare these influences apart. This work will not only rely on novel technological platforms such as RNA-Seq and more 'holistic' approaches to data analysis, but on increased numbers of carefully characterized clinical cases to facilitate sufficiently powered analyses. The NSW TRC hopes to facilitate exactly this type of research over the next 20 years.

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