Medical Hypotheses 116 (2018) 10–21

Contents lists available at ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Pathways from epigenomics and glycobiology towards novel biomarkers of addiction and its radical cure

Albert Stuart Reece[a,b,*], Wei Wang[b], Gary Kenneth Hulse[a,b]

[a] Division of Psychiatry, University of Western Australia, Crawley, Western Australia 6009, Australia
[b] School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, 6027, Australia

ARTICLE INFO

Keywords:
Epigenomics
Biomarker
Opioid
Glycobiology
Single cell assays
Aging

ABSTRACT

The recent demonstration that addiction-relevant neuronal ensembles defined by known master transcription factors and their connectome is networked throughout mesocorticolimbic reward circuits and resonates harmonically at known frequencies implies that single-cell pan-omics techniques can improve our understanding of Substance Use Disorders (SUD's). Application of machine learning algorithms to such data could find diagnostic utility as biomarkers both to define the presence of the disorder and to quantitate its severity and find myriad applications in a developmental pipeline towards therapeutics and cure. Recent epigenomic studies have uncovered a wealth of clinically important data relating to synapse-nucleus signalling, memory storage, lineage-fate determination and cellular control and are contributing greatly to our understanding of all SUD's. Epigenetics interacts extensively with glycobiology. Glycans decorate DNA, RNA and many circulating critical proteins particularly immunoglobulins. Glycosylation is emerging as a major information-laden post-translational protein modification with documented application for biomarker development. The integration of these two emerging cutting-edge technologies provides a powerful and fertile algorithmic-bioinformatic space for the development both of SUD biomarkers and novel cutting edge therapeutics. Hypotheses: These lines of evidence provide fertile ground for hypotheses relating to both diagnosis and treatment. They suggest that biomarkers derived from epigenomics complemented by glycobiology may potentially provide a bedside diagnostic tool which could be developed into a clinically useful biomarker to gauge both the presence and the severity of SUD's. Moreover they suggest that modern information-based therapeutics acting on the epigenome, via RNA interference or by DNA antisense oligonucleotides may provide a novel 21st century therapeutic development pipeline towards the radical cure of addictive disorders. Such techniques could be focussed and potentiated by neurotrophic vectors or the application of interfering electric or magnetic fields deep in the medial temporal lobes of the brain.

Introduction

Opioid Use Disorder (**OUD, see glossary**) is a classical scourge of human health and has presently reached high community prevalence in both developing [1] and industrialized nations [2]. Computational formulation of a biomarker for opioid dependency would be useful for diagnosis and staging of the severity of the disorder, treatment selection, treatment comparison and for monitoring progress on treatment.

Central to the question of peripheral biomarker development is the non-trivial issue of the metric against which it is to be standardized. From the myriad mechanistic papers on OUD it appears that there are some central brain mechanisms involved which are then reflected in other more peripheral phenomena that manifest systemically by both direct and indirect routes. It seems appropriate in this paper to give some brief consideration to the central key mechanisms including the neuronal ensembles and their connectomes to provide a context within which to place discussion of the peripheral biomarkers which may be secondarily derived.

Reflecting our major current research interests our group has considered the potential of emerging epigenetic and glycobiological techniques for application and further assessment in OUD. This will therefore form the substance of the present discussion. Not only is there significant cross-talk between epigenomic and glycobiological regulatory systems but both areas also touch on other fields such as the immunostimulation of **substance use disorders (SUD's)** [3] and the endocrinopathy – including sex differences – so that these subjects are mentioned *en passant* albeit in such a way so as not to distract from the main thread of discussion. This broad approach is contributory to the

* Corresponding author at: 39 Gladstone Rd., Highgate Hill, Brisbane, Queensland, Australia.
E-mail address: sreece@bigpond.net.au (A.S. Reece).

https://doi.org/10.1016/j.mehy.2018.04.011
Received 16 September 2017; Accepted 11 April 2018

main discussion as a useful biomarker should have some predictive relationship with the diverse and disparate systemic phenomenology of OUD [4–6].

Importantly many of the central changes are reflected in the blood [7–9]. Hence the new findings on OUD suggest that some central changes are reflected in peripheral phenomena. Moreover since both epigenomics [9,10] and glycobiology [11–14] have been used to derive highly predictive clinical biomarker indices for complex disorders our hypothesis is that their combination should provide enhanced power for discrimination of the presence or absence of OUD-SUD and for severity ascertainment.

Neuronal ensembles

Classical authors including Donald Hebb had suggested that memories were likely to be encoded by a sparse and diffusely located network of neurons which were called cell assemblies [15] and are now usually known as neuronal ensembles. This is of relevance to addiction because clinical SUD syndromes are often described as subversions of normal reward and memory processes [16]. Complementing this work on memory and motivation in general an elegant series of studies has been conducted by the National Institute of Drug Abuse Intramural Research Program in recent years using optogenetic and stereotactic techniques in transgenic rats demonstrating that drug dependency syndromes related to nicotine [17], alcohol [18], amphetamines [19] cocaine [20], food [21] and opioids [22] are related to neuronal ensembles distributed across the ventral prefrontal cortex, the hippocampus, the basolateral amygdala, Nucleus Accumbens (**NAc**) and ventral tegmental area (**VTA**). Only about 1% by volume of the number of neurons in each area is involved in forming the neuronal ensemble. Neurons are believed to become incorporated into the ensemble based on receiving the most active input [23] albeit this is an issue of ongoing discussion and enquiry. Rodent neurons engaged in the neuronal ensemble (Fig. 1) are marked both by master transcription factors (**TF's**) [24] and by the activation of immediate early genes of which the most notable is ***cFos*** (gene) and its protein product the TF fos and the products of its various mRNA splice variants [23]. Neurons can be involved in multiple ensembles in which they partner with different networks of cells [23].

Importantly interruption of these neuronal ensembles has been shown to abrogate rodent behavioural states relevant to addictive behaviour involving both cocaine and opioids [22,25–27]. Interdiction of addiction-relevant behaviours has been achieved by inactivation of the – very few – hippocampal cells concerned [22], their VTA [27] or amygdala [25] counterparts, or re-allocation of hippocampal place cells to erroneously confound a previously naturally encoded drug-place preference in rats [26]. Moreover silencing of neurones in the rat orbitofrontal cortex has been shown to interrupt both context-induced relapse to heroin [22] and the incubation of heroin craving [28].

Neuronal connectomics

Activity dependent (**Hebbian**) changes at the synapse have been described to endorse the finding that *"cells that wire together fire together"* in many species both vertebrate and invertebrate [15,29]. Long-term synaptic potentiation and depression in various forms has been shown to be a key organic substrate of rodent memory [30,31]. Given that activity dependent processes in the synapse have been shown to control plasticity it would follow that there must be a coordination between the nucleus and the machinery of the synapse to make the changes long-lasting [29]. This key coordination is thought to be controlled in the nucleus epigenetically [29] by mechanisms which are still being explored.

Since neurons have a refractory period after action potential firing, and frequently receive inhibition, their mutual connections naturally engender oscillations in neuronal networks which occur at certain

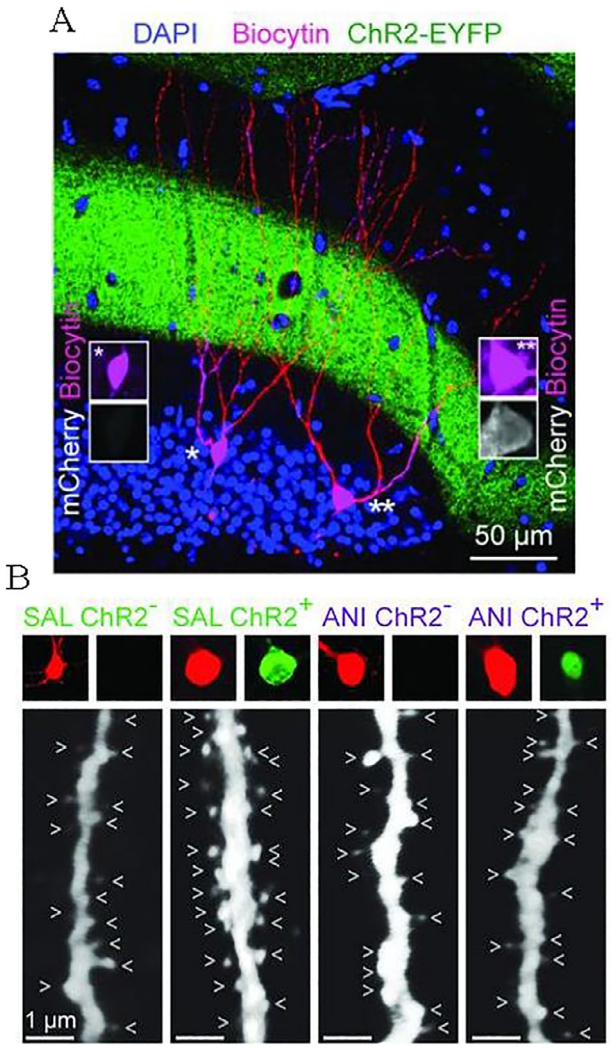

Fig. 1. Identifying Hippocampal Engram cells and their (B) synaptic connectivity. Sal – Saline; ChR2 – Channelrhodopsin; Ani – Anisomycin. From: Ryan TJ, Memory. "Engram Cells Retain Memory under Retrograde Amnesia." Science 2015; 348 (6238): 1007–1013. Used by Permission.

defined frequencies over a wide dynamic range in many species [32]. Gamma (25–100 Hz) and theta (4–10 Hz) waves have been shown to be particularly important [33]. Moreover significant theta-gamma modulation occurs such that the phase interaction (or interference) of the two waves carries information and has been linked to movement initiation and percept [34] and memory formation [35,36] in many mammalian species including rodents and primates. These pre-clinical findings have also been validated in the human: neocortex [37], medial prefrontal cortex (**mPFC**) [38], temporal cortex [33], somatosensory cortex [39], cingulate cortex [40], occipital cortex [41], **nucleus accumbens** [42], amygdala [43], insula [39] and hippocampus [44] many of which are components of the mesolimbic reward circuitry.

These earlier studies were elegantly combined in a recent paper studying affiliative bonding in monogamous prairie voles [45]. These workers showed that the theta-gamma modulation of the circuit between the mPFC and the NAc controlled female affiliative behaviour with dramatic and sudden slowing of the theta (5–6 Hz)-gamma (80–84 Hz) coupling during and after mating. Moreover larger increases in net theta-gamma modulation caused faster displays of affiliative behaviour. This slowed mPFC-NAc activity persisted after mating and was predictive of social behaviors. mPFC-generated oscillatory synaptic plasticity altered NAc-based partner responsiveness which had previously been shown in this species to be controlled by epigenomic

mechanisms through oxytocin and prolactin signalling [46]. Although this work has not been replicated in the SUD field to our knowledge, this detailed dissection of reward neurophysiology carries obvious significant implications for understanding, studying, modulating and potentially one day treating reward related disorders.

Epigenomics

The recent explosion of studies on the epigenetics of SUD's has yielded many insights into what were previously poorly understood pathophysiological mechanisms. This work has been done mainly in rats with limited validation in human post-mortem brain tissue [47–49]. Cytosine methylation at **CpG** dinucleotides usually suppresses DNA transcription. Histone acetylation negates the positive charge on the ε-lysine tails rupturing the hydrogen bonds with DNA and moving them away from the double helix, opening up the double helix and effectively making genes available to the transcription machinery [8]. Multiple pathways exist coordinating DNA methylation and histone post-translational modifications [50–53].

It is widely believed that the epigenome is a key locus of gene: environment interactions [9] in many SUD's [54,55]. Cell-wide changes in (**non-Hebbian**) excitability have been shown to be controlled epigenomically [29] by several mechanisms. Synaptic insertion of **AMPA**, **NMDA** and **metabotropic** glutamate receptors in the rat NAc is controlled epigenomically after chronic morphine correlating closely with anxiety-like behaviours [56]. Epigenomic mechanisms account for heightened neuronal excitability in the rat VTA exposed to chronic opioids mediated via by **AMPAR's**, **GABAAR's** (**GABA** A receptors) and potassium channels [57]. Epigenomically reduced rat VTA μ-opioid receptors and GABAAR's cause increased tolerance to both opioids and benzodiazepines characteristic of clinical OUD [57]. This tolerance was also related to epigenomically-regulated mechanistic target of rapamycin (mTOR) -mediated reduction in the size of VTA neuronal somas [48]. NAc spinogenesis after chronic morphine and cocaine is controlled epigenomically [47].

These findings have prompted several interventional studies in rats. Histone deacetylase administration attenuates morphine withdrawal [49] and modulates behavioural and reward responses to morphine [58,59]. Addiction-relevant behaviours can be modified by artificial manipulation of NAc histone post-translational modifications and chromosomal state (particularly histone 3 lysine 9 dimethylation (**H3K9me2**)) using bioengineered transcription factors targeted to the *FosB* promoter in a rodent model of chronic cocaine exposure [51].

For example dramatic downregulation of **H3K9me2** in the rodent NAc occurs at splice sites in *FosB* gene at exon-4 increasing transcription of **ΔFosB** which is an unusually long-acting transcription factor with a half-life of around 12 days as it is phosphorylated and lacks the 101 amino acid C-terminal degron [7,56]. DNA methylation has also been shown to control rat memory formation [53,60] and inhibition of the DNA methyltransferases (**DNMT**) **DNMT1** and **DNMT3a** reduces neuronal membrane excitability through reduction in potassium channels in cultured rat neurons [61]. Cortical DNA methylation has been proposed as a biomarker of rat hippocampal memory [60].

As an example relevant to clinical addiction a useful predictive whole blood biomarker of current alcohol consumption was derived from the DNA methylation state of 144 genes amongst Europeans and 165 genes amongst African-ancestry cohorts with many epigenome wide association study hits occurring in **CpG** island-shores and enhancers [9]. Methylcytosine can be oxidized to **hydroxymethylcytosine**, formylcytosine [62] and carboxycytosine by the serial action of the ten-eleven translocase (**TET**) 1–3 DNA oxidizing enzymes and others, which has been shown to be important for brain function and memory formation [63,64], anxiety [65] and cocaine action [66]. Rat potassium channel upregulation and its accompanying heightened membrane excitability is TET-3 dependent [61].

RNA forms a further layer of fascinating and complex regulation within the mechanisms of cellular information control. Cellular RNA's occur in both long and short forms. As RNA can bind to itself, it often folds over and can perform catalytic functions on proteins and other RNA's. RNA's routinely undergo both post-transcriptional splicing in large spliceosome machines [67] and post-transcriptional modification using 111 modifications most of which are believed to carry information [68,69]. Long non-coding RNA's can be expressed from enhancers [70] and can form regulatory scaffolds governing the transcriptional availability of long DNA segments including formation of hybrid DNA-RNA triplex scaffolds [70–72], arrange nuclear architecture and coordinate chromosomal silencing [73].

An epigenomic age biomarker-algorithm has been used to study numerous neurological and other conditions relevant to SUD in humans including stress and PTSD [74], neurodegenerative disease [75], HIV dementia [76], Parkinson's disease [77], Huntingdon's disease [78], Alzheimer's disease [75,76,79] alcoholism [80,81], insomnia [82] and menopause [83].

BOX 1

Immune Activation.

Immune activation is a hallmark of addictive disorders. This is induced by several mechanisms including addictive drugs themselves [3] and the use of non-sterile injecting equipment and has been seen in both virally infected and non-infected patients [91,92,137,138]. Importantly immune activation is also a major cause of ageing processes [139] and immunosenescence [140,141] and its related "inflamm-aging" [142] is also a major peripheral biomarker of it [140,141]. The polyclonal gammopathy of SUD [92,137,143] and its related changes likely reproduces immunosenescence [4,5,137,144,145]. This peripheral immune activation is shown in Fig. 2 redrawn and updated from [92,137,145] which compares the globulin fraction of human plasma, which is largely composed of immunoglobulins, in opioid dependent and control patients over both age and time (see Supplementary data for statistical analysis). Importantly NF-κB is the master TF of the immune system in many tissues [146] and is also involved in sculpting brain networks and synaptic and dendritic pruning [147,148], and is importantly involved in cell survival/death and stem cell decisions [146]. Interestingly there are now several reports of the direct involvement of key epigenomic regulators (ten-eleven translocation methylcytosine dioxygenase (TET2) and DNMT3a) in the modulation of immune response [149–151].

The immunostimulatory aspects of drug dependency syndromes [3] assumes further importance in view of the interaction of glycosylation processes with the immune system and particularly circulating immunoglobulins, especially with regard to their valence determination.

Recent research has revealed intimate interaction at numerous points between neuronal activity and immune regulation for not only do neurons have many cytokine and immune modulator receptors but so too immunocytes carry high density receptors for neurotransmitters including opioids and dopamine [152]. This arrangement sets up neuroimmune reflexes with vagal control of systemic immune resolution including peritoneal antibacterial activity, and splenic nerve control of systemic inflammatory tone in rheumatoid arthritis, metabolic syndrome, essential hypertension, Crohn's disease and immunosuppression accompanying spinal cord trauma [152].

Interestingly it was the recently demonstrated that the DNA methylation status of GABA receptor genes was associated with the activity state of the promoters of immune

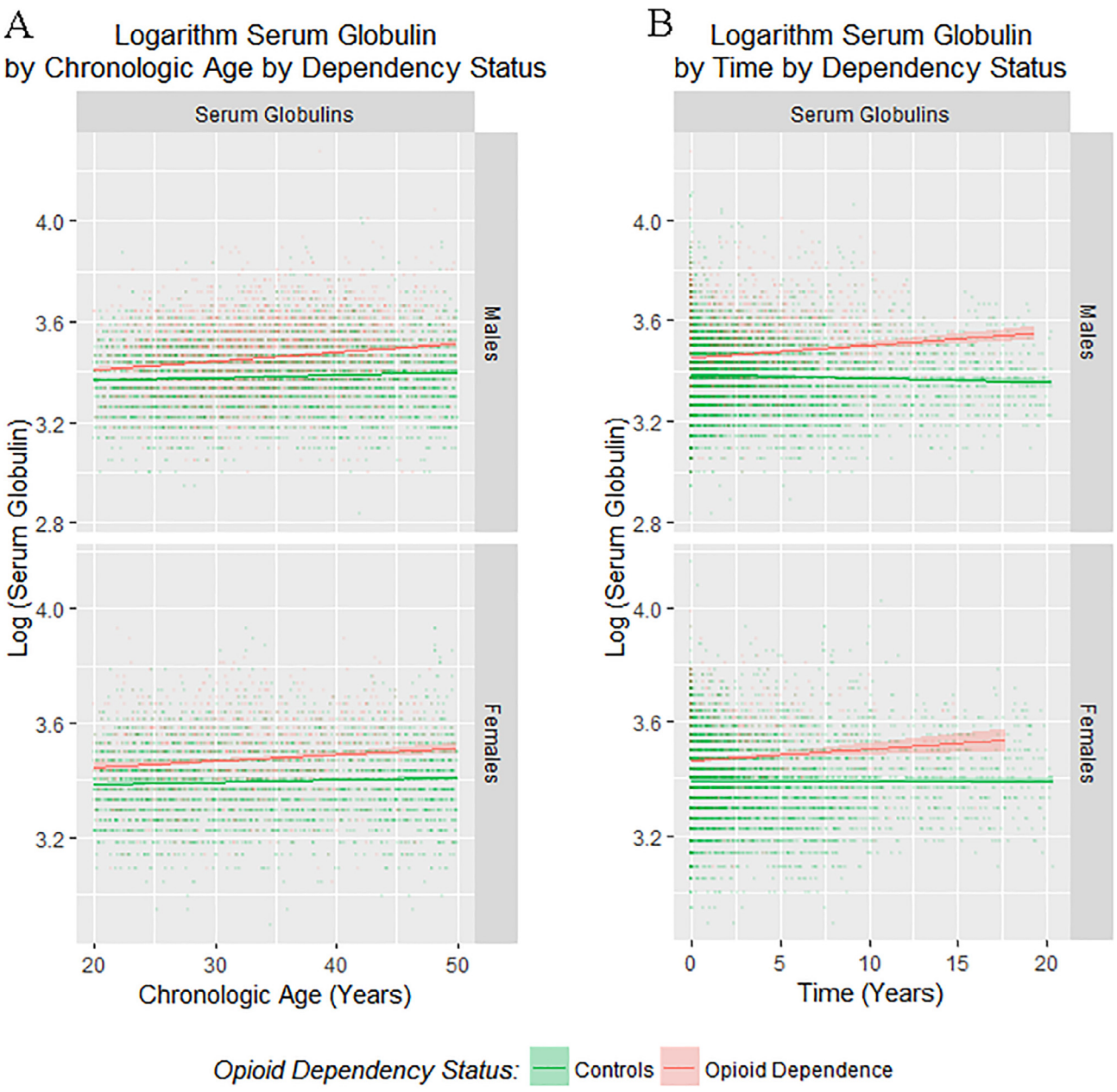

Fig. 2. Variation in Serum Globulins with (A) Chronological Age and (B) Time in opioid dependent and control patient groups.

response genes in circulating monocytes in a manner predictive of clinical alcohol use [9]. This finding relates the epigenetic regulation of synaptic activity to a well characterized and readily accessible major peripheral pathophysiological process – immunoactivation. These important findings suggest that peripheral epigenomic biomarkers are reflective of central neuroinflammatory processes by several pathways.

In conclusion we feel that it is likely that an unbiased hypothesis free approach to screening of peripheral epigenomic markers will likely identify genes involved in immune pathways, and immune-related genes such as the GABA genes previously implicated in and reflective of alcoholism [9] may demonstrate useful discriminative value in defining a peripheral biomarker in OUD/SUD. Similarly we feel that glycomic changes identified in previous studies in disorders characterized by immune activation [153–155] may have some overlap with drug dependency syndromes.

Glycosylation

For over a century OUD has been linked with hyperglycaemia and there are also many points of interaction between glycobiology and epigenomics. Hyperglycaemia is both more common [84–87] and more severe [88] in OUD, a point well recognized by Claude Bernard in 1877 working in dogs [85]. Mechanisms validated in man include physical inactivity [88], stress hormone signalling [87,89], hypothalamically mediated preference for fatty and sweet foods [88,90], the proinflammatory milieu both centrally [3] and systemically [91,92], a likely pro-senescent state (since atherosclerosis [93,94] and diabetes [85,88] share common genome wide association study (GWAS) hits at the senescence locus on chromosome 9q21.3 [95]), acute cerebral hypoxia [96] and an inhibition of insulin receptor substrate signalling both in mouse VTA neurons [97] and mouse pancreatic islets [98].

This is relevant pathophysiologically because more than half of all proteins are glycosylated including cytokines and their receptors and

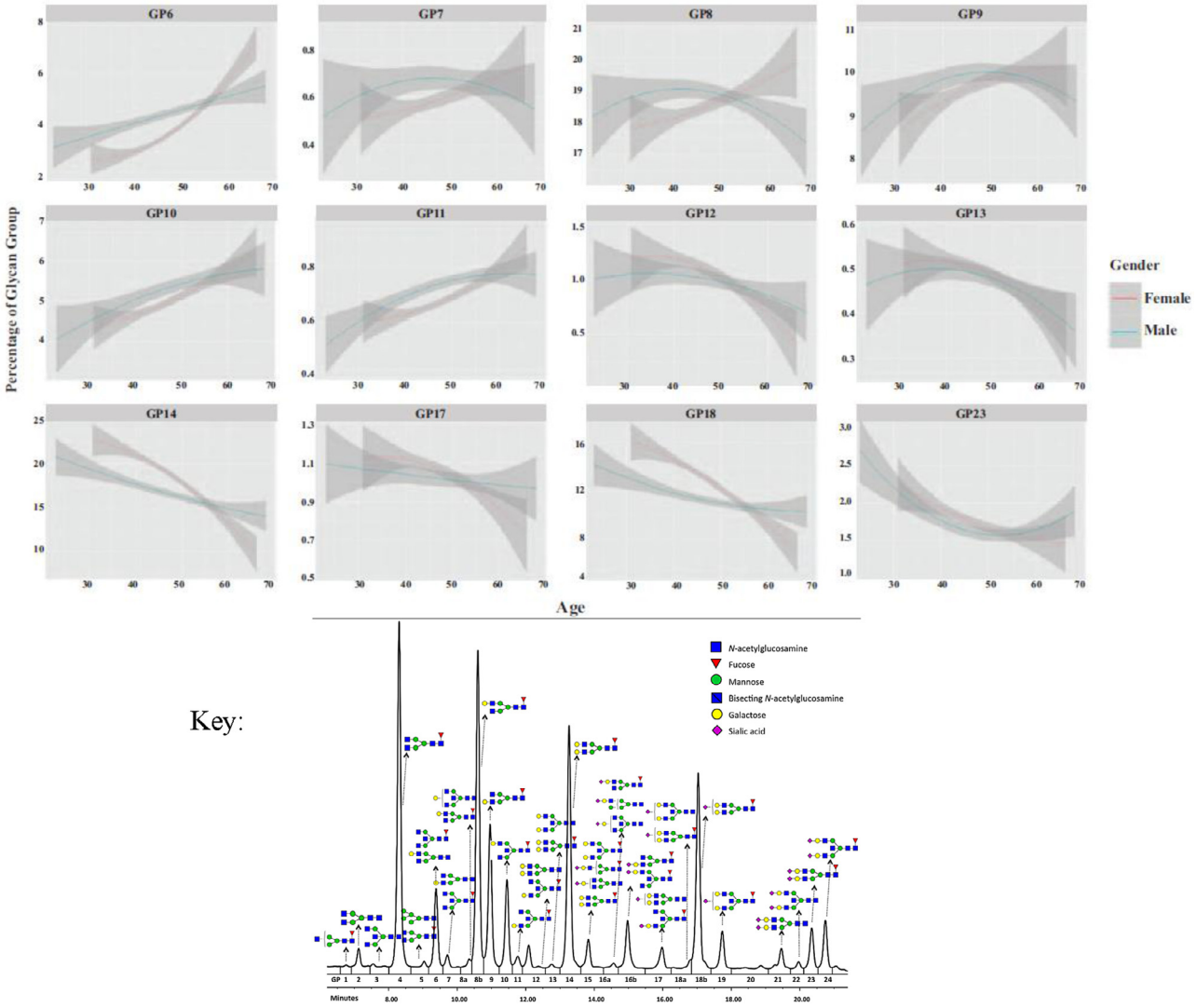

Fig. 3. Variation in Various Circulating Plasma Glycans by Age and Sex. From: Yu X et al. "Profiling IgG N-glycans as potential biomarker of chronological and biological ages. A community based study in a Han Chinese population." Medicine (2016); 95 (28): e4112-e4122. Used by Permission. Key taken from: Lauc G. et al. (2013), Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. Plos Genetics 2013 9 (1) e1008225. Used by Permission.

neurotransmitter receptors [99] which may occur either passively and non-enzymatically, or mediated by dedicated enzyme control systems for glycan attachment and removal. O-Glucose-N-Acetyl transferase (OGT) is one of the major glycosyl transferase enzymes and has been shown to be essential for embryonic survival in mice [100]. Glycosylation is often reciprocal with phosphorylation [100]. Glycosylation is the most complex post-translational modification. There are 120 sugars in the biological glycan "alphabet" and these often form long and branched polymers and may bind in multiplex [101,102]. Since sugar chains are often long and highly charged, they can change all the biophysical and chemical properties of proteins including their charge, size, solubility, binding affinities, and polarity (stimulatory to inhibitory), protein turnover, calcium handling and transcription [99]. Unlike simpler post-translational modifications such as acetylation, methylation and phosphorylation, the enormous diversity and inherent complexity of glycosylation reactions makes available to biological systems nuanced and graded signalling subtlety [99]. Glycans also bind to DNA and RNA [103,104] and control stem cell proliferation and migration [105,106] via transmembrane receptors [105] and are implicated in aging [103]. Extracellular glycans can signal to immune genes via the epigenome [107]. Gene transcription is downregulated by interaction of the epigenomic (including sirtuin-histone deacetylase and Polycomb Repressive Complex, PRC) repressive and glycosylation machinery, and indeed OGT has been noted to be part of the PRC [100,108].

Some of the key proteins which are glycosylated and downregulated include mTOR [109] which coordinates protein synthesis and cell growth; Mediator kinase complex which transduces signals from promoter and enhancer TF binding to RNA polymerase II; RNA polymerase II [100,110] which transcribes DNA; and all the histone proteins [111] which form the core of the nucleosomes around which DNA is wound.

BOX 2

Feminine Hormonal Factors.

> The lateral hypothalamus is involved in morphine reward mediation [90,156,157] and its neurohormonal output provides a useful readout on limbic system function albeit contingent upon multiple peripheral feedback loops [4,158]. The lateral hypothalamus also coordinates female hormonal cycles in all mammals including humans [139,158]. Gonadotrophin releasing hormone has been implicated in lifespan regulation

in the mouse [159]; and FSH has been shown to control mouse oocyte viability [160] which is also known to determine rodent lifespan [161,162]. FSH has also been shown to have metabolic activity and rodent control central visceral fat mass [163]. During a woman's reproductive years the LH exceeds the FSH and this ratio then reverses in the post-menopausal period implying that the cross-over point is a key measure of the premenopause. This suggests that the 58% reduction in the fecund period defined by this ratio in clinical OUD has far reaching implications [158]. These data are consistent with the heightened human female sensitivity to OUD noted by several authors [7,93,158].

There are also significant interactions between gonadotrophic hormone regulation and glycosylation pathways. LH, FSH and their receptors are all glycosylated and down regulated in women [164]. Plasma glycans in women have also been found to change dramatically with both the menstrual cycle [165] and with age with significant changes occurring across the menopausal period in human females [11].

In conclusion we therefore predict that unbiased investigation of the circulating glycomic profile of OUD/SUD patients and in particular of their immunoglobulin G subfractions will reflect in some measure age- and menopausal-specific changes identified in earlier studies [11,116]. Moreover it would appear likely that specific investigation of the glycosylation states of key female hormones such as LH, FSH, estradiol, progesterone and their respective receptors in circulating leukocytes, may yield further diagnostic insights and increase the discriminative power of peripheral biomarkers on ROC analysis.

Our hypothesis that plasma glycans may be useful biomarkers for OUD draws support from other literatures. For example as suggested in Fig. 3 plasma glycans have been shown to vary with human age, they have been used for development of a biomarker of ageing and important sex differences have been noted [11]. Plasma glycan profiles have also been used clinically to develop a biomarker for Parkinson's disease where four glycans were selected to derive a biomarker profile with an 87% sensitivity and 92% specificity for the presence of Parkinsons disease [13].

Important glycosylation changes on plasma immunoglobulins have also been shown with inflammatory diseases. Circulating immunoglobulins of the G class (IgG) are usually glycosylated in various ways (Fig. 4) forming over 30 glycovariants which may make them either proinflammatory or immunosuppressive [112]. Principal component analysis has been used in biomarker development to separate systemic lupus erythematosus patients from controls where a receiver operator analysis coefficient of 0.842 was achieved [113].

The majority of our biological processes rely on *N*-glycosylation of human proteins. *N*-glycans affect protein structure and function, and glycosylation events are known to alter with environmental changes, age and disease [99]. Variations in *N*-glycosylation of the IgG complex have adverse downstream effects on inflammatory pathways known to be associated with ageing and chronic disease aetiopathogenesis. IgG *N*-glycan traits are only partly determined by genetics and so represent signatures of joint genetic predisposition and environmental influences across the life-course on overall immune function and wellbeing [99]. The early detection and intervention of disease processes have become increasingly important to prevent life-long complications associated with SUD's, chronic diseases and the subsequent burden on global health. Studies have shown that selective combinations of IgG *N*-glycan structures associate with biological hallmarks of pre-chronic disease states when biological age exceeds chronological age [11,114]. Studies have also verified the validity of these IgG *N*-glycan combinations as predictive risk profiles and biomarkers of biological ageing in several human population cohorts of European [114], Chinese [11] and African descent [113].

The first GWAS of the human *N*-glycome combined high-throughput protein glycosylation analysis with GWAS in a mixed European population cohort study of almost 3000 Croatian and Scottish adults [115]. This performed the most comprehensive association analysis of common genetic variants with typical glycan profiles and identified that these genetic variants adversely influence the relative proportions of different types of *N*-glycans on human plasma proteins and thereby a course of events leading to metabolic and inflammatory disorders [113,115]. In particular, common variants of Hepatocyte Nuclear Factor 1α (HNF1α) regulate the expression of key fucosyltransferase and fucose biosynthesis genes, both master regulators of plasma protein fucosylation influencing *N*-glycan levels in human plasma. These findings characterised nine genetic loci associated with specific IgG *N*-glycan patterns. These replicated and validated findings further support the notion that genetic mutations and consequent alterations in the molecular and enzymatic processes of glycosylation can change glycoprotein function, triggering inflammatory or autoimmune conditions. The central role of HNF1α in the regulation of multiple genes involved in fucosylation may be the molecular mechanism behind the reported association between common variants of HNF1α and inflammatory markers, such as C-Reactive Protein, and conditions in which inflammation plays a key pathogenic role, such as ageing and ageing-related chronic diseases including autoimmune diseases (arthritis and systemic lupus erythematosus), haematological cancers, coronary artery disease, hypertension and metabolic syndrome [12,14,113–118]. These data are summarized in Fig. 5 depicting the utility of composite glycan-derived clinical biomarkers for rheumatoid arthritis, systemic lupus erythematosus and Parkinsons disease as receiver-operator curves.

For drug abuse syndromes, it is essential that an accurate diagnosis is obtained and disease progression can be monitored. Immunoglobulin G (IgG) has the ability to exert both anti-inflammatory and pro-inflammatory effects, and the N-glycosylation of the fragment crystallizable portion of IgG is involved in this process. Although there is no yet a population-based study on profiling of IgG glycan among drug abuse cases, our study on whether the IgG glycome could be a candidate biomarker for neurodegenerative disease, i.e., Parkinson's disease, could be a good example on such application. Ninety-four community-based individuals with Parkinson's disease and a sex-, age- and ethnically-matched cohort of 102 individuals with mixed phenotypes, representative of a "normally" aged Caucasian controls, were investigated [13]. Plasma IgG glycans were analysed by ultra-performance liquid chromatography. Overall, seven glycan peaks and 11 derived traits had statistically significant differences ($P < 8.06 \times 10^{-4}$) between Parkinson's disease cases and healthy controls. Out of the seven significantly different glycan peaks, four were selected by Akaike's Information Criterion to be included in the logistic regression model, with a sensitivity of 87.2% and a specificity of 92.2% [13]. The study suggested that there is a reduced capacity for the IgG to inhibit Fcγ-RIIIa binding, which would allow an increased ability for the IgG to cause antibody-dependent cell cytotoxicity and a possible state of low-grade inflammation in individuals with Parkinson's disease. The peripheral IgG glycome profile changes in PD patients infer an increased pro-inflammatory capacity. Although it is yet to be determined whether the immunoglobulins in blood plasma correlate with those in cerebrospinal fluid, it is evident that the study of systemic inflammation in PD may provide important information about the neurodegenerative process for other neurodegenerative disease syndromes such as drug abuse and provides potential as biomarkers from the perspectives of preventive, predictive and personalized medicine. In this way it seems likely that glycomic parameters will comprehend the endocrinopathy, pro-aging and pro-inflammatory aspects of OUD/SUD's and significantly enhance the predictive power of epigenomic peripheral biomarkers.

It is interesting to consider the routes by which central changes of

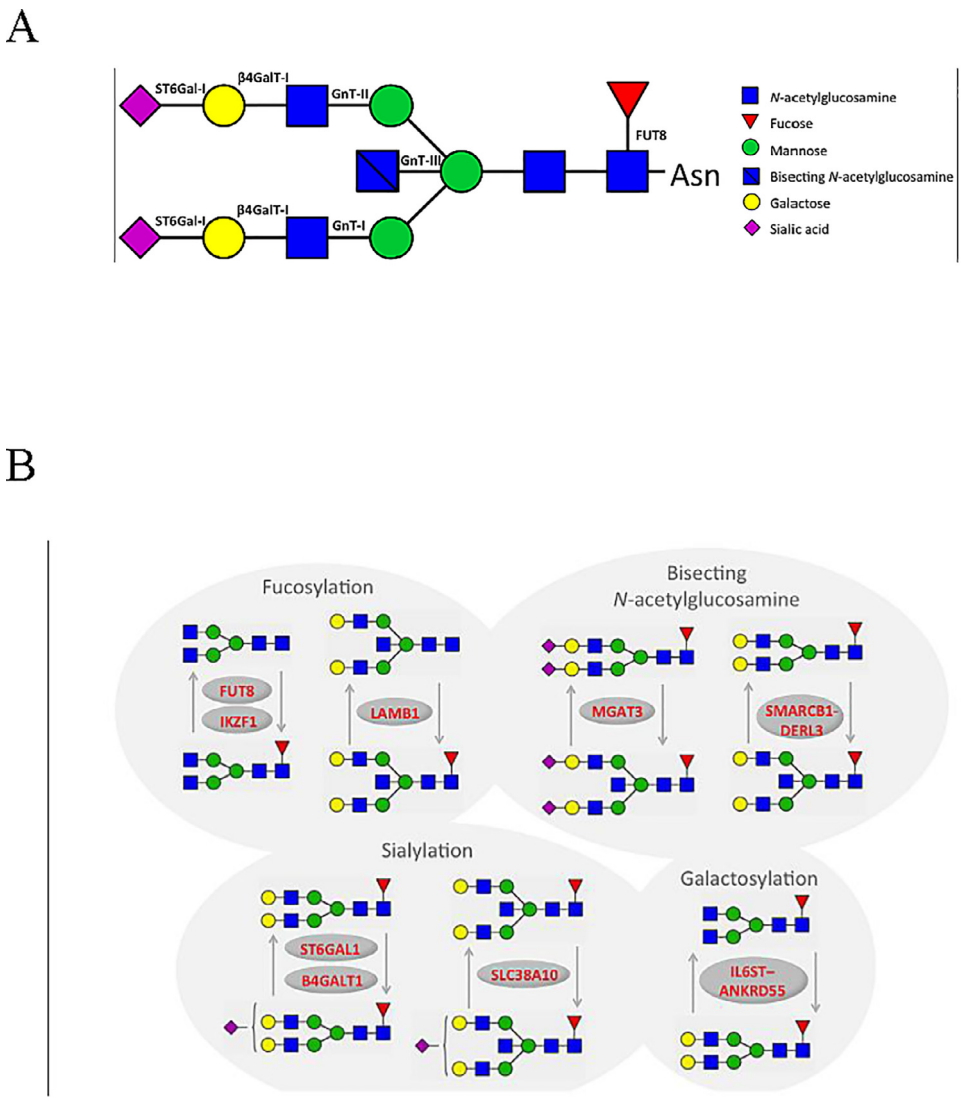

Fig. 4. Structural summary of circulating Plasma IgG N-glycans used in (B) Systemic Lupus Erythematosus Biomarker Development. From: Lauc G. et al. (2013), "Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers." Plos Genetics 2013 9 (1) e1008225. Used by Permission.

Parkinson's disease might be reflected peripherally. Oxidative stress, immune and epigenetic pathological pathways and immune: stem cell interactions provide possible clues which may act centrally and also be detected peripherally.

Therapeutic considerations

These concepts also suggest several very exciting novel therapeutic pathways into addiction treatment which have not been previously been explored. These pathophysiological and mechanistic insights into the fundamental wiring of addictive cells and circuits in turn suggest several novel potential applications with possible therapeutic implications. The cells involved are of known cell type - particularly pyramidal cells in the cortex and hippocampus and medium spiny neurons in the Nucleus Accumbens. Moreover the ensemble cells have been extensively characterized by single cell techniques [22,119–123] and contrasted with surrounding non-activated cells [21]. Each cell type is controlled by a master transcription factor [24,124]. By definition these cells are activated by the specific drug of choice. This suggests that the master transcription factor of these cells could be specifically targeted by the peripheral injection of the antisense oligonucleotide (ASO's) which is complementary to the master TF concerned or targeted by RNA interference (RNAi) technology and either temporarily or permanently inactivated. Such a strategy has already been trialled in large scale phase III clinical trials for the medium and longer term modification of cholesterol metabolism and the atherogenic process by interfering with the ANGPTL9 cascade and also targeting coagulation factors 8 and 9 in haemophilia and for spinal muscular atrophy and reported in leading medical journals [125–130]. Since the cells are active there should be a way to make the uptake of the ASO's dependent on cell activity as has already been achieved experimentally [121].

Moreover powerful new drugs are being developed by several major pharmaceutical companies which act on the epigenome [131,132]. They are being developed for disparate applications including weight reduction the prevention of atherosclerosis, treatment of diabetes and as powerful new cancer agents which are remarkable for minimally usually minimally toxic [131,132].

Alternatively new techniques are being developed which allow deep areas within the substance of the human brain to be specifically targeted by separate alternating electric or magnetic fields in such a way that the two fields interfere with each other cancelling out in the more superficial regions, but reinforcing in the deepest areas of the brain. Such strategies can be used strategically to map and to target various brain structures non-invasively within the living awake human brain

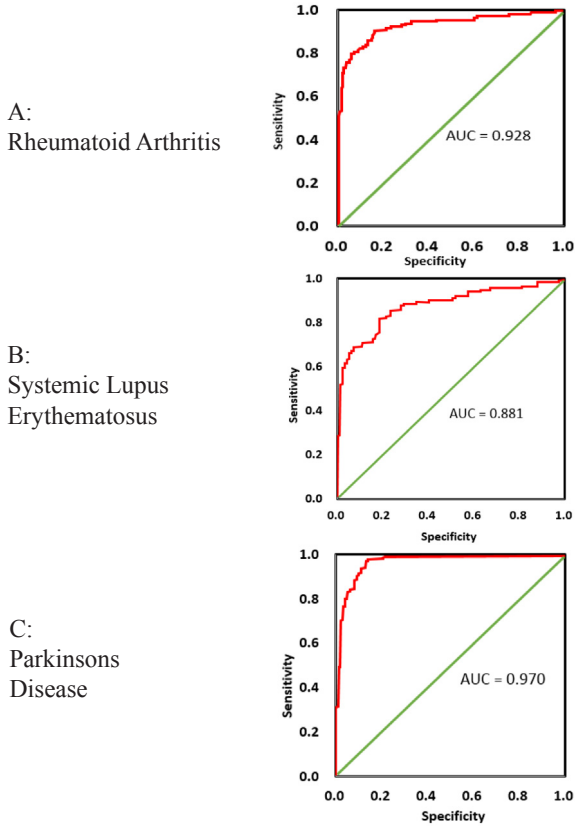

Fig. 5. Receiver Operating Characteristic Curve for Glycans as Biomarkers of (A) Rheumatoid Arthritis (coefficient = 0.881), (B) Systemic Lupus Erythematosus (coefficient = 0.842) and (C) Parkinsons disease (coefficient = 0.970). From: (A) Sebastian A. et. al. "Glycan Biomarkers for Rheumatoid Arthritis and its Remission Status in Han Chinese Patients." OMICS: A journal of Integrative Biology. 2016, (6): 343–351; (B) Lauc G. et al., "Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers." Plos Genetics 2013, 9: (1) e1008225; (C) Russell A.C. et al. "The N-glycosylation of immunoglobulin G as a novel biomarker of Parkinson's disease." Glycobiology. 2017; 27 (5):501–510. All Used by Permission.

[133]. The proof of concept has been established in rats, and such techniques are now under development for humans [133]. It may be possible to coordinate targeted information-based therapeutics such as ASO's and RNAi and have them uncaged after nanomolecular delivery in the areas of interest and thus change brain neurophysiology in a precise and controlled manner for a duration which could be programmed. It has been demonstrated that cultured neurons can take up micron-sized particles including nanospheres containing 6000 atoms of iron in ferritin, and this has been considered as a novel neuroimaging agent [134]. Clearly such techniques could be re-purposed from diagnostic application to therapeutic implementation.

The demonstration that addictions – and all memories – depend on delicately balanced harmonically-tuned cellular networks as their organic substrate – implies that such finely balanced cellular ensembles should be susceptible to targeted interference and thus disruption– effectively re-programming the thinking patterns of the brain! Obviously the potential to do so – by a technique as simple as a subcutaneous injection and/or the subsequent application of targeted field potentials – would require stringent ethical oversight to prevent its inappropriate application. The coordination of such cutting-edge techniques clearly opens major new treatment vista's and may significantly increase the power of the therapeutic armamentarium of tomorrow.

Hypotheses.

The central hypotheses of this enlarged pathophysiological understanding are that exciting new pathways to both diagnostic and treatment modalities may be developed in the near future. We feel that diagnostics based on epigenetics and glycomics hold unusual promise for potential bedside application both for the presence of substance dependency disorders and their severity. Moreover the concepts described above hold exciting power for therapeutic application to design and deliver in a fashion targeted over both time and anatomical structure information-based treatments to disrupt delicate reward-motivational circuits which form the biological substrate of numerous dependency syndromes.

In terms of specifying and subsequently testing the hypotheses more exactly the following guiding remarks may be made:

Diagnostic Biomarkers:

1) Type of future Study: prospective longitudinal – controls and opioid dependence; also need to include non-smoking and smoking controls to differentiate out effects of tobacco
2) Direction – both addiction longitudinally and coming off drugs – e.g. by antagonist supported abstinence such as long acting naltrexone implant maintenance
3) Aims – to describe in detail epigenomic and glycomic profiles of opioid addiction
4) Samples – blood – because these are best validated and can readily be re-sampled
5) Methods to be applied –
   i) Epigenomic Next generation sequencing
   ii) Glycomics
   iii) Genomic – for Mendelian randomization to interrogate causation
6) Outcomes

Machine learning could then be applied to develop:

i) An Epigenomic signature for opioid dependence possibly controlling the μ-opioid receptor
ii) An Epigenomic signature for tobacco dependence
iii) A Glycomic signature for opioid dependence – possibly involving β-linked N-acetylglucosamine.
iv) A Glycomic signature for tobacco dependence
v) Causal inferences from both forward and reverse opioid dependence longitudinal processes
vi) Causal inferences from genomic Mendelian randomization

Biomarkers for ageing, opioid, and tobacco use may be identified by the application of artificial intelligence machine learning approaches to epigenomic and glycomic datasets derived from peripheral blood and the optimization of receiver-operator curve characteristics against dose-duration of substance exposure. β-linked N-acetylglucosamine is a glycan of particular interest and merits specific attention. Similarly the μ-opioid receptor gene and its epigenomic and non-coding DNA regulatory regions merit particular attention and would comprise a useful focus for Mendelian randomization studies. The use of Mendelian randomization together with longitudinal samples studying addiction in both the forward and reverse directions (patients going into addiction and also coming out of it) would provide a particularly powerful framework within which to investigate potentially causal relationships.

Treatments

Emerging from (6) (i) above would be epigenomic targets for therapeutic interdiction in addiction. Since some studies show correlations between biomarkers circulating in the blood and those in the reward circuitry of the brain it may be that key insights can be gained

by studies of peripheral biomarkers which reflect key central pathophysiological processes implicated in the neurocircuitry of opioid dependence.

Adeno- Associated virus No 9 has been shown to allow the delivery of therapies to the brain and across the blood brain barrier [129,130]. Treatments could be targeted direct to the deep brain structures of the limbic reward system both by the use of AAV9 vectors and by the use of electromagnetic uncaging of nanoparticle structures in the circulation of the limbic reward system [133].

Such targets could then be tested in preclinical animal models prior to their consideration in humans.

Concluding remarks

These theoretical considerations suggest multidimensional-multidirectional networked interactions between OUD, epigenomics, glycomics, immunoactivation and endocrine read-outs of central corticolimbic status. As the neuronal ensemble networks including their connectomics are increasingly characterized in the mesocorticolimbic reward system the application of machine learning algorithms to the systemic phenomenology of OUD should enable the development of peripheral-epiphenomenological biomarker sets as was recently demonstrated for clinical alcoholism [9]. Importantly, central corticolimbic neuroinflammation [3] has been shown to be predictively relatable to epigenomic neuroimmune biomarkers within readily accessible human circulating peripheral monocytes [9]. Since glycan-derived biomarkers have demonstrated utility as predictive clinical discriminators of several chronic diseases [11–14] their incorporation along with epigenomic indices should refine and increase the power of peripherally-sourced algorithms in OUD. Including genomic data for Mendelian randomization would allow the interrogation of causality [135] suggesting a comprehensive **pan-omic** approach to comparative central-peripheral biomarker algorithm development. As both epigenomics and glycomics are emerging as powerful multilayered combinatorial biological control systems reflecting gene-environment interactions the prospect of their computational combination from peripherally accessible tissues implies the dawning not only of a new era of diagnostic insight but also the potential to assess central mechanistically significant processes. Coordinated and precisely targeted application of new and emerging therapeutic techniques suggests that the concepts described herein may find novel application in exciting new therapies for substance dependency conditions which were previously considered intransigent and refractory. In time it is conceivable that other psychiatric disorders such as refractory depression and possibly post-traumatic stress disorder, may find similar remediation. All of this suggests a bright future for substance dependence treatment and the dawning of a new age of diagnostic sophistication and targeted therapeutic assistance to facilitate change.

Note added in proof

The PINS Prize in Science was awarded by the American Academy for the Advancement of Science to both the Winner and the Runner up for execution and demonstration of some of the concepts described in this paper particularly optogenetic studies in neural engrams of memory and molecular uncaging of nanoparticles by electromagnetic fields deep in the brain while this paper was under consideration [136].

Conflict of interest

The authors have no conflict of interest to declare.

Funding statement

No funding was received for the conduct of this study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.mehy.2018.04.011.

References

[1] Degenhardt L, et al. Global burden of disease attributable to illicit drug use and dependence: findings from the Global Burden of Disease Study 2010. Lancet 2013;382(9904):1564–74.
[2] Murthy VH. Ending the opioid epidemic — a call to action. N Engl J Med 2016;375(25):2413–5.
[3] Hutchinson MR, et al. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. Pharmacol Rev 2011;63(3):772–810.
[4] Reece AS, Hulse GK. Hypothalamic pathophysiology in the neuroimmune, dysmetabolic and longevity complications of chronic opiate dependency. J. Forensic Toxicol Pharmacol 2014;3:3–46.
[5] Reece AS. Epidemiologic and molecular pathophysiology of chronic opioid dependence and the place of naltrexone extended-release formulations in its clinical management. Subst Abuse 2012;6:115–33.
[6] Degenhardt L, et al. Mortality among clients of a state-wide opioid pharmacotherapy program over 20 years: Risk factors and lives saved. Drug Alcohol Depend 2009;105(1–2):9–15.
[7] Robison AJ, Nestler EJ. Transcriptional and epigenetic mechanisms of addiction. Nat Rev Neurosci 2011;12(11):623–37.
[8] Borrelli E, et al. Decoding the epigenetic language of neuronal plasticity. Neuron 2008;60(6):961–74.
[9] Liu C, et al. A DNA methylation biomarker of alcohol consumption. Mol Psychiatry 2016.
[10] Huang H, et al. Fine-mapping inflammatory bowel disease loci to single-variant resolution. Nature 2017;547(7662):173–8.
[11] Yu X, et al. Profiling IgG N-glycans as potential biomarker of chronological and biological ages: a community-based study in a Han Chinese population. Medicine (Baltimore) 2016;95(28):e4112.
[12] Vuckovic F, et al. Association of systemic lupus erythematosus with decreased immunosuppressive potential of the IgG glycome. Arthritis Rheumatol 2015;67(11):2978–89.
[13] Russell AC, et al. The N-glycosylation of immunoglobulin G as a novel biomarker of Parkinson's disease. Glycobiology 2017;27(5):501–10.
[14] Sebastian A, et al. Glycan biomarkers for rheumatoid arthritis and its remission status in Han Chinese patients. OMICS 2016;20(6):343–51.
[15] Hebb DO. The organization of behavior: a neuropsychological theory. Lawrence Erlbaum Associates; 1949.
[16] Calipari ES, et al. Dopaminergic dynamics underlying sex-specific cocaine reward. Nat Commun 2017;8:13877.
[17] Xue YX, et al. Selective inhibition of amygdala neuronal ensembles encoding nicotine-associated memories inhibits nicotine preference and relapse. Biol Psychiatry 2017.
[18] Leao RM, et al. Chronic nicotine activates stress/reward-related brain regions and facilitates the transition to compulsive alcohol drinking. J Neurosci 2015;35(15):6241–53.
[19] Caprioli D, et al. Role of dorsomedial striatum neuronal ensembles in incubation of methamphetamine craving after voluntary abstinence. J Neurosci 2017;37(4):1014–27.
[20] Cruz FC, et al. Role of nucleus accumbens shell neuronal ensembles in context-induced reinstatement of cocaine-seeking. J Neurosci 2014;34(22):7437–46.
[21] Whitaker LR, et al. Bidirectional modulation of intrinsic excitability in rat prelimbic cortex neuronal ensembles and non-ensembles after operant learning. J Neurosci 2017;37(36):8845–56.
[22] Bossert JM, et al. Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. Nat Neurosci 2011;14(4):420–2.
[23] Cruz FC, et al. Using c-fos to study neuronal ensembles in corticostriatal circuitry of addiction. Brain Res 2015;1628(Pt A):157–73.
[24] Zeisel A, et al. Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science 2015;347(6226):1138–42.
[25] Hsiang HL, et al. Manipulating a “cocaine engram” in mice. J Neurosci 2014;34(42):14115–27.
[26] Trouche S, et al. Recoding a cocaine-place memory engram to a neutral engram in the hippocampus. Nat Neurosci 2016;19(4):564–7.
[27] Koya E, et al. Targeted disruption of cocaine-activated nucleus accumbens neurons prevents context-specific sensitization. Nat Neurosci 2009;12(8):1069–73.
[28] Fanous S, et al. Role of orbitofrontal cortex neuronal ensembles in the expression of incubation of heroin craving. J Neurosci 2012;32(34):11600–9.
[29] Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. Science 2001;294(5544):1030–8.
[30] Nabavi S, et al. Engineering a memory with LTD and LTP. Nature 2014;511(7509):348–52.
[31] Nestler EJ. Transgenerational epigenetic contributions to stress responses: fact or fiction? PLoS Biol 2016;14(3):e1002426.
[32] Buzsaki G, Draguhn A. Neuronal oscillations in cortical networks. Science 2004;304(5679):1926–9.
[33] Yaffe RB, et al. Cued memory retrieval exhibits reinstatement of high gamma power on a faster timescale in the left temporal lobe and prefrontal cortex. J

Neurosci 2017;37(17):4472–80.
[34] Tomassini A, et al. Theta oscillations locked to intended actions rhythmically modulate perception. Elife 2017;6:e25618.
[35] Tort AB, et al. Theta-gamma coupling increases during the learning of item-context associations. Proc Natl Acad Sci U S A 2009;106(49):20942–7.
[36] Buzsaki G, Moser EI. Memory, navigation and theta rhythm in the hippocampal-entorhinal system. Nat Neurosci 2013;16(2):130–8.
[37] Canolty RT, et al. High gamma power is phase-locked to theta oscillations in human neocortex. Science 2006;313(5793):1626–8.
[38] Cohen MX, et al. Oscillatory activity and phase-amplitude coupling in the human medial frontal cortex during decision making. J Cogn Neurosci 2009;21(2):390–402.
[39] Garcia-Cordero I, et al. Attention, in and out: scalp-level and intracranial EEG correlates of interoception and exteroception. Front Neurosci 2017;11:411.
[40] Zhou F, et al. Frequency-dependent changes in local intrinsic oscillations in chronic primary insomnia: a study of the amplitude of low-frequency fluctuations in the resting state. Neuroimage Clin 2017;15:458–65.
[41] Sato W, et al. Rapid, high-frequency, and theta-coupled gamma oscillations in the inferior occipital gyrus during face processing. Cortex 2014;60:52–68.
[42] Cohen MX, et al. Good vibrations: cross-frequency coupling in the human nucleus accumbens during reward processing. J Cogn Neurosci 2009;21(5):875–89.
[43] Sato W, et al. Bidirectional electric communication between the inferior occipital gyrus and the amygdala during face processing. Hum Brain Mapp 2017;38(9):4511–24.
[44] Axmacher N, et al. Cross-frequency coupling supports multi-item working memory in the human hippocampus. Proc Natl Acad Sci U S A 2010;107(7):3228–33.
[45] Amadei EA, et al. Dynamic corticostriatal activity biases social bonding in monogamous female prairie voles. Nature 2017;546(7657):297–301.
[46] Wang H, et al. Histone deacetylase inhibitors facilitate partner preference formation in female prairie voles. Nat Neurosci 2013;16(7):919–24.
[47] Ferguson D, et al. Essential role of SIRT1 signaling in the nucleus accumbens in cocaine and morphine action. J Neurosci 2013;33(41):16088–98.
[48] Mazei-Robison MS, Nestler EJ. Opiate-induced molecular and cellular plasticity of ventral tegmental area and locus coeruleus catecholamine neurons. Cold Spring Harb Perspect Med 2012;2(7):a012070.
[49] Rehni AK, et al. Modulation of histone deacetylase attenuates naloxone-precipitated opioid withdrawal syndrome. Naunyn Schmiedebergs Arch Pharmacol 2012;385(6):605–19.
[50] Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 2009;10(5):295–304.
[51] Heller EA, et al. Locus-specific epigenetic remodeling controls addiction- and depression-related behaviors. Nat Neurosci 2014;17(12):1720–7.
[52] Miller CA, et al. DNA methylation and histone acetylation work in concert to regulate memory formation and synaptic plasticity. Neurobiol Learn Mem 2008;89(4):599–603.
[53] Sweatt JD. Neural plasticity and behavior - sixty years of conceptual advances. J Neurochem 2016;139(Suppl 2):179–99.
[54] Heller EA, et al. Morphine and cocaine increase serum- and glucocorticoid-inducible kinase 1 activity in the ventral tegmental area. J Neurochem 2015;132(2):243–53.
[55] Nestler EJ. Reflections on: “A general role for adaptations in G-Proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function”. Brain Res 2016;1645:71–4.
[56] Sun H, et al. Morphine epigenomically regulates behavior through alterations in histone H3 lysine 9 dimethylation in the nucleus accumbens. J Neurosci 2012;32(48):17454–64.
[57] Koo JW, et al. Epigenetic basis of opiate suppression of Bdnf gene expression in the ventral tegmental area. Nat Neurosci 2015;18(3):415–22.
[58] Sanchis-Segura C, et al. Selective boosting of transcriptional and behavioral responses to drugs of abuse by histone deacetylase inhibition. Neuropsychopharmacology 2009;34(13):2642–54.
[59] Wang R, et al. The extinction of morphine-induced conditioned place preference by histone deacetylase inhibition. Neurosci Lett 2010;483(2):137–42.
[60] Miller CA, et al. Cortical DNA methylation maintains remote memory. Nat Neurosci 2010;13(6):664–6.
[61] Meadows JP, et al. Dynamic DNA methylation regulates neuronal intrinsic membrane excitability. Sci Signal 2016;9(442):ra83.
[62] Song CX, et al. Genome-wide profiling of 5-formylcytosine reveals its roles in epigenetic priming. Cell 2013;153(3):678–91.
[63] Kumar D, et al. Tet1 oxidase regulates neuronal gene transcription, active DNA hydroxy-methylation, object location memory, and threat recognition memory. Neuroepigenetics 2015;4:12–27.
[64] Heyward FD, et al. Obesity weighs down memory through a mechanism involving the neuroepigenetic dysregulation of Sirt1. J Neurosci 2016;36(4):1324–35.
[65] Feng J, et al. Tet1 in nucleus accumbens opposes depression- and anxiety-like behaviors. Neuropsychopharmacology 2017;42(8):1657–69.
[66] Feng J, et al. Role of Tet1 and 5-hydroxymethylcytosine in cocaine action. Nat Neurosci 2015;18(4):536–44.
[67] Yan C, et al. Structure of a yeast step II catalytically activated spliceosome. Science 2017;355(6321):149–55.
[68] Gilbert WV, et al. Messenger RNA modifications: form, distribution, and function. Science 2016;352(6292):1408–12.
[69] Roundtree IA, et al. Dynamic RNA modifications in gene expression regulation. Cell 2017;169(7):1187–200.
[70] Savell KE, et al. Extra-coding RNAs regulate neuronal DNA methylation dynamics. Nat Commun 2016;7:12091.
[71] Yap KL, et al. Molecular interplay of the noncoding RNA ANRIL and methylated histone H3 lysine 27 by polycomb CBX7 in transcriptional silencing of INK4a. Mol Cell 2010;38(5):662–74.
[72] Tripathi V, et al. Long noncoding RNA MALAT1 controls cell cycle progression by regulating the expression of oncogenic transcription factor B-MYB. PLoS Genet 2013;9(3):e1003368.
[73] Chen CK, et al. Xist recruits the X chromosome to the nuclear lamina to enable chromosome-wide silencing. Science 2016;354(6311):468–72.
[74] Boks MP, et al. Longitudinal changes of telomere length and epigenetic age related to traumatic stress and post-traumatic stress disorder. Psychoneuroendocrinology 2015;51:506–12.
[75] Li Y, et al. An epigenetic signature in peripheral blood associated with the haplotype on 17q21.31, a risk factor for neurodegenerative tauopathy. PLoS Genet 2014;10(3):e1004211.
[76] Levine AJ, et al. Systems analysis of human brain gene expression: mechanisms for HIV-associated neurocognitive impairment and common pathways with Alzheimer's disease. BMC Med Genomics 2013;6:4.
[77] Horvath S, Ritz BR. Increased epigenetic age and granulocyte counts in the blood of Parkinson's disease patients. Aging (Albany NY) 2015;7(12):1130–42.
[78] Horvath S, et al. Huntington's disease accelerates epigenetic aging of human brain and disrupts DNA methylation levels. Aging (Albany NY) 2016;8(7):1485–512.
[79] Levine ME, et al. Epigenetic age of the pre-frontal cortex is associated with neuritic plaques, amyloid load, and Alzheimer's disease related cognitive functioning. Aging (Albany NY) 2015;7(12):1198–211.
[80] Ando B, et al. Personality traits and coping compensate for disadvantageous decision-making in long-term alcohol abstinence. Alcohol Alcohol 2012;47(1):18–24.
[81] Khalid O, et al. Gene expression signatures affected by alcohol-induced DNA methylomic deregulation in human embryonic stem cells. Stem Cell Res 2014;12(3):791–806.
[82] Carroll JE, et al. Epigenetic aging and immune senescence in women with insomnia symptoms: findings from the women's health initiative study. Biol Psychiatry 2016.
[83] Levine ME, et al. Menopause accelerates biological aging. Proc Natl Acad Sci U S A 2016;113(33):9327–32.
[84] Ceriello A, et al. Opiate addict as diabetic patient? Diabetes Care 1988;11(5):443.
[85] Claude Bernard M. Lecons Sur Le Diabete et la Glycogenese Animale. Cours Med 1877.
[86] Passariello N, et al. Glucose tolerance and hormonal responses in heroin addicts. A possible role for endogenous opiates in the pathogenesis of non-insulin-dependent diabetes. Metabolism 1983;32(12):1163–5.
[87] Ceriello A, et al. Impaired glucose metabolism in heroin and methadone users. Horm Metab Res 1987;19(9):430–3.
[88] Reece AS. Manifold implications of forgotten hyperglycaemia in clinical opiate dependence. Drug Chem Toxicol 2013;36(1):55–66.
[89] Passariello N, et al. Impaired insulin response to glucose but not to arginine in heroin addicts. J Endocrinol Invest 1986;9(5):353–7.
[90] Olson VG, et al. Regulation of morphine reward and feeding by CREB in the lateral hypothalamus. Synapse 2007;61(2):110–3.
[91] Neri S, et al. Randomized clinical trial to compare the effects of methadone and buprenorphine on the immune system in drug abusers. Psychopharmacology (Berl) 2005;179(3):700–4.
[92] Reece AS. Relative and age dependent stimulation of soluble and cellular immunity in opiate dependence. J Addict Med 2012;6(1):10–7.
[93] Reece AS, Hulse GK. Impact of lifetime opioid exposure on arterial stiffness and vascular age: cross-sectional and longitudinal studies in men and women. BMJ Open 2014;4(6):1–19.
[94] Sadeghian S, et al. The association of opium with coronary artery disease. Eur J Cardiovasc Prev Rehabil 2007;14(5):715–7.
[95] Pasmant E, et al. ANRIL, a long, noncoding RNA, is an unexpected major hotspot in GWAS. FASEB J 2010;25(2):444–8.
[96] Solis Jr. E, et al. Intravenous heroin induces rapid brain hypoxia and hyperglycemia that precede brain metabolic response. eNeuro 2017;4(3).
[97] Russo SJ, et al. IRS2-Akt pathway in midbrain dopamine neurons regulates behavioral and cellular responses to opiates. Nat Neurosci 2007;10(1):93–9.
[98] Li Y, et al. Morphine induces desensitization of insulin receptor signaling. Mol Cell Biol 2003;23(17):6255–66.
[99] Wang Y, et al. Glycomics and its application potential to precision medicine in precision medicine in China. Science 2016;354(6319):36–9.
[100] Yang X, et al. Recruitment of O-GlcNAc transferase to promoters by corepressor mSin3A: coupling protein O-GlcNAcylation to transcriptional repression. Cell 2002;110(1):69–80.
[101] Czjzek M. Biochemistry: a wine-induced breakdown. Nature 2017;544(7648):45–6.
[102] Ndeh D, et al. Complex pectin metabolism by gut bacteria reveals novel catalytic functions. Nature 2017;544(7648):65–70.
[103] Sun C, et al. O-GlcNAcylation: a bridge between glucose and cell differentiation. J Cell Mol Med 2016;20(5):769–81.
[104] Peres GB, et al. Impact of high glucose and AGEs on cultured kidney-derived cells. Effects on cell viability, lysosomal enzymes and effectors of cell signaling pathways. Biochimie 2017;135:137–48.
[105] Wong TY, et al. Hyaluronan keeps mesenchymal stem cells quiescent and maintains the differentiation potential over time. Aging Cell 2017;16(3):451–60.
[106] Lee HJ, et al. Glycerol-3-phosphate acyltransferase-1 upregulation by O-GlcNAcylation of Sp1 protects against hypoxia-induced mouse embryonic stem cell apoptosis via mTOR activation. Cell Death Dis 2016;7:e2158.
[107] Novakovic B, et al. beta-Glucan reverses the epigenetic state of LPS-induced

immunological tolerance. Cell 2016;167(5). 1354-1368 e14.
[108] Gambetta MC, et al. Essential role of the glycosyltransferase sxc/Ogt in polycomb repression. Science 2009;325(5936):93–6.
[109] Costa C, et al. Abnormal protein glycosylation and activated PI3K/Akt/mTOR pathway: role in bladder cancer prognosis and targeted therapeutics. PLoS One 2015;10(11):e0141253.
[110] Kelly WG, et al. RNA polymerase II is a glycoprotein. Modification of the COOH-terminal domain by O-GlcNAc. J Biol Chem 1993;268(14):10416–24.
[111] Ronningen T, et al. Prepatterning of differentiation-driven nuclear lamin A/C-associated chromatin domains by GlcNAcylated histone H2B. Genome Res 2015;25(12):1825–35.
[112] Ahmed AA, et al. Structural characterization of anti-inflammatory immunoglobulin G Fc proteins. J Mol Biol 2014;426(18):3166–79.
[113] Lauc G, et al. Loci associated with N-glycosylation of human immunoglobulin G show pleiotropy with autoimmune diseases and haematological cancers. PLoS Genet 2013;9(1):e1003225.
[114] Kristic J, et al. Glycans are a novel biomarker of chronological and biological ages. J Gerontol A Biol Sci Med Sci 2014;69(7):779–89.
[115] Lauc G, et al. Genomics meets glycomics-the first GWAS study of human N-Glycome identifies HNF1alpha as a master regulator of plasma protein fucosylation. PLoS Genet 2010;6(12):e1001256.
[116] Lu JP, et al. Screening novel biomarkers for metabolic syndrome by profiling human plasma N-glycans in Chinese Han and Croatian populations. J Proteome Res 2011;10(11):4959–69.
[117] Wang Y, et al. The association between glycosylation of immunoglobulin G and hypertension: a multiple ethnic cross-sectional study. Medicine (Baltimore) 2016;95(17):e3379.
[118] McLachlan F, et al. A case-control study in an Orcadian population investigating the relationship between human plasma N-glycans and metabolic syndrome. J Glycomics Lipidomics 2016;6:139.
[119] Fanous S, et al. Unique gene alterations are induced in FACS-purified Fos-positive neurons activated during cue-induced relapse to heroin seeking. J Neurochem 2013;124(1):100–8.
[120] Guez-Barber D, et al. FACS purification of immunolabeled cell types from adult rat brain. J Neurosci Methods 2012;203(1):10–8.
[121] Marchant NJ, et al. Role of ventral subiculum in context-induced relapse to alcohol seeking after punishment-imposed abstinence. J Neurosci 2016;36(11):3281–94.
[122] Pickens CL, et al. Neurobiology of the incubation of drug craving. Trends Neurosci 2011;34(8):411–20.
[123] Suto N, et al. Distinct memory engrams in the infralimbic cortex of rats control opposing environmental actions on a learned behavior. Elife 2016;5:e21920.
[124] Lobo MK, et al. FACS-array profiling of striatal projection neuron subtypes in juvenile and adult mouse brains. Nat Neurosci 2006;9(3):443–52.
[125] Dewey FE, et al. Genetic and pharmacologic inactivation of ANGPTL3 and cardiovascular disease. N Engl J Med 2017;377(3):211–21.
[126] Graham MJ, et al. Cardiovascular and metabolic effects of ANGPTL3 antisense oligonucleotides. N Engl J Med 2017;377(3):222–32.
[127] Keaney Jr. JF. CHIP-ping away at atherosclerosis. N Engl J Med 2017;377(2):184–5.
[128] Pasi KJ, et al. Targeting of Antithrombin in Hemophilia A or B with RNAi therapy. N Engl J Med 2017;377(9):819–28.
[129] Mendell JR, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. N Engl J Med 2017;377(18):1713–22.
[130] Kaiser J. Gene therapy triumph. Science 2017;358(6370):1526.
[131] Filippakopoulos P, et al. Selective inhibition of BET bromodomains. Nature 2010;468(7327):1067–73.
[132] Loven J, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell 2013;153(2):320–34.
[133] Lozano AM. Waving hello to noninvasive deep-brain stimulation. N Engl J Med 2017;377(11):1096–8.
[134] Wallace VJ, et al. Neurons internalize functionalized micron-sized silicon dioxide microspheres. Cell Mol Neurobiol 2017;37(8):1487–99.
[135] Birney E, et al. Epigenome-wide association studies and the interpretation of disease -omics. PLoS Genet 2016;12(6):e1006105.
[136] American Academy for Advancement of Science. PINS Prize in Science of AAAS 2017. http://www.sciencemag.org/new-research-offers-blueprint-tackling-addiction (2017) (accessed 15th January 2018).
[137] Reece AS. Evidence of accelerated ageing in clinical drug addiction from immune, hepatic and metabolic biomarkers. Immun Ageing 2007;4(1):6–15.
[138] Reece AS. Chronic viral hepatitis is a significant contributor to the immunosenescent phenotype of parenteral drug addiction. Addict Biol 2008;14(2):214.
[139] Fauci AS et al. (eds.). Harrison's Principles of Internal Medicine, seventeenth ed. McGraw Hill; (2008).
[140] Pattabiraman G, et al. Aging-associated dysregulation of homeostatic immune response termination (and not initiation). Aging Cell 2017;16(3):585–93.
[141] Fulop T, et al. From inflamm-aging to immune-paralysis: a slippery slope during aging for immune-adaptation. Biogerontology 2016;17(1):147–57.
[142] Pangrazzi L, et al. "Inflamm-aging" influences immune cell survival factors in human bone marrow. Eur J Immunol 2016;47(3):481–92.
[143] Brunton LL, editor. Goodman and Gilman's the pharmacologic basis of therapeutics. McGraw Hill; 2006.
[144] Reece AS. Clinical implications of addiction related immunosuppression. J Infect 2008;56(6):437–45.
[145] Reece AS. Chronic viral hepatitis is a significant contributor to the immunosenescent phenotype of parenteral drug addiction. Addict Biol 2009;14(2):214–26.
[146] Saggu R, et al. Astroglial NF-kB contributes to white matter damage and cognitive impairment in a mouse model of vascular dementia. Acta Neuropathol Commun 2016;4(1):76.
[147] Carpentier PA, Palmer TD. Immune influence on adult neural stem cell regulation and function. Neuron 2009;64(1):79–92.
[148] Deverman BE, Patterson PH. Cytokines and CNS development. Neuron 2009;64(1):61–78.
[149] Leoni C, et al. Dnmt3a restrains mast cell inflammatory responses. Proc Natl Acad Sci U S A 2017;114(8):E1490–9.
[150] Zhang Q, et al. Tet2 is required to resolve inflammation by recruiting Hdac2 to specifically repress IL-6. Nature 2015;525(7569):389–93.
[151] Fuster JJ, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science 2017;355(6327):842–7.
[152] Chavan SS, et al. Mechanisms and therapeutic relevance of neuro-immune communication. Immunity 2017;46(6):927–42.
[153] Hayes JM, et al. Fc gamma receptors: glycobiology and therapeutic prospects. J Inflamm Res 2016;9:209–19.
[154] Bennun SV, et al. Systems glycobiology: integrating glycogenomics, glycoproteomics, glycomics, and other 'omics data sets to characterize cellular glycosylation processes. J Mol Biol 2016;428(16):3337–52.
[155] Sackstein R. Translational glycobiology: patient-oriented glycoscience research. Glycobiology 2016;26(6):544–5.
[156] Georgescu D, et al. Involvement of the lateral hypothalamic peptide orexin in morphine dependence and withdrawal. J Neurosci 2003;23(8):3106–11.
[157] Sharpe MJ, et al. Lateral hypothalamic GABAergic neurons encode reward predictions that are relayed to the ventral tegmental area to regulate learning. Curr Biol 2017;27(14). 2089-2100.e5.
[158] Reece AS, et al. Dramatic acceleration of reproductive aging, contraction of biochemical fecundity and healthspan-lifespan implications of opioid-induced endocrinopathy-FSH/LH ratio and other interrelationships. Reprod Toxicol 2016;66:20–30.
[159] Zhang G, et al. Hypothalamic programming of systemic ageing involving IKK-[bgr], NF-[kgr]B and GnRH. Nature 2013;497(7448):211–6.
[160] Ben-Meir A, et al. Coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging. Aging Cell 2015;14(5):887–95.
[161] Partridge L, et al. Sex and death: what is the connection? Cell 2005;120(4):461–72.
[162] Kirkwood TB. Understanding the odd science of aging. Cell 2005;120(4):437–47.
[163] Kohrt WM, Wierman ME. Preventing fat gain by blocking follicle-stimulating hormone. N Engl J Med 2017;377(3):293–5.
[164] Nunez Miguel R, et al. Glycosylation pattern analysis of glycoprotein hormones and their receptors. J Mol Endocrinol 2017;58(1):25–41.
[165] Juric J, et al. Immunoglobulin G Glycosylation in Menstrual Cycle. In Poster Presentation at 12th Jenner Glycobiology and Medicine Genos-Genes Conference Symposium. Translational Glycobiology, Dubrovnik, Croatia, 2017, p. 1.

Glossary

Ac: Acetyl group (on Histones)
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AMPAR: AMPA Receptor
CpG: - Cytosine-phosphate-guanosine – sites of DNA methylation
ΔFosB: Long lasting splice variant of FosB – lacks terminal 101 amino acids
EWAS: Epigenome-wide association study
Fos: Immediate early gene TF activating cells and neurons
FosB: Immediate early gene TF activating cells and neurons – splice variant
GABA: Gamma-alpha-butyric acid
GABAAR: GABA A Receptor
GWAS: Genome-Wide Association Studies
H3: Histone 3
H4: Histone 4
HAT: Histone Acetyl Transferase
HDAC: Histone Deacetylase
Hebbian synapse: Activity dependent synaptic changes
hmC: Hydroxymethylcytosine
K3: Lysine 3
K4: Lysine 4
K27: Lysine 27
me: Methyl group (on DNA)
me2: Dimethyl group (on DNA)
me3: Trimethyl group (on DNA)
Metabotropic: A membrane receptor which acts through a second messenger often via membrane-linked transduction machinery
mPFC: Medial prefrontal cortex
mTOR: Mechanistic Target of Rapamycin
OGT: O-GlcNAc transferase
OUD: Opioid Use Disorder
mC: Methylcytosine
NAc: Nucleus Accumbens
NIDA: National Institute of Drug Abuse, part of the National Institutes of Health, Bethesda, Maryland, USA.
NMDA: N-methyl-D-aspartate
NMDAR: N-methyl-D-aspartate receptor
Non-Hebbian Synapse: Non-activity dependent synaptic changes

Pan-omics: Genomics, transcriptomics including the fascinating and important subject of non-coding RNA's, epitranscriptomics including cytosine 1 methylation, glycomics, DNA methylation, histone lysine methylation and acetylation and arginine methylation and Hi-C
PTM: Post-translational modification
Rodent: Rat and/or mouse
SUD: Substance Use Disorder
TET1: Ten-Eleven (methylcytosine dioxygenase) Translocation 1
TET2: Ten-Eleven (methylcytosine dioxygenase) Translocation 2
TET3: Ten-Eleven (methylcytosine dioxygenase) Translocation 3
VTA: Ventral Tegmental Area