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

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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

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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

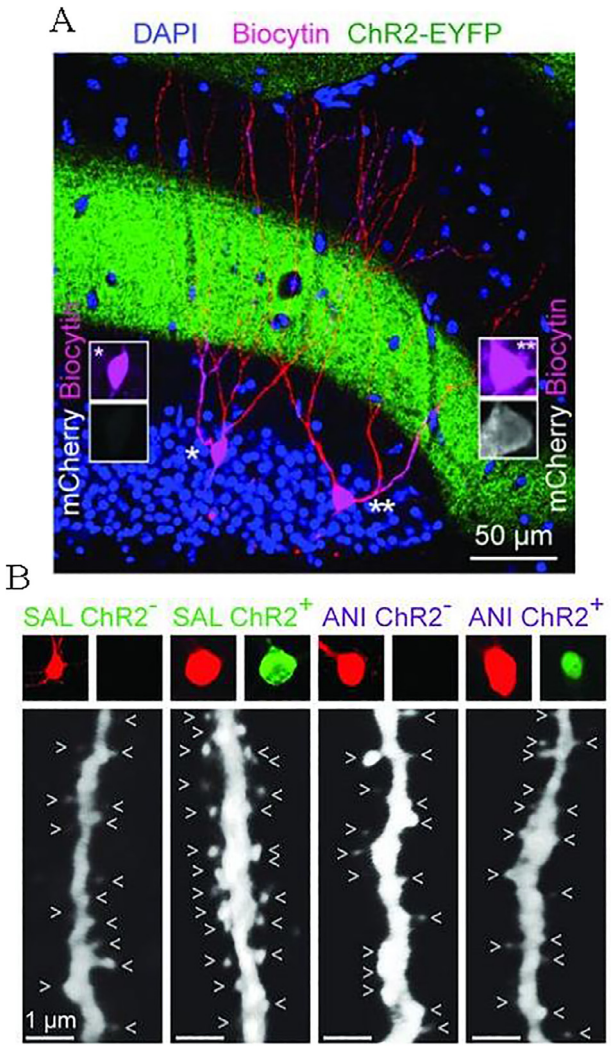

Fig. 1. Identifying Hippocampal Engram cells and their (B) synaptic connectivity. Sal – Saline; ChR2 – Channelrhodopsin; Ani – Anisomycin. From: Ryan TJ, Memory. "Engram Cells Retain Memory under Retrograde Amnesia." Science 2015; 348 (6238): 1007–1013. Used by Permission.

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