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Invited review

Cortical and amygdalar neuronal ensembles in alcohol seeking, drinking and withdrawal

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ABSTRACT

Alcohol induces many alterations in the brain that are thought to contribute to alcohol addiction. Most of the known alterations are induced in all neurons of a brain area or all neurons of a given cell type, regardless of whether they were activated during behavior. While these alterations can have important modulatory effects on behavior, they cannot explain why animals respond specifically to alcohol-paired cues as opposed to all other non-paired cues and evoke highly specific goal-directed learned responses in models of drug craving. As an alternative, we hypothesize another class of alterations that are induced only within sparsely distributed patterns of neurons, called neuronal ensembles, that are selectively activated by alcohol-specific cues during behavior and encode the long-term memories underlying these learned behaviors in animal models of alcohol addiction. Here we review recent studies and techniques used to identify the role of neuronal ensembles in animal models of different phases of the alcohol addiction cycle.

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1. Introduction

Alcoholism is a chronic relapsing disorder characterized by increased overall motivation to seek alcohol, increased alcohol intake, loss of control over alcohol intake, and compulsive alcohol seeking and taking. Three major components have been identified in the alcohol addiction cycle—*binge/intoxication*, *withdrawal/negative affect*, and *preoccupation/anticipation* (craving)—and incorporate the constructs of impulsivity and compulsivity with varying contributions of positive and negative reinforcement (Dhaher et al., 2008; Doremus-Fitzwater et al., 2010; Koob, 2013, 2015; Kwako et al., 2016; Kyzar et al., 2016; Leung et al., 2017). Stimulus-Outcome (SO) and Stimulus-Reward (SR) learning processes play critical roles in all three of these addiction cycle

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components. In the case of positive reinforcement, cues and contexts associated with alcohol drinking acquire incentive salience after repeated association with alcohol drinking (Robinson and Berridge, 2000). With further binge/intoxication experience, these cues and contexts can acquire a greater degree of incentive salience that may contribute to compulsive alcohol seeking and drinking (Flagel et al., 2009). In the case of negative reinforcement, it is the motivational value of the negative states of alcohol withdrawal during the *withdrawal/negative affect* phase that may induce craving. This stage then leads to the *preoccupation/anticipation* phase where craving is intensified by the anticipation of access to alcohol resulting in compulsive alcohol seeking and drinking (George et al., 2014). While many studies have uncovered a number of important neural mechanisms that are altered by alcohol experience, few of them explain how specific cues or contexts paired with these stages of the addiction cycle are encoded in the brain. In this review, we focus on the hypothesis that neuronal ensembles encode and mediate the recall of learned associations among the specific cues, contexts, and behaviors during operant alcohol seeking and drinking.

Cue- or context-dependent reinstatement of alcohol seeking is often used as an animal model of alcohol relapse (Le and Shaham, 2002; Martin-Fardon and Weiss, 2013; Rodd et al., 2004). Re-exposure to cues or contexts after extinction of alcohol drinking produces robust reinstatement of alcohol seeking that can be observed during each phase of the alcohol addiction cycle (Burattini et al., 2006; Liu and Weiss, 2002; Mantsch et al., 2015; Zironi et al., 2006). Brain imaging using immediate early genes as a marker of neuronal activity indicate that neurons are strongly activated by alcohol-associated cues in the prefrontal cortex, amygdala, hippocampus, lateral hypothalamus, and nucleus accumbens (Hamlin et al., 2007, 2009; Marinelli et al., 2007; Millan et al., 2010). Context-induced reinstatement of alcohol seeking is prevented by glutamate, dopamine, and opioid receptor antagonists into these brain areas (Burattini et al., 2006; Chaudhri et al., 2008, 2009a; Sinclair et al., 2012). Moreover, the use of disconnection procedures, non-selective inactivation, or receptor antagonists to disrupt normal neurotransmission indicate an important role for cortico-subcortical projections (Marchant et al., 2015) in context-induced reinstatement of alcohol seeking as well as the prelimbic and infralimbic cortex (Willcocks and McNally, 2013), basolateral amygdala (Millan et al., 2010; Sinclair et al., 2012), hippocampus (Marinelli et al., 2007), thalamus (Hamlin et al., 2009), lateral hypothalamus (Hamlin et al., 2007), and nucleus accumbens (Chaudhri et al., 2008, 2009a). For cue-induced reinstatement of alcohol seeking, the nucleus accumbens core subregion appears more important than the shell subregion (Chaudhri et al., 2009b). Thus, alcohol seeking requires activation of neurons in a number of key corticolimbic areas.

Many different neuroadaptations within these neural circuits and neurotransmitter systems have been identified and shown to alter cue- and context-induced reinstatement of alcohol seeking during the different stages of the addiction cycle (Le and Shaham, 2002; Mantsch et al., 2016). However, these neuroadaptations were identified by assessing molecular or cellular alterations in whole brain regions or in specific cell types, including electrophysiological alterations of randomly selected neurons, regardless of whether neurons were activated during behavior. While these 'global' alterations can indirectly modulate behavior, they do not have the required resolution to encode and distinguish among the different learned associations activated by the highly specific cues and contexts that control alcohol seeking and drinking. For example, overall increases or decreases of glutamatergic or dopaminergic neurotransmission cannot explain why one specific tone-light combination used as the alcohol-paired cue induces reinstatement while another tone-light combination used as the unpaired cue does not induce reinstatement. Instead, we hypothesize that specific 'patterns' of sparsely distributed neurons, called neuronal ensembles, are selectively activated by the specific cues, contexts, and rewards during alcohol-related behavior to encode these learned associations. Considering there are millions of neurons in a brain area, that only 1–5% of these neurons are required to be part of a neuronal ensemble (see below), and that different ensembles can use partially overlapping sets of neurons, there is an immense number of possible ensembles to encode all alcohol-related and other learned associations. These neuronal ensembles are not defined by region or by cell type – indeed research has shown they are composed of all cell types found in a given brain area. The only defining characteristic of a neuronal ensemble is their selective activation by cues and contexts during alcohol seeking and drinking.

2. Approaches to identifying neuronal ensembles in animal models of alcohol addiction

Techniques for examining these ensembles have only recently become available to study learned associations, including those in alcohol research (Cruz et al., 2013; Josselyn et al., 2015; Mayford and Reijmers, 2015; Sorensen et al., 2016; Tonegawa et al., 2015). Nearly all of the techniques for identifying recently activated neurons within neuronal ensembles depended on activation of the promoter for the immediate early gene (IEG) c-*fos*, although other IEG (*arc*, *egr1*) promoters have also been used. As described in more detail in Cruz et al. (2014b), strong persistent activation of neurons leads to sufficiently high sustained levels of calcium influx that activates the MAP kinase pathway and activation of transcription factors (SRF/Elk-1 and CREB) on the promoter of Fos (and other IEGs). Cue- and context-specific information is conveyed by specific patterns of excitatory afferent inputs to receiving neurons that integrate the combined activities of these inputs. Only the small subset of neurons that receives the strongest and most persistent integrated inputs during behavior produces sufficiently high sustained levels of calcium influx to activate the MAP kinase pathway and activation of the c-*fos* promoter. We identify these Fos-expressing neurons using immunohistochemical labeling for Fos protein or in situ hybridization for Fos mRNA and hypothesize that they form a functional unit that we define as Fos-expressing ensembles. Less activated neurons that do not express Fos are not part of this type of ensemble. In general, only 1–5% of all neurons in a brain area are sufficiently activated during behavior to induce expression of Fos mRNA or protein (Cruz et al., 2013, 2014a).

Cue- and context-induced reinstatement of alcohol seeking induces expression of c-*fos* and other IEGs as markers of neuronal activity in discrete populations of neurons in the prefrontal cortex, amygdala, hippocampus, lateral hypothalamus, and nucleus accumbens (Hamlin et al., 2007, 2009; Marinelli et al., 2007; Millan et al., 2010). *In vivo* electrophysiology studies indicate selective activation of different neurons in the nucleus accumbens by alcohol versus water self-administration and by alcohol-related cues (Janak et al., 1999; Robinson and Carelli, 2008). While many more activated neurons can be detected using *in vivo* electrophysiology, it is thought that only a small subset of them are sufficiently activated to undergo molecular alterations such as c-*fos* induction. Nevertheless, while *in vivo* electrophysiology and c-*fos* and other IEG markers are useful for identifying these ensembles, they provide only correlational evidence of their relevance to alcohol-related behaviors.

To assess causal roles for Fos-expressing ensembles, we developed the Daun02 inactivation procedure for selectively manipulating only the Fos-expressing neurons that are strongly activated