Optogenetics reduces alcohol consumption in rats

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Low-frequency (5Hz) optical activation of dopamine neurons significantly delays the first drink and reduces a drinking binge by 54%.

Alcohol consumption has been shown to be implicated in more than 60 human medical conditions, including malignant cancers, neuropsychiatric and cardiovascular disorders, gastrointestinal disease, fetal alcohol syndrome, and intentional and unintentional injuries. Despite the prevalence of alcohol consumption and abuse, the underlying neurochemical alterations that play a primary role in developing and maintaining such abuse are not known.

Over the last two decades, research has pointed to dopamine (DA) as the primary neurotransmitter that is involved in positive reward and pleasurable conditions. The VTA (ventral tegmental region) is an important region of the dopamine system that is connected by dopaminergic neurons to another brain area, the nucleus accumbens (NA). This area plays an integral role in the regulation of motivated behaviors, and alterations of this circuit have long been hypothesized to contribute to the cause of alcoholism. However, until recently technical limitations have made it impossible to investigate the causal role of DA transmission in mediating these behaviors. Using newly developed optogenetic tools, we have explored the causal relationship between specific patterns of VTA dopamine cell activation using light and abusive drinking behaviors in rats.

Many studies have implicated a DA mechanism in the regulation of ethanol drinking. However, it is not possible to selectively stimulate DA release from discrete DA neurons using electrical or chemical stimulation. This is absolutely necessary to test the causal link between DA neuron activation and drinking. Optogenetics has allowed us to optically stimulate dopaminergic cells with exceptional selectivity and temporal precision.

While several transgenic strategies have been developed to target opsins (light-sensitive proteins) to dopaminergic neurons in rodents, we have developed a novel viral approach that can be used in any mammalian species and potentially even humans. Here, the expression of channelrhodopsin-2 (ChR2), a mutated cell membrane protein that opens ion channels in response to light, was driven by a tyrosine hydroxylase (TH) promoter. The TH promoter confined production of ChR2 to only dopamine neurons, and not any other type of neurons. We applied laser light to the VTA region to stimulate DA transmission.

We showed that we could deliver the ChR2 gene to dopamine neurons, which allowed us to use the light to selectively control the timing/amount of DA released in the NA without affecting DA dynamics in the neighboring area (in the bordering brain region). We also showed that we could use optogenetic activation of the VTA to reduce the consumption of ethanol in rats. Finally, we demonstrated that only specific optogenetic light pulses (frequency and duration) succeeded in affecting drinking behavior.

We used two different optogenetic protocols, which mimicked natural DA concentration changes, and which we labeled phasic and tonic (see Figure 1). Using 4ms laser pulses at 473nm in both protocols, light pulses (50 pulses at 50Hz) are produced in the phasic protocol: see Figure 1(A). Light pulses (250 pulses at 5Hz) are produced in the tonic protocol: see Figure 1(B).

Figure 1. Light activation of ventral tegmental area (VTA) dopaminergic neurons can mimic (A) phasic and (B) tonic dopamine (DA) release. The data is presented as a standard error of mean (SEM) where the mean is in red and the two sets of SEM points are in black. (Reproduced with permission.)

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Figure 2. The top panel demonstrates ethanol and water-drinking patterns of a single rat during three separate sessions that were performed two days apart: (A, C) two sessions were with no stimulation; (B) one session was with optical stimulation of the VTA at 50Hz frequency. The bottom panel shows drinking patterns from analogous sessions with 5Hz frequency stimulation: (D, F) two sessions were with no stimulation; (E) one session was with VTA stimulation at 5Hz frequency. The blue bars indicate stimulation times. (Reproduced with permission.2)

Figure 1 shows the resulting DA concentration measured in the NA due to the light applied to the VTA region. The large difference in effect these two protocols have on the rat ethanol drinking behavior is well illustrated by a series of plots of the number of licks of ethanol that an ethanol-abusing rat would take when placed in a drinking cage for 30 minutes (see Figure 2). Comparing the number of licks taken after no stimulation, or after stimulation by either the phasic or tonic protocol, it is clear that a dramatic reduction in ethanol consumption occurred through tonic optogenetic control (see Figure 2). While the number of licks (consumption) of water remained unaffected by either protocol, the tonic protocol, applied during the first 10 minutes in the drinking cage, significantly reduced the number of ethanol licks (ethanol consumption) over the full 30 minutes. Note that the phasic protocol had no effect on ethanol consumption: see Figure 2(B). Using the tonic protocol reduced ethanol consumption by 54% and doubled the time to the first lick of ethanol.

In summary, we have shown the powerful potential of optogenetics to improve our understanding of neurocircuitry central to the risk-reward behavior of alcohol abuse. Future work will investigate several questions, including whether this effect is exclusively mediated in the NA, and whether it is dependent on changes in the signaling of either or both of the cell-surface dopamine receptors (D1R or D2R).

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Keith Bonin is a physics professor specializing in optics, microscopy, and biophotonics. His recent research has been in optogenetics, fluorescent imaging methods (fluorescence recovery

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Evgeny Budygin is an assistant professor specializing in neurochemistry and neuropharmacology. His recent research is in behavioral neuroscience applied to exploring dopaminergic mechanisms of alcohol addiction using optogenetics, and measuring dopamine dynamics in rodent brains in real time.

Jeff Weiner is a physiology and pharmacology professor. The focus of his research is to employ animal models along with sophisticated behavioral and neurobiological approaches to unravel the brain changes that contribute to alcohol addiction vulnerability.

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Caroline Bass is an assistant professor in pharmacology. Her research focuses on understanding the way the brain changes in response to substance abuse using advanced molecular manipulations in animal models. She specializes in the development of novel viral vector systems to manipulate gene expression, cell function, and targeted gene delivery.

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