PERSPECTIVE

Tipsy neural stem cells: chronic effects of alcohol on the brain

Neurogenesis occurs in the adult brain and is defined as the production of new neurons from a population of cells known as neural stem cells (NSCs) (Ming and Song, 2011). NSCs are also capable of self-renewal and differentiation into astrocytes or oligodendrocytes through processes known as astrogliogenesis and oligodendrogenesis, respectively. These properties of NSCs are crucial not only during development, where they drive the formation of neural connections and brain growth, but also throughout life to generate new neurons and glia in several key regions of the brain. The two main regions extensively studied for adult neurogenesis are the subventricular zone of the lateral ventricle and the subgranular zone of the hippocampus. NSCs have also been found in several other regions of the central nervous system, including a novel region recently identified as a neurogenic niche, the tanycyte layer of the hypothalamus (**Figure 1**) (Robins et al., 2013). Due to the integral role of NSCs in brain maintenance and repair, these cells have been investigated in neurodegenerative diseases, neurotrauma, aging, and addiction (Crews and Nixon, 2003; Zhang et al., 2017). However, the effects of drugs of abuse, specifically alcohol, on NSCs remains largely elusive.

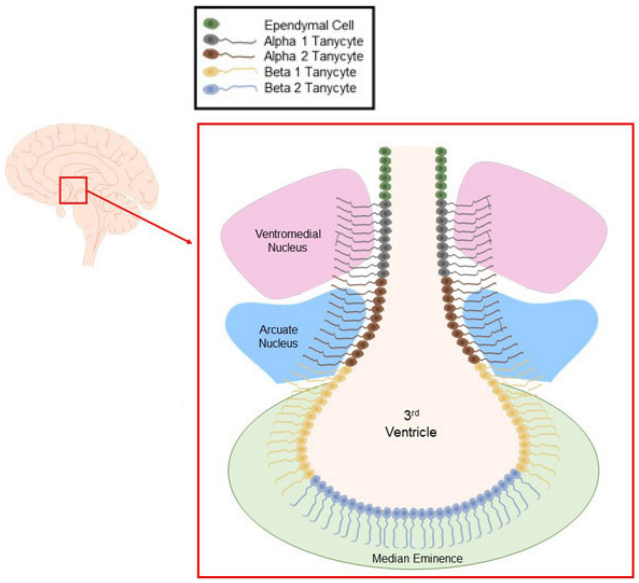

Figure 1 Representative image for the hypothalamic neurogenic niche.
The hypothalamus is highlighted by the red box and the enlarged image is a representative figure of the tanycyte layer of the third ventricle as shown by the red arrow.

Alcohol abuse is a highly prevalent substance use disorder with long-term consequences and increased risk for relapse following abstinence. It is estimated that in 2015, around 15 million adults (18 years or older) in the United States have alcohol use disorder. Alcohol is a small, 46 Da, hydrophilic molecule and as such, can easily cross the blood-brain barrier to have widespread effects in the brain. Once present, it is rapidly metabolized by enzymes, found primarily in the liver but also in the brain, into an active metabolite, acetaldehyde, which is carcinogenic and neurotoxic. Uncovering mechanisms by which NSCs are damaged by alcohol abuse has proven difficult. One such mechanism could be due to the production of reactive oxygen species following metabolism of alcohol into acetaldehyde. It has been shown that NSCs are susceptible to oxidative stress and this can lead to mitochondrial DNA damage (Wang et al., 2011). The resulting mitochondrial DNA damage, in combination with elevated reactive oxygen species levels, can alter NSC differentiation into glial rather than neuronal cells. Another mechanism could be related to cytotoxicity caused by increased intracellular calcium release, driven by activation of gamma-aminobutyric acid receptors. Overall, alcohol's role in the loss of NSCs still remains elusive and has not been narrowed down to a specific mechanism.

An additional layer of complexity when studying alcohol use is tolerance, where individuals who have long-term and repeated exposures will consume increasing amounts of alcohol over time in order to obtain comparable euphoric effects. Many studies have been conducted investigating the role of acute alcohol exposure on the NSC populations. However, due to alcohol consumption being repeated, often over long periods of time, it is of interest to focus on the effects of chronic alcohol exposure. To this end, rats with chronic exposure of alcohol have been shown to exhibit significant brain degeneration and impairment of neurogenic capabilities (He et al., 2005). Decreased proliferation of NSCs was measured by bromodeoxyuridine (BrdU) labeling, which has been used extensively to investigate NSCs because it binds to DNA of the proliferating cells. More recently, our group also confirmed the inhibitory effect of chronic alcohol consumption on NSC proliferation in transgenic mice that were induced to express a genetic reporter, yellow fluorescent protein, specifically in NSCs (McGrath, 2017). Furthermore, chronic alcohol exposure can also alter neurotransmitters and drive dependence, which leads to addictive phenotypes and relapse following abstinence (Crews and Nixon, 2003). An example of this is alterations in serotonergic neurotransmission following chronic alcohol exposure, in which decreases in neurogenesis are correlated with secondary depressive-like phenotypes in rodents (Crews and Nixon, 2003).

Similar to clinical data showing behavioral deficits after chronic alcohol exposure in humans, studies focusing on chronic exposure in rodents have shown comparable trends. Accompanying behavioral changes include cognitive decline and increased psychological deficits. One such consequence is detrimental impacts to learning and memory, which has been partially attributed to the loss of hippocampal neurogenesis (He et al., 2005). Additionally, stress is an extrinsic environmental factor, driving the development of alcohol use disorder, binge drinking, or relapse following abstinence (Crews and Nixon, 2003). However, stress can be a confounding variable when studying alcohol use because stress reduced neurogenesis in rodents even in the absence of alcohol (Crews and Nixon, 2003). Therefore, stress should be taken into consideration when using rodent models to study alcohol use, and should be minimized whenever possible.

Chronic alcohol use has been studied with multiple models, including administration of alcohol *via* oral gavage (intragastric administration), liquid diet containing alcohol, and others (Crews and Nixon, 2003; McGrath et al., 2017). Along with this, dosing during chronic administration affects NSCs, as seen by decreased proliferation, loss of NSCs, and changes in differentiation. Changes in differentiation could be the result of reduction of all cell types or shifts from a neuronal to a glial cell fate. Studies with mild to moderate doses of alcohol (blood alcohol

concentrations at 50–150 mg/dL) show decreases in NSC survival and proliferation without changes in differentiation. However, in moderate to high doses of chronic alcohol treatment, there are significant decreases in both survival and differentiation of NSCs (McGrath et al., 2017). Moderate to high doses of alcohol are considered to be the amount of alcohol that produces a blood alcohol concentration of 200–300 mg/dL, which is three to four times the legal limit in humans. While acute administration has not been shown to affect NSC survival, chronic administration of alcohol does impact survival of NSCs at multiple doses ranging from mild to moderate exposure.

The primary area previously investigated following chronic alcohol exposure is the subgranular zone of the hippocampus, due to the fundamental role it plays in learning and memory (Crews and Nixon, 2003; He et al., 2005). Recently a new neurogenic area, the tanycyte layer along the third ventricle, has gained increased scrutiny due to the discovery that certain subpopulations of tanycytes were shown to have neurogenic capabilities *via* BrdU staining and other advanced molecular techniques (**Figure 1**) (Haan et al., 2013; Robins et al., 2013). Tanycytes were divided into subpopulations, alpha 1, alpha 2, beta 1 and beta 2, due to their morphology. More research is needed to discover if all tanycytes have neurogenic capabilities or only a select population. One group has shown the alpha 2 subpopulation to express NSC-like capabilities, with these cells differentiating into primarily new astrocytes and less frequently, new neurons. However, Haan et al. (2013) showed that the beta1 cells were also capable of differentiating into new neurons, however this was a temporal increase between P4–P28 in mice. Since then, other groups have confirmed the neurogenic properties of tanycytes, or more recently, they are now known as hypothalamic neural stem cells (Zhang et al., 2017). Due to their location, lining the third ventricle, they have been primarily studied for their role in energy homeostasis and neuroendocrine functions (Rodríguez et al., 2005). The hypothalamic NSCs lining the third ventricle are exposed directly to the cerebrospinal fluid and therefore act as sensors, relaying the information back to the mediobasal hypothalamus. Our study is the first to investigate the potential role of addictive substances on the tanycyte layer, and shows that distinct NSC responses are region-dependent (McGrath et al., 2017).

Most studies conducted previously have solely focused on the response of alcohol exposure in male animals. It was of interest to us to examine both males and females, due to the fact that clinically, male and female patients respond differently to alcohol (Erol and Karpyak, 2015; McGrath et al., 2017). Therefore, we included male and female transgenic mice and were able to show significant sex differences following chronic alcohol exposure, with females being more sensitive to alcohol-induced damages in NSC survival and differentiation (McGrath et al., 2017). The transgenic mice employed in this study allowed for fate-mapping of endogenous adult NSCs using a Cre-Lox mouse model. Following administration of tamoxifen, there was constitutive expression of yellow fluorescent protein in all nestin-expressing cells. Nestin is an intermediate filament protein expressed in quiescent and proliferative NSCs, which allowed for tracking of NSCs despite proliferation or differentiation.

Further investigation is needed to understand the mechanism by which alcohol is affecting these cells, either directly or indirectly. Interestingly, we identified regionally distinct responses of NSCs to alcohol which warrants further exploration, with our study being the first to document changes in hypothalamic NSCs following alcohol exposure (McGrath et al., 2017). We also showed that NSCs in the subventricular zone are more sensitive to the effects of alcohol. Furthermore, we discovered sex differences in regards to cell differentiation, with down-regulation of astrogliogenesis as seen in females but not males. Further investigation is needed to understand hypothalamic NSCs and their role in brain function. One group has shown that loss of these cells leads to an advanced aging phenotype and decreased cognition (Zhang et al., 2017). Due to the critical role of NSCs in brain regeneration, understanding their role in addiction, as a neurodegenerative mechanism, is a key first step. By protecting or stimulating regeneration of this crucial population, identified therapeutics could then be applied to further impact human health.

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