



Cross-Species Alterations in Synaptic Dopamine Regulation After Chronic Alcohol Exposure

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Contents

1	Introduction	214
1.1	Alcohol Use Disorder	214
1.2	The Role of Dopamine in Alcohol Use Disorder Symptomology	215
1.3	Preclinical Models of Alcohol Abuse	216
1.4	Importance of Consistent Cross-Species Results	217
1.5	Alcohol Exposure Protocols Across Species	218
1.6	Tonic and Phasic Dopamine Signaling	221
2	Dopamine Signaling Following Chronic Alcohol Exposure	222
2.1	Acute Effects of Alcohol on Dopamine Release	222
2.2	Effects of Chronic Alcohol Exposure on Dopamine Release During Abstinence ...	223
2.3	Dopamine Uptake	224
2.4	Autoreceptors	226
2.5	Kappa Opioid Receptors	227
3	Conclusions	230
	References	231

Abstract

Alcohol use disorders are a leading public health concern, engendering enormous costs in terms of both economic loss and human suffering. These disorders are characterized by compulsive and excessive alcohol use, as well as negative affect and alcohol craving during abstinence. Extensive research has implicated the dopamine system in both the acute pharmacological effects of alcohol and the

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symptomology of alcohol use disorders that develop after extended alcohol use. Preclinical research has shed light on many mechanisms by which chronic alcohol exposure dysregulates the dopamine system. However, many of the findings are inconsistent across experimental parameters such as alcohol exposure length, route of administration, and model organism. We propose that the dopaminergic alterations driving the core symptomology of alcohol use disorders are likely to be relatively stable across experimental settings. Recent work has been aimed at using multiple model organisms (mouse, rat, monkey) across various alcohol exposure procedures to search for commonalities. Here, we review recent advances in our understanding of the effects of chronic alcohol use on the dopamine system by highlighting findings that are consistent across experimental setting and species.

Keywords

Alcohol · Autoreceptors · Cross-species · Dopamine · Kappa Opioid receptors · Monkey · Mouse · Nonhuman primate · Rat · Uptake

1 Introduction

1.1 Alcohol Use Disorder

Alcohol use disorder (AUD) is a medical diagnosis describing a cluster of symptoms characterized by compulsive and excessive alcohol use despite negative health and social outcomes of drinking (Dziegielewski 2010; Hagman and Cohn 2011; O'Brien 2011). AUD is a leading public health concern, affecting roughly 14% of the adult population in the United States (Grant et al. 2015). The prognosis for AUD is often poor, and it is estimated that alcohol use leads to around 100,000 deaths per year in the United States alone, and 4% of deaths worldwide, making it the third leading preventable cause of death (Mokdad et al. 2004; Rehm et al. 2009). Beyond mental and physical deterioration, alcoholism and the larger spectrum of AUD also negatively affect civil and social responsibilities and interpersonal relationships. AUDs are associated with higher incidences of stress, anxiety, depression, and other mood disorders (Hasin et al. 2007), which may contribute to the maintenance of alcohol use as an anxiolytic (Blaine and Sinha 2017). In addition, AUD is a chronically relapsing disease (Dawson et al. 2007; Pickens et al. 1985). Given the severe negative outcomes of this prevalent brain disorder, the alcohol research field has focused its efforts on understanding the neurobiology of AUD in an effort to develop effective therapeutic strategies. Here we will review recent advances in understanding the mechanisms by which alcohol exposure affects dopamine signaling to produce aberrant behaviors seen in AUD.

1.2 The Role of Dopamine in Alcohol Use Disorder Symptomology

Many drugs of abuse exert their subjective effects (euphoria, or “high”), in part, via actions on the mesolimbic dopamine system (Di Chiara and Imperato 1988; Siciliano et al. 2015b; Volkow et al. 1997). Alcohol, like many abused substances, increases extracellular dopamine concentrations in the ventral striatum, an area known to be involved in reward and motivation (Humphries and Prescott 2010; Imperato and Di Chiara 1986). Acutely, alcohol increases dopamine signaling via directly targeting ion channels expressed on dopamine neurons, which alter the currents that shape cellular communication, and result in increased firing of dopamine neurons in the ventral tegmental area (VTA), leading to increased dopamine release downstream in the ventral striatum (Brodie 2002; Brodie et al. 1990, 1999; Nimitvilai et al. 2016). Alcohol enhances hyperpolarization-activated depolarizing cation currents (I_h), which increase intrinsic activity in dopamine neurons (Okamoto et al. 2006). In addition, alcohol modulates various potassium channel (K^+)-mediated aspects of hyperpolarization (Appel et al. 2003). For example, alcohol has been shown to modulate large-conductance potassium (BK) channels (Chu et al. 1998), G-protein-coupled inwardly rectifying K^+ channels (GIRK) (Aryal et al. 2009), and K^+ -channel mediated M currents (Koyama et al. 2007). Additionally, alcohol has been shown to alter L-type Ca^{2+} channels (Hendricson et al. 2003), which may alter dopamine cell firing. While it is important to note that alcohol acutely modulates dopaminergic activity, this review will focus on the adaptations to the dopamine system induced by chronic alcohol use. Further, this review will focus on receptor and circuit level analyses; for more in-depth discussions of the molecular mechanisms underlying these alterations we point the reader towards several other helpful reviews (Morikawa and Morrisett 2010; Abrahao et al. 2017; Morisot and Ron 2017; Ron and Barak 2016).

Changes in dopamine signaling are particularly important in the context of alcohol abuse, as the dopamine system plays a key role in mediating adaptive decision-making. Dopamine neurons in the VTA are required for adaptive encoding of reward-predictive cues, which allows organisms to successfully navigate complex environments and acquire rewards, such as food (Nicola 2010; Phillips et al. 2003; Schultz et al. 1997). Dynamic activity of dopamine neurons, and dopamine release downstream in areas such as the striatum, are critical to both initial learning of reinforcement contingencies and updating the value of these contingencies as they shift over time (Cools et al. 2009; Schultz 2013). Continued alcohol use can, in some individuals, induce a maladaptive shift in contingency valuation such that the motivational saliency of alcohol is increased, leading to behaviors aimed at acquiring alcohol at the expense of more adaptive rewards. Because alcoholics show a decreased ability to make adaptive decisions – and instead continue maladaptive behavioral strategies such as alcohol seeking – the dopamine system has been implicated as a probable locus of these behavioral aberrations.

In humans, it is known that chronic alcohol use dysregulates the dopamine system. For example, alcohol abusers show greatly reduced dopamine signaling in

the ventral striatum (Diana et al. 1993; Martinez et al. 2005; Volkow et al. 1996, 2007). This reduced dopamine signaling is often referred to as a “hypodopaminergic state” and is also observed in other addictive disorders such as psychostimulant addiction (Melis et al. 2005). It is hypothesized that this low-dopamine state results in deficits in reward processing, which contributes to anhedonia during withdrawal from alcohol (Danjo et al. 2014; Schulteis et al. 1995). Because anhedonia, defined as a reduced ability to experience pleasure or reward, is thought to occur primarily in relation to non-alcohol stimuli, this may bias choices towards previously reinforced alcohol seeking behaviors over alternative options (Pierce et al. 1990; Rebec et al. 1997; Twining et al. 2015), resulting in continued and persistent alcohol use. Anhedonia, maladaptive decision-making, and alcohol seeking are *sine qua non* symptoms of AUD. The precise mechanisms by which alcohol disrupts dopamine system function are difficult to study in humans, and thus have been a major focus of preclinical alcohol abuse research.

1.3 Preclinical Models of Alcohol Abuse

A great deal of research into the neurobiological basis of AUD has been conducted in animal models, where hypotheses can be readily tested via direct measurements and manipulations of the receptors and circuits involved. Preclinical studies have overwhelmingly leveraged rodent (rat and mouse) and nonhuman primates as model organisms. Rodent and nonhuman primate models each offer specific advantages in exploring alcohol’s effects on the brain. For example, rodents can be procured and bred quickly, and are thus more practical for studies which require brain tissue to be harvested (e.g., for protein analysis, or *ex vivo* slice preparations) or where high-risk, invasive methods are needed (e.g., intracranial implants). Further, there are a wide array of tools that have been developed for use in rodents, including genetic modifications, *in vivo* microscopy, neurotransmitter sensors, and tools for manipulating neural circuit activity (Boyden et al. 2005; Flusberg et al. 2008; Koller and Smithies 1992; Wightman 1988; Wightman et al. 1976). Many of these techniques have been adapted for, and implemented in, nonhuman primates; however, they are generally less developed and often pose greater challenges (Ariansen et al. 2012; Eldridge et al. 2016; Stauffer et al. 2016).

Nonhuman primate models offer many advantages over rodents, although invasive approaches are often not feasible. First, they are genetically much closer to humans, as macaque monkeys share 95% gene homology with humans while mice share only 75% homology (Church et al. 2009). This genetic similarity manifests itself in, among other things, a high degree of correspondence in neuroanatomical structures between monkeys and humans (Seress 2007). High levels of homology increase the likelihood that discoveries will generalize from nonhuman primates to humans, compared to lower organisms. The advantages of nonhuman primates’ similarity to humans is even greater in studies of alcohol drinking, as the patterns of alcohol consumption in nonhuman primates are similar to humans (Grant et al. 2008; Majchrowicz and Mendelson 1970). While rodents metabolize alcohol at a

much faster rate than humans, nonhuman primates have similar alcohol metabolism as humans. Further, AUD is a chronic disorder which can take years to develop, and lasts a lifetime (Dawson et al. 2008). Not only is AUD long lasting, but there are also interactions between drinking and age/developmental periods. For example, age at first drink is strongly predictive of problematic drinking behaviors later in life (Dawson et al. 2008). The large disparity between the human and rodent life span can make examining the effects of alcohol exposure over long periods of time or during specific developmental periods challenging (Silberberg and Silberberg 1954). Macaques, which have been used most frequently for alcohol studies in the nonhuman primate literature, have a lifespan of 25–35 years in captivity, and thus are often more appropriate for longitudinal experimental questions (Tigges et al. 1988).

1.4 Importance of Consistent Cross-Species Results

Rodent and nonhuman primate models have provided valuable insight into the neurobiological and pharmacological basis of AUD; here, we posit that the most important insights from these literatures are the consistencies that can be observed across species. Searching for these consistencies is particularly important because the effects of chronic alcohol exposure in preclinical models is extremely sensitive to experimental parameters such as alcohol concentration, exposure length, route of administration, withdrawal length, strain of rodent or species of nonhuman primate, and many other variables that can have large impacts on the observed effects (Bonthius and West 1990; Budygin et al. 2003; Hwa et al. 2011; Kashem et al. 2012; Rimondini et al. 2003; Siciliano et al. 2016b, 2017). However, the core behavioral symptomology of AUD (excessive alcohol consumption, craving/seeking, and withdrawal behaviors) is relatively consistent across experimental parameters (Green and Grahame 2008; Le et al. 1998; Macey et al. 1996; Venniro et al. 2016), suggesting that neurochemical adaptations that are only observed under very specific experimental settings may not be driving the primary symptomology of AUD. Instead, it is likely that some of the alterations that are inconsistent across experimental setting may be “epiphenomenon” of the specific paradigm or model organism used. The large number of methodological differences across laboratories makes determining the source of any inconsistent effects difficult.

Comparing across species, strain, sex, and experimental parameters to search for consistent adaptations induced by chronic alcohol exposure is a powerful approach for filtering “noise” out of large sets of studies. Here, we will review commonalities and disparities in studies examining chronic alcohol-induced alterations to the dopamine system across multiple model organisms.

1.5 Alcohol Exposure Protocols Across Species

Each species utilized as a preclinical model of AUD described in this chapter provides unique assets to address and examine specific facets of alcoholism. The three model organisms discussed here (mouse, rat, and monkey) are distinct in terms of alcohol metabolism and intake pattern; thus, species-specific alcohol exposure protocols are often used. These protocols are described in the sections below and depicted in Fig. 1.

1.5.1 Chronic Intermittent Alcohol Exposure in Mice and Rats

Rodents (both mice and rats) will voluntarily consume alcohol under certain conditions; however, due to relatively low intakes and fast metabolism of alcohol, environmental or genetic manipulations are often required to produce high blood alcohol levels in these animals (Li et al. 1979; Penn et al. 1978; Rhodes et al. 2005, 2007). For example, selectively breeding animals with high alcohol intake has resulted in alcohol preferring strains of rats and mice (Penn et al. 1978). Environmental manipulations often include removing access to water or allowing alcohol access at specific times during the light cycle. Another approach to exposing animals to alcohol is a noncontingent method of alcohol administration in which rodents inhale tightly controlled levels of vaporized alcohol (for review see Gilpin et al. 2008). Because the levels are experimenter controlled (i.e., not dependent on the actions of the animal) this allows for titration of blood alcohol levels around a desired amount. Vapor alcohol exposure is often used to rapidly induced alcohol dependence by inducing very high blood alcohol levels (≥ 200 mg/dL) for extended periods of time (Anderson et al. 2016a; Diaz et al. 2011; Rose et al. 2016). This blood alcohol level is approximately three times the legal limit for motor vehicle operation in the United States; it is important to achieve such high blood alcohol concentrations intermittently in order to drive dependence in rodents (Griffin et al. 2009; Rose et al. 2016).

The primary utility of the vapor exposure model is that dependence-like symptoms can be induced in short periods of time, relative to models that require the animal to voluntarily drink. The exposure protocol used most often involves repeated exposures to vaporized alcohol separated by withdrawal periods. Typically, this is referred to as the chronic intermittent alcohol (CIE) vapor exposure model (Griffin et al. 2009). While the exact procedure varies between laboratories, the CIE exposure procedure often consists of exposure to vaporized alcohol for a large portion of the day (12–16 h) followed by withdrawal period (8–12 h) where room air is pumped into the chamber. This procedure is repeated daily (usually 4 consecutive days) before a longer withdrawal period (often 3 days) is imposed, for a total time of 1 week (referred to as one “cycle”). The CIE procedure in mice has typically been utilized to deliver one, two, three, four, or five cycles of alcohol vapor exposure, depending on the requirements of the study (Fig. 1a). Because mice metabolize alcohol at a high rate, to achieve desired blood alcohol levels it is necessary to inhibit the metabolic pathway of alcohol, and expose the animals to a “loading” dose of alcohol. This is typically achieved via systemic injection of the

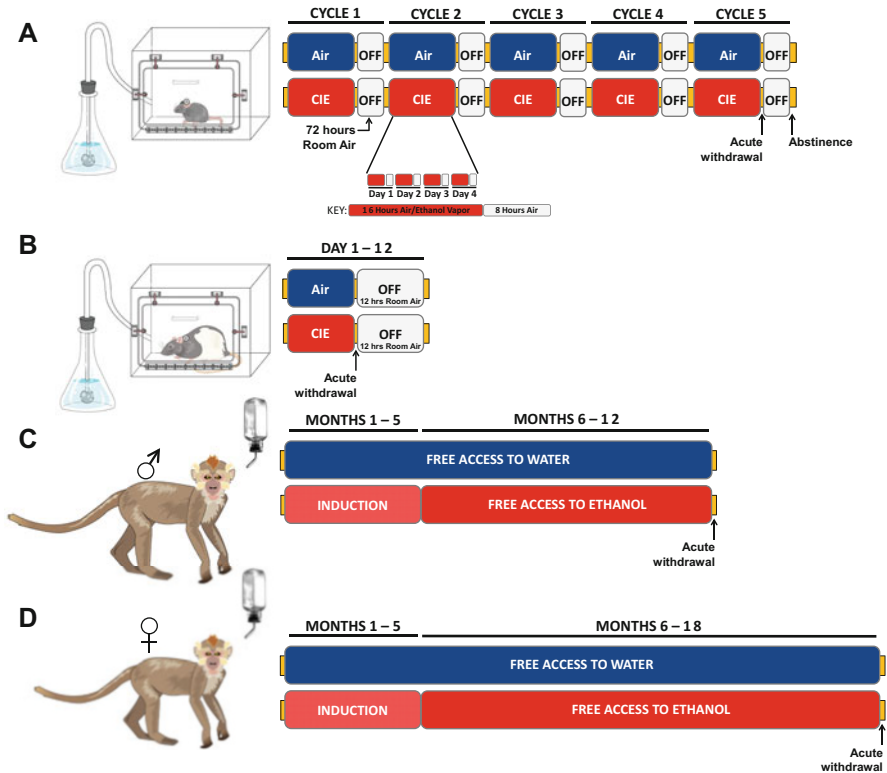


Fig. 1 Example paradigms of chronic ethanol administration across species. Schematic outline of ethanol administration in (a) mice, (b) rats, (c) male cynomolgus monkeys, and (d) female rhesus monkeys (note that many parameters vary slightly across lab and study depending on the specific hypotheses being addressed). (a, b) In order to precisely regulate blood ethanol levels and achieve high levels of ethanol intake, vaporized ethanol administration paradigms are utilized for noncontingent chronic exposure in rodents. (a) Mice undergo multiple cycles, ranging from 1 to 5, of intermittent ethanol vapor exposure (inset: daily ethanol exposure during one cycle) separated by 3 days of abstinence. In many of the experiments discussed in this review, behavioral and physiological measurements were taken during acute withdrawal, immediately following removal from the vapor chamber, or during abstinence – typically 72 h after last exposure. (b) Rats undergo a similar vapor exposure paradigm; however, daily ethanol exposure is continuous and not divided into cycles by days of abstinence. (c, d) The pattern of ethanol consumption by nonhuman primates is similar to that of humans and volitional intake paradigms offer greater insight to consummatory behaviors and subsequent physiological alterations. In many of the experiments discussed in this review, following a 5-month schedule-induced polydipsia induction period, nonhuman primates were given free access to ethanol and water for 22 h/day for either 6 or 12 months, and neurophysiological experiments examining the dopamine system are performed during acute withdrawal

alcohol dehydrogenase inhibitor, pyrazole, mixed with alcohol. In this model, it is important to include a control group that is housed in a similar chamber and given

injections of pyrazole, but not exposed to alcohol, to control for the effects of pyrazole as well as housing condition. Even though CIE exposure is a noncontingent exposure method (i.e., the animal has no choice but to be exposed to alcohol), it has been shown to drive augmented compulsive/anxiety-like behavior and an increase in alcohol drinking when animals are later given volitional access to alcohol, which suggests recapitulation of at least some aspects of alcohol dependence in humans (Anderson et al. 2016a, b; Rose et al. 2016).

Most protocols for exposing rats to alcohol vapor are very similar to mice. Rats are typically exposed to alcohol vapor for 12 h followed by 12 h of room air. This procedure is often repeated daily for 10–12 consecutive days (Gilpin et al. 2008) (Fig. 1b). Because rats do not metabolize alcohol as fast as mice, administration of pyrazole and a loading dose of alcohol is not required.

1.5.2 Chronic Alcohol Self-administration in Nonhuman Primates

Similar to rodents, there are many different procedures used to study nonhuman alcohol exposure. The most commonly used nonhuman primate model of alcohol consumption, and what we will focus on in this review, involves training animals to volitionally consume alcohol. Volitional consumption, as opposed to noncontingent exposure such as a vapor chamber or alcohol injection, is an important distinction. Indeed, humans consume alcohol volitionally, giving this approach strong face validity. Further, pattern of drug exposure and rate of onset/clearance are important factors in the pharmacological action of drugs, and can often affect the neurochemical adaptations induced by drug exposure (Allain et al. 2015; Calipari et al. 2013). Thus, allowing the animal to consume the drug in a self-imposed pattern is more likely to result in effects similar to those in humans.

The method of inducing alcohol self-administration often varies across laboratory and/or study. Generally, animals are trained to pull a lever or activate a finger-poke to receive access to a sipper containing alcohol (Grant et al. 2008; Vivian et al. 2001). In some cases, schedule-induced polydipsia is used to augment alcohol consumption during the initial exposure and training phase (Grant et al. 2008; Vivian et al. 2001). Schedule-induced polydipsia involves simply delivering small amounts of food at spaced intervals. Because most mammals tend to increase fluid consumption during times of feeding, the increase in number of feeding bouts produces a robust increase in fluid consumption (Falk 1966). Once trained to consume alcohol, animals are then allowed to drink, either freely with continuous access to alcohol in the home cage, or in daily sessions where alcohol becomes available. In this review we will primarily discuss studies in which nonhuman primates were given 22 h/day access to alcohol in the home cage, for a period of 6–18 months (Fig. 1c, d). Importantly, this model produces robust individual differences in alcohol intake between animals and between days within each animal. This allows for determining the effects of alcohol across a range of intake, as well as the factors that may predict individual differences in alcohol consumption (Cuzon Carlson et al. 2011; Grant and Bennett 2003; Grant et al. 2008; Nimitvilai et al. 2017; Pleil et al. 2015b; Vivian et al. 2001; Baker et al. 2014, 2017).

1.6 Tonic and Phasic Dopamine Signaling

Dopamine neurons originating from the VTA have two distinct types of firing patterns, tonic or phasic firing. Tonic firing is characterized by the periodic occurrence of single action potentials (2–5 Hz), while phasic firing is characterized by bursts of action potentials (10–25 Hz) occurring in close temporal proximity (Grace and Bunney 1983, 1984). These two types of signaling are critical in regulating reward processing and internal state, and can be controlled by both changes in VTA firing and local modulatory mechanisms directly at dopamine terminals in the nucleus accumbens (NAc; a subregion of the ventral striatum) (Exley and Cragg 2008). Below we will discuss the different methods for measuring tonic and phasic dopamine signaling.

Tonic dopamine levels, often referred to as extracellular levels, are comparatively low (usually 5–20 nM) and can be measured with relatively low temporal resolution over several minutes using techniques such as *in vivo* microdialysis. To conduct microdialysis, a concentric perforated probe is implanted into the area of interest. Artificial cerebrospinal fluid is perfused into the region of interest; neurotransmitters, such as dopamine, diffuse down their concentration gradient across this perforated membrane. This fluid is then collected over 5–30 min and analyzed using detection methods such as high performance liquid chromatography or mass spectrometry, which allow for quantification of analytes (e.g., neurotransmitters) within the sample. Changes in tonic dopamine levels have been shown to predictably alter thresholds for intracranial self-stimulation, which is used to monitor the function of brain reward systems and measure the motivational state of the animal, suggesting that tonic dopamine levels are involved in reward sensitivity and affective states (Carlezon and Chartoff 2007; Hernandez et al. 2012; Kokkinidis and McCarter 1990; Negus and Miller 2014; see Dobrossy et al. 2015 for review).

While microdialysis can give information about relative levels of synaptic neurotransmitters, it is important to understand how receptors and local regulation of dopamine neurons are influenced by alcohol exposure. *Ex vivo* fast-scan cyclic voltammetry (FSCV), typically performed in coronal brain slices, allows for measuring experimenter-stimulated dopamine release when the dopamine terminal is isolated from its endogenous inputs (due to severing these connections in the slicing process). FSCV detects electroactive analytes (including dopamine) by applying voltage to a microelectrode, which drives oxidation of dopamine to dopamine-o-quinone; the oxidation of dopamine results in the loss of electrons which are detected at the electrode as a change in current which is proportional to the concentration of dopamine molecules near the surface of the electrode. Thus, based on the electroactive properties of dopamine, FSCV is able to detect dopamine levels with high specificity, even within a heterogeneous environment of transmitter signaling. This detection can occur quickly (typically sampled at 10 Hz) allowing for information to be obtained about real-time dopamine release and clearance kinetics. With this technique, receptors expressed on dopamine terminals can be pharmacological targeted and their effects on the kinetics of dopamine signaling can be assessed (for review of the utility of *ex vivo* voltammetry see Ferris et al. 2013).

Phasic firing refers to bursts of activity that result in high concentrations (estimated to be around 100 μM) of dopamine within the synapse (Grace et al. 2007). These phasic dopamine signals are particularly important in the case of addiction where they act to encode information not just about rewards, but also about cues that predict their availability. For example, reward conditioning experiments have shown that phasic dopamine responses occur immediately following presentation of unexpected rewards; however, after multiple pairings of cue and reward, phasic dopamine responses shift to the cue predicting the reward instead of the reward itself (Phillips et al. 2003; Schultz 1998). Thus, understanding how this type of signaling is dysregulated by alcohol has implications not only for subsequent alcohol use, but also for decision-making and reward seeking outside of alcohol-related contexts.

Thus, understanding phasic and tonic dopamine signaling is crucially important as their interplay controls the execution of motivated behaviors, and the examination of these two aspects of dopaminergic signaling in tandem allows a greater understanding of the alcohol-induced maladaptive responses to external stimuli.

2 Dopamine Signaling Following Chronic Alcohol Exposure

2.1 Acute Effects of Alcohol on Dopamine Release

Acute alcohol administration has distinct, regionally specific effects on dopamine system activity. Systemic alcohol administration transiently increases extracellular tonic levels of dopamine in the NAc of rodents and monkeys as measured by microdialysis (Bradberry 2002; Karkhanis et al. 2016; Weiss et al. 1993; Yim et al. 1998). Similarly, an *in vivo* FSCV study in awake, behaving rats showed that alcohol administration resulted in an increase in phasic dopamine release (Shnitko and Robinson 2015). In contrast, *ex vivo* FSCV studies show that acute application of alcohol to brain slices results in a *reduction* of phasic dopamine release in the NAc of both rodents and monkeys (Siciliano et al. 2016b; Yorgason et al. 2014, 2015), which is dependent on alcohol concentration and frequency of stimulation. Reductions in dopamine release in these experiments were observed only at high concentrations of alcohol (80 mM and above) and during high frequency of stimulation (20 Hz and above) (Yorgason et al. 2015). While these findings may seem in opposition, the inconsistencies between *in vivo* (enhanced release) and *ex vivo* (reduced release) are likely driven by the fact that dopamine terminals are separated from the cell body in *ex vivo* slice preparations. In the *ex vivo* slice preparation, effects that are observed represent only the synaptic connections that are maintained within the slice, and do not assess the full spectrum of circuit connectivity between the region of interest and the rest of the brain. Without the contribution of alcohol-mediated excitation of VTA neurons to augment dopaminergic signaling, NAc terminals are inhibited by alcohol. However, the net effect of alcohol in the intact animal (i.e., inhibitory actions at the terminal and excitatory actions at the cell body) is increased extracellular dopamine levels in the

striatum. These findings highlight both the complexity of this system and the need for multiple levels of exploration (from the slice to the whole animal) in order to fully appreciate the pharmacological actions of alcohol on the dopaminergic system. For more information on alcohol's presynaptic actions, including non-dopaminergic systems, see Lovinger (2017) elsewhere in this volume.

2.2 Effects of Chronic Alcohol Exposure on Dopamine Release During Abstinence

While the section above outlines the acute effects of alcohol on dopamine release, chronic use leaves a lasting impact on dopamine release during alcohol-free periods, which may contribute to maladaptive decision making. In this section we will outline literature which has examined dopamine release in animals with a history of alcohol exposure, when there is no alcohol "on board" (i.e., during withdrawal).

Studies examining the role of chronic alcohol exposure on dopamine release have generally yielded mixed results, with species, sex, and experimental design appearing to have a strong influence on the findings. For example, stimulated dopamine release in *ex vivo* slices preparations was attenuated following three to five cycles of CIE exposure in mice (Karkhanis et al. 2015; Rose et al. 2016; but see Melchior and Jones 2017). However, in rats, shorter exposure to alcohol vapor over 5 or 10 days did not alter dopamine release as compared to control animals (Budygin et al. 2007). Data from nonhuman primates further "muddies the waters" in regard to interpreting the effects of alcohol exposure on stimulated dopamine release. In contrast to the decreased release observed in mice, male cynomolgus macaques were found to have increased stimulated dopamine release following 6 months of alcohol self-administration (Siciliano et al. 2015a). Complicating matters further, female rhesus macaques showed no change in dopamine release after 12 months of alcohol self-administration (Siciliano et al. 2016b). The driving factor underlying the inconsistency within the nonhuman primate studies is currently unclear, as sex, length of exposure (6 vs 12 months), and species (cynomolgus vs rhesus) were all divergent between the two studies.

Although these seemingly inconsistent results may suggest that alterations in dopamine release are not related to the primary pathology of AUD, it should also be noted again that *ex vivo* measurements of dopamine release can only give insight into certain aspects of the system. Indeed, many *in vivo* studies of dopamine release in response to stimuli have demonstrated that release can encode many different aspects of learned behaviors and drug associated cues, and is plastic throughout the formation of these associations (Wanat et al. 2009). Because the afferent inputs that drive dopamine release *in vivo* are severed in an *ex vivo* slice preparation, *ex vivo* approaches provide insight to the size of the readily releasable pool of dopamine, but do not capture the complexity of dopaminergic encoding of these behaviors. It is also important to note that the tonic, extracellular level of dopamine is dependent on many factors (discussed below); when tonic dopamine levels are measured via microdialysis, multiple studies have found them to be decreased following alcohol

exposure in rats (Rossetti et al. 1992, 1999). Further, metabolic markers of dopaminergic activity are reduced in macaques after chronic alcohol use (Cervera-Juanes et al. 2016).

2.3 Dopamine Uptake

Extracellular dopamine levels are a complex interaction between dopamine release and its reuptake via the dopamine transporter (DAT) (Ferris et al. 2014). Not only is the DAT a major factor in determining tonic extracellular levels, but it is also thought to tightly regulate the “sphere of influence” and duration of dopamine effects at postsynaptic receptors as it flows out of release sites (Cragg and Rice 2004). Thus, DAT function and expression is an integral component in guiding dynamic dopamine neurotransmission.

Exposure to chronic alcohol modulates the DAT, both in terms of its function and expression. In mice, repeated cycles of CIE exposure augments dopamine reuptake, which is a primarily DAT mediated process, in the NAc (Karkhanis et al. 2015, 2016; Rose et al. 2016; Melchior and Jones 2017). In CIE models, uptake rate is enhanced immediately following cessation of the final alcohol exposure and are maintained for at least until 72 h into withdrawal, suggesting that this effect may be long lasting, though later time-points have not yet been tested (Karkhanis et al. 2015; Rose et al. 2016) (Fig. 2). Further, DAT density in the NAc is increased after CIE (Healey et al. 2008). Enhanced dopamine uptake rate likely contributes to reduced tonic dopamine levels by increasing the speed of dopamine removal from the extracellular space.

Enhanced dopamine uptake rate following alcohol exposure is a phenomenon that is strongly conserved across species. Similar to mice, dopamine uptake rate is increased in rats exposed to CIE vapor (Budygin et al. 2007). In rats exposed to an alcohol-containing liquid diet for 1 year, DAT protein expression was increased in both the ventral and dorsal striatum (Rothblat et al. 2001), suggesting that increased functional uptake is a result of increased protein levels, although other mechanisms such as conformational alterations or changes in the affinity state of the DAT could also be at play. These findings have been extended to nonhuman primates, where dopamine uptake rate has been found to be increased in the NAc of male cynomolgus macaques and female rhesus macaques after 6 and 12 months of volitional access to alcohol, respectively (Siciliano et al. 2015a, 2016b). Together, these data demonstrate that, in the NAc, dopamine uptake is increased across species and experimental setting, suggesting that it may be an important factor in driving the core symptomology of AUD.

One exception is that in male cynomolgus macaques, uptake rate in the dorsolateral caudate (a subregion of the striatum typically thought to be involved in motor control and habit learning (Graybiel 1995, 2008; Porrino et al. 2004) were *reduced* after chronic access to alcohol (Siciliano et al. 2015a)). This decrease in dopamine uptake rate likely produces increases in extracellular dopamine levels. Interestingly, the ratio of uptake rates between the dorsolateral caudate and NAc was highly

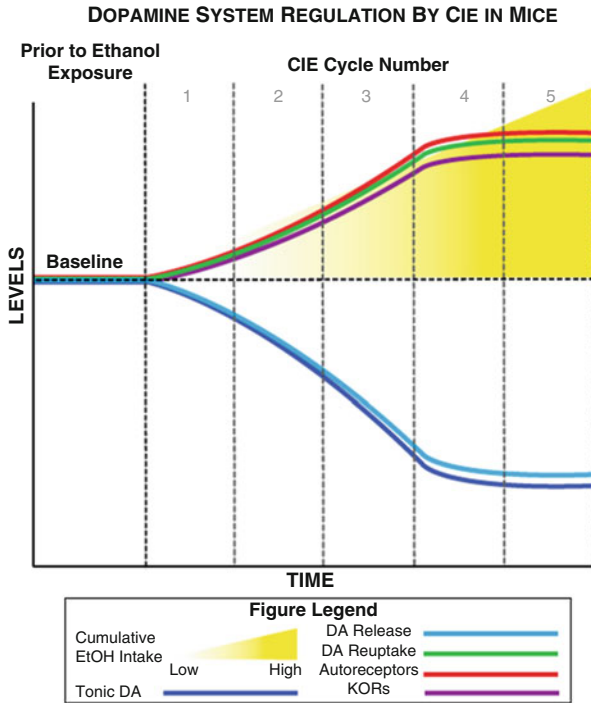


Fig. 2 Alterations in NAc dopamine signaling in mice following successive cycles of CIE. This timeline represents schematized alterations in dopamine system regulation with increasing exposures to chronic intermittent ethanol cycles. With increasing cumulative ethanol exposure, tonic dopamine levels (measured via microdialysis) and stimulated dopamine release (measured via FSCV) are diminished, but dopamine uptake rate is enhanced, resulting in faster clearance of dopamine from the synaptic cleft. In addition, KOR and D2/D3 autoreceptor function is also augmented. Together these factors give rise to a systemic hypodopaminergic state in animals exposed to chronic intermittent ethanol. These effects appear to be dose-dependent on total cumulative ethanol exposure until they plateau after approximately three to five cycles. *DA* dopamine, *EtOH* ethanol, *KORs* kappa opioid receptors

correlated with alcohol intake across animals. While the behavioral relevance of differential changes in dopamine uptake between these two regions remains to be determined, there is evidence that dysregulation of dopamine-mediated communication between these areas can lead to habitual and addiction-like behaviors (Belin and Everitt 2008; Everitt and Robbins 2013). Thus, enhancement of NAc dopamine reuptake and blunting of dorsolateral caudate dopamine reuptake together may contribute to maladaptive alcohol seeking and intake following chronic exposure.

Not only is dopamine uptake altered by chronic alcohol administration in pre-clinical models, there is also evidence that abnormal dopamine uptake may contribute to disease states in the human population. Genetic association studies point to alterations in the DAT in a subset of individuals with an AUD diagnosis, which may confer a heightened vulnerability towards development of the disease (Köhnke et al.

2005). The relationship between DATs and alcoholism in humans is not entirely clear, however. Alcoholism is associated with both increases and decreases in DAT availability as measured by positron emission tomography (PET) or single photon emission computed tomography (SPECT), depending on the study (Laine et al. 1999; Tiihonen et al. 1995; Tupala et al. 2006; Yen et al. 2015, 2016). These discrepancies may be due, at least in part, to different durations of withdrawal between studies and limitations of lower resolution, noninvasive approaches which are typically employed in human studies. However, given the abundant preclinical data showing alterations in uptake rate following chronic alcohol exposure discussed above, and implications for genetic DAT alterations in the clinical population, it is clear that the DAT plays a significant role in the etiology of AUD that should be further examined in future studies.

2.4 Autoreceptors

In the NAc, dopamine D2-type (D2, D3, D4) receptors are found on medium spiny projection neurons, local interneurons, and presynaptic terminals from afferent inputs (Alcantara et al. 2003; Ford 2014; Levey et al. 1993). D2-type receptors that are located on presynaptic dopamine terminals are autoreceptors, which function in a feedback-inhibitory manner, binding released dopamine and inhibiting future release. D2-type dopamine autoreceptors are G-protein coupled receptors expressed at both cell bodies and presynaptic terminals of dopamine neurons, where they inhibit action potential firing activity, release and synthesis of dopamine. Thus, in the NAc, when extracellular dopamine levels are high, autoreceptors inhibit dopamine release and synthesis, driving the system towards homeostasis. For this reason, D2-type autoreceptors are often conceptualized as the “brakes” on the dopamine system. Of the members of the D2-type receptor family, D2 receptors themselves have been found to mediate the majority of autoreceptor activity in the striatum, although D3 and D4 receptors are also present (Bello et al. 2011; Meador-Woodruff et al. 1994; Meller et al. 1993; Rubinstein et al. 1997).

Most studies examining D2-type autoreceptor sensitivity following chronic alcohol exposure have shown functional increases in activity/sensitivity, contributing to a reduction in dopamine signaling. Multiple cycles of CIE exposure result in greater autoregulation of release in the NAc in mice (Karkhanis et al. 2015), but shorter exposure times did not change the ability of autoreceptors to inhibit dopamine release in rats (Budygin et al. 2007) or their ability to inhibit dopamine synthesis in mice (Siciliano et al. 2017). Repeated CIE-induced increases in autoreceptor sensitivity appears to be relatively short-lived during abstinence, however, with sensitivity returning to control levels within a few days (Karkhanis et al. 2015). Typically, the sensitivity of these receptors has been assessed by examining the ability of D2-type dopamine receptor agonists to inhibit dopamine release *ex vivo*. When greater effects of D2-type specific agonists are observed, it is interpreted as an increase in the sensitivity of these receptors, which translates to increased inhibitory feedback when endogenous dopamine interacts with these receptors. Thus, increased

sensitivity of D2-type autoreceptors likely contributes to a hypodopaminergic state via decreased probability of dopamine release from presynaptic terminals.

Similar findings have been observed in nonhuman primate models of AUD. In one study of male cynomolgus macaques it was found that, after 6 months of volitional alcohol drinking, there was no change in overall autoreceptor-mediated inhibition of dopamine release; however, there was a shift in the relative contribution of D2 vs D3 dopamine autoreceptors towards D2 receptors (Siciliano et al. 2016b). In other words, D2 sensitivity was increased, and D3 sensitivity was decreased such that the sum of autoreceptor inhibition remained the same while the contribution of the two receptor subtypes was shifted. In two studies of monkeys exposed to longer, 12- to 18-month periods of alcohol drinking, D2-type autoreceptor sensitivity was increased, but the relative contributions of receptor subtypes were not queried (Budygin et al. 2003; Siciliano et al. 2016a). Thus, it appears that, across species, overall changes in autoreceptor sensitivity occur after extended exposure to alcohol, but not after modest exposure lengths. These consistent cross-species findings of dopamine autoreceptor changes, with consistent relative time-courses and direction of change towards greater dopamine inhibition, provide confidence that autoreceptor changes may be functionally related to the core symptomatology of AUD.

2.5 Kappa Opioid Receptors

2.5.1 Dopamine and Kappa Opioid Receptor Interactions

Like D2/D3 autoreceptors, kappa opioid receptors (KORs) are located on dopamine terminals (Ebner et al. 2010; Svingos et al. 2001; Werling et al. 1988) and act to reduce dopamine release. KOR activation results in reduced tonic dopamine levels as well as both decreased probability and magnitude of phasic release events (Steiner and Gerfen 1996). Given the role of dopamine in reward, it is not surprising that the activation of KORs, which inhibit dopamine release, is generally aversive (Land et al. 2009). Dynorphin, the endogenous ligand of KORs, is released during exposure to painful, noxious, or stressful stimuli (Bruchas and Chavkin 2010; Land et al. 2009; Nabeshima et al. 1992). KORs are believed to exert their behavioral and subjective effects in part through inhibition of dopaminergic signaling (Ebner et al. 2010; Svingos et al. 2001; Werling et al. 1988).

In the NAc, dopamine, dynorphin, and KORs have an intimate relationship of interconnectivity and feedback regulation. GABAergic medium spiny neurons (MSNs) account for more than 90% of all neurons in the striatum and are the major projection population. Striatal MSNs consist of two distinct populations, which are interspersed and equally distributed across the dorsal and ventral striatum, and differentiated based on the expression of dopamine receptors and opioid peptides. Approximately half of the MSN population expresses excitatory D1-type dopamine receptors and dynorphin peptides, and the other half of MSNs express inhibitory D2-type receptors and enkephalin peptides (ligand for mu and delta opioid receptors) (Gerfen et al. 1990). Dynorphin is generated in D1-MSNs in response to D1 receptor activation, and its release inhibits further dopamine release from the

presynaptic terminal (Gerfen and Surmeier 2011; Steiner and Gerfen 1996). Dynorphin peptides are transported to recurrent collateral axons within the NAc and decrease dopamine release via presynaptic KORs (Steiner and Gerfen 1993). In this way, a feedback loop wherein dopamine release can increase the probability of dynorphin release, which in turn reduces dopamine release, is propagated.

2.5.2 Kappa Opioid Receptors and Chronic Alcohol Exposure

In humans, activation of KORs has been associated with feelings of dysphoria, and both KORs and dynorphin mRNA are upregulated in patients suffering from AUD (Bazov et al. 2013). In preclinical models, a single injection of alcohol in naïve rats results in a transient increase in dynorphin levels (Kuzmin et al. 2013; Lam et al. 2008; Marinelli et al. 2006). Following CIE exposure, inhibition of KORs has been shown to successfully mitigate negative affect-like behavioral alterations observed in mice and rats, without altering these behaviors in alcohol-naïve animals (Anderson et al. 2016b; Pleil et al. 2015a; Rose et al. 2016; Kissler et al. 2014). Interestingly, the effect of KOR activation on alcohol intake behaviors appears to be strongly influenced by the animal's history of alcohol intake and state of dependence. Pharmacological blockade of KORs does not change alcohol consumption in non-alcohol-dependent animals; however, in animals that have been made dependent through CIE exposure, and show high dependence-induced volitional alcohol intake, KOR blockade reduces alcohol consumption to control levels (Rose et al. 2016; Walker et al. 2011; see Anderson and Becker 2017 for review). Conversely, in naïve animals, pretreatment with KOR agonists drives an increase in alcohol consumption comparable to animals that have been previously made dependent, again suggesting that alcohol-induced increased activity of these receptors is associated with excessive alcohol intake (Anderson et al. 2016b; Rose et al. 2016).

Behavioral studies, outlined above, have suggested that KORs are functionally altered by previous alcohol exposure, and subsequent studies directly measuring KOR regulation of dopamine signaling after chronic alcohol exposure support this hypothesis (Karkhanis et al. 2016; Rose et al. 2016; Siciliano et al. 2016a). Indeed, in mice and rats, the dopamine-decreasing effects of KOR activation, observed using *ex vivo* FSCV, are heightened dramatically following CIE exposure (Karkhanis et al. 2016; Rose et al. 2016). Thus, alcohol exposure augments the ability of KORs to reduce dopamine, contributing further to hypodopaminergia following chronic alcohol exposure. Importantly, dependence-induced increases in alcohol consumption can be reduced via microinfusion of a KOR antagonist directly into the NAc (Nealey et al. 2011). This suggests a causal role for increased KOR function in the NAc in aberrant alcohol consumption.

Augmentation of KOR sensitivity is conserved across species. Following 6 months of drinking in male cynomolgus macaques, KOR-mediated inhibitory regulation of dopamine signaling in the NAc is increased (Siciliano et al. 2016a). Further, KOR-mediated inhibition of dopamine release in the dorsolateral caudate is augmented after alcohol drinking (Siciliano et al. 2015a). Interestingly, while both regions appear to develop KOR hyper-function, KOR activity in the NAc, but not the dorsolateral caudate, is correlated with alcohol intake (Siciliano et al. 2015a), again

suggesting that KOR signaling in the NAc is a critical node in driving excessive alcohol intake. Further, these effects are consistent across sex, as 1 year of alcohol self-administration also increased KOR regulation of dopamine release in the NAc of female rhesus macaques (Siciliano et al. 2016a). Together, these studies demonstrate that KOR regulation of dopamine release is increased across species as well as several other experimental parameters, including route of administration, exposure length, and withdrawal time, suggesting an integral role in the etiology of AUD. We hypothesize that alcohol dependence-induced increases in KOR function contribute to a hypodopaminergic state possibly leading to craving and excessive consumption of alcohol. These alterations in KOR changes at the dopamine terminal are illustrated in Fig. 3.

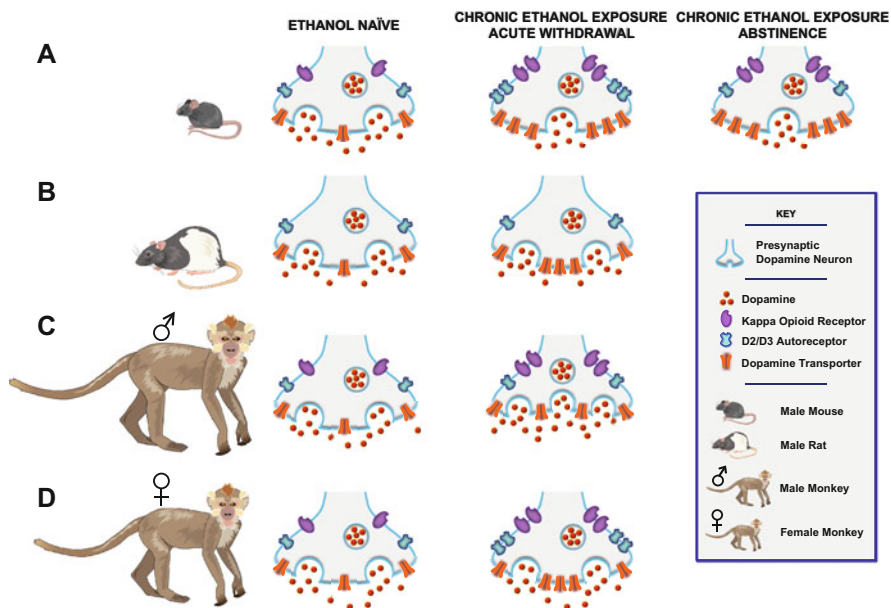


Fig. 3 Putative synaptic alterations associated with chronic ethanol exposure across species. Synaptic changes in the NAc associated with withdrawal from chronic alcohol exposure in (a) male mice, (b) male rats, (c) male cynomolgus monkeys, and (d) female rhesus monkeys. (a) In mice, acute withdrawal from chronic intermittent ethanol exposure is associated with reduced dopamine release and augmented reuptake, D2R/D3R autoreceptor, and KOR sensitivity. With the exception of autoreceptor sensitivity, most of these effects are relatively long lasting, and remain altered 72 h following the final ethanol exposure. (b) In rats, dopamine release and autoreceptor function are unaffected during acute withdrawal; however, enhanced D1R and DAT sensitivity are observed. (c) Male cynomolgus monkeys show synaptic alterations remarkably similar to male mice during acute withdrawal from chronic ethanol intake, whereby DAT and KOR functions are augmented. However, dopamine release is increased and total autoreceptor function remains unaltered. (d) In female rhesus monkeys, while there is no change in dopamine release, the DAT, KOR, and autoreceptor function is increased during acute withdrawal

The extensive alcohol-induced reductions in presynaptic dopamine terminal activity in the NAc would be predicted to result in differential signaling onto downstream, postsynaptic targets. In the NAc, these consist primarily of D1 and D2 receptor expressing MSNs, which are also altered in AUD models. These other aspects of alcohol-induced alterations in striatal activity are beyond the scope of the current chapter, but have been extensively characterized elsewhere and continue to be areas of intense investigation (Clarke and Adermark 2015; Engel and Jerlhag 2014; Koob 2014; Renteria et al. 2017; Soderpalm et al. 2009; Tupala and Tiihonen 2004).

3 Conclusions

It is clear that factors such as alcohol exposure length, species, route of administration, and withdrawal time-point can be important variables in determining the effects of alcohol on the dopamine system. However, in this chapter, we highlight some of the robust findings that transcend these variables and may hint at common neurobiological substrates following chronic exposure to alcohol. Primary examples of these commonalities include increased functionality/sensitivity of multiple negative regulators of dopamine signaling, such as DATs, dopamine autoreceptors, and KORs. The combination of increased clearance of dopamine from the synapse, via upregulation of DATs, with enhanced KOR and autoreceptor function, likely combines to produce hyper-inhibitory regulation of dopamine signaling. Thus, deviations from “healthy” function of the mesolimbic dopaminergic system following chronic exposure to alcohol appear to be a universal adaptation that may be driving maladaptive behaviors associated with repeated alcohol exposure.

The use of multiple species and paradigms offers insight into possible treatment strategies that could alleviate suffering in individuals with an AUD. As illustrated above, such diverse experimental designs help to “separate the wheat from the chaff” by identifying consistent, key neurobiological changes following exposure. To this end, we argue that successful pharmacological strategies may lie in a combinatorial pharmacotherapy that would quiet both DAT and KOR systems (e.g., a DAT inhibitor and KOR antagonist or partial agonist). Such a treatment could suppress two key contributors to dopamine signal reduction following chronic alcohol exposure, and may alleviate negative affective states that are pervasive in abstinent AUD patients. Resultant normalization of affect during withdrawal would be predicted to both improve quality of life and decrease likelihood of relapse in individuals striving to maintain abstinence.

Taken together, it is clear that key alterations in mesolimbic dopamine signaling are consistently observed following chronic alcohol exposure across species and administration paradigms. Although there is far more work to be done in order to fully elucidate the role of dopaminergic signaling in neural and behavioral adaptations following alcohol exposure, pharmacological strategies targeting these adaptations are a potentially important treatment avenue for alleviating suffering in individuals with an AUD.

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