

# GDNF and alcohol use disorder

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## ABSTRACT

Glial cell line-derived neurotrophic factor (GDNF) has been extensively studied for its role in the development and maintenance of the midbrain dopaminergic system, although evidence suggests that GDNF also plays a role in drug and alcohol addiction. This review focuses on the unique actions of GDNF in the mechanisms that prevent the transition from recreational alcohol use to abuse. Specifically, we describe studies in rodents suggesting that alcohol acutely increases GDNF expression in the ventral tegmental area, which enables the activation of the mitogen-activated protein kinase signaling pathway and the gating of alcohol intake. We further provide evidence to suggest that GDNF acts in the ventral tegmental area via both nongenomic and genomic mechanisms to suppress alcohol consumption. In addition, we describe findings indicating that when this endogenous protective pathway becomes dysregulated, alcohol intake levels escalate. Finally, we describe the potential use of GDNF inducers as a novel therapeutic approach to treat alcohol use disorder.

**Keywords** addiction, alcohol, ethanol, GDNF, mesolimbic system, nucleus accumbens, ventral tegmental area.

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## INTRODUCTION

Alcohol use disorder (AUD) is characterized by compulsive consumption of increasing quantities of alcohol despite detrimental consequences. AUD is a devastating, life-threatening psychiatric disorder affecting up to 15 percent of the population (Grant *et al.* 2017; Nutt, King, Phillips *et al.* 2010; Sridhar 2012; World Health Organization 2014). Loss of life, decreased workplace productivity and exacerbated healthcare costs due to illnesses associated with excessive drinking all contribute to significant societal and financial liabilities (McGinnis & Foege 1999; Rehm 2011; Whiteford *et al.* 2013). Substantial relief from these burdens has been delayed, as pharmacotherapeutic approaches for the treatment of AUD, which are questionably effective, are currently very limited (Bouza *et al.* 2004; Koob & Volkow 2010; American Psychiatric Association 2013). Characterizing the neuroadaptations underlying the transition from controlled social drinking to compulsive, excessive alcohol intake may therefore lead to the development of improved drug treatments for AUD. Although alcohol is widely

consumed worldwide, only a minority of consumers drink alcohol excessively, and an even smaller portion develop alcohol dependence (World Health Organization 2014; Grant *et al.* 2017). This suggests the existence of innate and/or acquired mechanisms that protect against the transition from moderate to excessive, uncontrolled, compulsive alcohol use. Here, we discuss data indicating precisely such a role for the growth factor glial cell line-derived neurotrophic factor (GDNF).

GDNF is a secreted growth factor, initially identified in a glial-derived cell line (Lin *et al.* 1993). *Gdnf* is expressed throughout the central nervous system during development, and in the adult brain, *Gdnf* is highly expressed in the striatum, thalamus, cortex and hippocampus (Pochon *et al.* 1997; Ortega-de San Luis & Pascual 2016). GDNF signals through the receptor tyrosine kinase Ret (Durbec *et al.* 1996), with the activation of Ret by GDNF also requiring the presence of the co-receptor GDNF family receptor  $\alpha 1$  (GFR $\alpha 1$ ; Jing *et al.* 1996; Airaksinen & Saarna 2002). Ligation of GDNF to Ret and GFR $\alpha 1$  leads to the activation of several intracellular signaling cascades: the mitogen-activated protein kinase

(MAPK) extracellular signal-regulated kinase 1/2 (ERK1/2), the phosphatidylinositol 3-kinase, and phospholipase C $\gamma$  (PLC $\gamma$ ) cascades (Airaksinen & Saarma 2002; Sariola & Saarma 2003). *Ex vivo* and *in vivo* data indicate that GDNF signaling depends on the presence of GFR $\alpha$ 1 and Ret in lipid raft compartments within the plasma membrane (Tansey et al. 2000; Paratcha et al. 2001; Tsui et al. 2015). Interestingly, Ret expression is restricted to the midbrain (Trupp et al. 1997; Glazner, Mu, & Springer 1998), whereas the distribution of GDNF and GFR $\alpha$ 1 are much more widespread (Ortega-de San Luis & Pascual 2016). In brain regions deficient in Ret, GDNF acts through alternate receptors, such as the adhesion proteins syndecan-3 (Bespalov et al. 2011) and neuronal cell adhesion molecule (NCAM) (Paratcha, Ledda, & Ibanez 2003).

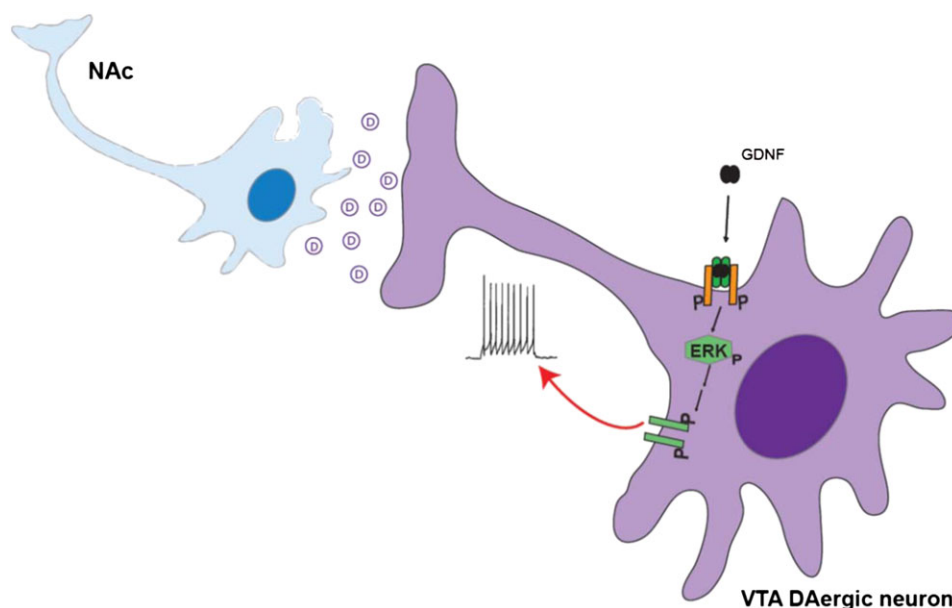
### GDNF functions

GDNF has been shown to play a vital role in peripheral neuron development (Airaksinen & Saarma 2002; Bespalov & Saarma 2007). For example, GDNF is a chemoattractant in neuronal growth cones (Dudanova, Gatto, & Klein 2010), and it plays an important role in axon guidance (Bonanomi et al. 2012). Moreover, GDNF promotes axonal growth of hippocampal and cortical neurons by signaling through NCAM (Paratcha et al. 2003), and promotes neurite outgrowth by signaling via syndecan-3 (Bespalov et al. 2011). GFR $\alpha$ 1 has been

reported to be important for development of cortical GABA neurons (Pozas & Ibanez 2005). GDNF also plays a role in the survival and maintenance of spinal neurons in the dorsal root ganglia (Molliver et al. 1997), and in nociception (Salio et al. 2014). Importantly, GDNF regulates the function of midbrain dopaminergic (DAergic) neurons, discussed in detail in the following section.

### GDNF in the mesolimbic dopaminergic system

GDNF is produced by striatal neurons (Pochon et al. 1997; Barroso-Chinea et al. 2005) and is retrogradely transported via DAergic neurons to the midbrain, namely, the substantia nigra (Tomac et al. 1995; Kordower et al. 2000), and ventral tegmental area (VTA; Wang et al. 2010), where the Ret receptor is abundant (Trupp et al. 1997). GDNF has been shown to positively regulate DAergic activity in both nigrostriatal and mesolimbic projections (Yang et al. 2001; Wang et al. 2010; Kumar et al. 2015; Fig. 1). For example, infusion of the growth factor into the VTA rapidly increased the spontaneous activity of DA neurons, resulting in increased extracellular DA levels in the nucleus accumbens (NAc), as measured by *in vivo* microdialysis (Wang et al. 2010; Fig. 1). The rapid increase in DA release in the NAc is mediated by GDNF activation of the MAPK pathway (Wang et al. 2010). Likewise, MEN2B mice, in which the Ret receptor is constitutively active, show higher levels of DA in the NAc compared with WT controls (Mijatovic et al. 2007; Kopra



**Figure 1** Glial cell line-derived neurotrophic factor (GDNF) signaling in the ventral tegmental area (VTA)-nucleus accumbens (NAc) system. GDNF produced in the NAc is retrogradely transported by dopamine neurons to the VTA, where the GDNF receptors Ret and GFR $\alpha$ 1 are expressed. Ligand of GDNF to its receptors in the midbrain results in the activation of ERK1/2, which in turn promotes the spontaneous firing of VTA DA neurons. Increase in the firing of DA neurons increases DA release in the NAc

*et al.* 2017). Moreover, short hairpin RNA (shRNA) -mediated knockdown of *Gdnf* levels in the NAc suppressed, whereas intra-NAc infusion of GDNF increased, the firing rate of VTA DA neurons (Wang *et al.* 2010). Contrary to these findings, Ret phosphorylation of the exchange protein Vav-2 negatively regulated the trafficking and function of the DA transporter, thereby contributing to the maintenance of DA homeostasis in the mesolimbic system (Zhu *et al.* 2015). Together, these data demonstrate an integral role for GDNF in regulating the activity of mesolimbic DAergic neurons.

Interestingly, data obtained *ex vivo* suggest that, in addition to GDNF regulating DAergic activity, DA signaling regulates GDNF expression. Treatment of DAergic-like SH-SY5Y cells or rat midbrain slices with the DA D2 receptor (D2R) agonist quinpirole led to a transient increase in the expression of the DNA-binding transcription factor zinc-finger protein 268 (*Zif268*) (Ahmadiantehrani & Ron 2013), followed by an increase in *Gdnf* expression that was prevented by D2R antagonist treatment or by short hairpin RNA-mediated knockdown of *Zif268*. Furthermore, the D2R-mediated induction of *Gdnf* and *Zif268* expression was dependent on G $\beta$  $\gamma$ -mediated signaling, and on activation of ERK1/2. Thus, D2R activation increases the level of *Zif268* via G $\beta$  $\gamma$  and ERK1/2, which functions to directly upregulate *Gdnf* expression (Ahmadiantehrani & Ron 2013).

### GDNF and psychiatric disorders

GDNF has been implicated in several neuropsychiatric disorders in which the mesolimbic system plays an important role, including depression, anxiety, stress and schizophrenia (Ibanez & Andressoo 2017). In the NAc, epigenetic modifications within the *Gdnf* gene have been associated with increased susceptibility to mild stressors (Uchida *et al.* 2011). Polymorphism within the *Gdnf* gene has been reported to be associated with increased risk of depression (Ma *et al.* 2013) and anxiety disorders (Kotyuk *et al.* 2013). Moreover, in a post-mortem study, GFR $\alpha$ 1 levels were reduced in the basolateral amygdala of patients that suffered from depression and correlated with the increased expression of microRNAs that target the receptor (Maheu *et al.* 2015). Interestingly, serum GDNF levels have been correlated with mood assessments. For instance, levels of GDNF were lower in patients suffering from depression as compared with healthy controls (Diniz *et al.* 2012; Lin & Tseng 2015), and increased serum GDNF levels were reported during the manic phase in bipolar patients (Tunca *et al.* 2015). Unfortunately, administration of GDNF into the cerebral ventricles had no antidepressant effects in a genetic model of depression (Naumenko *et al.* 2013). Notably, intracerebral administration of GDNF was shown to induce

side effects in primates (Zhang *et al.* 1997) and human Parkinson's disease patients (Nutt *et al.* 2003). Nonetheless, continued elucidation of GDNF's contribution to psychiatric disorders is of great interest, particularly with respect to the misuse of drugs and alcohol. Finally, GDNF has been implicated as a key player in the regulation of intake of abused drugs, reward and relapse, as reviewed elsewhere (Carnicella & Ron 2009; Ghitza *et al.* 2010). Here, we focus on GDNF as a regulator of alcohol-drinking behaviors.

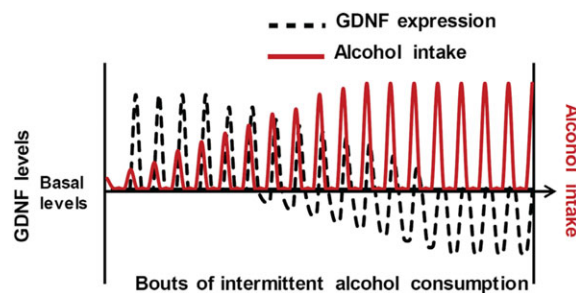
## GDNF AND ALCOHOL

### GDNF is an alcohol-responsive gene

Similar to other genes whose expression is altered by alcohol (Ron & Barak 2016), studies in rodents indicate that *Gdnf* levels fluctuate in response to different regimens of alcohol exposure. For instance, 1 week of intermittent access 2-bottle choice (IA2BC), in which rodents have concurrent access to one bottle containing 20 percent alcohol and a second bottle containing water, increased *Gdnf* mRNA levels in the VTA of rats when assessed at the end of the last drinking session, relative to water-only controls. Similarly, a single systemic administration of a non-hypnotic dose of alcohol increased *Gdnf* mRNA expression in the VTA (Ahmadiantehrani, Barak, & Ron 2014). Interestingly, the levels of *Gdnf* in the NAc were unaltered in response to alcohol exposure (Ahmadiantehrani *et al.* 2014). *Gdnf* expression was still elevated in the VTA when measured immediately after a 30-minute binge-like drinking session following 7 weeks of IA2BC training, as compared with rats drinking only water (Ahmadiantehrani *et al.* 2014). However, *Gdnf* levels in the VTA were reduced below baseline in response to 7 weeks of IA2BC when tested after a 24-hour withdrawal period (Ahmadiantehrani *et al.* 2014). Together, these findings suggest that *Gdnf* is an alcohol-responsive gene upregulated during short-term alcohol intake, but downregulated during withdrawal from excessive alcohol intake (Fig. 2). Notably, studies in humans reported elevated serum GDNF levels in individuals displaying mild to severe AUD (Lhullier *et al.* 2015), but reduced levels in alcohol-dependent individuals experiencing withdrawal (Heberlein *et al.* 2010), supporting the notion that the GDNF system is sensitive to voluntary alcohol intake and that dysregulation of GDNF expression can be detected across species.

### GDNF is a negative regulator of alcohol intake

Interestingly, GDNF in the mesolimbic system plays a unique role in neuroadaptations underlying AUD. As *Gdnf* is an alcohol-responsive gene in the VTA, an essential component of the brain reward circuitry (Volkow &



**Figure 2** Use-dependent dysregulation of alcohol-induced *Gdnf* expression and the development of excessive drinking. Initial bouts of alcohol drinking (solid red line) result in the upregulation of *Gdnf* expression (dashed black line), which acts to limit subsequent intake. Over time, repeated cycles of increasing amounts of alcohol consumption lead to an eventual decrease in the basal level of *Gdnf* (arrowhead), manifesting in abnormally low levels of the neurotrophic factor that are measured during withdrawal periods. A bout of alcohol consumption, while sufficient to restore *Gdnf* expression to its previous basal levels, no longer induces the enhanced level of expression that would effectively curb subsequent drinking

Morales 2015), a logical assumption was that GDNF participates in mechanisms that drive alcohol-drinking behaviors. Surprisingly, activation of the GDNF pathway in the VTA produced a very rapid and robust suppression of alcohol-drinking behaviors in rats. Specifically, infusion of recombinant GDNF (rGDNF) into the VTA reduced alcohol-reinforced lever pressing, regardless of previous levels of alcohol intake (Carnicella et al. 2008). Furthermore, rGDNF infusion into the VTA also inhibited reacquisition of operant alcohol self-administration upon renewed alcohol access in rats whose alcohol-directed operant responding had previously been extinguished (Carnicella et al. 2008), suggesting that the growth factor decreases alcohol seeking during relapse. Importantly, rGDNF did not affect the self-administration of sucrose (Carnicella et al. 2008), implying that the growth factor does not alter the general motivation to seek and consume natural rewards.

To further elucidate the role of GDNF in regulating alcohol drinking, the IA2BC procedure was employed to determine GDNF's ability to curb binge-like drinking. Infusion of rGDNF into the VTA rapidly suppressed binge-like home cage alcohol intake, and this suppressive effect persisted 24 and even 48 hours after a single administration of the growth factor (Carnicella, Amamoto, & Ron 2009; Barak et al. 2011b). Moreover, viral-mediated overexpression of the growth factor in the NAc or VTA blocked escalation from moderate to excessive alcohol drinking, as measured in the IA2BC procedure (Barak et al. 2015). Together, these data suggest that activation of the GDNF signaling pathway in the VTA produces a robust reduction of alcohol-drinking behaviors.

To test the contribution of the endogenous GDNF system to alcohol-drinking phenotypes, GDNF expression in the VTA or NAc was knocked down by adenoviral delivery of shRNA directed against *Gdnf* (AdV-shGDNF). *Gdnf* downregulation in either the VTA or NAc facilitated

the escalation of IA2BC alcohol drinking compared with rats infected with a non-specific control sequence in the same brain regions (Ahmadiantehrani et al. 2014; Barak et al. 2015). Similarly, GDNF heterozygote knockout mice, in which 50 percent of the GDNF is knocked out (Griffin et al. 2006), consumed more alcohol than their WT littermates after a period of abstinence and exhibited increased alcohol conditioned place preference (CPP), a measure of alcohol reward (Carnicella, Ahmadiantehrani, Janak et al. 2009). These findings could be linked to the observation that *Gdnf* heterozygote knockout mice exhibit higher levels of DA in the striatum (Airavaara et al. 2004). Taken together, these data suggest that endogenous GDNF protects against the development of excessive alcohol drinking during the early stages of alcohol consumption (Fig. 2). However, long-term, excessive alcohol intake may lead to breakdown of this protective response, resulting in the escalation of alcohol intake (Fig. 2).

Interestingly, a recent study showed that the re-exposure to a context previously associated with nicotine caused a 50 percent reduction in *Gdnf* expression in the VTA, coupled with pronounced and long-lasting (3 month) increases in operant alcohol self-administration and relapse (Zipori et al. 2017). These findings suggest that breakdown of the GDNF pathway is a common adaptation that may underlie co-morbidity of nicotine and alcohol misuse.

Another important piece of evidence for the crucial role of endogenous GDNF in controlling alcohol intake is the differential expression of *Gdnf* in low and high alcohol drinkers. Specifically, although the IA2BC procedure generally produces a high number of excessively drinking rats (approximately 60–70 percent), not all rats develop this typical pattern of alcohol consumption (Carnicella, Ron, & Barak 2014). Thus, alcohol-drinking rats can be segregated into two distinct groups: those whose alcohol intake progressively increases over

time (excessive drinkers), and those who maintain moderate alcohol consumption (low drinkers). Interestingly, although binge-like alcohol drinking increased *Gdnf* expression in the VTA in both groups, the effect was considerably stronger among low drinkers (Ahmadiantehrani *et al.* 2014). While this effect is confounded by the fact that excessive drinkers consumed higher levels of alcohol during the binge-drinking period (Ahmadiantehrani *et al.* 2014), nonetheless, these results suggest that GDNF is more functional, and thus more responsive to alcohol, in low drinkers compared with excessive drinkers. This conclusion was further supported by a negative correlation between alcohol intake levels and *Gdnf* expression in the VTA (Barak *et al.* 2015), indicating that the lower *Gdnf* expression was, the more alcohol was consumed. Interestingly, these data are in line with human data indicating that serum GDNF is reduced in humans undergoing alcohol withdrawal (Heberlein *et al.* 2010), suggesting that low serum levels of GDNF may be an indicator of susceptibility to relapse. Taken together, these findings suggest that the sensitivity of the GDNF expression system to alcohol determines the profile of drinking. Thus, variation in the *Gdnf* gene, particularly modifications that would impair the *Gdnf* response to alcohol, should be investigated further as a possible marker of AUD susceptibility.

### POTENTIAL MECHANISMS OF ACTION UNDERLYING GDNF REGULATION OF ALCOHOL INTAKE

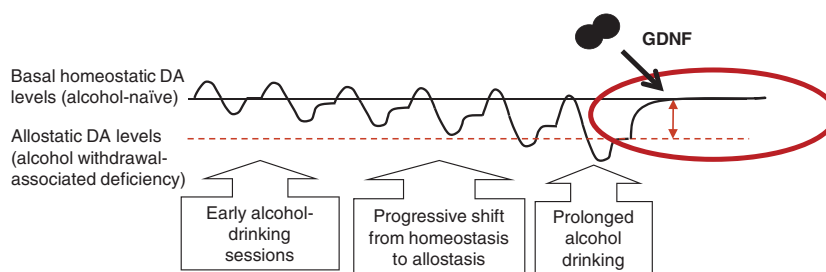
As mentioned in the introduction, GDNF binding to the receptor tyrosine kinase Ret and the co-receptor GFR $\alpha$ 1 can activate several downstream signaling cascades. GDNF-dependent regulation of alcohol intake is mediated by the ERK1/2 pathway in the VTA (Carnicella *et al.* 2008). Specifically, intra-VTA infusion of GDNF activated ERK1/2 in the VTA, and specific inhibition of the MAPK pathway, but not of PI3K-, prevented GDNF-mediated suppression of alcohol self-administration (Carnicella *et al.* 2008). PLC $\gamma$  inhibition reduced alcohol self-administration on its own, making it impossible to conclude whether the pathway contributes to the effects of GDNF on alcohol self-administration (Carnicella *et al.* 2008). It is possible that protein kinase C, the downstream target of PLC $\gamma$ , is activated under basal conditions, and this kinase has been implicated in the enhancement of alcohol-drinking behavior (Ron & Barak 2016). The rapid suppression of alcohol self-administration in response to intra-VTA GDNF infusion suggests that a non-genomic mechanism, such as post-translational modification, modulates alcohol drinking downstream of GDNF activation of ERK1/2 signaling. A limited number of extra-nuclear neuronal substrates for

ERK1/2 have been identified, of which the A-type potassium channel Kv4.2 was shown to be phosphorylated by ERK1/2 (Adams *et al.* 2000; Schrader *et al.* 2006). ERK1/2-mediated phosphorylation of Kv4.2 results in the inhibition of channel activity, thereby increasing neuronal excitability (Schrader *et al.* 2006; Yuan *et al.* 2006). Interestingly, GDNF increased evoked firing of primary midbrain DA neurons via the inhibition of an A-type potassium channel in a MAPK-dependent manner (Yang *et al.* 2001), and GDNF-dependent enhancement of VTA DA neuron firing also depends on ERK1/2 (Wang *et al.* 2010). Further studies are required to confirm the involvement of an A-type potassium channel phosphorylation in the GDNF-dependent, ERK1/2-mediated enhancement of VTA DA neuron activity.

### Nongenomic, rapid effects of GDNF on alcohol intake

One source of GDNF that modulates the increase in VTA DA neuron activity is the NAc, because GDNF produced in the NAc is retrogradely transported by DA VTA neurons (Wang *et al.* 2010; Fig. 1). Upon binding to its receptors in the VTA, GDNF produces a rapid increase in the spontaneous firing of VTA DA neurons (Wang *et al.* 2010; Fig. 1). The ability of GDNF to increase VTA DA neuron activity has important therapeutic implications in light of the well-established reduction in VTA DA neuronal activity during acute and protracted alcohol withdrawal (Diana *et al.* 1993; Bailey *et al.* 2001; Shen 2003; Shen, Choong, & Thompson 2007; Barak *et al.* 2015). Infusion of GDNF into the VTA reversed the deficiency in rat VTA DA neuron firing induced by long-term IA2BC alcohol intake, assessed 24 hours after cessation of alcohol drinking (Barak *et al.* 2015). Together, these results support the possibility that GDNF reduces alcohol intake by increasing VTA DA neuronal excitability, thereby reversing maladaptive changes in the VTA that may support alcohol dependence.

Decreased VTA DA neuron firing during withdrawal from chronic exposure to high levels of alcohol (Diana *et al.* 1993; Shen *et al.* 2007; Barak *et al.* 2015) concomitantly impacts DA release in the NAc, resulting in an allostatic reduction in NAc DA levels (Koob & Le Moal 2001; Koob 2003; Barak *et al.* 2011a) that has been associated with alcohol-seeking behaviors (Rossetti *et al.* 1992; Diana *et al.* 1993; Weiss *et al.* 1996). This relationship between reduced NAc DA input and elevated alcohol seeking suggested that GDNF might suppress alcohol consumption by reversing the alcohol withdrawal-associated DA deficiency in the mesolimbic system (Fig. 3). In line with this possibility, a single intra-VTA administration of rGDNF reversed DA overflow deficiencies in the NAc of rats following 24-hour withdrawal from



**Figure 3** Alcohol, glial cell line-derived neurotrophic factor (GDNF) and the mesolimbic dopaminergic system. Withdrawal from excessive alcohol consumption or downregulation of GDNF in the nucleus accumbens results in deficient dopaminergic function in the mesolimbic system. This deficiency enhances alcohol seeking and intake, and alcohol temporarily alleviates the dopamine (DA) deficiency (Barak et al. 2011a). Activating the GDNF signaling pathway, either by infusion of recombinant GDNF or by overexpressing GDNF in the mesolimbic system, reverses withdrawal-induced DA deficiency and consequently suppresses alcohol seeking and drinking

7 weeks of IA2BC drinking (Barak et al. 2011a). Together, these results suggest that GDNF can reverse allostatic alterations in the mesolimbic DA system associated with withdrawal from long-term consumption of high levels of alcohol (Fig. 3).

GDNF-regulated modulation of DA neuronal activity, in combination with the finding that GDNF triggers DA release in the NAc of both alcohol-naïve and IA2BC-withdrawn rats (Wang et al. 2010; Barak et al. 2011a), raised the possibility that GDNF is rewarding, hence reducing alcohol consumption by substituting for alcohol reward. However, intra-VTA GDNF infusion did not induce the expression of CPP (Barak et al. 2011a), suggesting that the growth factor is not rewarding. Moreover, intra-VTA infusion of GDNF abolished both the acquisition and the expression of alcohol CPP, suggesting that, rather than generating rewarding effects, the growth factor suppresses alcohol's reinforcing effects. GDNF infusion into the VTA also produced a downward shift in the dose–response curve for alcohol self-administration (Barak et al. 2011a), implying that the growth factor does not substitute for or augment the rewarding effects of alcohol, but rather suppresses the motivation for alcohol seeking and drinking. Together, these studies point to rectification of alcohol withdrawal-induced deficits in VTA DA firing rate and NAc DA release, and the mechanism underlying the rapid actions of GDNF involving activation of ERK1/2 signaling. These actions result in an as-yet unidentified posttranslational modification to suppress alcohol intake.

#### Genomic, long-term effects of GDNF on alcohol intake

Interestingly, although GDNF-mediated reduction of alcohol intake is very rapid, it persists for a long time (Carnicella et al. 2009). Several pieces of data suggest that the GDNF-mediated reduction in alcohol intake is prolonged through an autoregulatory cycle in which the ligation of GDNF to its receptor leads to the induction of GDNF expression. Specifically, GDNF treatment of the

DAergic-like SH-SY5Y cell line induced long-lasting increases in the growth factor's levels, resulting in long-lasting activation of Ret and Ret-mediated activation of ERK1/2 (He & Ron 2006). Furthermore, this autoregulatory positive feedback loop was observed to contribute to GDNF expression *in vivo*. A single infusion of rGDNF into the VTA increased the expression of GDNF mRNA and protein for at least 48 hours (Barak et al. 2011b). This long-term upregulation of *Gdnf* mRNA expression was eliminated by inhibition of protein synthesis, as well as by viral mediated downregulation of *Gdnf* mRNA using AdV-shGDNF (Barak et al. 2011b). Together, these findings indicate that GDNF positively regulates its own expression and that this process depends on *de novo* transcription and translation of the growth factor. Importantly, the long-lasting attenuation of excessive alcohol consumption by intra-VTA administration of GDNF was prevented by inhibition of protein synthesis, as well as by downregulation of *Gdnf* mRNA levels in the VTA (Barak et al. 2011b). Together, these findings suggest that GDNF can amplify and prolong its own signaling in the VTA to produce a long-lasting suppression of alcohol intake.

#### THERAPEUTIC APPLICATIONS

Given the robust effects of GDNF on alcohol-drinking behaviors, as well as the observations of abnormal circulating levels of the growth factor in humans suffering from AUDs (Heberlein et al. 2010; Lhullier et al. 2015), targeting GDNF signaling may be a novel approach for the development of new pharmacotherapies to treat AUD. One attractive pharmacological means to reduce drinking via elevated GDNF is through use of a GDNF inducer, which increases endogenous levels of GDNF to generate a long-lasting activation of GDNF signaling. Interestingly, the FDA-approved drug Cabergoline, which is prescribed for hyperprolactinemia (Webster et al. 1994), increased GDNF levels in DAergic-like SH-SY5Y cells, resulting in activation of the GDNF signaling pathway

(Carnicella, Ahmadiantehrani, He *et al.* 2009). Systemic administration of a single dose of Cabergoline reduced alcohol drinking in rodents through a GDNF-dependent mechanism (Carnicella, Ahmadiantehrani, He *et al.* 2009). Thus, GDNF mimetics like Cabergoline may be developed as medications to suppress alcohol drinking and relapse, as well as to prevent escalation to excessive alcohol consumption in individuals with a predisposition to develop AUD. Further exploration of these and other mechanisms for restoring the protection conferred by endogenous GDNF in alcohol-dependent individuals represents an intriguing new avenue for medication development to ameliorate AUDs. In addition, advances in the speed, reliability and cost of genetic sequencing, coupled with the knowledge of the specific genomic mechanisms underlying alcohol-induced expression of endogenous GDNF, represent a promising and powerful approach to identify persons at risk of developing AUDs and implement individually tailored treatment regimens.

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### Authors Contribution

SB, SA, MLL and DR wrote the manuscript.

### References

Adams JP, Anderson AE, Varga AW, Dineley KT, Cook RG, Pfaffinger PJ, Sweatt JD (2000) The A-type potassium channel Kv4.2 is a substrate for the mitogen-activated protein kinase ERK. *J Neurochem* 75:2277–2287.

Ahmadiantehrani S, Barak S, Ron D (2014) GDNF is a novel ethanol-responsive gene in the VTA: implications for the development and persistence of excessive drinking. *Addict Biol* 19:623–633.

Ahmadiantehrani S, Ron D (2013) Dopamine D2 receptor activation leads to an up-regulation of glial cell line-derived neurotrophic factor via G $\beta$  $\gamma$ -Erk1/2-dependent induction of Zif268. *J Neurochem* 125:193–204.

Airaksinen MS, Saarma M (2002) The GDNF family: signalling, biological functions and therapeutic value. *Nat Rev Neurosci* 3:383–394.

Airavaara M, Planken A, Gaddnas H, Piepponen TP, Saarma M, Ahtee L (2004) Increased extracellular dopamine concentrations and FosB/DeltaFosB expression in striatal brain areas of heterozygous GDNF knockout mice. *Eur J Neurosci* 20:2336–2344.

American Psychiatric Association (2013) The diagnostic and statistical manual of mental disorders: DSM 5. bookpointUS.

Bailey CP, O'Callaghan MJ, Croft AP, Manley SJ, Little HJ (2001) Alterations in mesolimbic dopamine function during the abstinence period following chronic ethanol consumption. *Neuropharmacology* 41:989–999.

Barak S, Carnicella S, Yowell QV, Ron D (2011a) Glial cell line-derived neurotrophic factor reverses alcohol-induced allostasis of the mesolimbic dopaminergic system: implications for alcohol reward and seeking. *J Neurosci* 31(27):9885–9894.

Barak S, Ahmadiantehrani S, Kharazia V, Ron D (2011b) Positive autoregulation of GDNF levels in the ventral tegmental area mediates long-lasting inhibition of excessive alcohol consumption. *Transl Psychiatry* 1:e60.

Barak S, Wang J, Ahmadiantehrani S, Ben Hamida S, Kells AP, Forsayeth J, Bankiewicz KS, Ron D (2015) Glial cell line-derived neurotrophic factor (GDNF) is an endogenous protector in the mesolimbic system against excessive alcohol consumption and relapse. *Addict Biol* 20:629–642.

Barroso-Chinea P, Cruz-Muros I, Aymerich MS, Rodriguez-Diaz M, Afonso-Oramas D, Lanciego JL, Gonzalez-Hernandez T (2005) Striatal expression of GDNF and differential vulnerability of midbrain dopaminergic cells. *Eur J Neurosci* 21:1815–1827.

Bespalov MM, Saarma M (2007) GDNF family receptor complexes are emerging drug targets. *Trends Pharmacol Sci* 28:68–74.

Bespalov MM, Sidorova YA, Tumova S, Ahonen-Bishopp A, Magalhaes AC, Kuleskiy E, Paveliev M, Rivera C, Rauvala H, Saarma M (2011) Heparan sulfate proteoglycan syndecan-3 is a novel receptor for GDNF, neurturin, and artemin. *J Cell Biol* 192:153–169.

Bonanomi D, Chivatakarn O, Bai G, Abdesselem H, Lettieri K, Marquardt T, Pierchala BA, Pfaff SL (2012) Ret is a multifunctional coreceptor that integrates diffusible- and contact-axon guidance signals. *Cell* 148:568–582.

Bouza C, Angeles M, Munoz A, Amate JM (2004) Efficacy and safety of naltrexone and acamprosate in the treatment of alcohol dependence: a systematic review. *Addiction* 99:811–828.

Carnicella S, Ahmadiantehrani S, He DY, Nielsen CK, Bartlett SE, Janak PH, Ron D (2009) Cabergoline decreases alcohol drinking and seeking behaviors via glial cell line-derived neurotrophic factor. *Biol Psychiatry* 66:146–153.

Carnicella S, Ahmadiantehrani S, Janak PH, Ron D (2009) GDNF is an endogenous negative regulator of ethanol-mediated reward and of ethanol consumption after a period of abstinence. *Alcohol Clin Exp Res* 33:1012–1024.

Carnicella S, Amamoto R, Ron D (2009) Excessive alcohol consumption is blocked by glial cell line-derived neurotrophic factor. *Alcohol* 43:35–43.

Carnicella S, Kharazia V, Jeanblanc J, Janak PH, Ron D (2008) GDNF is a fast-acting potent inhibitor of alcohol consumption and relapse. *Proc Natl Acad Sci U S A* 105:8114–8119.

Carnicella S, Ron D (2009) GDNF—a potential target to treat addiction. *Pharmacol Ther* 122:9–18.

Carnicella S, Ron D, Barak S (2014) Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. *Alcohol* 48(3):243–52.

Diana M, Pistis M, Carboni S, Gessa GL, Rossetti ZL (1993) Profound decrement of mesolimbic dopaminergic neuronal activity during ethanol withdrawal syndrome in rats: electrophysiological and biochemical evidence. *Proc Natl Acad Sci U S A* 90:7966–7969.

- Diniz BS, Teixeira AL, Miranda AS, Talib LL, Gattaz WF, Forlenza OV (2012) Circulating Glial-derived neurotrophic factor is reduced in late-life depression. *J Psychiatr Res* 46:135–139.
- Dudanova I, Gatto G, Klein R (2010) GDNF acts as a chemoattractant to support ephrinA-induced repulsion of limb motor axons. *Curr Biol* 20:2150–2156.
- Durbec P, Marcos-Gutierrez CV, Kilkenny C, Grigoriou M, Wartiovaara K, Suvanto P, Smith D, Ponder B, Costantini F, Saarma M, et al. (1996) GDNF signalling through the Ret receptor tyrosine kinase. *Nature* 381:789–793.
- Ghitza UE, Zhai H, Wu P, Airavaara M, Shaham Y, Lu L (2010) Role of BDNF and GDNF in drug reward and relapse: a review. *Neurosci Biobehav Rev* 35:157–171.
- Glazner GW, Mu X, Springer JE (1998) Localization of glial cell line-derived neurotrophic factor receptor alpha and c-ret mRNA in rat central nervous system. *J Comp Neurol* 391:42–49.
- Grant BF, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, Huang B, Jung J, Zhang H, Fan A, Hasin DS (2017) Prevalence of 12-month alcohol use, high-risk drinking, and DSM-IV alcohol use disorder in the United States, 2001–2002 to 2012–2013: Results from the National Epidemiologic Survey on Alcohol and Related Conditions. *JAMA Psychiatr* 74:911–923.
- Griffin WC 3rd, Boger HA, Granholm AC, Middaugh LD (2006) Partial deletion of glial cell line-derived neurotrophic factor (GDNF) in mice: effects on sucrose reward and striatal GDNF concentrations. *Brain Res* 1068:257–260.
- He DY, Ron D (2006) Autoregulation of glial cell line-derived neurotrophic factor expression: implications for the long-lasting actions of the anti-addiction drug, Ibogaine. *FASEB J* 20:2420–2422.
- Heberlein A, Muschler M, Wilhelm J, Frieling H, Lenz B, Groschl M, Kornhuber J, Bleich S, Hillemacher T (2010) BDNF and GDNF serum levels in alcohol-dependent patients during withdrawal. *Prog Neuropsychopharmacol Biol Psychiatry* 34:1060–1064.
- Ibanez CE, Andressoo JO (2017) Biology of GDNF and its receptors—relevance for disorders of the central nervous system. *Neurobiol Dis* 97:80–89.
- Jing S, Wen D, Yu Y, Holst PL, Luo Y, Fang M, Tamir R, Antonio L, Hu Z, Cupples R, Louis JC, Hu S, Altrock BW, Fox GM (1996) GDNF-induced activation of the ret protein tyrosine kinase is mediated by GDNFR-alpha, a novel receptor for GDNF. *Cell* 85:1113–1124.
- Koob GF (2003) Alcoholism: allostasis and beyond. *Alcohol Clin Exp Res* 27:232–243.
- Koob GF, Le Moal M (2001) Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology : Offic Publ Am Coll Neuropsychopharmacol* 24:97–129.
- Koob GF, Volkow ND (2010) Neurocircuitry of addiction. *Neuropsychopharmacology : Offic Publ Am Coll Neuropsychopharmacol* 35:217–238.
- Kopra J, Villarta-Aguilera M, Savolainen M, Weingerl S, Myohanen TT, Rannanpaa S, Salvatore MF, Andressoo JO, Piepponen TP (2017) Constitutive Ret signaling leads to long-lasting expression of amphetamine-induced place conditioning via elevation of mesolimbic dopamine. *Neuropharmacology* 128:221–230.
- Kordower JH, Emborg ME, Bloch J, Ma SY, Chu Y, Leventhal L, McBride J, Chen EY, Palfi S, Roitberg BZ, Brown WD, Holden JE, Pyzalski R, Taylor MD, Carvey P, Ling Z, Trono D, Hantraye P, Deglon N, Aebischer P (2000) Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 290:767–773.
- Kotyuk E, Keszler G, Nemeth N, Ronai Z, Sasvari-Szekely M, Szekely A (2013) Glial cell line-derived neurotrophic factor (GDNF) as a novel candidate gene of anxiety. *PLoS One* 8:e80613.
- Kumar A, Kopra J, Varendi K, Porokuokka LL, Panhelainen A, Kuure S, Marshall P, Karalija N, Harma MA, Vilenius C, Lillevali K, Tekko T, Mijatovic J, Pulkkinen N, Jakobson M, Jakobson M, Ola R, Palm E, Lindahl M, Stromberg I, Voikar V, Piepponen TP, Saarma M, Andressoo JO (2015) GDNF overexpression from the native locus reveals its role in the nigrostriatal dopaminergic system function. *PLoS Genet* 11:e1005710.
- Lhullier AC, Moreira FP, da Silva RA, Marques MB, Bittencourt G, Pinheiro RT, Souza LD, Portela LV, Lara DR, Jansen K, Wiener CD, Oses JP (2015) Increased serum neurotrophin levels related to alcohol use disorder in a young population sample. *Alcohol Clin Exp Res* 39:30–35.
- Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F (1993) GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 260:1130–1132.
- Lin PY, Tseng PT (2015) Decreased glial cell line-derived neurotrophic factor levels in patients with depression: a meta-analytic study. *J Psychiatr Res* 63:20–27.
- Ma XC, Chen C, Zhu F, Jia W, Gao CG (2013) Association of the GDNF gene with depression and heroin dependence, but not schizophrenia, in a Chinese population. *Psychiatry Res* 210:1296–1298.
- Maheu M, Lopez JP, Crapper L, Davoli MA, Turecki G, Mechawar N (2015) MicroRNA regulation of central glial cell line-derived neurotrophic factor (GDNF) signalling in depression. *Transl Psychiatry* 5:e511.
- McGinnis JM, Foege WH (1999) Mortality and morbidity attributable to use of addictive substances in the United States. *Proc Assoc Am Physicians* 111:109–118.
- Mijatovic J, Airavaara M, Planken A, Auvinen P, Raasmaja A, Piepponen TP, Costantini F, Ahtee L, Saarma M (2007) Constitutive Ret activity in knock-in multiple endocrine neoplasia type B mice induces profound elevation of brain dopamine concentration via enhanced synthesis and increases the number of TH-positive cells in the substantia nigra. *J Neuroscience: Offic J Soc Neuroscience* 27:4799–4809.
- Molliver DC, Wright DE, Leitner ML, Parsadanian AS, Doster K, Wen D, Yan Q, Snider WD (1997) IB4-binding DRG neurons switch from NGF to GDNF dependence in early postnatal life. *Neuron* 19:849–861.
- Naumenko VS, Bazovkina DV, Semenova AA, Tsybko AS, Il'chibaeva TV, Kondaurova EM, Popova NK (2013) Effect of glial cell line-derived neurotrophic factor on behavior and key members of the brain serotonin system in mouse strains genetically predisposed to behavioral disorders. *J Neurosci Res* 91:1628–1638.
- Nutt DJ, King LA, Phillips LD, Independent Scientific Committee on D (2010) Drug harms in the UK: a multicriteria decision analysis. *Lancet* 376:1558–1565.
- Nutt JG, Burchiel KJ, Comella CL, Jankovic J, Lang AE, Laws ER Jr, Lozano AM, Penn RD, Simpson RK Jr, Stacy M, Wooten GF (2003) Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology* 60:69–73.
- Ortega-de San Luis C, Pascual A (2016) Simultaneous detection of both GDNF and GFRalpha1 expression patterns in the mouse central nervous system. *Front Neuroanat* 10:73.



- Paratcha G, Ledda F, Baars L, Couplier M, Besset V, Anders J, Scott R, Ibanez CF (2001) Released GFRalpha1 potentiates downstream signaling, neuronal survival, and differentiation via a novel mechanism of recruitment of c-Ret to lipid rafts. *Neuron* 29:171–184.
- Paratcha G, Ledda F, Ibanez CF (2003) The neural cell adhesion molecule NCAM is an alternative signaling receptor for GDNF family ligands. *Cell* 113:867–879.
- Pochon NA, Menoud A, Tseng JL, Zurn AD, Aebischer P (1997) Neuronal GDNF expression in the adult rat nervous system identified by in situ hybridization. *Eur J Neurosci* 9:463–471.
- Pozas E, Ibanez CF (2005) GDNF and GFRalpha1 promote differentiation and tangential migration of cortical GABAergic neurons. *Neuron* 45:701–713.
- Rehm J (2011) The risks associated with alcohol use and alcoholism. *Alcohol Res Health* : J Nat Inst Alcohol Abuse and Alcohol 34:135–143.
- Ron D, Barak S (2016) Molecular mechanisms underlying alcohol-drinking behaviours. *Nat Rev Neurosci* 17:576–591.
- Rossetti ZL, Melis F, Carboni S, Diana M, Gessa GL (1992) Alcohol withdrawal in rats is associated with a marked fall in extraneuronal dopamine. *Alcohol Clin Exp Res* 16:529–532.
- Salio C, Ferrini F, Muthuraju S, Merighi A (2014) Presynaptic modulation of spinal nociceptive transmission by glial cell line-derived neurotrophic factor (GDNF). *J Neurosci* 34:13819–13833.
- Sariola H, Saarma M (2003) Novel functions and signalling pathways for GDNF. *J Cell Sci* 116:3855–3862.
- Schrader LA, Birnbaum SG, Nadin BM, Ren Y, Bui D, Anderson AE, Sweatt JD (2006) ERK/MAPK regulates the Kv4.2 potassium channel by direct phosphorylation of the pore-forming subunit. *Am J Physiol Cell Physiol* 290:C852–C861.
- Shen RY (2003) Ethanol withdrawal reduces the number of spontaneously active ventral tegmental area dopamine neurons in conscious animals. *J Pharmacol Exp Ther* 307:566–572.
- Shen RY, Choong KC, Thompson AC (2007) Long-term reduction in ventral tegmental area dopamine neuron population activity following repeated stimulant or ethanol treatment. *Biol Psychiatry* 61:93–100.
- Sridhar D (2012) Health policy: regulate alcohol for global health. *Nature* 482:302.
- Tansey MG, Baloh RH, Milbrandt J, Johnson EM Jr (2000) GFRalpha-mediated localization of RET to lipid rafts is required for effective downstream signaling, differentiation, and neuronal survival. *Neuron* 25:611–623.
- Tomac A, Widenfalk J, Lin LF, Kohno T, Ebendal T, Hoffer BJ, Olson L (1995) Retrograde axonal transport of glial cell line-derived neurotrophic factor in the adult nigrostriatal system suggests a trophic role in the adult. *Proc Natl Acad Sci U S A* 92:8274–8278.
- Trupp M, Belluardo N, Funakoshi H, Ibanez CF (1997) Complementary and overlapping expression of glial cell line-derived neurotrophic factor (GDNF), c-ret proto-oncogene, and GDNF receptor-alpha indicates multiple mechanisms of trophic actions in the adult rat CNS. *J Neuroscience: Offic J Soc Neuroscience* 17:3554–3567.
- Tsui CC, Gabreski NA, Hein SJ, Pierchala BA (2015) Lipid rafts are physiologic membrane microdomains necessary for the morphogenic and developmental functions of glial cell line-derived neurotrophic factor in vivo. *J Neuroscience: Offic J Soc Neuroscience* 35:13233–13243.
- Tunca Z, Kivircik Akdede B, Ozerdem A, Alkin T, Polat S, Ceylan D, Bayin M, Cengizcetin Kocuk N, Simsek S, Resmi H, Akan P (2015) Diverse glial cell line-derived neurotrophic factor (GDNF) support between mania and schizophrenia: a comparative study in four major psychiatric disorders. *Eur Psychiatry* 30:198–204.
- Uchida S, Hara K, Kobayashi A, Otsuki K, Yamagata H, Hobara T, Suzuki T, Miyata N, Watanabe Y (2011) Epigenetic status of Gdnf in the ventral striatum determines susceptibility and adaptation to daily stressful events. *Neuron* 69:359–372.
- Volkow ND, Morales M (2015) The brain on drugs: from reward to addiction. *Cell* 162:712–725.
- Wang J, Carnicella S, Ahmadianehrani S, He DY, Barak S, Kharazia V, Ben Hamida S, Zapata A, Shippenberg TS, Ron D (2010) Nucleus accumbens-derived glial cell line-derived neurotrophic factor is a retrograde enhancer of dopaminergic tone in the mesocorticolimbic system. *J Neuroscience: Offic J Soc Neuroscience* 30:14502–14512.
- Webster J, Piscitelli G, Polli A, Ferrari CI, Ismail I, Scanlon MF (1994) A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhea. Cabergoline Comparative Study Group. *N Engl J Med* 331:904–909.
- Weiss F, Parsons LH, Schulteis G, Hyytia P, Lorang MT, Bloom FE, Koob GF (1996) Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. *J Neuroscience: Offic J Soc Neuroscience* 16:3474–3485.
- Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, Burstein R, Murray CJ, Vos T (2013) Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet* 382:1575–1586.
- World Health Organization (2014) Global status report on alcohol and health—2014. World Health Organization.
- Yang F, Feng L, Zheng F, Johnson SW, Du J, Shen L, Wu CP, Lu B (2001) GDNF acutely modulates excitability and A-type K(+) channels in midbrain dopaminergic neurons. *Nat Neurosci* 4:1071–1078.
- Yuan LL, Chen X, Kunjilwar K, Pfaffinger P, Johnston D (2006) Acceleration of K+ channel inactivation by MEK inhibitor U0126. *Am J Physiol Cell Physiol* 290:C165–C171.
- Zhang Z, Miyoshi Y, Lapchak PA, Collins F, Hilt D, Lebel C, Kryscio R, Gash DM (1997) Dose response to intraventricular glial cell line-derived neurotrophic factor administration in parkinsonian monkeys. *J Pharmacol Exp Ther* 282:1396–1401.
- Zhu S, Zhao C, Wu Y, Yang Q, Shao A, Wang T, Wu J, Yin Y, Li Y, Hou J, Zhang X, Zhou G, Gu X, Wang X, Bustelo XR, Zhou J (2015) Identification of a Vav2-dependent mechanism for GDNF/Ret control of mesolimbic DAT trafficking. *Nat Neurosci* 18:1084–1093.
- Zipori D, Sadot-Sogrin Y, Goltseker K, Even-Chen O, Rahamim N, Shaham O, Barak S (2017) Re-exposure to nicotine-associated context from adolescence enhances alcohol intake in adulthood. *Sci Rep* 7:2479.