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The role of fibroblast growth factor 2 in drug addiction

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Abstract

Fibroblast growth factor 2 (FGF2) is a member of the FGF-family, which consists of 22 members, with four known FGF receptors (five in humans). Over the last 30 years, FGF2 has been extensively studied for its role in cell proliferation, differentiation, growth, survival and angiogenesis during development, as well as for its role in adult neurogenesis and regenerative plasticity. Over the past decade, FGF2 has been implicated in learning and memory, as well as in several neuropsychiatric disorders, including anxiety, stress, depression and drug addiction. In this review, we present accumulating evidence indicating the involvement of FGF2 in neuroadaptations caused by drugs of abuse, namely, amphetamine, cocaine, nicotine and alcohol. Moreover, evidence suggests that FGF2 is a positive regulator of alcohol and drug-related behaviors. Thus, although additional studies are yet required, we suggest that reducing FGF2 activity may provide a novel therapeutic approach for substance use disorders.

Introduction

Drug addiction is a chronic and relapsing psychiatric disorder that affects millions worldwide (American Psychiatric Association, 2013; World Health Organization, 2014) and is characterized by excessive, out of control, compulsive drug seeking and consumption, as well as the emergence of withdrawal symptoms during abstinence (American Psychiatric Association, 2013). Over the last two decades, it has become widely accepted that these addiction phenotypes may result from neuroadaptations, caused by prolonged, excessive exposure to the drugs (Nestler, 2001; Russo *et al.*, 2010; Koob, 2013). These neuroadaptations occur mainly in the brain reward system, consisting of the mesocorticolimbic dopaminergic (DAergic) pathway, which projects from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), hippocampus, amygdala and prefrontal cortex (PFC) (Koob & Volkow, 2009; Spanagel, 2009); and the nigrostriatal DAergic pathway, which projects from the substantia nigra pars compacta (SNc) to the dorsal striatum, and which plays a key role in the progression of goal-directed behaviors to the habitual, compulsive nature of drug addiction (Wise, 2009; Everitt & Robbins, 2013).

Characteristics of drug addiction, such as psychological and physical dependence and withdrawal syndrome, usually appear only after prolonged and excessive drug use. This suggests that additional protective mechanisms may prevent or delay the behavioral expression of the neuroadaptations underlying these phenotypes. Several growth factors, such as brain-derived neurotrophic factor (BDNF) (Ghitza *et al.*, 2010; Jeanblanc *et al.*, 2009; Ron & Messing, 2013; Logrip *et al.*, 2015; Ron & Barak, 2016) and glial cell line-derived neurotrophic factor (GDNF) (Carnicella *et al.*, 2008; Carnicella & Ghitza *et al.*, 2010; Ron, 2009; Ron & Messing, 2013; Barak *et al.*, 2018), have been identified for their role in the protective mechanisms of several drugs, including alcohol, cocaine, methamphetamine and morphine. Here, we review evidence that suggests a role of fibroblast growth factor 2 (FGF2; also known as bFGF) in drug addiction, and argue for further mechanistic inquiry.

Fibroblast growth factor 2 has been extensively studied for its role during development - in cell proliferation, differentiation, growth, survival and angiogenesis (Dono *et al.*, 1998; Ford-Perriss *et al.*, 2001; Reuss & von Bohlen und Halbach, 2003). In recent years, FGF2 has been implicated in learning and memory processes (Graham & Richardson, 2011), as well as in neuropsychiatric disorders, including anxiety (Perez *et al.*, 2009; Eren-Kocak *et al.*, 2011; Turner *et al.*, 2012), depression (Mallei *et al.*, 2002; Maragnoli *et al.*, 2004; Riva *et al.*, 2005; Elsayed *et al.*, 2012; Turner *et al.*, 2012), stress-related disorders (Molteni *et al.*, 2001a,b; Fumagalli *et al.*, 2005; Xia *et al.*, 2013) and schizophrenia (Klejbor *et al.*, 2006; Terwisscha van Scheltinga *et al.*, 2010). It has also been shown to play a part in neurodegenerative diseases, such as Parkinson's disease (Claus *et al.*, 2004; Timmer *et al.*, 2007) and Alzheimer's disease (Cummings *et al.*, 1993; Kiyota *et al.*, 2011). In addition, as surveyed below, accumulating evidence suggests that FGF2 may play a role in the behavioral and neurobiological effects of drugs of abuse.

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FGF 2 - General information

Fibroblast growth factor 2 is a member of the FGF-family, which consists of 22 members. A single *Fgf2* transcript can be translated to several isoforms: the secreted low molecular weight isoform (18 kDa) (Reuss & von Bohlen und Halbach, 2003) and the high molecular weight isoforms (21 and 23 kDa), which have a nuclear localization signal. Upon entering the nucleus, the high molecular weight isoforms affect nuclear function such as gene expression (Chlebova *et al.*, 2009; Forthmann *et al.*, 2015). From the early stages of embryonic development, FGF2 is extensively expressed throughout the neuronal tube (Dono *et al.*, 1998; Ford-Perriss *et al.*, 2001). In adulthood, FGF2 is expressed in both neurons and astrocytes (Gonzalez *et al.*, 1995; Reuss & von Bohlen und Halbach, 2003) in many brain regions, including the frontal cortex, striatum, hippocampus, amygdala, thalamus, hypothalamus SNc, VTA and pons (Gonzalez *et al.*, 1995; Reuss & von Bohlen und Halbach, 2003).

There are four known receptors to the FGF ligand family (five in humans), with FGF2 having the highest affinity to FGF receptor 1 (FGFR1), a receptor tyrosine kinase (Ford-Perriss *et al.*, 2001). The binding of FGF2 to this receptor leads to receptor dimerization and activates the receptor via autophosphorylation (Eswarakumar *et al.*, 2005). As a result, the FGF2–FGFR1 complex recruits and activates intracellular second messenger molecules, mainly through three signal transduction pathways: phospholipase C gamma (PLCγ), mitogen-activated protein kinase (MAPK) (Numakawa *et al.*, 2002; Reuss & von Bohlen und Halbach, 2003), and phosphatidylinositol-3-kinases (PI3K)/protein kinase B (PKB, also known as Akt) (Eswarakumar *et al.*, 2005; Peltier *et al.*, 2007).

FGF2 and dopamine

The crosstalk between FGF2 and the DAergic system is well-established (Reuss & Unsicker, 2000; Reuss & von Bohlen und Halbach, 2003; Grothe & Timmer, 2007; Clinton *et al.*, 2012; Terwisscha van Scheltinga *et al.*, 2013). FGF2 is expressed in the ventral midbrain and striatal regions (Bean *et al.*, 1991; Chadi *et al.*, 1993; Claus *et al.*, 2004). Within the SNc, FGFR1 expression is mainly found in DAergic neurons (Yazaki *et al.*, 1994; Gonzalez *et al.*, 1995; Belluardo *et al.*, 1997; Grothe & Timmer, 2007).

Application of FGF2 to embryonic day 12 DAergic culture cells resulted in increased proliferation and delayed cell differentiation (Reuss & von Bohlen und Halbach, 2003). FGF2 also enhanced the survival of DAergic neurons in striatal astrocytic cultures (Li *et al.*, 2006; Grothe & Timmer, 2007), and was found to protect DAergic neurons against lesions induced by rotenone *ex vivo* (Hsuan *et al.*, 2006), 1-metyl-1,2,3,6 – tetrahydropyridine (MPTP) *in vivo* (Date *et al.*, 1993; Zhang *et al.*, 2009), and by 6-hydroxydopamine (6-OHDA) both *in vitro* (Mayer *et al.*, 1993a) and *in vivo* (Mayer *et al.*, 1993b; Timmer *et al.*, 2007). The latter lesion also increased FGF2 protein levels in the striatum, SNc and VTA (Chadi & Gomide, 2004), presumably as a neuroprotective mechanism.

In the other direction, FGF2 expression is enhanced by DAergic activity. Specifically, activation of dopamine (DA) D1 or D2 receptors promotes FGF2 synthesis and release in astrocytic cultures (Li *et al.*, 2006) and *in vivo* (Fumagalli *et al.*, 2003; Zhang *et al.*, 2009). Taken together, these findings suggest that FGF2 and DA constitute a positive regulatory feedback mechanism in the nigrostriatal system (Reuss & Unsicker, 2000; Grothe & Timmer, 2007).

Finally, endogenous FGF2 plays an important role in promoting the survival of DAergic neurons in adulthood. For example, DAergic cell death was increased in FGF2 knockout mice with 6-OHDA-lesion, but was decreased in FGF2 overexpressing mice, compared to wild-type controls (Timmer *et al.*, 2007). Interestingly, mice overexpressing FGF2 had fewer DAergic neurons, whereas FGF2 knockout mice had an increased number of DAergic neurons (Timmer *et al.*, 2007; Ratzka *et al.*, 2012), increased striatum volume (Rumpel *et al.*, 2016), and increased DAergic innervation from the midbrain to the striatum (Baron *et al.*, 2012; Rumpel *et al.*, 2016). It is possible that these apparent paradoxical findings were due to compensative mechanisms by other FGF-family ligands (Timmer *et al.*, 2007; Baron *et al.*, 2012; Ratzka *et al.*, 2012). This overcompensation could have led to increased proliferation of DA precursors during the late stages of embryonic development, as well as reduced apoptotic bodies in newborn FGF2 knockout mice (Ratzka *et al.*, 2012). Together, these findings suggest that *Fgf2* deletion results in abnormal nigrostriatal development and reduced protection of DAergic neurons in adulthood.

FGF2 and Glutamate

The glutamatergic system plays an established role in drug-induced neuroadaptation and behavioral phenotypes, as well as in the regulation of DA transmission (Kalivas, 2009; Kalivas *et al.*, 2009; Spencer *et al.*, 2016). Glutamate was shown to increase FGF2 immunoreactivity in cultured astrocytes (Pechan *et al.*, 1993). FGF2 administration increased the levels of the AMPA receptor subunit GluR1 and enhanced Ca^{2+} influx after AMPA receptor activation (Cheng *et al.*, 1995). In addition, FGF2 application prevented NMDA receptor-mediated cell death in cultured cells (Fernandez-Sanchez & Novelli, 1993), presumably by reducing NR2A/NR2C NMDA subunits expression (Brandoli *et al.*, 1998). Furthermore, cultured cortical neurons treated with FGF2 showed enhanced glutamate release and synaptic plasticity (Numakawa *et al.*, 2002).

In vivo studies showed that FGF2 knockout mice had fewer glutamatergic neurons in the PFC (Korada *et al.*, 2002) and less glutamatergic innervation of the striatum (Fadda *et al.*, 2007), as compared to wild-type controls. Moreover, FGF2 increased the expression of the astrocytic glutamate transporters GLAST and GLT-1 and promoted glutamate uptake both *in vivo* (Feng *et al.*, 2015) and in cultured astrocytes (Suzuki *et al.*, 2001). Together, these finding strongly indicate that FGF2 is a regulator of the glutamatergic system.

FGF2 and drugs of abuse

All drugs of abuse increase DA transmission in the mesolimbic system, and the DAergic system has been identified for its critical role in many behavioral aspects of drug addiction (Wise, 1987, 2009; Berridge & Robinson, 1998; Hyman *et al.*, 2006; Koob & Volkow, 2009). The well-established interaction between the FGF2 and the DAergic systems, as described above, aroused interest in the involvement of FGF2 in the effects of several drugs of abuse. Most studies have shown that drugs of abuse can affect the expression of FGF2 in several brain regions. Moreover, several studies have also suggested that FGF2 plays an important regulatory role in behavioral phenotypes caused by these drugs (Table 1).

Nicotine

A single nicotine injection (1 mg/kg i.p) increased *Fgf2* mRNA in the rat striatum (Roceri *et al.*, 2001). Likewise, acute treatment with the nicotinic acetylcholine receptor agonists (±)-epibatidine (50 μg

TABLE 1. FGF2 in studies with drugs of abuse. (A) Effects of drugs on FGF2 expression. (B) Effects of FGF2-related manipulations on drug-related behaviors

Drug	Treatment regimen	FGF2 expression	Effect	Reference
(A)				
Nicotine	Acute intermittent (3 injections × 1 mg/kg, administered with 30-min intervals)	*mRNA*		Roceri *et al.* (2001)
		Striatum	↑↑↑	
		Entorhinal cortex, parietal cortex	↑	
	A single injection (1 mg/kg)	*mRNA*		Roceri *et al.* (2001)
		Striatum	↑↑	
		Protein		
		Striatum (LMW:18 and HMW: 21 kDa)	↑↑	
	Oral gavage on PND 1–7 6 mg/kg a day	*mRNA*		Son & Winzer-Serhan (2009)
		Hippocampus (neurons and astrocytes)	↑	
	Acute intermittent (3 injections × 1 –2 mg/kg, administered with 30-min intervals)	*mRNA*		Belluardo *et al.* (1998)
		Cortex, hippocampus: CA1, dentate gyrus, ventral midbrain, striatum (neurons)	↑↑↑	
		Protein		
		SNc, hippocampus, cortex	↑↑	
	Acute intermittent (3 injections × 1 mg/kg, administered with 30-min intervals)	*mRNA*		Maggio *et al.* (1998)
		Striatum	↑↑	
	Chronic exposure (0.125–0.3 mg/kg/hour, over 14 days)	*mRNA*		Blum *et al.* (1996)
		Ventral midbrain	↓↓↓	
		No change in 6-OHDA treated rats		
	Chronic exposure (0.125 mg/kg/hour, over 14 days)	*mRNA*		Blum *et al.* (1996)
		Hippocampus, striatum	-	
Amphetamine	Intermittent exposure (3 days × 3 mg/kg)	*Protein*		Flores *et al.* (1998)
		VTA, SNc (astrocytes)	↑↑	
		NAc and dorsal striatum (astrocytes)	-	
	Intermittent exposure (4 days × 1.5 mg/kg)	*Protein*		Flores *et al.* (2000)
		VTA, SNc (astrocytes)	↑↑	
	Escalating exposure (1–4 mg/kg over 2 weeks)	*Protein*		Flores & Stewart (2000b)
		VTA, SNc (astrocytes)	↑↑	
		NAc and PFC (astrocytes) (1 week after the last amphetamine injection)	↑	
	Escalating exposure (1–9 mg/kg over 5 weeks)	*Protein*		Flores & Stewart (2000b)
		VTA, SNc (astrocytes) (1 week after the last amphetamine injection)	-	
		VTA, SNc (astrocytes) (5 weeks after the last amphetamine injection)	↓↓	
	Early life intermittent exposure (PND 10, 12 and 14, 2 mg/kg)	*Protein*		Mueller *et al.* (2006)
		VTA (on PND 21)	↑	
		VTA (on PND 30)	-	
Cocaine	Acute injection (dose response 5–20 mg/kg)	*mRNA*		Fumagalli *et al.* (2006)
		Striatum, PFC (5–20 mg/kg)	↑↑	
		Primary and secondary motor cortex (20 mg/kg)	↑↑	
		Hippocampus (5–20 mg/kg)	-	
	Acute injection (time response 5 mg/kg)	*mRNA*		Fumagalli *et al.* (2006)
		Hippocampus (30 min after injection)	↑↑	
		PFC, ventral midbrain (2 h after injection)	↑↑	
		Striatum (2 or 6 h after injection)	↑↑	
		Hippocampus, PFC, striatum (24 h after injection)	-	
	Sub-chronic exposure (5 mg/kg once a day for 5 days)	*mRNA*		Fumagalli *et al.* (2006)
		Striatum and PFC (2 and 72 h after the last injection)	↑↑	
		Ventral midbrain (72 h after the last injection)	↑↑↑	
		Primary and secondary motor cortex, hippocampus (2 and 72 h after the last injection)	-	
	Chronic exposure (5 mg/kg once a day for 14 days)	*mRNA*		Fumagalli *et al.* (2006)
		Primary and secondary motor cortex, hippocampus (2 h after the last injection)	↑↑	
		PFC (72 h after the last injection)	↑↑	
		Striatum (2 h, 72 h, 14 days after the last injection)	↑↑	
		Ventral midbrain (72 h after the last injection)	-	
	Adolescent chronic exposure (20 mg/kg once a day PND 28–42)	*mRNA*		Giannotti *et al.* (2013)
		Hippocampus (PND 45)	↑↑	
		PFC (PND 90)	↑↑	
		Dorsal striatum, NAc (both PND 45 and 90)	-	
	Adolescent chronic exposure (20 mg/kg once a day PND 28–42)	*mRNA*		Giannotti *et al.* (2016)
		VTA, infralimbic PFC, NAc core, ventral hippocampus, central amygdala (24 h after the last injection)	↓	
	Acute injection (10 mg/kg)	*mRNA*		Fumagalli *et al.* (2008)
		Striatum, PFC and primary and secondary motor cortex	↑↑	
		Hippocampus	↑	

(continued)

Table 1 (continued)

Drug	Treatment regimen	FGF2 expression	Effect	Reference
	Operant self-administration (a single session)	*mRNA* NAc, hypothalamus, hippocampus, Primary and secondary motor cortex, striatum, midbrain, medial PFC	-	Fumagalli *et al.* (2009)
	Prolong operant self-administration (16–20 sessions)	*Protein* Infralimbic PFC (after 9 withdrawal days)	↑↑	Hafenbreidel *et al.* (2015)
Alcohol	Acute exposure (2.5 g/kg)	*mRNA* Dorsal striatum, NAc, hippocampus	↑	Even-Chen *et al.* (2017)
	Sub-chronic exposure (7 × 2.5 g/kg)	*mRNA* DMS and DLS	↑↑	Even-Chen *et al.* (2017)
	Voluntary consumption (IA2BC 20% alcohol)	*mRNA* DMS	↑↑	Even-Chen *et al.* (2017)
Natural rewards (sucrose/saccharin)	Sucrose operant self-administration	*Protein* Infralimbic PFC (after 9 withdrawal days)	-	Hafenbreidel *et al.* (2015)

Drug	FGF2 manipulation	Behavioral procedure	Effect	Reference
(B)				
Amphetamine	Anti-FGF2 neutralizing antibody in the VTA	Amphetamine-induced locomotor activity during training	↓↓	Flores *et al.* (2000)
		Amphetamine-induced locomotor sensitization during challenge test	↓↓↓	
Cocaine	Selectively bred rat to have high novelty response (bHR, higher FGF2 expression in VTA, SNc, NAc, hippocampus compared to bLR)	Cocaine sensitization	↑↑↑	Clinton *et al.* (2012)
		Operant cocaine self-administration	↑↑↑	Kabbaj *et al.* (2001)
		Break point in progressive ratio operant schedule	↑↑↑	Cummings *et al.* (2011)
		Cue- and drug-induced reinstatement of operant self-administration	↑↑↑	Flagel *et al.* (2016)
		Extinction of operant cocaine self-administration	↓↓↓	Flagel *et al.* (2016)
	Neonatal (PND 2) systemic FGF2 injection (20 μg/kg)	Cocaine-induced locomotor activity	↑↑↑	Clinton *et al.* (2012)
		Cocaine sensitization * Only in bLR rats that express low levels of FGF2 as compared to bHR	↑↑↑	Clinton *et al.* (2012)
		Acquisition of operant cocaine self-administration (PND 67)	↑↑↑	Turner *et al.* (2009)
	Anti-FGF2 neutralizing antibody in the infralimbic PFC	Extinction retention in operant cocaine self-administration	↑↑↑	Hafenbreidel *et al.* (2015)
	FGF2 knockout mice	Locomotor activity in a novel environment	↑↑↑	Fadda *et al.* (2007)
		Cocaine-induced locomotor activity	↑↑↑	
Alcohol	Systemic FGF2 injection (80 μg/kg)	Alcohol consumption and preference (two-bottle choice)	↑↑	Even-Chen *et al.* (2017)
		Total fluids consumption	-	
	Intra-DMS FGF2 infusion (200 ng/hemisphere)	Alcohol consumption and preference (two-bottle choice)	↑↑↑	Even-Chen *et al.* (2017)
		Total fluids consumption	-	
	Anti-FGF2 neutralizing antibody in the DMS	Alcohol consumption and preference (two-bottle choice)	↓↓↓	Even-Chen *et al.* (2017)
		Total fluids consumption	-	
Natural rewards (sucrose/saccharin)	Systemic FGF2 injection (80 μg/kg)	Sucrose and saccharin consumption and preference (two-bottle choice)	-	Even-Chen *et al.* (2017)

↑, mild increase; ↑↑, moderate increase; ↑↑↑, strong increase. ↓, mild decrease; ↓↓, moderate decrease; ↓↓↓, strong decrease. 6-OHDA, 6-hydroxydopamine; bHR, basal high responders to novel environment; bLR, basal low responders to novel environment; DLS, dorsolateral striatum; DMS, dorsomedial striatum; HMW, high molecular weight; LMW, low molecular weight; NAc, nucleus accumbens; PFC- prefrontal cortex; PND, postnatal day; SNc, substantia nigra pars compacta; VTA, ventral tegmental area.

/kg) and ABT-594 (0.3 μmol/kg), increased *Fgf2* mRNA levels in the rats hippocampus, frontal cortex, SNc, and striatum (Belluardo *et al.*, 1999a,b). Acute intermittent treatment with nicotine (3–4 injections of 1–2 mg/kg, administered in 30 min intervals) produced a more widespread effect, leading to upregulation in neuronal *Fgf2* mRNA expression in several rat brain regions, namely the hippocampus, cerebral cortex, entorhinal cortex, parietal cortex, striatum, and ventral midbrain (that included the VTA and SNc) (Belluardo *et al.*, 1998; Maggio *et al.*, 1998; Roceri *et al.*, 2001). Importantly, this mRNA upregulation was followed by an increase in FGF2 protein levels (Belluardo *et al.*, 1998; Maggio *et al.*, 1998; Roceri *et al.*, 2001). Finally, nicotine treatment (6 mg/kg/day) given to developing rats by oral gavage in postnatal days (PND) 1–7 increased hippocampal *Fgf2* mRNA levels (Son & Winzer-Serhan, 2009). Thus, short-term or early-life treatment with nicotine increases the expression of FGF2 in several brain regions.

Nicotine-induced *Fgf2* upregulation was blocked by the DA D1-like and D2-like antagonists (SCH 23390 and haloperidol, respectively) (Roceri *et al.*, 2001), suggesting that this effect is mediated by DAergic transmission. In contrast, blocking NMDA and non-NMDA glutamatergic receptors, as well as cholinergic muscarinic receptors with MK-801, CNQX and scopolamine, respectively, had no effect on nicotine-induced *Fgf2* mRNA expression (Belluardo *et al.*, 1998).

Contrary to the acute and early-life treatments, chronic (0.0125–0.3 mg/kg/hour, over 14 days) nicotine treatment produced a dose-dependent reduction in *Fgf2* mRNA levels in the rat ventral midbrain, with no effect on *Fgf2* levels in the dorsal hippocampus or striatum (Blum *et al.*, 1996). Interestingly, rats with a 6-OHDA DAergic lesion had normal levels of *Fgf2* mRNA after nicotine treatment, suggesting that an intact DAergic system is required to mediate the effects of nicotine on FGF2 (Blum *et al.*, 1996).

Together, these data suggest that short nicotine exposure leads to upregulation in FGF2 expression in several brain regions, whereas longer chronic treatment reduces midbrain FGF2 levels, and that these opposite effects are both mediated by the DAergic system.

Amphetamine

Early-life repeated treatment with amphetamine (2 mg/kg on PND 10, 12 and 14) increased FGF2 levels in astrocytes in the rat VTA on PND 21, but not PND 30 (Mueller *et al.*, 2006). Moreover, a treatment protocol that leads to psychomotor sensitization (three injections of 1.5 or 3 mg/kg i.p, given every other day) increased FGF2 immunoreactivity in astrocytes in the rat ventral midbrain, but not in striatal DA terminal regions (Flores *et al.*, 1998, 2000). This upregulation was detected 24 h after the last amphetamine injection and lasted for at least a month (Flores *et al.*, 1998), indicating that amphetamine treatment results in long-lasting changes in the FGF2 system. Interestingly, blockade of glutamatergic receptors, which prevented the development of amphetamine sensitization, abolished the amphetamine-induced increase in FGF2 levels in the midbrain (Flores *et al.*, 1998, 2000). Thus, at least in the VTA and SNc, amphetamine upregulates FGF2 via a glutamate-dependent mechanism.

When amphetamine was administered in an escalating dose regimen (1–4 mg/kg over 2 weeks), FGF2 levels in astrocytes increased not only in the VTA and SNc, but also in the NAc and PFC (Flores & Stewart, 2000b). In contrast, longer escalating amphetamine treatment (1–9 mg/kg over a 5 weeks period) led to downregulation of astrocytic FGF2 levels in the rat VTA and SNc (Flores & Stewart, 2000a,b). Thus, long-term heavy exposure to amphetamine shifts its effect on FGF2 from upregulation to downregulation. Moreover, blocking FGF2 activity with an anti-FGF2 neutralizing antibody, infused intracisternally before amphetamine exposure, completely blocked the dendritic growth in VTA DAergic neurons induced by amphetamine (Mueller *et al.*, 2006). Together, the findings that repeated amphetamine treatment leads to long-lasting alterations in mesolimbic and nigrostriatal FGF2 expression raise the possibility that FGF2 is involved in amphetamine-induced neural adaptations.

In support of this assumption, the magnitude of amphetamine psychomotor sensitization correlated with the levels of FGF2 in midbrain astrocytes (Flores *et al.*, 2000). Moreover, blocking FGF2 activity in the VTA during repeated exposure to amphetamine reduced the immediate locomotor response to amphetamine and prevented the expression of sensitization after an amphetamine challenge injection (0.75 mg/kg) that was given 1–2 weeks later (Flores *et al.*, 2000). Together, these findings suggest that FGF2 in the VTA is critical for the neuroadaptations that underlie amphetamine psychomotor sensitization.

To summarize, repeated amphetamine treatment results in upregulation in astrocytic FGF2 expression via glutamatergic receptor activation. In turn, elevated levels of FGF2 play a role in amphetamine-induced neuronal plasticity by promoting dendritic growth in DAergic VTA neurons. These long-lasting neuroadaptations may underlie amphetamine-induced psychomotor sensitization. Nevertheless, future studies should test the role of FGF2 at cell-specific reduction (astrocytes vs. neurons) in amphetamine-related behaviors, including psychomotor sensitization and self-administration.

Cocaine

Fgf2 mRNA expression increased after acute cocaine treatment (5 mg/kg) in the rat striatum, PFC, primary and secondary motor cortex and hippocampus, with different temporal dynamics (Fumagalli *et al.*, 2006). This increase was shown to be regulated by acute or chronic stress (Fumagalli *et al.*, 2008). Interestingly, the cocaine-induced increase in striatal *Fgf2* mRNA expression was further enhanced by chronic, but not acute, stress. In contrast, in the PFC only acute stress facilitated the increase in *Fgf2* expression, whereas chronic stress prevented this effect (Fumagalli *et al.*, 2008), suggesting that stress differentially modulates the cocaine-FGF2 interactions in a brain region-dependent manner.

While sub-chronic cocaine treatment (daily 5 mg/kg for 5 days) led to long-lasting increases in *Fgf2* expression in the striatum, ventral midbrain and PFC, it had no effect on *Fgf2* levels in the primary and secondary motor cortex and hippocampus (Fumagalli *et al.*, 2006). However, longer cocaine treatment (5 mg/kg for 14 days) led to more wide-spread effects and increased *Fgf2* mRNA levels in the striatum, PFC, primary and secondary motor cortex, and hippocampus (Fumagalli *et al.*, 2006). Interestingly, the increased *Fgf2* levels in the striatum and PFC were still detected 14 and 3 days, respectively, after the last cocaine injection (Fumagalli *et al.*, 2006). Thus, both short- and long-term treatments with cocaine increase *Fgf2* expression in cortical and subcortical brain regions.

Additional studies found that juvenile exposure to cocaine may have opposite short- and long-term effects on *Fgf2* expression. Cocaine (20 mg/kg) treatment during adolescence (PND 28–42) reduced *Fgf2* mRNA expression in the rat VTA, infralimbic PFC, NAc core, ventral hippocampus, and central amygdala, when measured 24 h after the last treatment (PND 43) (Giannotti *et al.*, 2016). However, after long-term withdrawal (PND 90), *Fgf2* mRNA levels were increased in the rat PFC (Giannotti *et al.*, 2013). Thus, early adolescent cocaine treatment may interfere with the expression of FGF2 in adulthood.

Voluntary cocaine operant self-administration was also shown to alter brain FGF2 levels. While a single session of operant cocaine self-administration was insufficient to affect *Fgf2* mRNA expression in rat brain (Fumagalli *et al.*, 2009), longer self-administration training (16–20 daily sessions) followed by 9 days of withdrawal increased FGF2 protein levels in the infralimbic PFC, and this effect was reversed by extinction training (Hafenbreidel *et al.*, 2015). Importantly, operant sucrose self-administration did not altered FGF2 protein expression (Hafenbreidel *et al.*, 2015).

Rats selectively bred to have a high novelty response (bHR) exhibit higher basal levels of FGF2 in the hippocampus, VTA, SNc, and NAc, compared to their low novelty responders (bLR) control counterparts (Perez *et al.*, 2009; Clinton *et al.*, 2012; Flagel *et al.*, 2014). bHR rats have shown a variety of addiction-related behavioral phenotypes, including increased locomotor response to a single cocaine injection (Clinton *et al.*, 2012), higher motivation to obtain cocaine in a progressive ratio schedule of reinforcement (Cummings *et al.*, 2011), higher levels of cocaine self-administration in adulthood (Kabbaj *et al.*, 2001), persistent cocaine seeking when cocaine is no longer available (extinction deficits), and higher levels of relapse in a reinstatement model (Flagel *et al.*, 2016). Interestingly, bLR rats were reported to have epigenetic modifications at the FGF2 promotor, which reduced *Fgf2* expression (Chaudhury *et al.*, 2014; Flagel *et al.*, 2016). Specifically, compared to bHR rats, bLRs had lower *Fgf2* mRNA levels, which correlated with increased association of a repressive mark on histones (H3K9me3) at the FGF2 promoter in the NAc, hippocampus and amygdala (Chaudhury *et al.*, 2014; Flagel *et al.*, 2016). Together, these findings suggest that FGF2 is a positive regulator of cocaine consumption (Flagel *et al.*, 2016).

Several studies suggested that FGF2 may mediate certain cocaine-related behaviors. Thus, compared with their wild-type littermates, FGF2 knockout mice showed enhanced locomotor response to novel environment, as well as an increased response to low (5 mg/kg), but not high (20 mg/kg), doses of cocaine injection (Fadda *et al.*, 2007). It is plausible that this effect was due to enhanced DAergic tone, as FGF2 knockout mice exhibit increased striatal DAergic innervation (Baron *et al.*, 2012; Rumpel *et al.*, 2016). Indeed, reducing DA transmission with a low dose of apomorphine (0.1 mg/kg) that selectively stimulates presynaptic inhibitory DA D2-like receptors restored locomotor activity of FGF2 knockout mice to levels similar to that of their wild-type littermates (Fadda *et al.*, 2007). On a similar note, neonatal FGF2 treatment (20 μg/kg) increased cocaine-induced behavioral sensitization in adulthood in the low-FGF2 expressing bLR rats, making their behavior more similar to the high FGF2-expressing bHR rats (Clinton *et al.*, 2012).

Finally, systemic neonatal (PND 2) administration of FGF2 (20 μg/kg) to wild-type rats enhanced their acquisition of cocaine self-administration in adulthood (PND 67) without affecting other types of learning (spatial learning, food self-administration) (Turner *et al.*, 2009). In contrast, blocking FGF2 activity in the infralimbic PFC with an anti-FGF2 neutralizing antibody before extinction training reduced cocaine seeking and enhanced extinction retention (Hafenbreidel *et al.*, 2015).

In summary, cocaine increases FGF2 levels in several mesocorticolimbic brain regions, and FGF2 levels positively correlate with cocaine consumption and sensitization. Thus, high levels of FGF2 may be a biomarker of vulnerability to addiction phenotypes, whereas low levels of FGF2 may indicate reduced risk for cocaine addiction-related behaviors. However, it is still unclear whether FGF2 plays a causal role in the regulation of cocaine-related behaviors. Therefore, future studies should aim to directly manipulate FGF2, which will allow the establishment of causality for FGF2 as a regulator of cocaine addiction-related behaviors.

Alcohol

Acute alcohol injection (2.5 g/kg) increased *Fgf2* mRNA levels in the mouse dorsal striatum, NAc, and hippocampus (Even-Chen *et al.*, 2017). However, longer alcohol exposure (2.5 g/kg injection once a day for 7 days) limited these changes to the dorsal striatum at both its sub-regions: the dorsomedial striatum (DMS) and the dorsolateral striatum (DLS), and these increases were found to be mediated by DA D2-like receptor activation (Even-Chen *et al.*, 2017).

Voluntary consumption of high levels of alcohol for 5–7 weeks via the intermittent access to 20% alcohol in two-bottle choice (IA2BC) procedure (Carnicella *et al.*, 2014) increased *Fgf2* levels in the DMS, but not the DLS, of both mice and rats (Even-Chen *et al.*, 2017). Thus, while short exposure to alcohol leads to widespread increases in the brain *Fgf2* expression, as the exposure to alcohol extends and becomes more robust the effects on *Fgf2* expression become more spatially specific.

In addition to alcohol affecting *Fgf2* expression, FGF2 was shown to mediate alcohol consumption. Specifically, systemic administration of recombinant FGF2 (80 μg/kg) to mice increased alcohol consumption and preference without affecting the consumption of water or natural rewards (sweetened solution: sucrose or saccharin) (Even-Chen *et al.*, 2017). Moreover, infusion of recombinant FGF2 (200 ng/hemisphere) into the dorsal striatum or the DMS of rats increased alcohol consumption and preference. In contrast, blocking FGF2 activity with an anti-FGF2 neutralizing antibody suppressed alcohol intake and preference (Even-Chen *et al.*, 2017). Thus, FGF2 acts as a positive regulator of alcohol consumption. The bidirectional effects of alcohol and FGF2 suggest that they constitute a positive feedback loop, which drives and maintains excessive alcohol intake. Hence, reducing striatal FGF2 activity can reduce alcohol drinking.

Summary

The data surveyed in this review suggests that *Fgf2* is a common responsive gene for nicotine, amphetamine, cocaine, and alcohol. While short-term exposure to drugs of abuse lead to general widespread increases in the expression of FGF2, longer-term exposure cause more region- and drug-specific effects, including downregulation of the growth factor in some brain regions (Fig. 1, Table 1A). Moreover, recent studies suggest that high levels of FGF2 are associated with increases in drug-related behaviors (Table 1B). Thus, high levels of endogenous FGF2, or administration of recombinant FGF2, enhances several behaviors associated with substance use disorder, including alcohol consumption and drug-induced psychomotor sensitization. In contrast, blocking the endogenous FGF2 activity suppresses drug/alcohol-related behavioral phenotypes. Importantly, while multiple studies tested the effects of drugs on FGF2 expression, only a few studies addressed the causal role of the growth factor in drug- and alcohol-related behaviors. This paucity of causal and mechanistic evidence is a major gap in the field, and should be addressed in future studies by directly manipulating the FGF2 system.

Interestingly, nicotine increases FGF2 expression in neurons, whereas amphetamine increases the expression of the growth factor in astrocytes (Table 1A). However, the specific cell-type in which the expression of FGF2 is affected by cocaine and alcohol is yet unknown. FGF2 expression in the striatum is upregulated by all the drugs surveyed here (Table 1A; Fig. 1). Striatal neurons mostly consist of GABAergic medium spiny neurons (MSN), which can be divided into two sub-populations as determined by the type of dopamine receptor they express: D1- or D2-expressing MSNs (Kreitzer & Malenka, 2008; Beaulieu & Gainetdinov, 2011; Gerfen & Surmeier, 2011; Calabresi *et al.*, 2014; Wei *et al.*, 2018). These neurons play opposite functional roles (Kreitzer & Malenka, 2008) in the so-called direct and indirect pathways, which were previously implicated in the action of drugs of abuse (Lobo & Nestler, 2011). Thus, manipulating FGF2 and/or FGFR1 at specific striatal cell type (D1-MSNs, D2-MSNs and astrocytes) is expected to significantly advance the understanding of the mechanism of FGF2-FGFR1 control over drug-related behaviors.

As mentioned above, the binding of FGF2 to its receptor activates several intracellular signaling pathways (Eswarakumar *et al.*, 2005). Activation of these signaling pathways has been implicated in the regulation of drug and alcohol addiction (Nestler *et al.*, 1996; Nestler, 2004; Kenny, 2011; Ron & Barak, 2016). Therefore, future studies addressing the mechanisms downstream of the FGF2–FGFR1 complex will be beneficial to elucidating those mechanisms that control the positive regulatory effects of FGF2 on drug and alcohol consumption and related behaviors.

Finally, a clear limitation of the current evidence for involvement of FGF2 in addiction is that they are derived from animal models (Table 1). Thus, genetic studies in humans, as well as postmortem studies in individuals with substance/alcohol use disorder that would reveal alterations in FGF2 expression as biomarkers of addiction, would establish and validate the FGF2–addiction link. Moreover, given the findings presented here, it is possible that reducing FGF2 activity or expression may possess anti-addictive effects, thus providing a novel pharmacological target to treat substance use disorders.

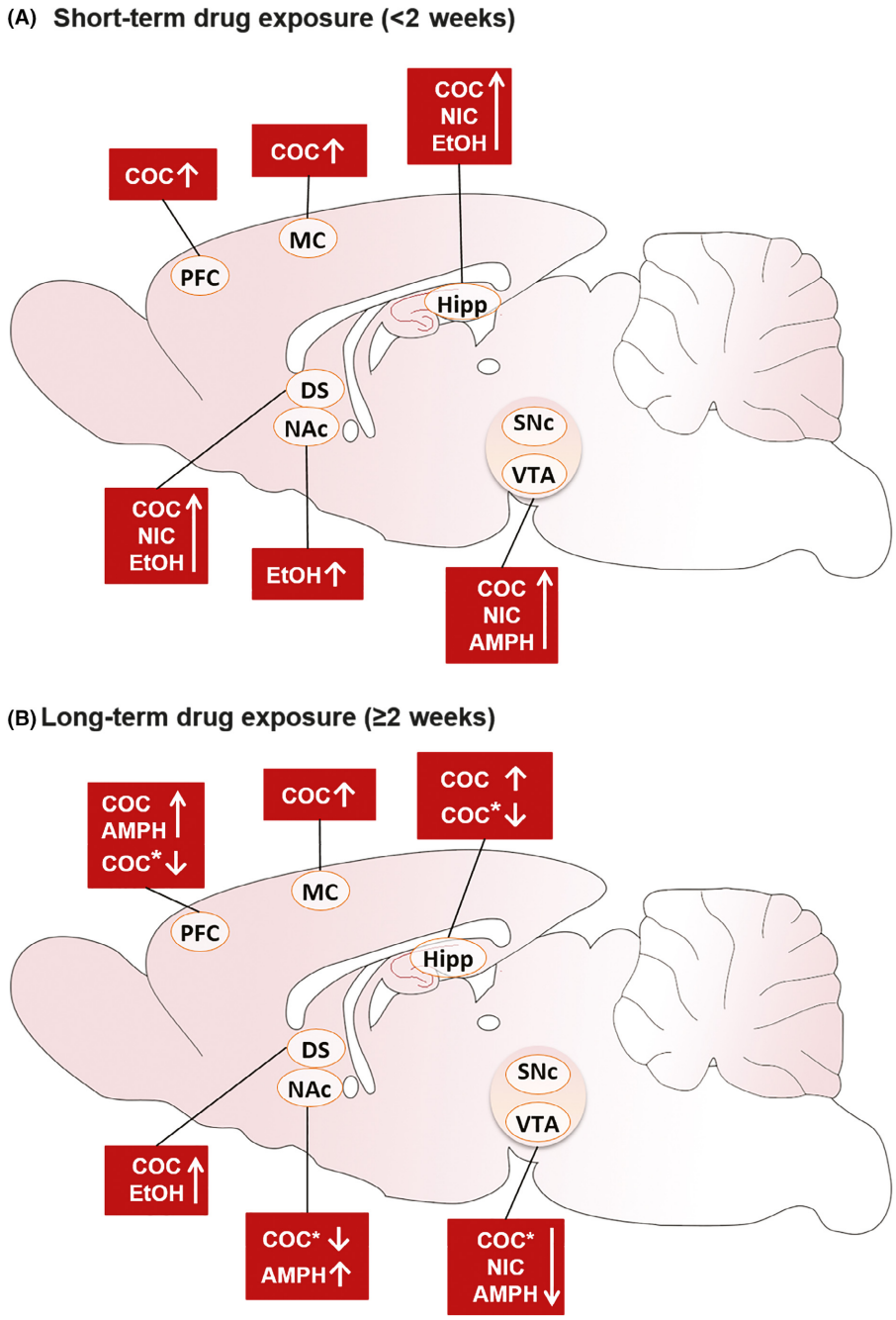

FIG. 1. Schematic representation of alterations in the brain FGF2 expression induced by drug exposure. Short-term (A) and long-term (B) drug exposure alter the mRNA or protein expression of FGF2. Up and down arrows represent upregulation and downregulation, respectively, in the expression of FGF2. COC, Cocaine; COC*, Cocaine treatment during adolescent (PND 28-42); AMPH, Amphetamine; NIC, nicotine; EtOH, alcohol; PFC, Prefrontal cortex; MC, Motor cortex; Hipp, Hippocampus; DS, Dorsal striatum; NAc, Nucleus accumbens; VTA, ventral tegmentum area; SNc, Substantia nigra pars compacta.

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Conflict of interest

The authors declare no competing financial interests.

Author contribution

OE-C and SB wrote the paper.

Abbreviations

6-OHDA, 6-hydroxydopamine; AMPH, Amphetamine; BDNF, Brain-derived neurotrophic factor; bHR, Basal high responders to novel environment; bLR, Basal low responders to novel environment; COC, Cocaine; DA, Dopamine; DAergic, Dopaminergic; DLS, Dorsolateral striatum; DMS, Dorsomedial striatum; DS, Dorsal striatum; EtOH, Ethyl alcohol; FGF2, Fibroblast growth factor 2; FGFR1, Fibroblast growth factor receptor 1; GDNF,

Glial cell line-derived neurotrophic factor; Hipp, Hippocampus; HMW, High molecular weight; IA2BC, Intermittent access to 20% alcohol in two-bottle choice; LMW, Low molecular weight; MAPK, Mitogen-activated protein kinase; MC, Motor cortex; MPTP, 1-metyl-1,2,3,6 – tetrahydropyridine; MSN, Medium spiny neurons; NAc, Nucleus accumbens; NIC, Nicotine; PFC, Prefrontal cortex; PI3K, Phosphatidylinositol-3-kinases; PKB, Protein kinase B; PLCγ, Phospholipase C gamma; PND, Postnatal day; SNc, Substantia nigra pars compacta; VTA, Ventral tegmental area.

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