Fibroblast Growth Factor 2 in the Dorsomedial Striatum Is a Novel Positive Regulator of Alcohol Consumption

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Abstract

Repeated alcohol intake leads to mesostriatal neuroadaptations, resulting in drinking escalation and addiction phenotypes. Fibroblast growth factor 2 (FGF2) has been shown to interact with the mesostriatal dopaminergic system, and has been implicated in the actions of psychostimulants in the brain, and in several psychiatric disorders. Here, we report on a positive regulatory feedback loop of alcohol and FGF2 in rodent models. Specifically, we found that acute alcohol exposure (2.5 g/kg, i.p.) increased the mRNA expression of Fgf2 in the dorsal hippocampus, nucleus accumbens, and dorsal striatum. Longer alcohol exposure (7 d × 2.5 g/kg, i.p.) restricted these increases to the dorsal striatum, and the latter effect was blocked by the dopamine D2-like receptor antagonist haloperidol. Voluntary prolonged and excessive alcohol consumption in a 2-bottle choice procedure increased Fgf2 expression selectively in dorsomedial striatum (DMS) of both mice and rats. Importantly, we found that systemic administration of recombinant FGF2 (rFGF2) in mice, or rFGF2 infusion into the dorsal striatum or DMS of rats, increased alcohol consumption and preference, with no similar effects on saccharin or sucrose consumption. Finally, we found that inhibition of the endogenous FGF2 function in the DMS, by an anti-FGF2 neutralizing antibody, suppressed alcohol consumption and preference. Together, our results suggest that alcohol consumption increases the expression of Fgf2 in the DMS, and that striatal FGF2 promotes alcohol consumption, suggesting that FGF2 in the DMS is a positive regulator of alcohol drinking.

Key words: addiction; alcohol; dopamine; dorsomedial striatum; fibroblast growth factor 2

Significance Statement

Long-term alcohol intake may lead to neuroadaptions in the mesostriatal reward system, resulting in addiction phenotypes. Fibroblast growth factor 2 (FGF2) is crucial for the development and maintenance of the mesostriatal dopaminergic system. Here, we provide evidence for the involvement of FGF2 in alcohol-drinking behaviors. We show that alcohol increases Fgf2 expression in the dorsal striatum, an effect mediated via dopamine D2-like receptors. Importantly, we show that infusion of recombinant FGF2 into the dorsomedial striatum increases alcohol consumption, whereas inhibiting the endogenous FGF2 function suppresses consumption. Thus, FGF2 is an alcohol-responsive gene constituting a positive regulatory feedback loop with alcohol. This loop leads to facilitation of alcohol consumption, marking FGF2 as a potential new therapeutic target for alcohol addiction.
McGough et al., 2004; Jeanblanc et al., 2006; Wang et al., 2007), and these alterations play a crucial role in subsequent alcohol-drinking behaviors (Wang et al., 2007, 2011; Jeanblanc et al., 2009; Logrip et al., 2014; Ron and Barak, 2016).

Fibroblast growth factor 2 (FGF2), a member of the FGF family consisting of 22 members, is implicated in brain development (Walicke et al., 1986; Ford-Perriss et al., 2001; Reuss and von Bohlen und Halbach, 2003), adult neurogenesis (Wagner et al., 1999), and regenerative plasticity (Gómez-Pinilla et al., 1995). In particular, FGF2 is involved in the development and maintenance of midbrain dopaminergic neurons (Reuss and Unsicker, 2000; Reuss and von Bohlen und Halbach, 2003; Grothe and Timmer, 2007).

The psychostimulants cocaine and amphetamine have been shown to increase FGF2 levels in several brain regions. Specifically, cocaine treatment upregulates Fgf2 mRNA levels in the striatum, PFC, and hippocampus (Fumagalli et al., 2006), and repeated administration of amphetamine leads to long-lasting increases in FGF2 immunoreactivity in the VTA and substantia nigra (Flores and Stewart, 2000a). FGF2 expression positively correlates with the magnitude of psychostimulants-induced locomotor sensitization (Flores and Stewart, 2000a), considered to model addiction-related neuroadaptations (Robinson and Berge, 2001). Furthermore, repeated administration of amphetamine results in an FGF2-mediated increase in dendritic growth of VTA dopaminergic neurons (Mueller et al., 2006). Together, these findings indicate that FGF2 is involved in the development of psychostimulant sensitization and may be involved in molecular mechanisms relevant to addiction.

Here, we tested whether alcohol exposure alters the expression of Fgf2 in mesolimbic and nigrostriatal brain regions, and if so, whether FGF2 regulates alcohol intake.

Materials and Methods

Animals

Male and female C57BL/6J mice and Wistar rats (20–25 g and 175–200 g at the beginning of experiments, respectively) were bred in Tel Aviv University animal facility and housed under a 12-h light/dark cycle (mice lights on at 4:00 A.M., rats lights on at 7:00 A.M.) with food and water available ad libitum. Animals were individually housed, except for alcohol-injection experiments, in which mice were housed 4 per cage. All experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University and of the National Institutes of Health. All efforts were made to minimize the number of animals and their suffering.

Reagents and drug preparation

Fast SYBR Green Master Mix (#4385617), TRIZol reagent (#13150101), and RevertAid kit (#0442) were supplied by Thermofisher Scientific. DNA oligonucleotides (qRT-PCR primers), haloperidol (#H1512), and isopropyl alcohol (99.5%) were obtained from Sigma-Aldrich. Isoflurane was obtained from Piramal Critical Care. Neutralizing antibody against FGF2 (05-117) and control IgG (12-371) were purchased from Millipore. FGF2 (05-117) and control IgG (12-371) were purchased from Millipore. rFGF2 (200 ng/0.75 μl) and control IgG (0.2% w/v) were used for solution instead of alcohol, and training lasted 3 weeks before rFGF2 treatment. Solutions of alcohol and water were infused in the target region.

Histology

Locations of cannulae were verified in 3 μm coronal sections of PFA-fixed tissue stained with cresyl violet. Only data from subjects with cannulae located in the region of interest were included in the analysis.

Experimental design and statistical analysis

Sex approximately equally distributed across experiments and was initially analyzed as a factor; however, all analyses did not yield a main effect.
of sex or any interaction with other factors (p values >0.05). Therefore, the data were collapsed across this factor. The rationale for the experiments is described in Results.

**Fgf2 expression experiments.** In these experiments, we tested the effects of alcohol exposure on Fgf2 expression in several brain regions. The mRNA expression of Fgf2 was normalized to Gapdh expression (Zipori et al., 2017), which we found to be unaffected by alcohol in the protocols we used (Fig. 1-1, available at https://doi.org/10.1523/JNEUROSCI.0890-17.2017.f1-1). Expression in each brain region was further normalized to its own control group (saline- or water-treated group).

First, we tested the effects of acute or subchronic alcohol administration on Fgf2 expression. Data were analyzed by one-way ANOVA, with a between-subjects factor of Time after the last alcohol injection. ANOVA was followed by Student-Newman-Keuls post hoc analyses throughout the study.

Next, we tested whether the effect of subchronic alcohol treatment on Fgf2 expression in the dorsal striatum was mediated by dopamine D2-like receptors. In this experiment, expression data were analyzed by two-way ANOVA, with between-subjects factors of Pretreatment (haloperidol, vehicle) and Treatment (alcohol, saline).

In the next set of experiments, we tested the effects of voluntary alcohol consumption on Fgf2 expression. Mice were trained to consume alcohol in the IA2BC drinking protocol, and Fgf2 levels were determined at the end of a 24-h alcohol drinking session or of a 24-h alcohol-withdrawal session. Data were analyzed by one-way ANOVA, with a between-subjects factor of Time point. Finally, we tested the effects of voluntary alcohol intake (IA2BC drinking protocol, in mice and rats) on Fgf2 expression in the dorsal striatum subregions: DMS and dorsolateral striatum (DLS). mRNA expression levels were measured at the end of a 24 h alcohol-withdrawal period and compared with a water-drinking control group by independent t tests with a between-subjects factor of Time point.

**Alcohol, saccharin, or sucrose consumption experiments.** In this set of experiments, we tested the effects of rFGF2, administered systemically in mice, or infused into the dorsal striatum in rats, on the consumption and preference of the reward, as well as on water and total fluid intake. First, we tested the effects of systemic rFGF2 or vehicle administration on alcohol (20%), saccharin (0.005%, 0.03%), or sucrose (0.2%, 1%) consumption. In the alcohol consumption experiment, data were analyzed by independent t tests (a between-subjects factor of Treatment). In the saccharin and sucrose experiments, data were analyzed by a 2 × 2 mixed-model ANOVA, with a between-subjects factor of Treatment (rFGF2, vehicle) and a within-subjects factor of solution Concentration.
The effects of intracerebral rFGF2 on alcohol intake and preference, as well as on water and total fluid intake, were tested in a counterbalanced within-subjects design. Data were analyzed by paired-sample t tests, with a within-subjects factor of Treatment.

Finally, we tested the effect of FGF2 inhibition on alcohol consumption. Anti-FGF2 neutralizing antibody and control IgG were infused into the rat DMS in a counterbalanced within-subjects design, and data were analyzed by paired-sample t tests (a within-subjects factor of Treatment).

Results
Acute and subchronic alcohol treatments increase Fgf2 mRNA expression
First, we tested whether acute alcohol treatment changes the mRNA expression of Fgf2. We focused on mesolimbic/striatal brain regions that were previously implicated in alcohol-drinking behaviors (Ron and Barak, 2016), namely, the NAc, dorsal striatum, and dorsal hippocampus. Mice received a single injection of alcohol (2.5 g/kg in a 20% alcohol v/v solution, i.p.) or saline, and we dissected these brain regions 2, 6, and 24 h later.

We found that alcohol increased Fgf2 mRNA levels in a time- and brain region-specific manner (Fig. 1A). Specifically, we observed an increase in Fgf2 expression in the dorsal striatum 2 h after alcohol injection, but the expression levels of the growth factor returned to baseline levels 6 h after alcohol injection (one-way ANOVA, F(3,33) = 6.46, p = 0.002; post hoc, 2 h vs saline, p = 0.027). In addition, alcohol increased Fgf2 expression in the NAc 2 and 24 h (but not 6 h) after alcohol injection (one-way ANOVA, F(3,34) = 7.15, p = 0.001; post hoc, 2 h vs saline, p = 0.003, or 24 h vs saline, p = 0.021). Finally, alcohol increased Fgf2 expression in the dorsal hippocampus 24 h after alcohol injection (one-way ANOVA, F(3,26) = 4.71, p = 0.01; post hoc, 24 h vs saline, p = 0.011).

Next, we determined the effects of a longer alcohol treatment on the expression of Fgf2. Mice were injected with alcohol (2.5 g/kg in a 20% alcohol v/v solution, i.p.) or saline once a day for 7 d. Mice were killed 2 or 24 h after the last alcohol injection, as we did not observe any changes in Fgf2 expression at the 6 h time point following the acute treatment. As shown in Figure 1B, we found that this subchronic alcohol treatment also increased Fgf2 mRNA levels in the dorsal striatum 2 h, but not 24 h after the last alcohol injection (one-way ANOVA, F(2,14) = 9.66, p = 0.002; post hoc, 2 h vs saline, p = 0.003). No significant changes in the growth factor’s expression were detected in the NAc or dorsal hippocampus (p values >0.15).

Together, these data indicate that short alcohol exposure increases Fgf2 expression in the NAc, dorsal striatum, and dorsal hippocampus, brain regions that were previously implicated in alcohol-drinking behaviors (Koob and Volkow, 2016; Ron and Barak, 2016). However, more extended alcohol exposure restricts the increases in Fgf2 expression to the dorsal striatum.

Dopamine D2-like receptors mediate the alcohol-induced increase in striatal Fgf2
Alcohol has been shown to increase mesolimbic and nigrostriatal dopamine levels (Di Chiara and Imperato, 1988; Gonzales et al., 2004), and we show here that striatal Fgf2 expression is increased by alcohol. Furthermore, treatment with a dopamine D2 receptor (D2R) agonist was shown to increase Fgf2 mRNA expression, whereas agonists of the D1 or D3 receptors had no such effect (Fumagalli et al., 2003). Thus, we next tested whether the effects of alcohol on Fgf2 expression in the dorsal striatum are mediated by dopamine D2-like activation.

To test this possibility, we used the repeated alcohol injection protocol as above, which yielded an ~50% increase in Fgf2 mRNA expression in the dorsal striatum 2 h after treatment (Fig. 1B). Therefore, we pretreated mice once a day for 7 d with the dopamine D2-like receptor antagonist haloperidol (1 mg/kg, i.p.) or vehicle, and 1 h later were treated with alcohol (2.5 g/kg, 20%, i.p.) or saline, once a day for 7 d. The dorsal striatum was collected 2 h after the last alcohol injection. Fgf2 mRNA levels were determined by qRT-PCR and normalized to Gapdh, which was not affected by alcohol on its own (Fig. 1-1), available at https://doi.org/10.1523/JNEUROSCI.0890-17.2017.f1-1). Bar graphs indicate mean ± SEM, normalized to the saline + vehicle control group, n = 5–7 per group, *p < 0.05.

Voluntary alcohol consumption increases the expression of Fgf2 in the dorsal striatum
Next, we determined the effects of voluntary alcohol drinking on the expression of Fgf2. Mice were trained to consume alcohol in the IA2BC drinking procedure (Neasta et al., 2010; Warnaut et al., 2013) for 5 weeks. In this drinking protocol, mice consume high quantities of alcohol, typically yielding an average alcohol consumption of 10–15 g/kg/24 h, which generates blood alcohol concentration of >100 mg/dl after binge-like drinking at the first hours of drinking (Hwa et al., 2011; Griffin, 2014; Ron and Barak, 2016). The NAc, dorsal striatum, and dorsal hippocampus of mice were collected at the end of a 24-h alcohol drinking session (mean alcohol consumption: ~10 ± 0.75 g/kg/24 h, alcohol pref-
Carnicella et al., 2014

alcohol or are due to a more generalized effect on reward compared with water control.

In addition, we found a trend toward an increase in alcohol preference at 80 g/kg rFGF2 (200 ng/0.75 l per hemisphere) and that systemic administration of the growth factor increased alcohol intake. To test whether the latter effect is mediated by the dorsal striatum, we next infused rFGF2 into the dorsal striatum of rats with a long-history of excessive alcohol drinking.

Although systemic administration of the growth factor increased alcohol intake, we next tested whether rFGF2 also affects the consumption of sweetened solutions (saccharin, which has no caloric value, or sucrose, which, like alcohol, has caloric value). Mice were trained to consume saccharin (0.005%, 0.03%) or sucrose (0.2%, 1%) in an intermittent access 2-bottle choice (water and saccharin/sucrose) procedure for 3 weeks. rFGF2 (80 μg/kg, s.c.) or vehicle was administered 1 h before the beginning of a drinking session. We found that rFGF2 had no effects on saccharin or sucrose intake or preference at any of the solution concentrations (Fig. 4G–J; p values >0.60). Importantly, our data indicated an increase in saccharin and sucrose consumption as a function of their concentration (mixed-model ANOVA, saccharin: F(4,22) = 26.22, p < 0.0001; sucrose: F(4,22) = 26.22, p < 0.0001), ruling out the possibility that rFGF2 failed to increase the consumption of the sweet solutions due to a ceiling effect.

Infusion of rFGF2 into the dorsal striatum increases voluntary alcohol consumption and preference

We found that a history of excessive alcohol consumption led to upregulation of Fgf2 expression selectively in the dorsal striatum and that systemic administration of the growth factor increased alcohol intake. To test whether the latter effect is mediated by the dorsal striatum, we next infused rFGF2 into the dorsal striatum of rats with a long-history of excessive alcohol drinking.

Rats were trained to consume alcohol in the IA2BC drinking procedure for 7 weeks (Carnicella et al., 2014). In this drinking protocol, Wistar rats typically show an average alcohol consumption of 3.5–5.5 g/kg/24 h, which generates blood alcohol concentration of >60 mg/dl after binge-like drinking during the first hours of drinking (Cippitelli et al., 2012; Carnicella et al., 2014). rFGF2 (200 ng/0.75 μl per hemisphere) and vehicle were infused into the dorsal striatum in a counterbalanced withins...
As depicted in Figure 5, we found that rFGF2 infusion increased alcohol consumption ($t_{(13)} = 3.67, p = 0.003$) and preference ($t_{(13)} = 2.60, p = 0.022$). rFGF2 did not alter water consumption ($p = 0.55$), further indicating that the growth factor increased alcohol consumption without affecting other consummatory behaviors. Because alcohol intake was increased by rFGF2 without an effect on water intake, the total fluid intake was increased ($t_{(13)} = 3.88, p = 0.002$). Interestingly, rFGF2 did not affect alcohol consumption or preference in the first 30 min or 4 h of drinking ($p$ values $>0.12$). These results indicate that FGF2 in the dorsal striatum is an enhancer of alcohol consumption and that its action involves slow-onset mechanisms.

The effect of excessive alcohol consumption on Fgf2 expression is localized to the DMS

The dorsal striatum is divided into two anatomical and functional subregions: the DMS and the DLS. The DMS is implicated in goal-directed behavior (Yin et al., 2005; Yin and Knowlton, 2006) and is important for the early stages of alcohol consumption (Corbit et al., 2012). The DLS is implicated in habit learning.
induced by excessive consumption of alcohol, are localized to the DMS (DMS: mice, $t_{(9)} = 2.67, p = 0.026$; rats, $t_{(10)} = 2.37, p = 0.039$; DLS: both species, $p$ values $>0.67$).

**Infusion of rFGF2 into the DMS increases voluntary alcohol consumption and preference**

Because we found that withdrawal from excessive alcohol consumption increases Fgf2 expression selectively in the dorsomedial subregion of the striatum, we next tested whether the effects of rFGF2 on alcohol intake were also localized to this brain region. Rats were trained to consume alcohol in the IA2BC procedure for 7 weeks, as described above. rFGF2 (200 ng/0.75 μl per hemisphere) and vehicle were infused into the DMS in a counterbalanced within-subjects design, 10 min before the beginning of a 24-h alcohol drinking session. As shown in Figure 7, we found that rFGF2 treatment increased alcohol consumption and preference, compared with vehicle (paired $t$ test; consumption: $t_{(7)} = 2.93, p = 0.022$; preference: $t_{(7)} = 4.11, p = 0.05$). Moreover, although rFGF2 reduced water intake ($t_{(7)} = 2.85, p = 0.025$), it did not affect...
the total fluid intake ($p = 0.118$). These results suggest that increasing the levels of FGF2 in the DMS enhances alcohol consumption.

**Inhibition of FGF2 activity in the DMS reduces alcohol consumption and preference**

Our results suggest that FGF2 in the DMS is a positive regulator of alcohol intake. If so, inhibition of FGF2 activity in this brain region is expected to reduce alcohol consumption. Therefore, we next determined the effects of inhibition of FGF2 function in the DMS on alcohol intake. We used a neutralizing antibody against FGF2 ([Mueller et al., 2006]; [Hafenbreidel et al., 2015]) to inhibit the function of the growth factor. This neutralizing antibody selectively targets FGF2, with no cross-reactivity to FGF1 ([Matsuzaki et al., 1989]; [Tao et al., 1997]).

Rats were trained to consume alcohol in the IA2BC procedure, as described above. Anti-FGF2 neutralizing antibody (750 ng/0.75 μl per hemisphere) and control IgG were infused into the DMS in a counterbalanced within-subjects design, 1 h before the beginning of a 24 h alcohol drinking session.

As detailed in Figure 8, we found that inhibition of FGF2 function in the DMS suppressed alcohol consumption and preference, compared with the control treatment (paired t test; consumption: $t_{(14)} = 2.81, p = 0.014$; preference: $t_{(14)} = 4.35, p = 0.001$). Although inhibition of FGF2 increased water intake ($t_{(14)} = 2.90, 0.012$), it did not affect the total fluid intake ($p = 0.54$). These results indicate that decreasing FGF2 activity in the DMS suppresses alcohol consumption, suggesting that the endogenous FGF2 positively regulates alcohol-drinking behavior.

**Discussion**

Our results demonstrate the involvement of FGF2 in alcohol-drinking behaviors, raising the possibility that this growth factor is involved in the escalation and maintenance of alcohol drinking. Our main findings are as follows: (1) a history of excessive alcohol drinking results in a sustained increase in $Fgf2$ mRNA levels in the DMS; (2) rFGF2 increases alcohol consumption and preference, and its action is localized to the DMS; and (3) inhibition of the endogenous FGF2 in the DMS suppresses alcohol intake. Based on these findings, we propose that excessive alcohol drinking leads to elevated FGF2 levels in the DMS, which in turn promote excessive alcohol consumption. Thus, these bidirectional effects provide a positive regulatory feedback loop of alcohol and FGF2, possibly contributing to the escalation in alcohol intake.
FGF2 is an alcohol-responsive gene

We show that acute alcohol exposure leads to increased expression of the \( Fgf2 \) transcript in the NAc, dorsal striatum, and dorsal hippocampus. Interestingly, as alcohol exposure extended, its effects on \( Fgf2 \) expression became more specific and spatially restricted. Specifically, short-term exposure to alcohol (7 daily injections) increased \( Fgf2 \) expression only in the dorsal striatum shortly after the last injection, but the expression levels returned to baseline after 24 h. Long-term voluntary consumption of excessive alcohol levels increased \( Fgf2 \) expression exclusively in the DMS. Thus, as the exposure to alcohol extends and becomes more robust, the effects on \( Fgf2 \) expression become more specific and stable.

We further show that the striatal increase in \( Fgf2 \) expression following repeated alcohol injections was blocked by haloperidol, suggesting that this effect is mediated via dopamine D2-like receptor activation. Given that alcohol injections increase dopamine levels in the dorsal striatum (Di Chiara and Imperato, 1988), it is likely that alcohol increases striatal \( Fgf2 \) levels by increasing dopamine levels, which activate dopamine D2-like receptors. As haloperidol was reported to have low affinity to additional receptors (e.g., sigma1 receptor and \( \alpha-1a \) andrenoreceptor) (Walker et al., 1988; Richelson and Souder, 2000), we cannot rule out the involvement of these receptors in the effects of alcohol on \( Fgf2 \). Moreover, withdrawal from prolonged excessive alcohol drinking leads to an allostatic reduction in dopamine levels (Weiss et al., 1996; Barak et al., 2011a). Therefore, it is possible that, after a long period of alcohol exposure, additional mechanisms are involved in the upregulation of \( Fgf2 \) expression (e.g., glutamatergic or GABAergic neurotransmission), which were previously implicated in modulation of \( Fgf2 \) expression (Riva et al., 1994; Flores and Stewart, 2000a; Gómez-Pinilla et al., 2000).

Importantly, it has previously been reported that increases in the \( Fgf2 \) mRNA levels in the striatum, PFC, and hippocampus caused by nicotine (Roceri et al., 2001) and the D2R agonist quinpirole (Fumagalli et al., 2003) are accompanied by a similar increase in the \( Fgf2 \) protein levels. Thus, it is likely that the transcriptional changes in \( Fgf2 \), which we observed, translate into changes in the protein levels.

FGF2 positively regulates alcohol consumption

Upon showing that \( Fgf2 \) expression is increased in the DMS after a history of excessive alcohol consumption, we show that \( Fgf2 \)
regulates alcohol consumption. Specifically, systemic administration of rFGF2 to mice, as well as focal infusion of rFGF2 into the dorsal striatum, or more specifically into the DMS of rats, led to increased alcohol intake and preference. Importantly, the effect of rFGF2 was specific to alcohol, as it did not increase the consumption of water, saccharin, or sucrose. Interestingly, rFGF2 administration had no effects during the initial 4 h of the drinking session. This delayed-onset effect suggests that the actions of FGF2 may require transcriptional and/or translational mechanisms, which lead to increased alcohol intake in a delayed manner.

We further found that inhibition of the endogenous FGF2 in the rat DMS, by infusion of an anti-FGF2 neutralizing antibody, suppressed alcohol consumption and preference. These results indicate that the endogenous FGF2 function is required for consumption of high levels of alcohol. Together, our findings that bidirectional manipulations on FGF2 yield opposite effects on alcohol consumption provide strong evidence for the role of FGF2 in the DMS as a pivotal positive regulator of alcohol-drinking behaviors.

Relevantly, it was previously reported that inhibition of FGF2 in the VTA, by anti-FGF2 neutralizing antibody, blocked the development of amphetamine-induced psychomotor sensitization (Flores et al., 2000). Moreover, FGF2 inhibition in the infralimbic cortex enhanced extinction of operant cocaine self-administration (Hafenbreidel et al., 2015). Finally, rats selectively bred to show low novelty responses, which were shown to express reduced brain Fgf2 levels (Perez et al., 2009; Clinton et al., 2012), exhibited lower levels of operant cocaine self-administration (Cummings et al., 2011) and sensitization (Clinton et al., 2012), compared with controls. Together with the current findings, these findings suggest that the endogenous FGF2 regulates drug-related behaviors, including drug-seeking and consumption.

Interestingly, our finding that FGF2 is a positive regulator of alcohol consumption sets it apart from other growth factors that negatively regulate alcohol intake (Ghitza et al., 2010; Ron and Barak, 2016). Thus, downregulation of brain-derived neurotrophic factor (Bdnf) expression in the DLS (Jeanblanc et al., 2009; Logrip et al., 2015), or of glial cell line-derived neurotrophic factor (Gdnf) in the VTA or NAc (Ahmadiantehrani et al., 2014; Barak et al., 2015), increases alcohol consumption. Conversely, infusion of BDNF (Jeanblanc et al., 2009) or GDNF (Carnicella et al., 2008; Barak et al., 2011a, b) into these respective

Figure 8. Inhibition of FGF2 activity in the DMS decreases alcohol consumption and preference. Rats were trained to consume alcohol in the intermittent access to 20% alcohol in 2-bottle choice paradigm for 7 weeks before cannulation. Neutralizing antibody against FGF2 (750 ng/0.75 μl per hemisphere) or control IgG was infused into the DMS 1 h before the beginning of an alcohol-drinking session. Alcohol and water intake was measured at the end of the 24-h drinking session. A, Amount of alcohol (g/kg) consumed. B, Preference for alcohol, calculated as the ratio of the volume of alcohol solution intake/volume of total fluid intake. C, Water intake (ml/kg). D, Total fluid intake (alcohol + water; ml/kg). E, Schematic representation of the cannula tip placement in coronal sections (bregma + mm). Bar graphs indicate mean ± SEM adjusted for a within-subjects design (Cousineau, 2005). n = 15 per group. *p < 0.05; **p < 0.01.
brain regions attenuates alcohol intake. These opposite effects of FGF2 and other growth factors may be due to their action at different brain regions and/or via different downstream processes.

Alcohol and FGF2, a positive feedback regulatory loop for drinking escalation?

We used here an alcohol-drinking protocol that consists of repeated cycles of 24 h alcohol consumption and withdrawal sessions. Critically, our findings indicate that Fgf2 expression levels in the DMS are still elevated after a 24-h withdrawal session, a time point that corresponds to the beginning of the next alcohol-access session in this drinking protocol. Furthermore, administration of rFGF2 after a 24-h withdrawal period in the same drinking paradigm, either systemically to mice, or intra-dorsal striatum or intra-DMS to rats, increased alcohol drinking, whereas inhibition of FGF2 function at a similar time point had the opposite effect. These findings suggest that, throughout the alcohol drinking training, the increases in Fgf2 levels are likely leading to further increases in alcohol intake and preference in the next alcohol drinking sessions, possibly accounting for the escalation in drinking and maintaining high Fgf2 expression.

Interestingly, a positive feedback loop similar to the one we describe here with alcohol and FGF2 in the DMS has been reported with alcohol and AMPA receptor activity in the same brain region (Wang et al., 2012). Thus, it was shown that excessive alcohol consumption increases synaptic AMPA receptor levels and function in the DMS (Wang et al., 2012). Moreover, this AMPA receptor activity was shown to be vital for the expression of excessive alcohol consumption (Wang et al., 2012). Relatedly, FGF2 was reported to increase the expression of the AMPA receptor subunit GluR1 (Cheng et al., 1995; Chew et al., 1997), consequently increasing AMPA receptor function (Chew et al., 1997). Although these interactions remain to be tested, it is plausible that, within the DMS, alcohol increases FGF2 expression, which in turn increases AMPA receptor activity, resulting in excessive alcohol consumption.

We found that the bidirectional interaction between alcohol and FGF2 was localized to the DMS. This brain region was suggested to play a role in the early stages of alcohol consumption, which are governed primarily by goal-directed behavior, before the behavior becomes habitual (Corbit et al., 2012). Thus, the increase in FGF2 levels in the DMS, and its consequent enhancing effect on alcohol intake, may strengthen the response-outcome association, hence promoting engagement in alcohol-seeking and -drinking behaviors, and contributing to the development and maintenance of excessive alcohol-drinking behaviors.

Our results are in line with previous studies, which found that other drugs of abuse, such as amphetamine (Flores et al., 1998), cocaine (Flores and Stewart, 2000b; Fumagalli et al., 2006) and nicotine (Roceri et al., 2001), increase FGF2 levels in addiction-related brain regions. In addition, the midbrain levels of FGF2 positively correlated with the magnitude of amphetamine psychomotor sensitization (Flores et al., 2000). Moreover, Fgf2 mRNA expression and its epigenetic modifications in the rat NAc positively correlated with cocaine seeking and relapse (Flagel et al., 2016). Finally, neonatal rFGF2 treatment enhanced cocaine self-administration and sensitization in adulthood (Turner et al., 2009; Clinton et al., 2012). Together with our present findings, these findings suggest that various drugs of abuse increase the brain expression of FGF2 and that high levels of FGF2 likely promote drug sensitization and consumption.

In conclusion, our results show that alcohol consumption increases the expression of Fgf2 within the DMS and that FGF2 increases alcohol intake by acting in this brain region. Therefore, our findings suggest that alcohol and FGF2 constitute a positive feedback loop, which contributes to the facilitation and maintenance of alcohol intake. Finally, together with previous reports, our findings suggest that FGF2 is a positive regulator of the consumption not only of alcohol, but also of other drugs of abuse.

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