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FGF2 activity regulates operant alcohol self-administration and mesolimbic dopamine transmission

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ABSTRACT

Fibroblast growth factor 2 (FGF2) is involved in the development and maintenance of the brain dopamine system. We previously showed that alcohol exposure alters the expression of FGF2 and its receptor, FGF receptor 1 (FGFR1) in mesolimbic and nigrostriatal brain regions, and that FGF2 is a positive regulator of alcohol drinking. Here, we determined the effects of FGF2 and of FGFR1 inhibition on alcohol consumption, seeking and relapse, using a rat operant self-administration paradigm. In addition, we characterized the effects of FGF2-FGFR1 activation and inhibition on mesolimbic and nigrostriatal dopamine neuron activation using in vivo electrophysiology. We found that recombinant FGF2 (rFGF2) increased the firing rate and burst firing activity of dopaminergic neurons in the mesolimbic and nigrostriatal systems and led to increased operant alcohol self-administration. In contrast, the FGFR1 inhibitor PD173074 suppressed the firing rate of these dopaminergic neurons, and reduced operant alcohol self-administration. Alcohol seeking behavior was not affected by PD173074, but this FGFR1 inhibitor reduced post-abstinence relapse to alcohol consumption, albeit only in male rats. The latter was paralleled by the increased potency and efficacy of PD173074 in inhibiting dopamine neuron firing. Together, our findings suggest that targeting the FGF2-FGFR1 pathway can reduce alcohol consumption, possibly via altering mesolimbic and nigrostriatal neuronal activity.

1. Introduction

Alcohol use disorder (AUD) is a chronic, relapsing disease, characterized by compulsive alcohol use and loss of control over alcohol intake (American Psychiatric Association, 2013), causing a global economic and social burden and loss of health (Collins et al., 2011). Chronic consumption of high quantities of alcohol is thought to induce neuroadaptations in the brain reward system that lead to addiction phenotypes (Abrahao et al., 2017; Koob, 2013; Koob and Volkow, 2009; Ron and Barak, 2016; Spanagel, 2009; Vengeliene et al., 2008). These neuroadaptations occur mostly in the mesocorticolimbic and nigrostriatal pathways (Abrahao et al., 2017; Koob, 2013; Ron and Barak, 2016; Spanagel, 2009; Wise, 2009), that project from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), hippocampus, amygdala, and the prefrontal cortex, and from the substantia nigra (SN) to the dorsal striatum, respectively.

Fibroblast growth factor 2 (FGF2) is abundant throughout the brain, and participates in the development and maintenance of the dopamine mesolimbic and nigrostriatal system (Baron et al., 2012; Bean et al., 1991; Claus et al., 2004; Eckenstein et al., 1991; Grothe and Timmer, 2007; Ratzka et al., 2012; Timmer et al., 2007). As the dopamine system is involved in addiction to alcohol and drugs of abuse (Koob and Volkow, 2016; Robinson and Berridge, 2000; Ron and Barak, 2016), it is not surprising that FGF2 and its receptor, FGF receptor 1 (FGFR1) (Reuss

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et al., 2003; Turner et al., 2012) have been suggested to play a role in addiction (Clinton et al., 2012; Dremencov et al., 2021; Even-Chen and Barak, 2019b; Flagel et al., 2016; Hafenbreidel et al., 2017; Liran et al., 2020). We recently reported that activation of the FGF2-FGFR1 system in the dorsomedial striatum (DMS) positively regulates alcohol drinking (Even-Chen and Barak, 2019a,b; Even-Chen et al., 2022; Even-Chen et al., 2017). Specifically, we found that *Fgf2* and *Fgfr1* expression levels were increased in the DMS after prolonged alcohol intake in a home cage 2-bottle choice alcohol-drinking procedure (Even-Chen and Barak, 2019a; Even-Chen et al., 2017), and that this effect was mediated by activation of dopamine D2 receptors (Even-Chen et al., 2017). Infusion of recombinant FGF2 (rFGF2) into the DMS increased alcohol intake (Even-Chen et al., 2017) via activation of the PI3 kinase (PI3K) pathway downstream of FGFR1 (Even-Chen and Barak, 2019a), whereas the inactivation (Even-Chen et al., 2017) or genetic deletion (Even-Chen et al., 2022) of FGF2, as well as the inhibition of FGFR1 (Even-Chen and Barak, 2019a), reduced alcohol consumption. Together, these results indicate that there is a positive feedback loop between alcohol and FGF2, in which FGF2 increases alcohol consumption, and alcohol consumption increases FGF2 expression.

Our previous findings demonstrated the effects of rFGF2 and inhibition of FGFR1 on voluntary alcohol consumption in a non-operant, home cage consumption procedure. However, the effects of manipulations in the FGF2-FGFR1 system were not evaluated yet in an operant alcohol self-administration procedure, which can assess various addiction-related behaviors besides consumption, such as alcohol seeking and relapse. Thus, in the present study we set out to determine the effects of manipulations in the FGF2-FGFR1 system on operant alcohol self-administration, seeking and relapse. Importantly, given the regulatory role of FGF2 in the dopamine system, and the involvement of the latter in addiction, it is plausible that FGF2-FGFR1 modulates dopamine neuronal firing and transmission, which in turn affects alcohol consumption. Therefore, we also determined here the effects of rFGF2 and of FGFR1 inhibition on the excitability of dopamine neurons in the VTA and SN.

2. Materials and methods

Details on reagents, electrophysiological methods and their statistical analysis are in Supplemental Information

2.1. Animals

For behavioral experiments, male and female Wistar rats (175–250 g at the beginning of experiments) were bred in the Tel Aviv University animal facility for all the behavioral experiments. For the electrophysiology experiments, male and female Wistar rats were ordered from the Animal Breeding facility of the Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences (Dobra Voda, Slovakia). All rats were housed individually under a 12-h light/dark cycle (lights on at 7:00 am), with food and water available ad libitum. The light cycle in our vivarium is not inversed, so that the self-administration procedure was conducted during the light phase, as in previously published work (Barak et al., 2013, 2015; Zipori et al., 2017).

All the behavioral experimental protocols conformed to the guidelines of the Institutional Animal Care and Use Committee of Tel Aviv University and the Israeli Ministry of Health, and to the guidelines of the NIH (animal welfare assurance number A5010–01). All efforts were made to minimize the number of animals used. All electrophysiology experimental procedures were approved by the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic (Permit number Ro 2019/2022-220) and conformed to the Directive 2010/63/EU of the European Parliament and of the Council on the Protection of Animals Used for Scientific Purposes.

2.2. Behavioral procedures

2.2.1. Intermittent access to 20% alcohol in a 2-bottle choice (IA2BC)

After one week of habituation to individual housing, rats began training to consume alcohol in the intermittent access to the 2-bottle choice procedure, as previously described (Carnicella et al., 2014; Simms et al., 2008). Briefly, rats were given three 24-h sessions a week of access to two bottles, one containing 20% alcohol (v/v) and the other tap water, on Sundays, Tuesdays and Thursdays. Between each alcohol-drinking session, there were 24 or 48 h of alcohol-deprivation, during which rats received only one bottle of water. The position (left or right) of the two bottles was alternated between sessions to control side preference. Water and alcohol bottles were weighed before and after each alcohol-drinking session, and consumption levels were normalized to body weight. This training lasted 4–7 weeks.

2.2.2. Operant self-administration of alcohol or sucrose

For alcohol self-administration experiments, after 4–7 weeks of training in the IA2BC procedure, rats began training in operant alcohol self-administration, as previously described (Barak et al., 2015; Carnicella et al., 2014; Goltseker et al., 2021; Zipori et al., 2017). Briefly, rats were placed in operant self-administration chambers (Med Associates, Georgia, VT) containing two levers throughout the session (active and inactive), but no discrete cues. Pressing the active lever resulted in the administration of a reward (0.1 ml of 20% alcohol (v/v)), while pressing the inactive lever had no effect. The training began with a week of overnight sessions (14 h) in the chambers in a fixed ratio 1 (FR1) schedule of reinforcement – every press of the active lever resulted in reward administration. Following this week, rats were given daily sessions of 60 min in FR1 for three weeks, followed by seven weeks of training in an FR3 schedule of reinforcement (a reward is given for every third lever press). At this stage, we assured that the rats showed a stable level of lever pressing for at least three weeks (15 sessions). In experiments that used a between-subjects design, we assured similar average baseline levels of lever pressing and alcohol intake between groups during group allocation. In experiments that used a within-subjects design, we assured that rats always reached their average baseline response level (as observed before treatment) prior to the next treatment.

For operant self-administration of sucrose, the training began with overnight self-administration sessions without previous IA2BC training. The concentration of sucrose was gradually decreased in the first week from 3% to 0.5% (w/v) (Zipori et al., 2017). This was followed by one week of daily 60-min sessions in FR1, and an additional week in FR3.

2.2.2.1. Alcohol seeking test. This test assessed alcohol-seeking behavior after operant alcohol self-administration training. Rats were placed in the operant chambers for a 60-min test session under extinction conditions – both levers were presented but no alcohol rewards were delivered following lever pressing, and lever pressing was recorded.

2.2.2.2. Post-abstinence relapse (retention) test. To assess relapse to alcohol seeking after 10 days of abstinence from alcohol, rats were placed in the operant chambers for one 60-min session during which both levers were presented but no alcohol rewards were delivered following lever pressing. A prime of 0.1 ml 20% alcohol was placed in the chambers at the beginning of the session, and lever pressing was recorded.

2.2.2.3. Reacquisition test. One day after the retention test, rats were given another test for relapse to alcohol drinking, namely, a reacquisition test. Rats were placed in the operant chambers for one 60-min session identical to the previous training sessions. A prime of 0.1 ml 20% alcohol was placed in the chambers at the beginning of the session, and alcohol rewards were delivered in an FR3 schedule of reinforcement.

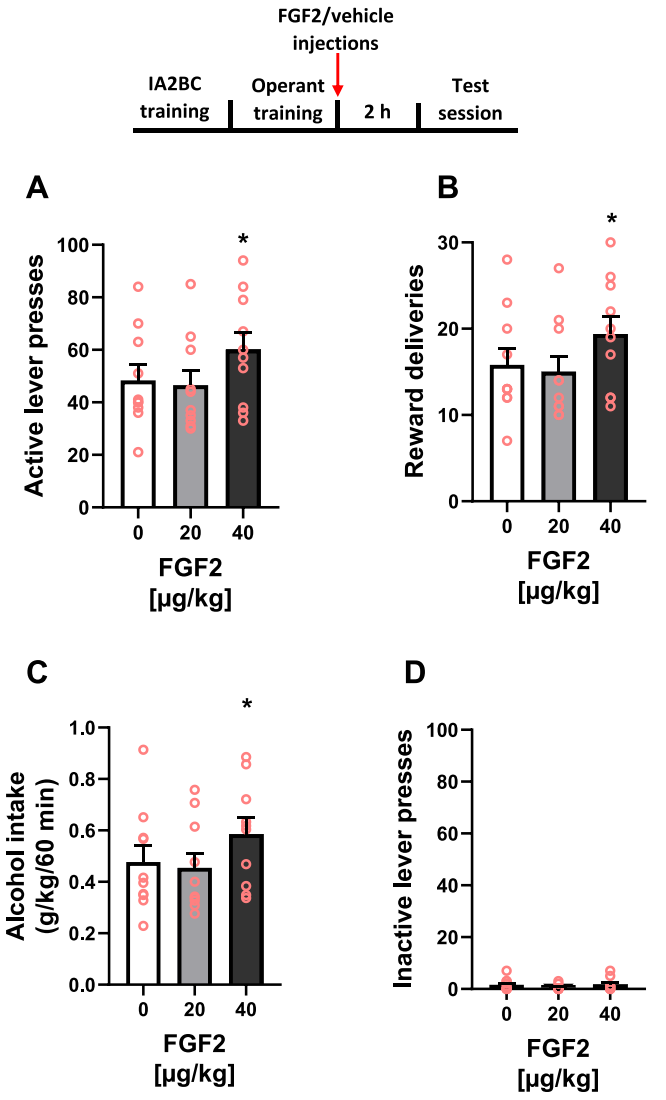

Fig. 1. Systemic recombinant FGF2 administration increases operant alcohol self-administration. Rats were pre-trained to consume alcohol in the home-cage intermittent access to 20% alcohol in 2-bottle choice (IA2BC) procedure, followed by operant alcohol self-administration training, for a total alcohol consumption period of 3–4 months. Recombinant FGF2 (20 or 40 µg/kg) or vehicle was injected 2 hours before the beginning of a 60-min operant alcohol self-administration test session. A-D. Means±SEM number of lever presses (A,D), number of reward deliveries (B) and alcohol intake normalized to body weight (C). A within-subjects design, n=10 per group as presented in the bar graphs; *p<0.05.

2.3. Experimental design

Each of the following experiments was performed on a separate batch of rats. Group sizes are specified in the figure legends.

2.3.1. Effects of rFGF2 on operant alcohol self-administration

Rats were trained in the IA2BC procedure, followed by training in the operant alcohol self-administration procedure, as described above. To test the effects of rFGF2 on operant alcohol self-administration, rats received injections of rFGF2 (20 µg/kg, 40 µg/kg or vehicle, s.c.), two hours before the session. Doses and injection intervals were chosen according to our previous studies with this compound (Even-Chen and Barak, 2019a; Even-Chen et al., 2017). This experiment was conducted in a within-subjects design, so that every rat received all three injections. The injections were given a week apart in a Latin square design, so that in each time point all doses were given (to different rats), and in the next weeks the doses were shifted for each rat for 3 cycles of injections, so that eventually each rat received all the doses, but the order of injections was counterbalanced across subjects. Numbers of lever presses and alcohol reward deliveries were recorded, and alcohol intake levels were calculated with normalization to body weight.

We previously used intracerebral injections of both rFGF2 and PD173074 directly into the DMS (Even-Chen and Barak, 2019a; Even-Chen et al., 2017). Here, we use a more translational approach, by demonstrating the effects of systemic administration of the drug on alcohol-drinking behaviors.

2.3.2. Effects of FGFR1 inhibition on operant alcohol self-administration

Rats were trained in the IA2BC procedure, followed by operant alcohol self-administration training, as described above. After this training, rats received injections of the FGFR1 inhibitor PD173074 (5 mg/kg, 15 mg/kg or vehicle, i.p.). All injections were in a volume of 0.5 ml/kg and administered eight hours before the operant self-administration test session. The doses and time of injections were chosen based on our previous findings that PD173074 affected alcohol intake mainly at the late stage of a 24-h 2-bottle choice drinking session (Even-Chen and Barak, 2019a). This experiment was conducted in a within-subjects design with the order of injections counterbalanced. Numbers of lever presses and alcohol reward deliveries were recorded, and alcohol intake levels were calculated with normalization to body weight.

2.3.3. Effects of FGFR1 inhibition on alcohol-seeking behavior

Rats were trained in the IA2BC procedure, followed by operant alcohol self-administration training, as described above. After reaching a stable level of lever pressing, rats were given an alcohol-seeking test. Eight hours before the test session, rats received an PD173074 injection (15 mg/kg, i.p.) or vehicle in a between-subjects design. The number of lever presses was recorded.

2.3.4. Effects of FGFR1 inhibition on relapse

Rats were trained in the IA2BC procedure, followed by operant alcohol self-administration training, as described above. Rats were then given 10 days of abstinence from alcohol, during which they were kept in the home cages. On the 11th day, rats were given a retention test to assess relapse to alcohol seeking. The next day, rats were given a reacquisition test, to assess relapse to alcohol drinking. Eight hours before each test, rats received an injection of PD173074 (15 mg/kg or vehicle, i.p.) in a between-subjects design. Numbers of lever presses and alcohol reward deliveries were recorded, and alcohol intake levels were calculated with normalization to body weight.

2.3.5. Effects of FGFR1 inhibition on operant self-administration of sucrose

To test the effects of FGFR1 inhibition on a natural reinforcer, rats were trained to consume a solution of 0.5% sucrose (w/v) in operant self-administration, as described above. In the test session, rats received a PD173074 injection (15 mg/kg or vehicle, i.p.) eight hours before the session, in a between-subjects design. Lever presses and sucrose reward deliveries were recorded, and sucrose intake was calculated and normalized to body weight.

2.3.6. Effects of FGF2 on midbrain dopamine neuron firing

Alcohol-naïve rats received s.c. injection of rFGF2 (80 µg/kg) or vehicle (0.1% BSA in PBS). An hour later, rats were anesthetized with chloral hydrate (0.4 g/kg, i.p.) and fixed in a stereotaxic frame. The electrode was lowered to the VTA or SN, six times for each brain region. Dopamine neurons were identified, using the previously described criteria (Grinchii et al., 2022), and the firing rate was recorded. Electrophysiological recordings lasted ~2 h, meaning that the neuronal firing activity was assessed on average 2 hours after rFGF2 administration.

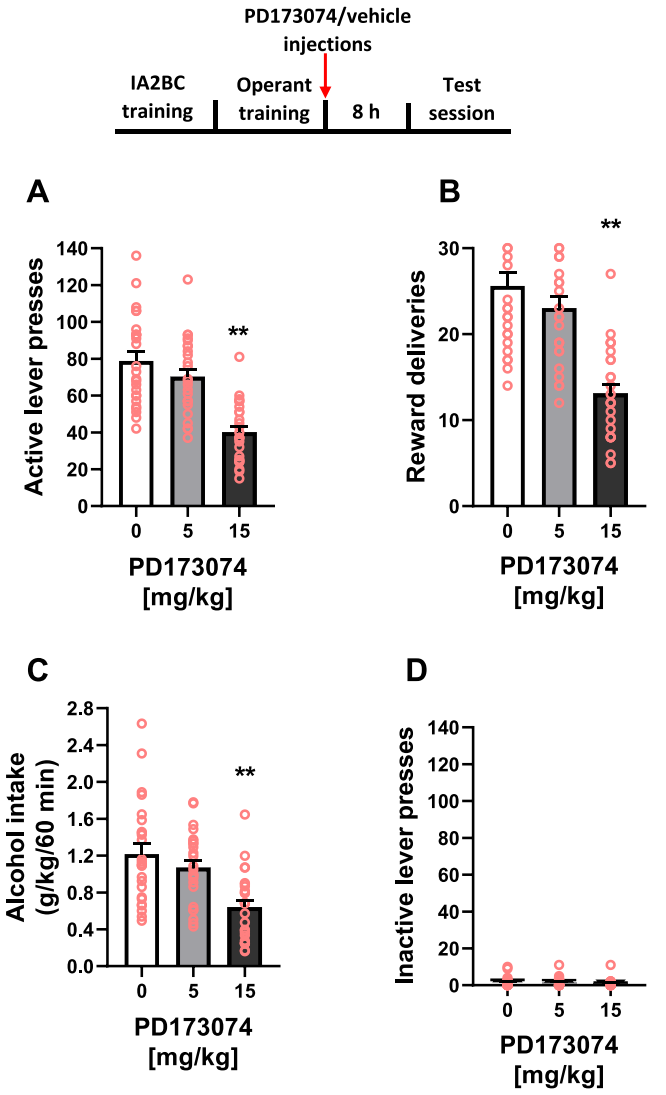

Fig. 2. FGFR1 inhibition decreases operant alcohol self-administration. Rats were pre-trained to consume alcohol in the home-cage intermittent access to 20% alcohol in 2-bottle choice (IA2BC) procedure, followed by operant alcohol self-administration training, for a total alcohol consumption period of 3–4 months. The FGFR1 inhibitor PD173074 (5 mg/kg or 15 mg/kg) or vehicle was injected 8 hours before the beginning of a 60-min operant alcohol self-administration test session. A-D. Means±SEM number of lever presses (A,D), number of reward deliveries (B) and alcohol intake normalized to body weight (C). A within-subjects design, n=25 per group as presented in the bar graphs; **p<0.001.

2.3.7. Effects of FGFR1 inhibition on midbrain dopamine neuron firing

Alcohol-naïve rats received i.p. injection of PD173074 (15 mg/kg) or vehicle (DMSO). Seven hours later, rats were anesthetized with chloral hydrate (0.4 g/kg, i.p.) and fixed in a stereotaxic frame. Similar to the previous experiment with rFGF2, the electrode was inserted into the VTA or the SN. Electrophysiological recordings lasted ~2 hours, meaning that the neuronal firing activity was assessed on average 8 hours after PD173074 administration. In a separate experiment, drug-naïve rats were anesthetized with chloral hydrate (0.4 g/kg, i.p.) and fixed in a stereotaxic frame. After the detection of spontaneously active dopamine neurons, their basal activity was recorded. Then, PD173074 was intravenously (i.v.) injected via a catheter placed in the femoral vein, at cumulative doses of 6,9,12 and 15 mg/kg, and thefiring rate was recorded.

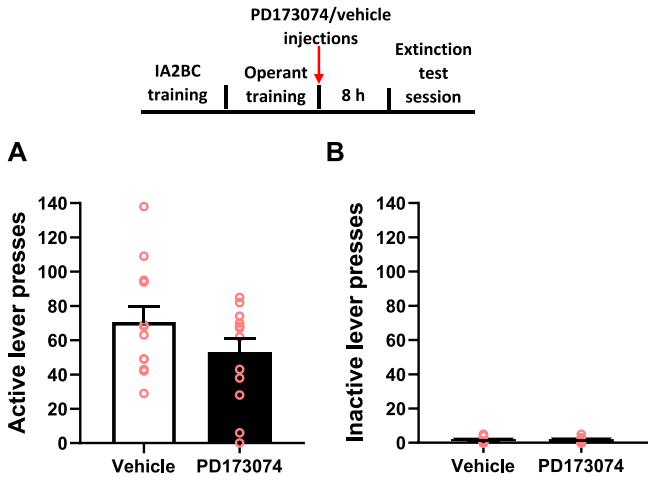

Fig. 3. FGFR1 inhibition does not alter alcohol seeking. Rats were pre-trained to consume alcohol in the home-cage intermittent access to 20% alcohol in 2-bottle choice (IA2BC) procedure, followed by operant alcohol self-administration training, for a total alcohol consumption period of 3–4 months. The FGFR1 inhibitor PD173074 (15 mg/kg) or vehicle was injected 8 hours before the beginning of a 60-min operant alcohol self-administration test session under extinction conditions (no alcohol delivery). A-B. Means ±SEM of the number of lever presses. A between-subjects design, n=6–7 per group as presented in the bar graphs.

2.4. Statistical analysis

Alcohol and sucrose intake levels were calculated using the number of reward deliveries and normalized to body weight. Lever presses, rewards and alcohol intake in the within-subjects experiments, results were analyzed with a mixed-model ANOVA, with a between-subjects factor of Sex and a repeated measures factor of Lever and Treatment dose. In the between-subjects experiments were analyzed with mixed-model ANOVA, with between-subjects factors of Sex and Treatment dose, and a within-subject factor of Lever. In experiments where the Sex factor did not interact with other experimental factors, the data were collapsed across this factor.

The effect of rFGF2 on dopamine neuronal firing activity in the VTA or SN was assessed using two-way ANOVA, with between-subjects factors of Sex and Treatment (rFGF2 or vehicle). The firing rate of dopamine neurons after PD173074 administration was expressed as a percentage of the basal firing activity of the same neurons. The effect of PD173074 on dopamine neuronal firing activity in the VTA or SN was analyzed using a mixed-model two-way ANOVA, with a between-subjects factor of Sex and a repeated measure factor of Treatment dose (basal firing activity and firing activity after the administration of 6, 9, 12, and 15 mg/kg of PD173074).

Significant effects in ANOVA were followed by LSD or Bonferroni post-hoc tests.

3. Results

3.1. rFGF2 increases operant alcohol self-administration

We began by testing whether systemic injections of rFGF2 (0, 20 or 40 μg/kg) would increase the operant response for alcohol, in an operant alcohol self-administration procedure.

We found that rFGF2 increased the number of active lever presses without affecting inactive lever pressing (Fig. 1A, D), alcohol-reward deliveries (Fig. 1B) and alcohol intake (Fig. 1C), in a dose-dependent manner, i.e., at 40 μg/kg, but not 20 μg/kg. The number of inactive lever presses was not affected by rFGF2 (Fig. 1D). Lever presses, two-way repeated-measures ANOVA: main effects of Treatment (F(2,18)=

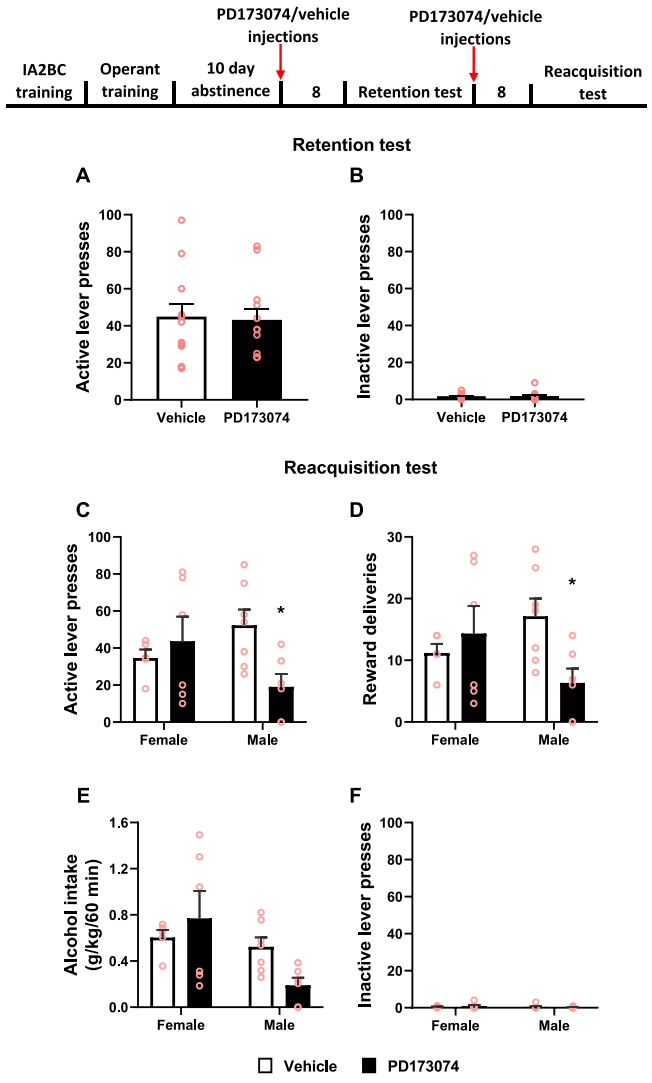

Fig. 4. FGFR1 inhibition decreases relapse to alcohol drinking in male rats. Rats were pre-trained to consume alcohol in the home-cage intermittent access to 20% alcohol in 2-bottle choice (IA2BC) procedure, followed by operant alcohol self-administration training, for a total alcohol consumption period of 3–4 months. After 10 days of abstinence, the FGFR1 inhibitor PD173074 (15 mg/kg) or vehicle was injected 8 hours before a retention test and before a reacquisition test. A-F. Means±SEM of the number of lever presses in the retention test (A, B) and reacquisition test (C,F), number of reward deliveries (D) and alcohol intake (E) in the reacquisition test. (A-B). A between-subjects design, n=6–7 per group as presented in the bar graphs (by sex); *p<0.05.

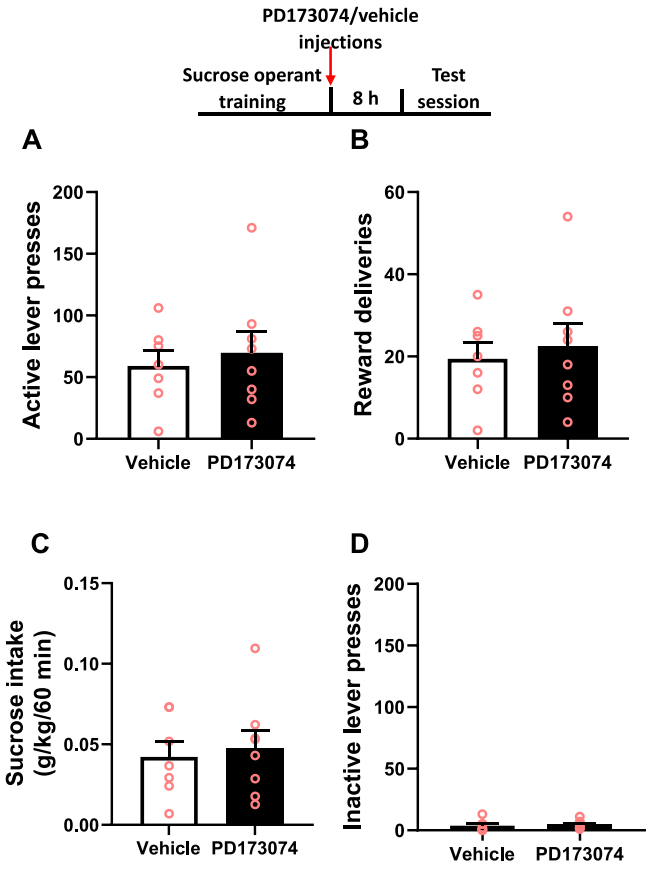

Fig. 5. FGFR1 inhibition has no effect on operant sucrose self-administration. Rats were trained to consume 0.5% sucrose in the operant sucrose self-administration procedure for 3 weeks. The FGFR1 inhibitor PD173074 (15 mg/kg.) or vehicle was given 8 hours before the beginning of a 60-min operant alcohol self-administration test session. A-D. Means±SEM of the number of lever presses (A,D), number of reward deliveries (B) and sucrose intake normalized to body weight (C). A between-subjects design, n=7–8 per group as presented in the bar graphs.

4.00, p=0.036), Lever (F(1,9)=93.95, p<0.0001), and a marginally significant Treatment X Lever interaction (F(2,18)=3.35, p=0.058). Rewards and alcohol intake, one-way repeated-measures ANOVA: a main effect of Treatment on reward deliveries (F(2,18)=3.66, p=0.046) and alcohol intake (F(2,18)=3.96, p=0.037). Post hoc analysis: significant differences between treatment of 40 μg/kg and vehicle in active lever presses (p=0.044) and alcohol intake (p=0.043), but no significant differences between 20 μg/kg and vehicle (p's>0.05).

These results indicate that FGF2 increases operant alcohol self-administration.

3.2. FGFR1 inhibition reduces alcohol self-administration

Having shown that rFGF2 increased operant alcohol self-administration, we next tested whether inhibition of the FGF2 receptor, FGFR1 using the FGFR1 inhibitor PD173074 (0, 5 or 15 mg/kg), produces the opposite effect, i.e., suppresses operant alcohol self-administration.

We found that injections of 15 mg/kg PD173074 decreased the number of active lever presses, without affecting inactive lever pressing (Fig. 2A, D), reward deliveries (Fig. 2B) and alcohol intake (Fig. 2C) compared to vehicle, with no effects at the 5 mg/kg dose. Lever presses, two-way repeated-measures ANOVA: main effects of Treatment (F(2,48)=30.21, p<0.001), Lever (F(1,24)=458.40, p<0.0001), and a Treatment X Lever interaction (F(2,48)=27.95, p<0.001). Rewards and alcohol intake, one-way repeated measures ANOVA: a main effect of Treatment on reward deliveries (F(2,48)= 29.86, p<0.0001) and alcohol intake (F(2,48)= 25.31, p<0.0001). Post hoc analysis: significant difference between treatment of 15 mg/kg and the other treatments in active lever presses, reward deliveries and alcohol intake (p's<0.0001).

These results indicate that a higher dose of PD173074 can reduce operant alcohol self-administration.

3.3. FGFR1 inhibition does not affect alcohol seeking

Next, we tested whether inhibition of FGFR1 affects alcohol-seeking behavior, by measuring the number of lever presses in a single extinction session, i.e., in the absence of alcohol, as previously described (Barak et al., 2015). We found no effect of PD173074 on lever presses (Fig. 3). Two-way mix model ANOVA: a main effect of Lever (F(1,23)=106.3,

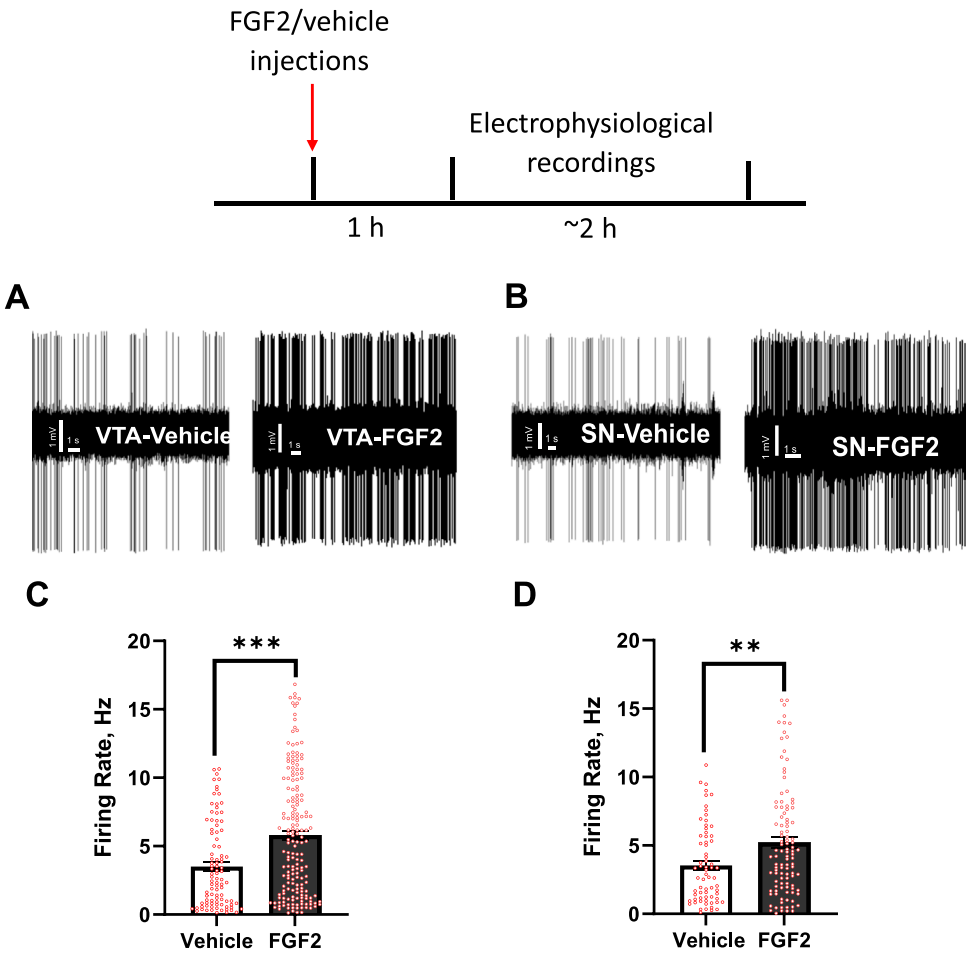

Fig. 6. Recombinant FGF2 increases the firing rate of dopamine neurons in the ventral tegmental area (VTA) and substantia nigra (SN). A-B. Representative recordings from the VTA (A) and SN (B). C-D. Summary effect from 49 neurons from 5 vehicle-treated, 78 neurons from 6 FGF2-treated male rats, 46 neurons from 5 vehicle-treated, and 102 neurons from 5 FGF2-treated female rats. **p<0.01 and ***p<0.001 in comparison with vehicle-treated rats, two-way ANOVA.

p<0.0001), But no effect of Treatment (F(1,23)=2.02, p=0.16) and no Treatment X Lever interaction (F(1,23)=2.215, p=0.15).

These results suggest that although PD173074 affects alcohol consumption in both operant and non-operant self-administration procedures, it does not affect alcohol-seeking behavior.

3.4. FGFR1 inhibition reduces relapse to alcohol drinking only in males

As inhibition of FGFR1 reduced operant alcohol self-administration, we next tested whether PD173074 also affects relapse after a period of abstinence from alcohol. We then conducted two tests for relapse, as we previously described (Barak et al., 2013, 2015), and as detailed in the Methods section.

We found that PD173074 had no effect on lever pressing in the retention test (Fig. 4A, D). Lever presses, two-way mixed model ANOVA: a main effect of Lever (F(1,24)=84.39, p<0.0001), but no effect of Treatment (F(1,22)=0.039, p=0.85), and no Treatment X Lever interaction (F(1,22)=0.033, p=0.86).

In the reacquisition test, PD173074 decreased the number of active lever presses (Fig. 4C) and reward deliveries (Fig. 4D) only in male rats, affecting their inactive lever presses (Fig. 4F). PD173074 had no effect on the reacquisition test in female rats. Moreover, PD173074 led to early termination of active lever pressing in males, compared to vehicle treatment, already from the first five minutes of the session (Supplemental Fig. 1). Lever presses, three-way mixed model ANOVA: a main effect of Lever (F(1,20)=63.86, p<0.0001), and significant interactions of Sex X Treatment (F(1,20)=5.33, p=0.032) and Sex X Treatment X Lever (F(1,20)=5.08, p=0.036) but no main effects of Treatment (F(1,20)=1.66, p=0.21) and Sex (F(1,20)=0.1668, p=0.69), and no interaction of Treatment X Lever (F(1,20)=1.74, p=0.20) or sex X lever (F(1,20)=0.12, p=0.73). Rewards and alcohol intake, two-way ANOVA: a main effect of Sex for alcohol intake (F(1,20)=5.97, p=0.024), but not for reward deliveries (F(1,20)=0.11, p=0.74); as well as a Treatment x Sex interaction for reward deliveries (F(1,20)=4.99, p=0.037), and a trend for alcohol intake (F(1,20)=3.4, p=0.08). Post hoc analysis: significant difference between the vehicle and PD173074-treated group in males for active lever presses (p=0.015) and reward deliveries (p=0.019).

These results indicate that inhibition of FGFR1 had no effect on relapse to alcohol seeking but reduces relapse to alcohol consumption only in males.

3.5. FGFR1 inhibition does not affect operant sucrose self-administration

Since we found that FGFR1 inhibition reduced the operant self-administration of alcohol, we next tested whether inhibition of this receptor also reduces the operant self-administration of a natural reward, sucrose (0.5% w/v).

We found that injections of PD173074 had no effect on the operant self-administration of sucrose (Fig. 5). Lever presses, two-way repeated-measures ANOVA: a main effect of Lever (F(1,13)=31.47, p<0.0001), But no effect of Treatment (F(1,13)=0.28, p=0.61) and no Treatment X Lever interaction (F(1,13)=0.21, p=0.65). Rewards and alcohol intake, one-way ANOVA: no effects of Treatment on reward deliveries (F

Table 1
Effect of recombinant FGF2 on the excitability of mesolimbic (A) and nigrostriatal (B) dopamine cells. ISI, inter-spike interval; ANOVA, analysis of variance, S: effect of sex, T: effect of treatment, I: sex × treatment interaction, ***p<0.001 in comparison with vehicle-treated rats of the same sex, and ##p<0.01 in comparison with the same treatment group of the opposite sex, Bonferroni post-hoc test. Data are means±SEM. Multi-level mixed linear model (MLM) corrections are available in Tables S1-S2 in the Supplementary Materials.

A: Dopamine neurons of the VTA

Characteristic	**Male-Vehicle** (49 cells/5 rats)	**Male-FGF2** (78 cells/6 rats)	**Female-Vehicle** (46 cells/5 rats)	**Female-FGF2** (102 cells/5 rats)	**Two-way ANOVA**
Number of active neurons per track	4.08±0.65	4.75±0.65##	3.83±0.86	8.58±1.50***	**S:** $F_{1,51}$=3.57, p=0.065 **T:** $F_{1,51}$=8.17, p=0.006**I:** $F_{1,51}$=4.64, p=0.036
Firing rate, s^{-1}	3.95±0.45	6.05±0.54	3.02±0.43	5.62±0.43	**S:** $F_{1,273}$=1.72, p=0.191**T:** $F_{1,273}$=20.44, p<0.001**I:** $F_{1,273}$=0.23, p=0.629
ISI coefficient of variation, %	183±19	175±14	144±14	151±7	**S:** $F_{1,273}$=5.63, p=0.065**T:** $F_{1,272}$=0.001, p=0.976 **I:** $F_{1,273}$=0.34, p=0.563
Bursts frequency, s^{-1}	0.40±0.05	0.51±0.05	0.35±0.06	0.59±0.04	**S:** $F_{1,273}$=0.10, p=0.754 **T:** $F_{1,273}$=13.21, p<0.001 **I:** $F_{1,273}$=0.20, p=0.203
Average number of spikes per burst	6.89±1.08	9.66±1.58	4.14±0.49	7.06±0.73	**S:** $F_{1,273}$=5.11, p=0.025 **T:** $F_{1,273}$=5.79, p=0.017 **I:** $F_{1,273}$=0.004, p=0.950
% of spikes occurring in bursts	52±4	63±3	42±4	68±3	**S:** $F_{1,273}$=0.53, p=0.467 **T:** $F_{1,273}$= 24.82, p<0.001 **I:** $F_{1,273}$=3.78, p=0.053

B: Dopamine neurons of the SN

Characteristic	**Male-Vehicle** (37 cells/5 rats)	**Male-FGF2** (71 cells/5 rats)	**Female-Vehicle** (30 cells/5 rats)	**Female-FGF2** (36 cells/5 rats)	**Two-way ANOVA**
Number of active neurons per track	2.92±0.56	3.50±0.54	2.73±0.52	3.00±0.43	**S:** $F_{1,\ 55}$=0.39, p=0.533 **T:** $F_{1,55}$= 0.59, p=0.447 **I:** $F_{1,\ 55}$=0.08, p=0.785
Firing rate, s^{-1}	3.46±0.43	4.94±0.46	3.66±0.56	5.74±0.73	**S:** $F_{1,174}$=0.75, p=0.388 **T:** $F_{1,17}$ =9.50, p=0.002 **I:** $F_{1,174}$=0.27, p=0.606
ISI coefficient of variation, %	356±90	204±12	166±13	142±16	**S:** $F_{1,174}$=8.53, p=0.004 **T:** $F_{1,174}$=4.15, p=0.043 **I:** $F_{1,174}$=2.22, p=0.138
Bursts frequency, s^{-1}	0.34±0.05	0.46±0.04	0.38±0.05	0.48±0.07	**S:** $F_{1,174}$=0.23, p=0.636 **T:** $F_{1,174}$=4.23, p=0.041 **I:** $F_{1,174}$=0.01, p=0.908
Average number of spikes per burst	9.37±2.22	10.39±1.97	5.99±1.08	9.06±2.03	**S:** $F_{1,174}$=1.15, p=0.285 **T:** $F_{1,174}$=0.87, p=0.352 **I:** $F_{1,174}$=0.22, p=0.640
% of spikes occurring in bursts	66±4	67±3	54±6	58±5	**S:** $F_{1,174}$=5.08, p=0.026 **T:** $F_{1,174}$=0.20, p=0.654 **I:** $F_{1,174}$=0.06, p=0.805

(1,13)=0.19, p=0.67) or on sucrose intake (F(1,13)=0.14, p=0.72).

These results suggest that the effects of FGFR1 inhibition are specific to alcohol self-administration and are not a result of a general effect on natural rewards.

3.6. FGF2 stimulates mesolimbic and nigrostriatal dopamine neurons

Since we previously showed that FGF2-FGFR1 activation during alcohol drinking occurs in the mesolimbic and nigrostriatal systems (Even-Chen and Barak, 2019a,b; Even-Chen et al., 2017), we next sought to determine the effects of rFGF2 and PD173074 on dopamine neuronal firing in the VTA and SN, the origin of dopaminergic projections to the mesolimbic and nigrostriatal systems, respectively.

We found that rats pretreated with rFGF2 had a higher firing rate of mesolimbic and nigrostriatal dopamine neurons, as well as the frequency of the bursts generated by these neurons, compared with vehicle-pretreated animals (Fig. 6, Table 1 for statistics). rFGF2 also increased the mean number of the spontaneously active dopamine neurons per electrode descent through the VTA, as well as the average number of spikes in bursts and percent of spikes occurring in bursts (Table 1). Interestingly, the increasing effect of rFGF2 on the density of the spontaneously active mesolimbic dopamine neurons was detected in females, but not in male rats (Table 1).

We further found that male rats had a significantly higher average number of action potentials per burst than females in the mesolimbic neurons, whereas in nigrostriatal neurons, male rats were characterized by a larger ISI coefficient of variation and higher percent of spikes occurring in bursts, compared with females. No other sex-related differences were detected (Table 1).

Thus, systemic rFGF2 administration increases the firing of dopamine neurons in both the mesolimbic and nigrostriatal neurons.

3.7. PD173074 inhibits mesolimbic and nigrostriatal dopamine neuronal firing in a sex-dependent manner

We showed that rFGF2 and FGFR1 inhibition yielded opposite outcomes on operant alcohol self-administration. Having shown that activation of the FGF2-FGFR1 system increases the firing of dopaminergic neurons in the VTA and SN, we next tested whether suppression of the FGF2-FGFR1 signal via inhibition of FGFR1, yields the opposite effect on dopaminergic neuronal firing.

We found that the i.p. administration of PD173074, eight hours prior to the electrophysiological recordings, led to a significant decrease in the firing rate and bursts frequency of mesolimbic dopamine neurons in the VTA (Fig. 7 and Table 2A). In the nigrostriatal dopamine neurons in the SN, the only characteristic affected by PD173074 was the coefficient of variation (CV), that was reduced in females, but not in males (Table 2B).

We also found that the i.v. administration of PD173074 (6–15 mg/kg) during the recording from spontaneously active midbrain neurons decreased the firing rate of dopamine neurons in the VTA and SN in a dose-dependent manner (Fig. 8).

Two-way repeated measures ANOVA demonstrated the main effect of Treatment dose (VTA: F(4,41)=8.09, p<0.001, data from 5 neurons from 5 male rats and 5 neurons from 5 female rats; SN: F(4,52)=7.12,

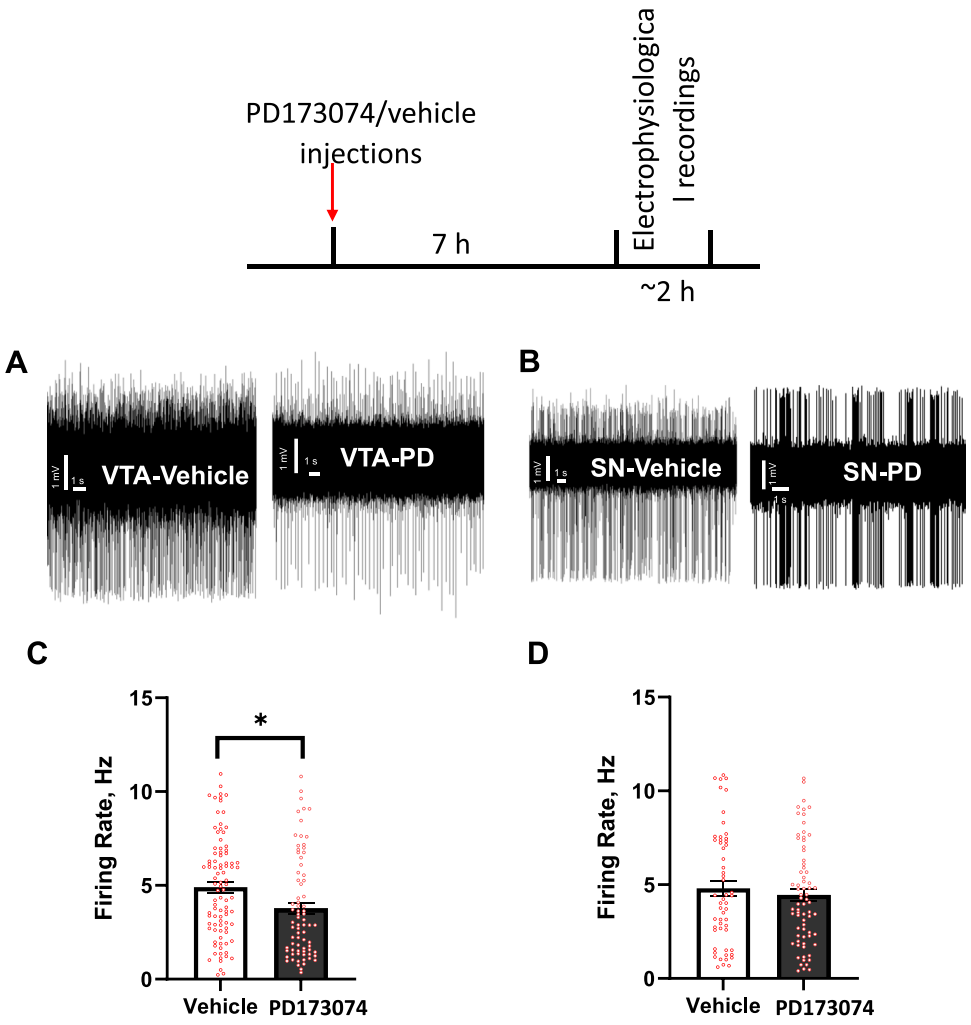

Fig. 7. PD173074 (PD, administered eight hours before recordings) decreases the firing rate of dopamine neurons in the ventral tegmental area (VTA), but not substantia nigra (SN). A-B. Representative recordings from the VTA (A) and SN (B). C-D. Summary effect from 68 neurons from 5 vehicle-treated, and 43 neurons from 5 FGF2-treated male rats, and 25 neurons from 5 vehicle-treated, and 33 neurons from 5 FGF2-treated female rats. *p<0.05 in comparison with vehicle-treated rats, two-way ANOVA.

p<0.001, data from 5 neurons from 5 male rats and 5 neurons from 4 female rats), but no effect of Sex (VTA: F(1,41)=3.76, p<0.12; SN: F(1,52)=0.85, p=0.38) and no Treatment dose x Sex interaction (VTA: F(1,41)=2.12, p=0.12; SN: F(1,52)=1.60, p=0.20).

Together, our results indicate that FGFR1 inhibition suppresses the neuronal firing of dopamine neurons in the VTA and SN, and that this effect emerges at lower doses in males.

4. Discussion

We show here that systemic administration of rFGF2, which increases the firing rate and burst firing activity of dopaminergic neurons of the mesolimbic and nigrostriatal systems, leads to increased operant self-administration and intake of alcohol. In contrast, inhibition of FGFR1, which inhibits the firing rate of these dopaminergic neurons, suppresses the operant self-administration and intake of alcohol. Moreover, we found that relapse to alcohol drinking in the operant model was affected in a sex-dependent manner by inhibition of FGFR1 whereas the latter manipulation had no effects on alcohol seeking, suggesting that alcohol seeking and consumption may have different underlying mechanisms, with FGF2-FGFR1 regulating only the latter. Together with our previous reports, our present results suggest that the FGF2-FGFR1 pathway may mediate the consummatory aspect of alcohol-drinking behaviors.

4.1. The FGF2-FGFR1 pathway regulates operant alcohol self-administration

We found that rFGF2 increased the operant self-administration of alcohol. This finding is consistent with our previous reports that FGF2 is a positive regulator of alcohol consumption (Even-Chen and Barak, 2019a,b; Even-Chen et al., 2022, 2017). In contrast, the FGFR1 inhibitor PD173074 reduced operant alcohol self-administration. These findings also expand our previous reports showing reduced alcohol consumption following FGFR1 inhibition using this compound (Even-Chen and Barak, 2019a) or by direct interference with the activity of FGF2 (Even-Chen et al., 2017), as well as knockout of the *Fgf2* gene (Even-Chen et al., 2022). In contrast, PD173074 had no effect on operant sucrose self-administration, suggesting that inhibition of FGFR1 affects alcohol in a specific manner that is not generalized to natural rewards, in line with our previous reports with FGF2-FGFR1 manipulations (Even-Chen and Barak, 2019a,b; Even-Chen et al., 2022, 2017; Liran et al., 2020).

It is important to note that our previous findings showed that FGF2-FGFR1 activation or inhibition affects alcohol consumption in non-operant alcohol-drinking procedures, i.e., 2-bottle choice home-cage consumption procedures in mice and rats. Here, we show that rFGF2 and PD173074 also affect alcohol self-administration in an operant setting, which allows assessment of different characteristics of alcohol-drinking behaviors related to addiction, including alcohol consumption, seeking and motivational aspects (Cunningham et al., 2000; Goltseker et al., 2019). Moreover, we recently showed that mice lacking

Table 2
Effect of PD173074 on excitability of mesolimbic (A) and nigrostriatal (B) dopamine cells. ISI, inter-spike interval; ANOVA, analysis of variance, S: effect of sex, T: effect of treatment, I: sex × treatment interaction, *p<0.05 in comparison with vehicle-treated rats of the same sex, Bonferroni post-hoc test. Data are means±SEM. Multi-level mixed linear model (MLM) corrections are available in the Tables S3-S4 in the Supplementary Materials.

A: Dopamine neurons of the VTA

Characteristic	Male-Vehicle (68 cells/5 rats)	Male-PD (43 cells/5 rats)	Female-Vehicle (25 cells/5 rats)	Female-PD (33 cells/5 rats)	Two-way ANOVA
Number of active neurons per track	5.67 ±0.86	3.31 ±0.68	2.50 ±0.37	2.54 ±0.48	**S:** $F_{1,47}$=9.22, p=0.004 **T:** $F_{1,47}$=3.21, p=0.080 **I:** $F_{1,47}$=3.42, p=0.071
Firing rate, s^{-1}	4.90 ±0.83	3.66 ±0.37	4.91 ±0.50	3.92 ±0.54	**S:** $F_{1,168}$=0.09, p=0.759 **T:** $F_{1,168}$=6.28, p=0.013 **I:** $F_{1,168}$=0.08, p=0.788
ISI coefficient of variation, %	131±8	131 ±10	91±7	95±6	**S:** $F_{1,168}$=14.80, p<0.001 **T:** $F_{1,168}$=0.03, p=0.854 **I:** $F_{1,168}$=0.02, p=0.888
Bursts frequency, s^{-1}	0.55 ±0.04	0.40 ±0.05	0.54 ±0.08	0.39 ±0.07	**S:** $F_{1,168}$=0.05, p=0.818 **T:** $F_{1,168}$=5.88, p=0.016 **I:** $F_{1,168}$=0.0004, p=0.948
Average number of spikes per burst	7.09 ±1.92	4.65 ±0.55	4.42 ±0.69	4.37 ±0.69	**S:** $F_{1,168}$=0.73, p=0.394 **T:** $F_{1,168}$= 0.52, p=0.472 **I:** $F_{1,168}$= 0.48, p=0.491
% of spikes occurring in bursts	55±3	43±4	42±5	38±5	**S:** $F_{1,168}$=4.38, p=0.038 **T:** $F_{1,168}$=3.48, p=0.064 **I:** $F_{1,168}$=0.76, p=0.383

B: Dopamine neurons of the SN

Characteristic	Male-Vehicle (33 cells/5 rats)	Male-PD (41 cells/5 rats)	Female-PD (22 cells/5 rats)	Female-FGF2 (31 cells/5 rats)	Two-way ANOVA
Number of active neurons per track	3.30 ±0.63	4.10 ±1.15	2.75 ±0.37	2.58 ±0.47	**S:** $F_{1,39}$=1.97, p=0.169 **T:** $F_{1,39}$=0.19, p=0.670 **I:** $F_{1,39}$=0.43, p=0.516
Firing rate, s^{-1}	5.16 ±0.51	4.57 ±0.43	4.26 ±0.70	4.30 ±0.49	**S:** $F_{1,126}$=1.24, p=0.267 **T:** $F_{1,126}$=1.29, p=0.594 **I:** $F_{1,126}$=0.36, p=0.552
ISI coefficient of variation, %	119±11	129±9	149±15	110 ±10*	**S:** $F_{1,126}$=0.30, p=0.583 **T:** $F_{1,126}$=1.50, p=0.224 **I:** $F_{1,126}$=4.67, p=0.033
Bursts frequency, s^{-1}	0.67 ±0.08	0.57 ±0.07	0.55 ±0.11	0.43 ±0.06	**S:** $F_{1,126}$=2.87, p=0.093 **T:** F_{1126}=2.13, p=0.147 **I:** F_{1126}=0.03, p=0.858
Average number of spikes per burst	5.13 ±0.71	5.27 ±0.70	5.88 ±0.63	5.21 ±0.69	**S:** $F_{1,126}$=0.22, p=0.640 **T:** $F_{1,126}$=0.14, p=0.640 **I:** $F_{1,126}$=0.32, p=0.575
% of spikes occurring in bursts	56±4	59±4	62±6	46±5	**S:** $F_{1,126}$=0.38, p=0.539 **T:** $F_{1,126}$=1.75, p=0.188 **I:** $F_{1,126}$=3.59, p=0.061

FGF2 (*Fgf2* knockout mice) took longer to recover from the loss of righting reflex and showed higher blood alcohol concentrations when challenged with an intoxicating alcohol dose (Even-Chen et al., 2022), suggesting that PD173074 might affect these capacities as well, which should be studied in the future.

We previously showed that administration of rFGF2 into the dorsal striatum of rats increased alcohol intake and preference in the 2-bottle choice alcohol-drinking paradigm (Even-Chen et al., 2017). Interestingly, this effect was visible after 24 hours of drinking, but was not seen in the first 4 hours of drinking. This indicates that the effects of rFGF2 on alcohol consumption occurs several hours after administration. This late effect of both FGF2 and PD173074 may be the result of transcriptional mechanisms involved in the function of FGF2 and FGFR1. Indeed, we recently found that the effects of rFGF2 on alcohol drinking are mediated via the PI3 kinase signaling pathway (Even-Chen and Barak, 2019a), whose activation has been implicated in excessive alcohol drinking, and in gene transcription and translation processes (Neasta et al., 2011; Ron and Barak, 2016).

4.2. *The FGF2-FGFR1 pathway modulates the excitability of mesolimbic and nigrostriatal dopamine neurons*

We found that activation and inhibition of the FGF2-FGFR1 pathway (by rFGF2 and PD173074, respectively) had opposite effects on midbrain dopaminergic neurons activation, with rFGF2 exciting and PD173074 inhibiting neuronal activation. Our findings that rFGF2 and PD173074 also have opposite effects on alcohol self-administration raise the possibility that FGF2-mediated increased dopaminergic firing is related to the increased alcohol intake, whereas PD173074-mediated suppression in dopaminergic firing is related to the suppression in alcohol intake. Moreover, as our previous studies localized the effects of FGF2-FGFR1 mostly to the DMS (Even-Chen and Barak, 2019a; Even-Chen et al., 2017), our finding here that FGF2 manipulations affect the firing of SN neurons that project to the DMS (i.e., nigrostriatal pathway), further suggests that dopaminergic activation or inhibition might mediate the effects on alcohol drinking, possibly by further activating the PI3K pathway.

We found that rFGF2 increased not only the firing rate, but also the burst firing of dopamine neurons. The burst mode of firing of dopamine neurons boosts the nerve terminal transmitter release, in comparison with the same amount of action potentials fired in a single-spike mode (Cooper, 2002). The results of the present study, therefore, suggest that FGF2 has a robust stimulatory effect on mesolimbic and nigrostriatal dopamine transmission. Since rFGF2 increased the mean number of spontaneously active dopamine neurons per electrode descent in the VTA, but not in the SN, the stimulatory effect of FGF2 on mesolimbic dopamine pathways might be more potent than on nigrostriatal ones. Since rFGF2 increased the density of the spontaneously active dopamine

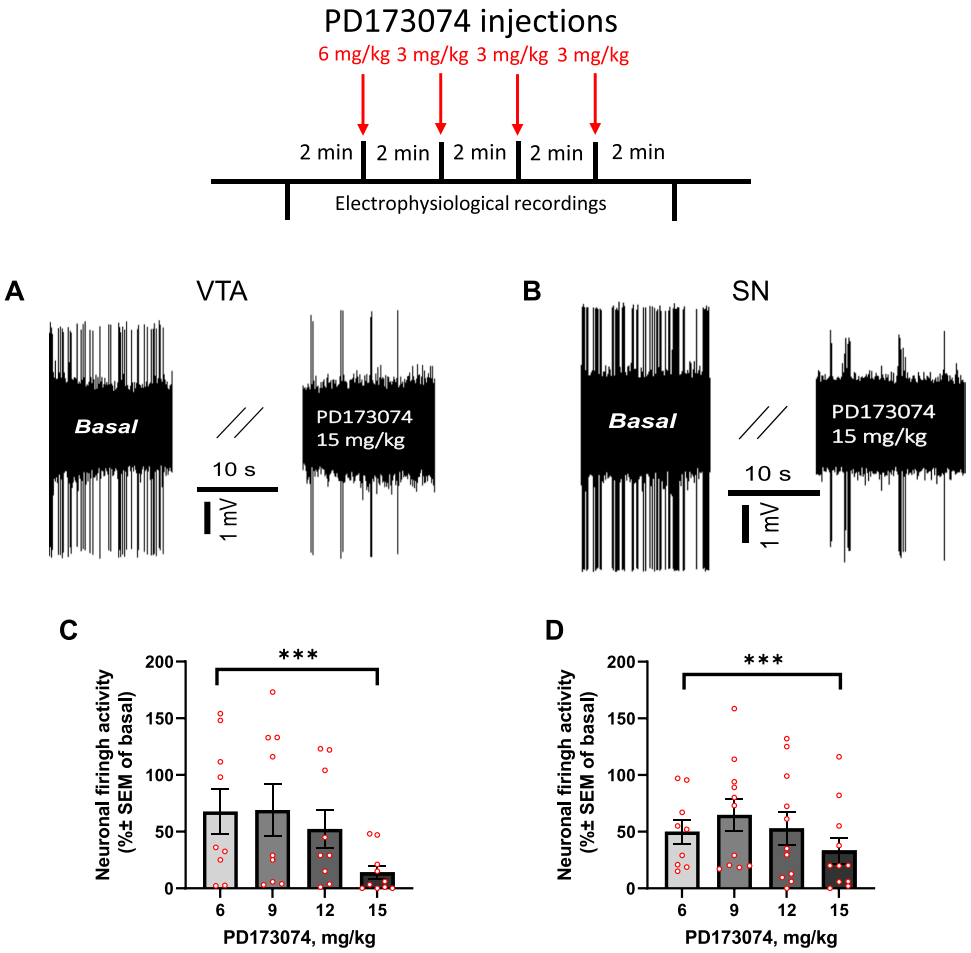

Fig. 8. PD173074 (administered during the recordings) suppresses the firing rate of dopamine neurons in the ventral tegmental area (VTA) and substantia nigra (SN). A-B. Representative recordings from the VTA (A) and SN (B). C-D. Summary effect from 5 neurons from 5 male rats and 5 neurons from 4 female rats. ***p<0.001 in comparison with baseline, two-way repeated measures ANOVA.

neurons in females, but not in males, the stimulatory effect of FGF2 on mesolimbic dopamine transmission might be more potent in females than males. This resemblance between behavioral and electrophysiological measures of reduced sensitivity to PD173074 in females compared to males, further suggests that the effects of FGF2-FGFR1 manipulations on alcohol self-administration may be mediated by their effects on nigrostriatal and mesolimbic dopamine neurons.

We found that pre-treatment with PD173074, eight hours before electrophysiological recordings (in concordance with the beahvioral effects of the compound), decreased the firing rate and burst activity of mesolimbic, but not nigrostriatal dopamine neurons. Regarding to the latter, the only characteristic affected by PD173074 was the CV. PD173074 induced decrease in the CV, meaning more regular and less stochastic pattern of the action potentials generation within nigrostriatal neurons, which might be linked with reduced efficacy of nigrostriatal dopamine transmission. We also observed that when PD173074 was administered i.v. during the recording from spontaneously active dopamine neurons, this FGFR1 inhibitor suppressed the firing activity of mesolimbic, as well as nigrostriatal dopamine neurons. It is therefore likely that FGFR1 mediates the effect of FGF2 on dopamine neurons in both nuclei, while its effect in the SN is more transient.

4.3. FGFR1 inhibition affects relapse and dopaminergic neuronal firing in a sex-dependent manner

We found that PD173074 administration reduced relapse to alcohol drinking, only in male rats. Specifically, we observed reduced lever presses and reward deliveries in male rats in the reacquisition test after PD173074 injections. Interestingly, we also found that PD173074 administration decreased dopaminergic neuronal activity in the SN and VTA, an effect that occurred already at the lowest dose tested, and was sex-dependent in some electrophysiological measures (Table 2).

Some of the effects of PD173074 on behavioral and on electrophysiological measures were sex-dependent. Critically, most previous animal studies on alcohol consumption and relapse for many decades were conducted on only male rodents, and therefore could not demonstrate sex-dependent differences. Recently, more studies are addressing this issue and differences between the sexes are emerging (Becker and Koob, 2016; Hilderbrand and Lasek, 2018; Lynch, 2018; Towers and Lynch, 2021). For example, it was shown that male rats displayed greater relapse to alcohol consumption than females (Randall et al., 2017), whereas female rats displayed elevated operant alcohol self-administration and alcohol seeking compared to males (Bertholomey et al., 2016). The latter finding is consistent with our previous finding in mice (Ziv et al., 2019), and with our current results, showing greater alcohol self-administration and seeking in female rats. The finding that the FGF2-FGFR1 pathway is implicated in the sex-dependent differences in alcohol relapse, plausibly via differential effects on dopaminergic activity should be further characterized in future studies, particularly as sex differences have been previously reported in the dopaminergic system, and sex steroids do not alter sex differences in tyrosine hydroxylase activity of dopaminergic neurons in vitro (Fernandezruiz et al., 1992; Ovtscharoff et al., 1992).

PD173074 did not affect alcohol-seeking behavior, tested after an

extended drinking period, as well as after a period of abstinence from alcohol. It is possible that inhibition of FGFR1 had no effect on alcohol seeking, because the effects of FGF2 and FGFR1 on alcohol consumption are localized to the DMS (Even-Chen et al., 2017). It was previously shown that following prolonged exposure to alcohol (over 8 weeks of self-administration training), alcohol-seeking behavior was no longer sensitive to the devaluation of alcohol, indicating habit formation (Corbit et al., 2012). While this type of response was affected by the inactivation of the dorsolateral striatum (DLS), it was unaffected by DMS inactivation (Corbit et al., 2012). In contrast, alcohol seeking was affected by the inactivation of the DMS at the earlier stages of alcohol consumption (Corbit et al., 2012). These findings indicate that alcohol seeking is prone to DMS manipulations only when conducted during the early stages of alcohol drinking, but not following extended drinking. Since FGFR1 acts in the DMS (Even-Chen and Barak, 2019a; Even-Chen et al., 2017), it is plausible that FGFR1 inhibition did not affect alcohol seeking here, because it was preceded by over 10 weeks of alcohol-drinking training. Thus, while FGF2 and FGFR1 act in the DMS to suppress alcohol consumption (Even-Chen and Barak, 2019a,b; Even-Chen et al., 2017; Liran et al., 2020), it is possible that they are also involved in alcohol seeking, but only at earlier stages of alcohol self-administration training.

In conclusion, the present study demonstrates that the FGF2-FGFR1 system plays a role in operant self-administration of alcohol, possibly by affecting the excitability of dopamine neurons in the VTA and SN. Inhibition of FGFR1 has stronger effects on males compared with females on some measures of dopaminergic neuronal activation and on relapse to alcohol drinking. This suggests that sex differences in the FGF2-FGFR1-dopamine interface may lead to differential outcomes when attempting to reduce alcohol drinking. Importantly, the association between the behavioral and electrophysiological effects in this study are correlative, and therefore we cannot conclude about the causal role of the dopaminergic effects on alcohol-associated behaviors. Together with our previous reports, our results here suggest that inhibition of FGFR1 may provide a potential treatment strategy to reduce excessive alcohol consumption. Importantly, while effective in reducing alcohol consumption, our results indicate that FGFR1 inhibition, at least with the inhibitor tested here and following extensive alcohol consumption, may have limited efficacy for relapse prevention as it may not be effective in females.

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CRediT authorship contribution statement

NL, DG, CG, ED and SB designed the research; NL, DG, RP, TK and MR performed the research; MLG, NL, TG, DG, CG, LH, ED and SB analyzed the data; MLG, NL, TG, LH, DG, ED and SB wrote the paper.

Declaration of Competing Interest

All authors declare no competing financial interests.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.drugalcdep.2023.109920.

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