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BDNF-mediated regulation of ethanol consumption requires the activation of the MAP kinase pathway and protein synthesis

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Abstract

We previously found that the brain-derived neurotrophic factor (BDNF) in the dorsolateral striatum (DLS) is part of a homeostatic pathway that gates ethanol self-administration [Jeanblanc *et al.* (2009). *J Neurosci*, **29**, 13494–13502)]. Specifically, we showed that moderate levels (10%) of ethanol consumption increase *BDNF* expression within the DLS, and that direct infusion of BDNF into the DLS decreases operant self-administration of a 10% ethanol solution. BDNF binding to its receptor, TrkB, activates the mitogen-activated protein kinase (MAPK), phospholipase C-γ (PLC-γ) and phosphatidylinositol 3-kinase (PI3K) pathways. Thus, here, we set out to identify which of these intracellular pathway(s) plays a role in the regulation of ethanol consumption by BDNF. We found that inhibition of the MAPK, but not PLC-γ or PI3K, activity blocks the BDNF-mediated reduction of ethanol consumption. As activation of the MAPK pathway leads to the initiation of transcription and/or translation events, we tested whether the BDNF-mediated reduction of ethanol self-administration requires *de novo* protein synthesis. We found that the inhibitory effect of BDNF on ethanol intake is blocked by the protein synthesis inhibitor cycloheximide. Together, our results show that BDNF attenuates ethanol drinking via activation of the MAPK pathway in a protein synthesis-dependent manner within the DLS.

Keywords

addiction; alcohol; dorsal striatum; growth factor; signal transduction

Introduction

Brain-derived neurotrophic factor (BDNF) is a growth factor that belongs to the nerve growth factor (NGF) family (Huang & Reichardt, 2001). BDNF secretion depends on neuronal depolarisation, and the growth factor is important for the survival, growth and maturation of neurons (Huang & Reichardt, 2001). Importantly, BDNF also plays a crucial role in synaptic plasticity, learning and memory (Kuipers & Bramham, 2006; Minichiello, 2009; Cowansage *et al.*, 2010). The binding of BDNF to the receptor tropomyosin-related kinase B (TrkB) results in the activation of the phospholipase C-γ (PLC-γ), phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways (Chao, 2003; Huang & Reichardt, 2003; Reichardt, 2006). Activation of these

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main pathways leads to intracellular events that underlie the functions of BDNF in the central nervous system (CNS; Reichardt, 2006; Minichiello, 2009; Cowansage *et al.*, 2010). For example, TrkB-mediated activation of Akt (also known as protein kinase B) by PI3K plays a role in cell survival and protein translation (Huang & Reichardt, 2003; Yoshii & Constantine-Paton, 2007), and activation of PLC-γ and MAPK via TrkB contributes to long-term potentiation in the hippocampus (Gartner *et al.*, 2006; Minichiello, 2009). Finally, activation of TrkB leads to the activation of the transcription machinery and to the upregulation of gene expression (Huang & Reichardt, 2003; Kuipers & Bramham, 2006; Minichiello, 2009; Wortzel & Seger, 2011).

BDNF has been shown to play both positive and negative roles in psychiatric disorders and addiction (Berton *et al.*, 2006; Russo *et al.*, 2009; Ghitza *et al.*, 2010; Ron & Messing, 2013). Studies in rodent models strongly support the possibility that BDNF is part of a homeostatic pathway that controls some of the adverse effects associated with ethanol exposure (Ron & Messing, 2013). Specifically, voluntary moderate ethanol consumption, modeled in the two-bottle choice and operant self-administration paradigms, leads to an increase in *BDNF* expression in the dorsal striatum of rodents (McGough *et al.*, 2004; Jeanblanc *et al.*, 2009; Logrip *et al.*, 2009). Global reduction of BDNF levels (Hensler *et al.*, 2003; McGough *et al.*, 2004), inhibition of the BDNF pathway (Jeanblanc *et al.*, 2006) and knockdown of *BDNF* expression specifically in the dorsolateral striatum (DLS) augment ethanol-drinking behaviors (Jeanblanc *et al.*, 2009). Conversely, global as well as dorsal striatal elevation of BDNF levels reduce ethanol intake (McGough *et al.*, 2004; Jeanblanc *et al.*, 2006, 2009). Similarly, infusion of BDNF into the medial amygdala also decreases ethanol intake, while inhibition of BDNF production via antisense oligonucleotides in either the medial or central nucleus of the amygdala increases ethanol preference (Pandey *et al.*, 2006). Finally, alcohol-preferring rat lines show reduced central and medial amygdala BDNF levels (Prakash *et al.*, 2008) and decreased synaptic density in those regions (Moonat *et al.*, 2011) as compared with alcohol non-preferring rats. Together these data support a beneficial role for BDNF in the negative modulation of ethanol intake. Here, we set out to identify the downstream pathway(s) mediating the regulation of ethanol consumption induced by activation of BDNF–TrkB signaling.

Materials and methods

Animals

Male Long–Evans rats (400–450 g at the time of surgery) were obtained from Harlan (Indianapolis, IN, USA). Animals were individually housed under a 12-h light:dark cycle, with lights on at 07:00 h, and food and water available *ad libitum*. All animal procedures in this report were approved by the Gallo Center Institutional Animal Care and Use Committee and were conducted in agreement with the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996.

Reagents

Recombinant BDNF, U0126, wortmannin, U73122 and cycloheximide (CHX) were purchased from Sigma–Aldrich, Inc. (Saint Louis, MO, USA).

Operant self-administration of ethanol

Rats were trained to self-administer ethanol as described previously (Jeanblanc *et al.*, 2009). Briefly, rats were habituated to drink ethanol (10% *v/v*) mixed with decreasing concentrations of sucrose (10, 5 and 0%, w/v) over 3 weeks in their home cages. Rats were then trained to self-administer a solution of 10% ethanol (v/v) in an operant self-administration procedure. The self-administration chambers contained two levers: an active

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lever (the ethanol-paired lever), for which presses resulted in delivery of 0.1 mL ethanol, and an inactive lever, for which presses were counted but no programmed events occurred. Rats were trained on an FR3 schedule (three presses are required to receive one reward) for 60-min sessions 5 days a week for a month. During the self-administration sessions, the number of lever presses and the number of ethanol deliveries were recorded using MED-PC IV software (Med Associates Inc., St. Albans, VT, USA).

Cannulae implantation into the DLS

Surgery was conducted after 1 month of operant self-administration training. Rats were continuously anaesthetised with isoflurane (Baxter, IL, USA) during the surgery. Four holes were drilled for screws and two additional holes were drilled for the placement of the cannulae (single cannula C315GA; 26G diameter; PlasticsOne, Roanoke, VA, USA). The coordinates for the DLS were +1.2 mm anterior to bregma and 3.5 mm lateral to the medial suture. The cannulae were implanted into the lateral part of the dorsal striatum (−4.2 mm from the skull surface; Fig. 1) and fixed with dental cement. Subject weights were monitored daily after the surgery to ensure the healthy recovery of each rat. One week after recovery, subjects returned to self-administration training and were habituated to the microinfusion procedure with two sham injections. Experimental microinjections began upon re-acquisition of a stable level of responding. The injectors used for each group extended 0.5 mm below the tip of the cannula.

BDNF and kinase inhibitor treatments

The MAPK/ERK kinase (MEK) inhibitor U0126 was dissolved in PBS containing 5% DMSO and 6% Tween-80 at a concentration of 1 μg/μl (Carnicella *et al*., 2008). The PLC-γ inhibitor U73122 was dissolved in DMSO at a concentration of 0.2 μg/μl (Carnicella *et al*., 2008). The PI3K inhibitor wortmannin was dissolved in PBS containing 25% DMSO at a concentration of 0.1 μg/μl (Neasta *et al*., 2011). BDNF was dissolved in PBS at a concentration of 0.75 μg/μl (Jeanblanc *et al*., 2009). One microlitre of the inhibitor, or its vehicle control, was infused 30 min before the injection of 1 μl of BDNF (0.75 μg/μl) or PBS. Each infusion lasted 2 min and the injectors remained in position for an additional 2 min. Rats were then returned to the home cage until the beginning of the self-administration session. Two hours after BDNF administration, rats underwent a 1-h self-administration session. The order of injections (inhibitor vs. vehicle and BDNF vs. PBS) was counterbalanced across all subjects over the 4-week, period with one injection per week.

BDNF and protein synthesis inhibitor treatments

The protein synthesis inhibitor CHX (1 μl of a 10 μg/μl solution) or its vehicle (PBS) was infused into the DLS immediately before the infusion of BDNF (1 μl of a 0.75 μg/μl solution). Infusions lasted 2 min with the injector kept in position for an additional 2 min, then the rats were returned to their home cages until the self-administration session. Three hours after the infusion of BDNF, rats underwent a 1-h self-administration session.

Histology

Rats implanted with cannulae were perfused transcardially with fixative (4% PFA), and 75-μm coronal slices were cut and examined for cannula placements (Fig. 1). As all cannula placements were appropriately located, no animals were omitted from the study.

Data analysis

Behavioral data were analysed by two-way ANOVA with repeated measures, followed by the Student–Newman–Keuls test when indicated by significant effects of treatments or interactions. Significance for all tests was set at $P < 0.05$.

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Results

Activation of the TrkB receptor leads to the activation of the MAPK, PI3K–Akt and PLC–PKC pathways (Huang & Reichardt, 2003; Reichardt, 2006). To address which signaling cascade mediates the inhibitory actions of BDNF on ethanol intake, we first tested the contribution of PI3K to the action of BDNF on ethanol intake. To do so, the specific PI3K inhibitor wortmannin (Bain *et al*., 2003) was infused at a concentration that attenuates ethanol intake when infused into the nucleus accumbens of rats (Neasta *et al*., 2011). We found that intra-DLS administration of wortmannin (0.1 μg per side) by itself did not affect ethanol self-administration, nor did it alter the BDNF (0.75 μg per side)-mediated inhibition of ethanol intake (Fig. 2). Specifically, infusion of wortmannin prior to the infusion of BDNF did not alter the number of ethanol deliveries (Fig. 2A); a two-way ANOVA with repeated measures revealed a main effect of the factor BDNF ($F_{1,10} = 13.76$; $P < 0.01$), with no effect of the factor wortmannin ($F_{1,10} = 2.28$; ns) and no interaction between the two factors ($F_{1,10} = 0.03$; ns). *Post hoc* analysis revealed a significant difference in the number of deliveries between the Vehicle–PBS and Vehicle–BDNF groups ($P < 0.01$) and between wortmannin–PBS and wortmannin–BDNF groups ($P < 0.01$). Wortmannin infusion also did not alter ethanol consumption (Fig. 2B); a two-way ANOVA with repeated measures revealed a main effect of the factor BDNF ($F_{1,10} = 11.17$; $P < 0.01$) but no effect of the factor wortmannin ($F_{1,10} = 2.15$; ns) and no interaction between the two factors ($F_{1,10} = 0.44$; ns). *Post hoc* analysis showed a significant difference in the number of deliveries between the Vehicle–PBS and Vehicle–BDNF groups ($P < 0.05$) and between the wortmannin–PBS and wortmannin–BDNF groups ($P < 0.05$).

Next, we tested the contribution of PLC-γ in the DLS to the action of BDNF in reducing ethanol self-administration. We used the PLC-γ inhibitor U73122 (Bleasdale *et al*., 1990), which we previously found to inhibit ethanol self-administration upon infusion into the ventral tegmental area (VTA) of rats (Carnicella *et al*., 2008). Administration of U73122 (0.2 μg per side) prior to the infusion of BDNF (0.75 μg per side) into the DLS did not reverse the inhibitory effect of BDNF on ethanol intake, but by itself decreased ethanol self-administration (Fig. 3A); a two-way ANOVA with repeated measures revealed a main effect of the factor BDNF ($F_{1,8} = 5.97$; $P < 0.05$), with no effect of the factor U73122 ($F_{1,8} = 5.10$; ns) but an interaction between the two factors ($F_{1,8} = 7.90$; $P < 0.05$). *Post hoc* analysis revealed a significant difference in the number of deliveries between the Vehicle–PBS and U73122–PBS groups ($P < 0.01$) and between the Vehicle–PBS and Vehicle–BDNF groups ($P < 0.01$). Ethanol intake in the presence and absence of U73122 revealed similar pattern (Fig. 3B); a two-way ANOVA with repeated measures revealed a main effect of the factor BDNF ($F_{1,8} = 5.65$; $P < 0.05$), with no effect of the factor U73122 ($F_{1,8} = 2.10$; ns) but an interaction between the two factors ($F_{1,8} = 9.86$; $P < 0.05$). *Post hoc* analysis showed a significant difference in the number of ethanol deliveries between the Vehicle–PBS and U73122–PBS groups ($P < 0.01$). *Post hoc* analysis also revealed a significant difference between the Vehicle–PBS and Vehicle–BDNF groups ($P < 0.01$)).

Finally, we set out to test the involvement of the MAPK pathway in the inhibitory actions of BDNF on operant self-administration of ethanol. To do so we used U0126, a specific inhibitor of the MEK, the kinase directly upstream of MAPK (Sweatt, 2001). We previously found that U0126 inhibits the actions of glial cell line-derived neurotrophic factor (GDNF) on ethanol intake in the VTA of rats (Carnicella *et al*., 2008). Intra-DLS infusion of U0126 (1 μg per side) prior to the infusion of BDNF (0.75 μg per side) reversed the BDNF-induced decrease in earned ethanol deliveries (Fig. 4A); a two-way repeated-measures ANOVA revealed a main effect of the factor BDNF ($F_{1,7} = 12.95$; $P < 0.01$), no effect of the factor U0126 ($F_{1,7} = 0.71$; ns) but an interaction between the two factors ($F_{1,7} = 24.86$; $P < 0.01$). *Post hoc* analysis revealed a significant difference between the PBS–PBS and PBS–BDNF groups (*P*

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< 0.001) and a difference between the PBS–BDNF and U0126–BDNF groups ($P < 0.05$). As a correlate, the amount of ethanol consumed was reduced after intra-DLS BDNF infusion, but this decrease was blocked when U0126 was co-infused with BDNF (Fig. 4B); a two-way ANOVA with repeated measures revealed a main effect of BDNF ($F_{1,7} = 12.4$, $P = 0.01$) and an interaction between BDNF and U0126 ($F_{1,7} = 21.9$, $P < 0.01$) but no main effect of the factor U0126 ($F_{1,7} = 0.8$, ns). Similarly, *post hoc* analysis revealed a significant difference between the PBS–PBS and PBS–BDNF groups ($P < 0.001$) and a difference between the PBS–BDNF and U0126–BDNF groups ($P < 0.05$). These results suggest that only the activation of MAPK, but not of PI3K or PLC-γ, in the DLS is required for the BDNF-mediated control of ethanol self-administration.

The main action of the MAPK pathway is to increase gene expression, accomplished by the translocation of a MAPK, such as the extracellular signal-regulated kinase 1/2 (ERK1/2), to the nucleus and the activation of the transcription machinery (Cargnello & Roux, 2011). We previously observed that the reduction in ethanol self-administration following BDNF infusion requires a 3-h delay (Jeanblanc *et al.*, 2009), suggesting a transcription and/or translation mechanism. We therefore hypothesised that the synthesis of new proteins is required for BDNF to alter ethanol intake. To test this possibility, the protein synthesis inhibitor CHX (10 μg/μl; Kesner *et al.*, 1981) was infused into the DLS immediately before BDNF (0.75 μg per side) administration. We found that CHX blocked the decrease in the number of rewards delivered in response to BDNF (Fig. 5A); a two-way ANOVA with repeated measures revealed a marginal effect of BDNF ($F_{1,6} = 4.32$, $P = 0.08$), with no main effect of CHX ($F_{1,6} = 1.7$, ns) but an interaction between BDNF and CHX ($F_{1,6} = 7.7$, $P < 0.05$). *Post hoc* analysis showed a significant difference between the PBS–PBS and PBS–BDNF groups ($P < 0.01$), demonstrating the effect of BDNF on the number of ethanol deliveries, as well as a significant difference between the PBS–BDNF and CHX–BDNF groups, revealing a blockade by CHX of the BDNF-induced reduction in ethanol deliveries ($P < 0.05$). As a consequence, the decrease in ethanol intake induced by BDNF was similarly blocked by CHX microinfusion (Fig. 5B); a two-way ANOVA with repeated measures revealed marginal effect of BDNF ($F_{1,6} = 4.75$, $P = 0.07$), no main effect of the injection of CHX ($F_{1,6} = 1.62$, ns) but an interaction between BDNF and CHX ($F_{1,6} = 7.7$, $P < 0.05$). *Post hoc* analysis revealed similar differences between the PBS–PBS and PBS–BDNF groups ($P < 0.01$) and between the PBS–BDNF and CHX–BDNF groups ($P < 0.05$). These results suggest that the effect of BDNF on ethanol consumption requires protein synthesis.

Discussion

Here, we have shown that the inhibitory actions of BDNF on ethanol consumption require the activation of the MAPK pathway and the synthesis of new proteins. Thus, while BDNF infusion into the DLS consistently reduced ethanol self-administration, it lost efficacy when either MAPK activity or protein synthesis was inhibited. These results are in agreement with previous studies in primary striatal neurons in which we observed BDNF-dependent ERK1/2 activation and nuclear translocation (Logrip *et al.*, 2008).

MAPK is required for the BDNF-mediated reduction of ethanol intake

We have demonstrated here that the MAPK pathway in the DLS mediates the beneficial actions of BDNF on ethanol intake. Whitfield *et al.* (2011) showed that MAPK activity in the dorsomedial prefrontal cortex (dmPFC) is required for the inhibitory actions of BDNF on cocaine-seeking. These authors blocked the suppressive BDNF effect on cue- and prime-induced reinstatement by microinfusing the MEK inhibitor U0126 into the dmPFC (Whitfield *et al.*, 2011). Moreover, the authors show that the BDNF-induced normalisation of the decreased phosphorylation levels of CREB and ERK following cocaine self-administration is blocked by U0126 (Whitfield *et al.*, 2011). Together, these results suggest

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that the MAPK pathway is responsible for the BDNF-mediated reduction of ethanol consumption as well as cocaine-seeking.

We have previously demonstrated an important role of the MAPK pathway in the regulation of ethanol consumption induced by another growth factor, GDNF, within the VTA (Carnicella *et al.*, 2008). Specifically, administration of GDNF directly into the VTA produces a rapid activation of the MAPK pathway, which underlies the reduction of ethanol consumption (Carnicella *et al.*, 2008). Thus, the same signaling pathway by two different neurotrophic factors in different brain regions produces a robust decrease in ethanol self-administration.

We found that inhibition of the PI3K pathway in the DLS does not alter the BDNF-mediated reduction in ethanol consumption, nor does it affect the level of intake when infused alone. In contrast, the PI3K pathway contributes to the development and/or maintenance of ethanol intake in the nucleus accumbens (Cozzoli *et al.*, 2009; Neasta *et al.*, 2011). It is also noteworthy that the inhibition of the PLC pathway in the DLS decreased ethanol self-administration, and a similar effect of PLC inhibition on ethanol self-administration was also observed in the VTA (Carnicella *et al.*, 2008). PLC is an upstream activator of protein kinase C (PKC). The PKC family includes nine isozymes, some of which have been implicated in phenotypes related to ethanol exposure, including ethanol intake (Ron & Messing, 2013). It is plausible that ethanol, by activating the PLC–PKC pathway in the DLS, contributes to the mechanisms that underlie ethanol intake. Alternatively, a basal level of activity of the PLC–PKC pathway may be necessary for the actions of ethanol in the DLS and other brain regions.

Interestingly, BDNF-induced activation of PLC-γ within the shell of the nucleus accumbens, but not within the dorsal striatum, has been shown to contribute to relapse in cocaine-seeking behavior (Graham *et al.*, 2007). Nevertheless, together these results suggest that specific activation of the MAPK pathway within the nigrostriatal system plays a role in the intake of drugs of abuse, whereas within the mesocorticolimbic system PLC-γ seems to be the key player.

The mechanism underlying the specificity of the BDNF–MAPK cascade in the DLS to reduce ethanol intake should be explored further. It is plausible that the compartmentalisation of the TrkB receptors with enzymes that participate in the MAPK, but not PI3K or PLC-γ, pathways in the DLS could account for the specificity of the BDNF–MAPK activity. Another possibility is that molecular adaptations in the DLS in response to long-term ethanol exposure may determine whether a signaling pathway is available to be activated in response to an exogenous application of BDNF. Finally, a recent study showing that arginine methylation determines the level and duration of ERK1/2 activation in response to different growth factors (Andreu-Perez *et al.*, 2011) may add another dimension to the brain region-specific actions of BDNF on ethanol and cocaine self-administration.

De novo protein synthesis is required for BDNF-mediated reduction of ethanol consumption

BDNF infused 10 min prior to the ethanol self-administration session did not modify the levels of ethanol consumption; however, when given 3 h prior to the session the growth factor significantly decreased ethanol self-administration (Jeanblanc *et al.*, 2009). This delay between the time of BDNF treatment and the effect on ethanol consumption suggests a transcriptional and/or translational mechanism. In support of this possibility, we have shown that the synthesis of protein(s) is necessary for BDNF to produce its effect on levels of ethanol intake. It is thus plausible that the MAPK pathway in the DLS initiates gene transcription followed by the synthesis of specific gene products. Our previous data

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demonstrating that an increase in the expression of dynorphin and dopamine D3 receptors in the dorsal striatum contributes, at least in part, to the actions of BDNF on ethanol intake (Jeanblanc *et al.*, 2006; Logrip *et al.*, 2008) support this option. Alternatively, the MAPK pathway in the DLS may directly control translation events which, in turn, mediate the reduction of ethanol intake by BDNF. Interestingly, BDNF plays an important role in dendritic translation of genes (Kuipers & Bramham, 2006), and this intriguing possibility should be further explored.

Implications

Taken together, these data strongly suggest that the MAPK signaling pathway is a key player in neurotrophic factor-induced decreases in the consumption of various drugs and within several brain regions, such as DLS, dmPFC and VTA. Thus, better understanding of how drug consumption could be altered by inhibition of this pathway is of great interest.

Alcoholism is a devastating chronic disease resulting in a large human and economic burden on society (Spanagel, 2009; Nutt *et al.*, 2010; Collins *et al.*, 2011). Here, we provide evidence that BDNF-mediated activation of the MAPK pathway, resulting in increased protein synthesis, is the molecular pathway underlying the beneficial regulation of ethanol intake by BDNF. The development of BDNF mimetics has been gaining interest (Nagahara & Tuszynski, 2011) and thus molecules that mimic the actions of BDNF by activating the BDNF signaling pathway, such as the TrkB receptor agonist 7,8-dihydroxyflavone (Jang *et al.*, 2010), may provide an attractive target for the development of medications to prevent alcohol use disorders.

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Abbreviations

BDNF	brain-derived neurotrophic factor
CHX	cycloheximide
CNS	central nervous system
DLS	dorsolateral striatum
dmPFC	dorsomedial prefrontal cortex
GDNF	glial cell line-derived neurotrophic factor
MAPK	mitogen-activated protein kinase
MEK	MAPK/ERK kinase
NGF	nerve growth factor
PI3K	phosphatidylinositol 3-kinase PLC-γ, phospholipase C-γ
TrkB	tropomyosin-related kinase B
VTA	ventral tegmental area.

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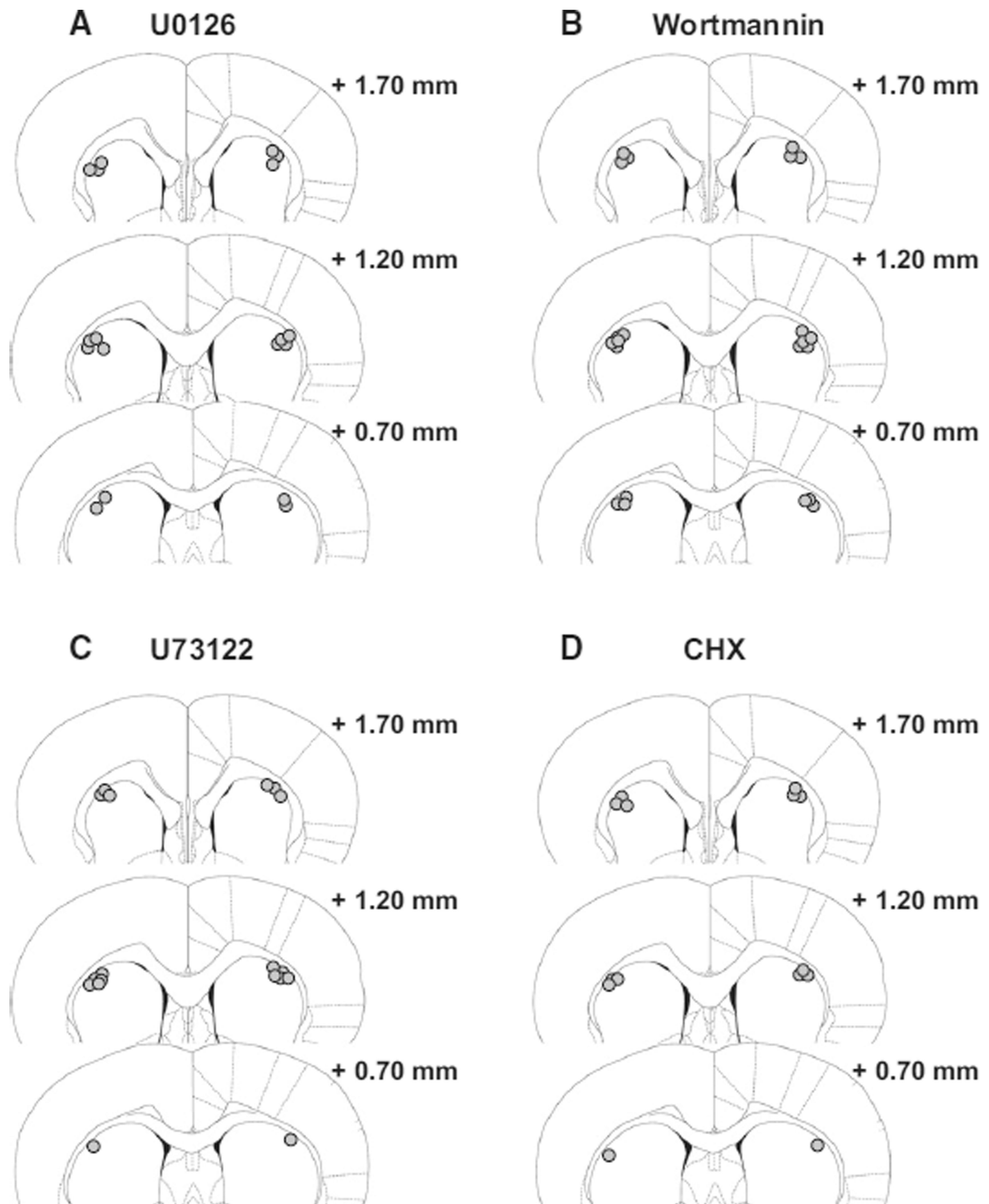

Fig. 1.

Schematic representation of coronal sections of the rat brain showing the placement of the injectors in the DLS corresponding to the experiments which used (A) U0126, (B) wortmannin, (C) U73122 and (D) CHX. No animals had to be excluded due to misplacement of the cannulae.

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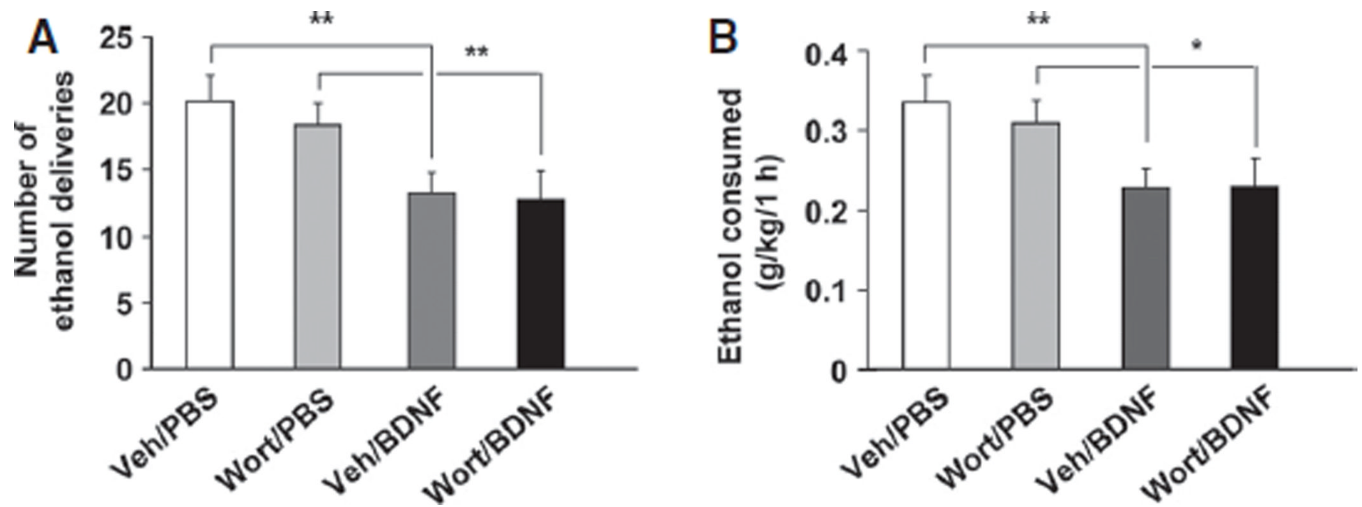

Fig. 2.

Inhibition of PI3K within the DLS did not block the effect of BDNF on ethanol self-administration. The PI3K inhibitor wortmannin (1 μl of a 0.1 μg/μl solution) was infused into the DLS 30 min prior to intra-DLS BDNF administration (1 μl of a 0.75 μg/μl solution), and lever presses were recorded 3 h later. (A) Results are expressed as mean ± SEM number of ethanol deliveries. (B) Results are expressed as mean ± SEM ethanol consumed (g/kg in 1 h). $^*P < 0.05$, $^{**}P < 0.01$, $n = 11$.

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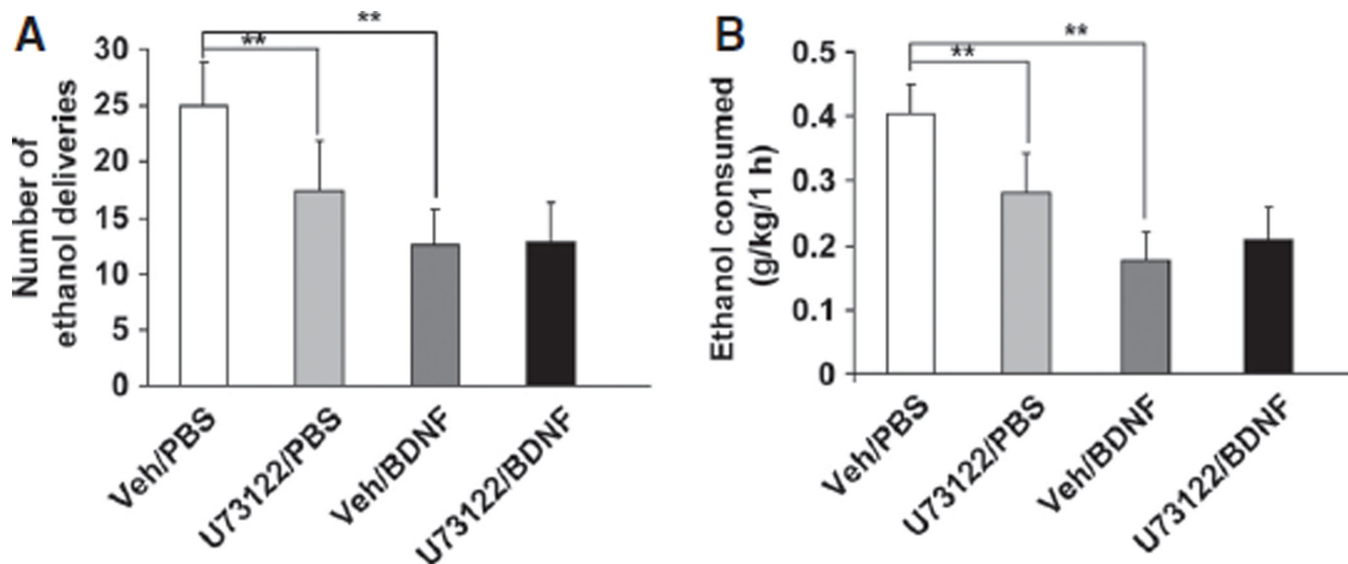

Fig. 3.

Inhibition of PLC-γ within the DLS did not block the effect of BDNF on ethanol self-administration. The PLC-γ inhibitor U73122 (1 μl of a 0.2 μg/μl solution) was infused into the DLS 30 min prior to intra-DLS BDNF administration (1 μl of a 0.75 μg/μl solution). (A) Results are expressed as mean ± SEM number of ethanol deliveries. (B) Results are expressed as mean ± SEM ethanol consumed (g/kg in 1 h). $^{**}P < 0.01$, $n = 9$.

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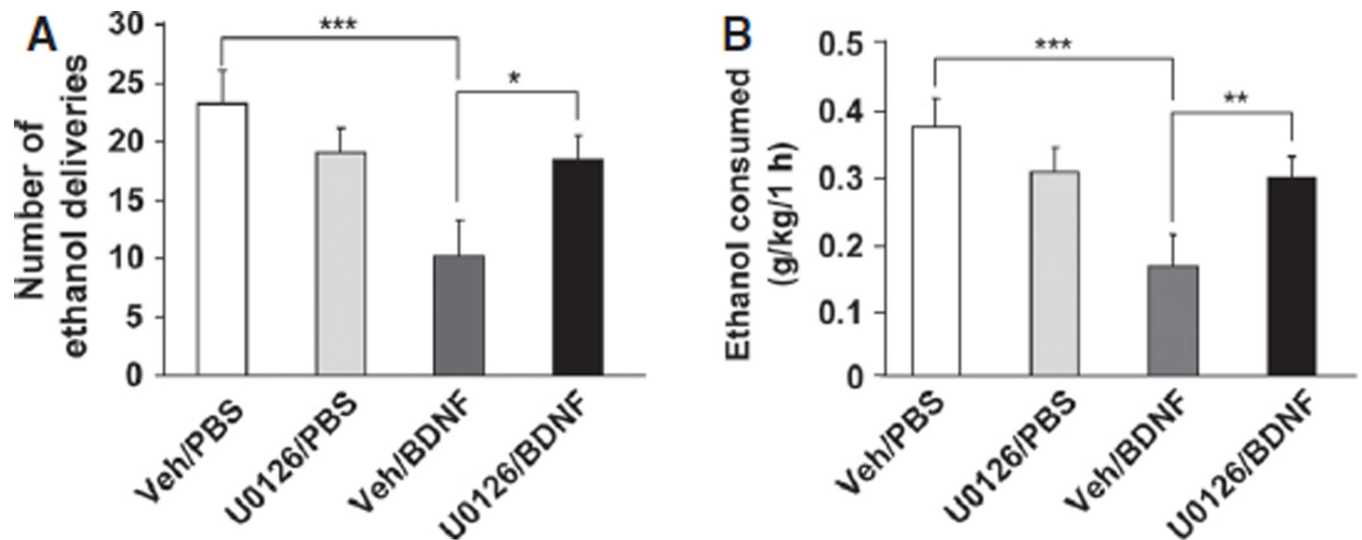

Fig. 4.

The MAPK pathway in the DLS was required for BDNF-mediated reduction of ethanol self-administration. The MEK inhibitor U0126 (1 μl of a 1 μg/μl solution) was infused into the DLS 30 min prior to intra-DLS infusion of BDNF (1 μl of a 0.75 μg/μl solution) and lever presses were recorded 3 hrs later. *A*, Results are expressed as mean ± SEM number of ethanol deliveries. *B*, Results are expressed as mean ± SEM ethanol consumed (g/kg in 1 h). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 8$.

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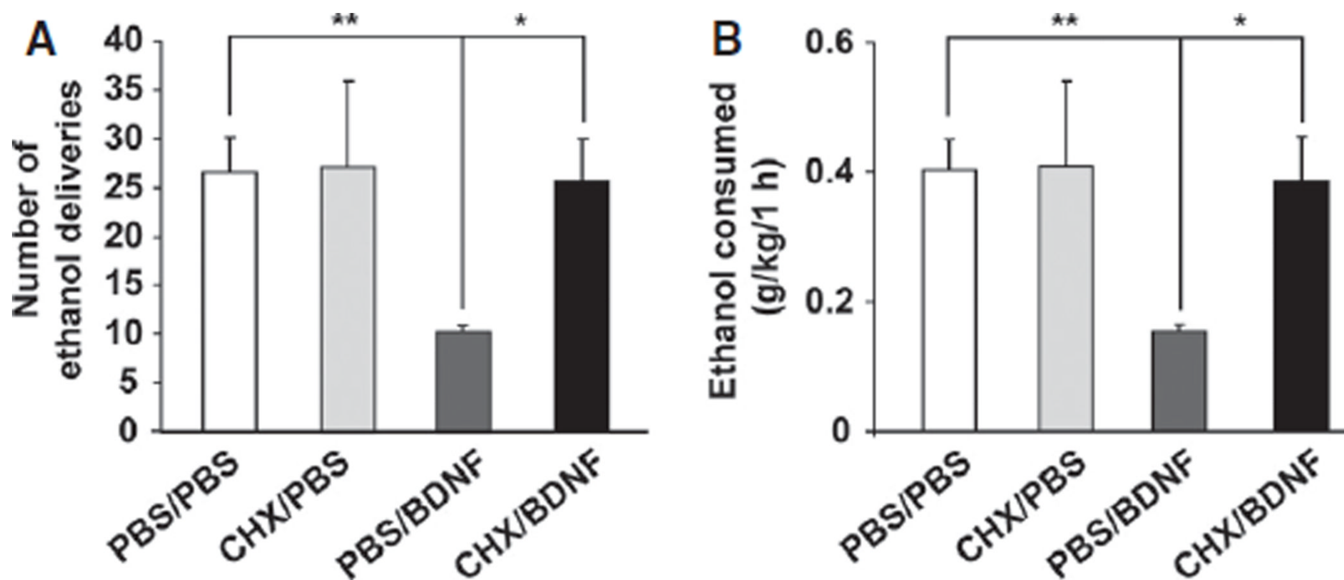

Fig. 5.

Protein synthesis within the DLS was necessary for BDNF to decrease ethanol self-administration. The protein synthesis inhibitor CHX (1 μl of a 10 μg/μl solution) and BDNF (1 μl of a 0.75 μg/μl solution) were co-infused into the DLS 3 h before the beginning of the operant self-administration session. (A) Results are expressed as mean ± SEM number of ethanol deliveries. (B) Results are expressed as mean ± SEM ethanol consumed (g/kg in 1 h). $*P < 0.05$, $**P < 0.01$, $n = 7$.