bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

IDENTIFICATION OF A NOVEL BDNF-SPECIFIC CORTICOSTRIATAL CIRCUITRY

Yann Ehinger, Drishti Soneja, Khanhky Phamluong, and Dorit Ron

Department of Neurology, University of California, San Francisco, USA

Correspondence:

Dorit Ron

Department of Neurology

University of California, San Francisco, 675 Nelson Rising Lane, San Francisco, CA 94143-0663, USA. Email: dorit.ron@ucsf.edu

Conflict of Interest: The authors declare no competing financial interests.

Acknowledgments: This research was supported by the National Institute of Alcohol Abuse and Alcoholism R37AA01684 (DR). We thank Dr. Zack Knight (UCSF) for the generous gift of the BDNF-2A-Cre knock-in mice and Dr. Jeffrey Moffat (UCSF) for helpful discussions.

Abstract

BDNF is released from axon terminals originating in the cerebral cortex onto striatal neurons. Here, we characterized BDNF in the corticostriatal circuitry. First, we utilized Bdnf-Cre and Ribotag transgenic mouse lines to label BDNF-positive cells in the cortex, and detected BDNF expression in the motor cortex, medial prefrontal cortex (mPFC) and the orbitofrontal cortex (OFC). Next, we used a retrograde viral tracing strategy, in combination with Bdnf-Cre knockin mice, to map the cortical outputs of BDNF neurons in the dorsal striatum. We found that the BDNF-positive prefrontal regions differentially project to the dorsal striatum. Specifically, BDNF-expressing neurons located in the mPFC project to both dorsolateral striatum (DLS) and

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

dorsomedial striatum (DMS), and those located in the motor cortex project to the DLS. Surprisingly however, the BDNF-expressing OFC neurons differentially target the dorsal striatum depending on their mediolateral location. Specifically, the DMS is mainly innervated by the medial part of the OFC (mOFC) whereas, the DLS receives projections specifically from the ventrolateral region of the OFC (vlOFC). Next, using an anterograde viral tracing strategy, we confirmed the presence of a BDNF-specific vlOFC-DLS circuit. Finally, we show that overexpression of BDNF in the vlOFC activates TrkB signaling specifically in the DLS but not in the DMS demonstrating the functionality of this circuit. Our study uncovers a previously unknown neural circuit composed of BDNF-positive vlOFC neurons projecting to the DLS. These findings could have important implications for the role of BDNF signaling in the OFC as well as in other corticostriatal circuitries.

Significance Statement

BDNF is released in axons upon neuronal depolarization. Surprisingly, careful mapping of BDNF projecting neurons in CNS has not been conducted. Using anterograde and retrograde viral strategies in combination with transgenic reporter mice, we mapped out corticostriatal BDNF circuits. We found that mPFC BDNF neurons project to both the DMS and DLS, and the motor cortex to the DLS only. In contrast, BDNF neurons in the OFC are anatomically segregated. Whereas BDNF in mOFC neurons project to the DMS, BDNF in vlOFC project specifically to the DLS. Our findings could be important to the study of BDNF in corticostriatal circuitries.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Introduction

Brain Derived Neurotrophic Factor (BDNF) is a member of the nerve growth factor family of neurotrophic factors, which is highly expressed in the central nervous system (CNS) (Hofer et al., 1990; Yan et al., 1997; Kowianski et al., 2018). The majority of BDNF in neurons is stored in presynaptic dense core vesicles and is released upon neuronal depolarization (Dieni et al., 2012; Song et al., 2017). Once BDNF is released at axon terminals, it binds the receptor tyrosine kinase, tropomyosin-related kinase B (TrkB). Activation of TrkB stimulates extracellular regulated kinase 1/2 (ERK1/2), Protein kinase C (PKC) and/or phosphoinositide 3 kinase (PI3K) signaling cascades resulting in the initiation of transcriptional and translational machineries (Huang and Reichardt, 2003; Leal et al., 2014; Zagrebelsky et al., 2020). In the adult brain, BDNF plays a crucial role in synaptic and structural plasticity (Panja and Bramham, 2014; De Vincenti et al., 2019), as well as in learning and memory (Bekinschtein et al., 2014; Miranda et al., 2019).

BDNF is highly expressed in the cerebral cortex of both rodents and humans (Hofer et al., 1990; Timmusk et al., 1993; Conner et al., 1997). BDNF in the cortex plays an important role in learning and memory (Miranda et al., 2019). For example, absence of BDNF in the prelimbic cortex alters fear expression in mice, indicating a role for consolidation and expression of learned fear (Choi et al., 2010). In the OFC, BDNF is critical for goal-directed decision making and in selecting actions based on their consequences (Gourley et al., 2013). In the motor cortex, BDNF contributes to motor learning (Andreska et al., 2020).

The cerebral cortex is the major source of BDNF in the striatum (Altar et al., 1997; Baquet et al., 2004; Strand et al., 2007). BDNF released from cortical terminals, binds to, and activates its receptor, TrkB, in the striatum (Altar et al., 1997; Baydyuk and Xu, 2014). BDNF/TrkB signaling in the striatum has important cellular and behavioral roles (Besusso et al., 2013; Engeln et al.,

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

2020). For example, TrkB signaling is required to control inhibition of locomotor behavior in enkephalin (ENK) positive MSNs (Besusso et al., 2013), and Lobo and colleagues have recently provided data to suggest that BDNF/TrkB signaling in D1 receptor MSNs plays a role in stereotypy behaviors (Engeln et al., 2020).

Finally, BDNF in corticostriatal circuitries also plays a role in addiction. Numerous studies investigated the role of BDNF in the prefrontal cortex in relation with cocaine use (Pitts et al., 2016). For example, cocaine exposure regulates BDNF signaling in the prefrontal cortex and in turn BDNF influences the development and maintenance of cocaine-related behaviors (Lu et al., 2010; Gourley et al., 2013; Zhang et al., 2015; Pitts et al., 2018). In addition, activation of BDNF signaling in the dorsolateral striatum (DLS) keeps alcohol intake in moderation (McGough et al., 2004; Jeanblanc et al., 2006; Jeanblanc et al., 2009; Jeanblanc et al., 2013), whereas malfunctioning of BDNF/TrkB signaling in the corticostriatal regions promotes compulsive heavy use of alcohol (Logrip et al., 2009; Darcq et al., 2015; Darcq et al., 2016; Warnault et al., 2016).

As detailed above, BDNF is crucial for cortical and striatal functions, yet careful characterization of BDNF neurons in corticostriatal circuitry is lacking. Here, using a combination of a transgenic BDNF reporter line together with anterograde and retrograde viral-mediated gene delivery approaches, we mapped out BDNF-specific corticostriatal projections. We found that mPFC BDNF neurons project to both the DMS and DLS, and BDNF neurons in the motor cortex project to the DLS only. Surprisingly, we discovered that OFC neurons projecting to the DLS are anatomically segregated; BDNF neurons in the mOFC project to the DMS, while BDNF neurons in the vlOFC project to the DLS.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Methods

Reagents

Mouse anti-NeuN antibody (MAB377) was obtained from Millipore (Billerica, MA). Rabbit anti-Phospho-ERK1/2 antibody (CST 4377) was purchased from Cell Signaling Technology (Danvers, MA). Chicken anti-GFP (A10262), donkey anti-mouse IgG AlexaFluor 594, donkey anti-rabbit IgG AlexaFluor 594 and donkey anti-chicken AlexaFluor 488 antibodies were purchased from Life Technologies (Grand Island, NY). Rabbit anti-VGUT1 antibody (VGT1-3) was purchased from Mab Technologies (Stone Mountain, GA). Other common reagents were from Sigma Aldrich (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Animals and Breeding

Male C57BL/6J mice (6-8 weeks old at time of purchase) were obtained from The Jackson Laboratory. Male Bdnf-Cre knockin mice, which express Cre recombinase at the endogenous *Bdnf* locus, were obtained from Zach Knight, UCSF (Tan et al., 2016). Ribotag mice (ROSA26CAGGFP-L10a), which express ribosomal subunit RPL10a fused to EGFP (EGFP-L10a) in Cre-expressing cells (Zhou et al., 2013), were purchased from The Jackson Laboratory (B6;129S4-Gt (ROSA)26Sortm9(EGFP/Rpl10a)Amc/J). Ribotag mice were crossed with Bdnf-Cre mice allowing EGFP-L10a expression in BDNF-expressing cells. Mouse genotype was determined by poly-chain reaction (PCR) analysis of tail DNA.

Mice were individually housed on paper-chip bedding (Teklad #7084), under a reverse 12-hour light-dark cycle (lights on 0100 to 2200 h). Temperature and humidity were kept constant at 22 ± 2°C, and relative humidity was maintained at 50 ± 5%. Mice were allowed access to food (Teklad Global Diet #2918) and tap water *ad libitum*. All animal procedures were approved by the

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

University of California, San Francisco Institutional Animal Care and Use Committee and were conducted in agreement with the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC, UCSF).

Virus information

Recombinant adeno associated virus (rAAV) retrograde EF1a Nuc-flox(mCherry)-EGFP (1 x 10^{12} vg/ml), which expresses nuclear-localized mCherry by default but switches to nuclear-localized EGFP expression in the presence of Cre, was purchased from Addgene (Watertown, MA) (Addgene viral prep # 112677-AAVrg). AAV1-pCAG-FLEX-EGFP (1x10^{13} vg/ml), expressing EGFP in the presence of Cre, was purchased from Addgene (Addgene viral prep # 51502-AAV1). AAV1/2-IRES-CMV-BDNF-GFP (AAV-BDNF; 1 x 10^{12} vg/ml) and AAV-IRES-CMV-GFP (AAV-GFP; 1 x 10^{12} vg/ml) were designed as described in (Warnault et al., 2016) and were produced by the University of North Carolina Viral Vector Core.

RT-PCR

Total RNAs were isolated using Trizol reagent (Invitrogen) and reverse transcribed (RT) using the Reverse Transcription System kit (Promega). The resulting cDNA samples were amplified by TaqMan quantitative PCR using commercially available primer/probe kits from Applied Biosystems for BDNF (Gene Expression Assay Mm00432069_m1) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Gene Expression Assay Mm99999915_g1) was used as the internal control.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Stereotaxic surgery and viral infection of the OFC, DLS and DMS

C57BL/6J (WT) or Bdnf-Cre mice underwent stereotaxic surgery as described in Ehinger et al (Ehinger et al., 2020). Specifically, mice were anesthetized by vaporized isoflurane and were placed in a digital stereotaxic frame (David Kopf Instruments, Tujunga, CA). Two holes were drilled above the site of viral injection. The injectors (stainless tubing, 33 gauges; Small Parts Incorporated, Logansport, IN) were slowly lowered into the target region. The injectors were connected to Hamilton syringes (10 µl; 1701, Harvard Apparatus, Holliston, MA), and the infusion was controlled by an automatic pump at a rate of 0.1 µl/minute (Harvard Apparatus, Holliston, MA). The injectors remained in place for an additional 10 minutes to allow the virus to diffuse and were then slowly removed. For experiments investigating BDNF+ efferent projections to the dorsal striatum, Bdnf-Cre animals were infused with 0.5 µl of retro-AAV-EF1aNuc-flox(mCherry)-EGFP per hemisphere targeting the DLS (AP: +1.1, ML: ± 2.3, DV: -2.85, infusion at -2.8 from bregma) or the DMS (AP: +1.1, ML: ± 1.2, DV: -3, infusion at -2.95 from bregma). For experiments investigating BDNF+ efferent projections from the OFC, Bdnf-Cre animals were infused with 0.5 µl of AAV1-pCAG-FLEX-EGFP per hemisphere targeting the DLS (AP: +1.1, ML: ± 2.3, DV: -2.85, infusion at -2.8 from bregma). To overexpress BDNF in the OFC, C57BL/6 mice were infused bilaterally with 1 µl of AAV-BDNF or AAV-GFP per injection site, with two injections per hemisphere (AP: +3 and +2.58, ML: ± 1.2, DV: -2.3, infusion at -2.2 and -2.55 and -2.5 from bregma for each injection, respectively).

Immunohistochemistry and Microscopy

Following intraperitoneal (IP) administration of euthasol (200 mg/kg), mice were transcardially perfused with phosphate buffered saline, followed by 4% paraformaldehyde (PFA), pH 7.4. Brains

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

were quickly removed post-perfusion and were fixed additionally for 24 hours in 4% PFA prior to cryoprotection in 30% sucrose solution for 3 days at 4°C. Brains were then sectioned to 50 µm by cryostat (CM3050, Leica, Buffalo Grove, IL), collected serially 1:6 in 6-well plates and stored in PBS with 0.01% sodium azide. Free-floating PFA-fixed sections were permeabilized and blocked in PBS containing 0.3% Triton and 5% donkey serum for 4 hours at 4°C. Sections were then incubated for 18 hours at 4°C on an orbital shaker with the primary antibodies anti-NeuN (1/500), anti-GFP (1/1000), anti-phospho-ERK1/2 (pERK1/2) (1:500) or anti-VGlut1 (1/1000) diluted in 3% bovine serum albumin (BSA) in PBS. Next, sections were washed in PBS and incubated for 4 hours at 4°C with Alexa Fluor 488-labeled donkey (1/500), Alexa Fluor 594-labeled donkey antibodies (1/500) or Alexa Fluor 647-labeled donkey antibodies (1/500) in 3% BSA in PBS. After staining, sections were rinsed in PBS and were cover slipped using Prolong Gold mounting medium. Images were acquired on Zeiss LSM 510 META laser confocal microscope (Zeiss MicroImaging, Jena Germany) or Olympus Fluoview 3000 Confocal microscope (Olympus America, Center Valley PA) using manufacture recommended filter configurations. For striatal quantification of ERK1/2 phosphorylation, sections containing the dorsal striatum were selected, stained, and quantified using Imaris Bitplane v. 8.1 imaging software (Zurich, Switzerland).

Data Analysis

Data were analyzed using Mann Whitney t-test Graphpad Prism 8. Analyses were run as two-sided. p value was set to 0.05.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Results

BDNF positive neurons are detected in medial prefrontal cortex, motor cortex and orbitofrontal cortex

We first assessed the distribution of BDNF-expression in cortical neurons. To do so, we used a Bdnf-Cre transgenic mouse line allowing Cre-recombinase expression only in BDNF expressing cells (Tan et al., 2016), which was crossed with a Ribotag mouse line expressing GFP-fused ribosomal subunit RPL10 in the presence of Cre-recombinase (Zhou et al., 2013) (**Figure 1a**). The presence of GFP fused ribosomal subunit RPL10 (EGFP-L10a) enabled the visualization of BDNF-expressing cells. As shown in **Figure 1b**, a large number of BDNF-expressing neurons were detected in the MC (motor cortex) and the mPFC (medial prefrontal cortex) as well as in the OFC. In contrast, we did not detect green fluorescence in the dorsal or ventral striatum (**Figure 1c**), which confirms previous data indicating that striatal neurons do not express BDNF (Baydyuk and Xu, 2014).

BDNF-expressing prefrontal neurons differentially project to the dorsal striatum

The cerebral cortex highly expresses BDNF (Hofer et al., 1990; Timmusk et al., 1993; Conner et al., 1997), and is the major source of BDNF in the striatum (Altar, Cai et al. 1997, Baquet, Gorski et al. 2004). The striatum is divided into the ventral and dorsal striatum which is further divided into the DMS and DLS (Hunnicutt et al., 2016). We focused on the dorsal striatum and assessed whether BDNF-expressing neurons in the prefrontal cortex send projections to different parts of the dorsal striatum. To do so, we used a retrograde viral strategy and infected the DMS or the DLS of Bdnf-Cre mice with a retrograde AAV-Nuc-flox-(mCherry)-EGFP viral

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

construct, which enables mCherry expression in BDNF negative neurons and EGFP expression in BDNF positive neurons (Back et al., 2019) **(Figure 2a).**

First, we assessed which BDNF-expressing cortical neurons project to the DMS by infecting the DMS of Bdnf-Cre mice with the retrograde AAV-Nuc-flox-(mCherry)-EGFP (**Figure 2b**). As shown in **Figure 2c,** the DMS contained cell nuclei labeled in red, corresponding to intrastriatal connections. Absence of EGFP-labeled neurons indicated once more a lack of *BDNF* message in the DMS. In the prefrontal regions, the mPFC and the mOFC show a high density of retrogradely EGFP-labeled neurons (**Figure 2c**). In contrast very few cell nuclei labeled in green were detected in the motor cortex and vlOFC, suggesting that BDNF neurons from the motor cortex and vlOFC do not project to the DMS. Additionally, we show that BDNF-positive prefrontal neurons project in an ipsilateral manner **Supplementary Figure 1**. Together these data indicate that the DMS receives projections mainly from BDNF neurons located in the mPFC and the medial part of the OFC.

Next, we assessed whether BDNF-expressing cortical neurons project to the DLS by infecting Bdnf-Cre mice with the retrograde AAV-Nuc-flox-(mCherry)-EGFP (**Figure 3a**). Like the DMS, the DLS contained only red cell nuclei, corresponding to intrastriatal connections, and indicating a lack of *BDNF* message in this brain region. However, we observed a high density of retrogradely EGFP labeled neurons in the prefrontal regions, the motor cortex and the mPFC (**Figure 3b**). The deep layers of the vlOFC contained cell nuclei labeled in green, in contrast with the mOFC containing few EGFP labeled neurons (**Figure3b**). These results suggest that BDNF-expressing neurons in the motor cortex and mPFC project to the DLS. These findings further reveal a BDNF-specific circuit between the vlOFC and the DLS.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

The DLS receives synaptic input from BDNF-positive vlOFC neurons

The OFC is anatomically and functionally subdivided into distinct subregions in rodents which are responsible for different behaviors (Izquierdo, 2017). Specifically, the vlOFC is involved in reward learning whereas the mOFC plays an important role in behavioral flexibility (Izquierdo, 2017). The majority of previous studies focused on the OFC to DMS circuit (Lipton et al., 2019), however very little is known about OFC to DLS projections. We therefore focused on further characterization of BDNF vlOFC neurons projecting to the DLS. First, to confirm the presence of a BDNF-specific circuit between the vlOFC and DLS, we infected the vlOFC of Bdnf-Cre mice with an AAV-Flex-GFP virus allowing visualization of projections extended by BDNF-positive vlOFC neurons to the dorsal striatum (**Figure 4a-b**). As shown in **Figure 4d**, projections from BDNF positive vlOFC neurons (Figure 4c) were localized in the DLS, and to a much lesser extent in the DMS suggesting that indeed BDNF neurons in the vlOFC extend projections specifically to the DLS.

Next, to determine whether the BDNF-positive vlOFC neurons extend excitatory presynaptic terminals within the DLS, we immunolabeled VGLUT1, a specific marker of cortical glutamatergic presynaptic compartment. We found co-labeling of BDNF-positive neuronal projections and VGLUT1 in the DLS (**Figure 5**), suggesting that BDNF-positive glutamatergic neurons from the vlOFC extend presynaptic terminals with neurons in the DLS.

BDNF in the OFC activates ERK1/2 only in the DLS

Finally, we reasoned that if BDNF-positive vlOFC neurons project to the DLS, then BDNF produced in vlOFC neurons should be released in the DLS and bind to and activate TrkB in the DLS (**Figure 6a**). Stimulation of TrkB results in the activation of Mitogen-activated protein kinase

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

kinase (MEK) which phosphorylates ERK1/2 (Leal et al., 2014). We therefore tested whether BDNF overexpression in the vlOFC increases ERK1/2 phosphorylation in the DLS. The vlOFC of C57BL/6 mice were infected with an AAV expressing BDNF (AAV-BDNF) and an empty vector control (AAV-GFP) (**Supplementary Figure 2**), and the activation of BDNF/TrkB signaling in the dorsal striatum was determined by measuring ERK1/2 phosphorylation (**Figure 6b**). As shown in **Figure 6c-d** overexpression of BDNF in the vlOFC produced a robust activation of ERK1/2 in the DLS suggesting that vlOFC neurons release BDNF and activate TrkB signaling in the DLS. In contrast, ERK1/2 phosphorylation was not detected in the DMS upon overexpression of BDNF in vlOFC neurons (**Figure 6c**), confirming that vlOFC BDNF neurons do not project to the DMS. Together these findings that BDNF secreted from axon terminals of OFC neurons activate BDNF/TrkB signaling selectively in the DLS.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Discussion

In the present study we mapped out cortical BDNF-expressing neurons projecting to the dorsal striatum. We found that BDNF-expressing neurons located in the mPFC project to both the DLS and the DMS, and the motor cortex projects to the DLS only (**Figure 7**). Interestingly, we observed an anatomical segregation of OFC BDNF neurons that project to the dorsal striatum. Specifically, we report that BDNF-expressing neurons located in the mOFC project to the DMS (**Figure 7**). In contrast, we show that BDNF-expressing vlOFC neurons project specifically to the DLS, and that BDNF in the vlOFC activates TrkB signaling in DLS (**Figure 7**). Thus, our data define a novel BDNF neural circuit connecting the vlOFC and the DLS.

Characterization of corticostriatal BDNF neurons

In 1990, Hofer et al. used *in situ* hybridization to characterize *BDNF* message in the brain and reported that *BDNF* mRNA is abundant in the mouse cerebral cortex (Hofer et al., 1990). In contrast, BDNF protein was detected in the striatum, even though *BDNF* mRNA was absent (Spires et al., 2004; Gharami et al., 2008). Indeed, BDNF protein found in the striatum is synthesized, anterogradely transported from the cell bodies located in the cerebral cortex and released presynaptically (Altar et al., 1997; Baquet et al., 2004). Although more recent BDNF expression surveys have been conducted (Singer et al., 2018; Leschik et al., 2019; Wosnitzka et al., 2020), a careful mapping of BDNF neurons in cortical areas, and their striatal projections, has not been previously undertaken. Crossing a Bdnf-Cre mouse line (Tan et al., 2016) with a Ribotag reporter line (Zhou et al., 2013) enabled labeling of BDNF-expressing neurons in the prefrontal cerebral cortex. Using this approach, which has superior accuracy compared to *in situ* hybridization and immunohistochemistry, we provide definitive proof that a majority of neurons

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

in prefrontal regions of the cortex express BDNF. Similar to what was previously reported (Hofer et al., 1990), we did not detect BDNF-positive neurons in the striatum. Interestingly, although basal *BDNF* levels in the rodent striatum are negligible, a robust increase in *BDNF* message is detected in response to behaviors such as voluntary alcohol intake (McGough et al., 2004; Jeanblanc et al., 2009), and cocaine (Liu et al., 2006; Harvey et al., 2019), as well as in response to exercise (Marais et al., 2009). Reconciling these potentially conflicting observations merits further investigation. One possibility is that alcohol, cocaine and/or exercise increase *BDNF* levels in cortical regions projecting to the striatum. Specifically, *BDNF* mRNA can be found in axon terminals (Lau et al., 2010), and recent studies show that presynaptic protein translation can occur within neuronal projections (Costa et al., 2019; Hafner et al., 2019). Thus, detectable BDNF in the striatum could be explained by the presence of *BDNF* mRNA within cortical neurons projecting to the striatum.

Using retrograde viral tracing, we analyzed the spatial profiles of BDNF projecting neurons in the prefrontal cortex. We observed that the mPFC exhibits a large number of BDNF-positive neurons projecting to the DMS. These results are in accordance with previous studies showing that the DMS receives projections from associative cortices such as the prelimbic cortex (Friedman et al., 2015; Hart et al., 2018; Shipman et al., 2019; Vicente et al., 2020). We also found that a subset of BDNF-positive neurons in the motor cortex and the mPFC extends projections to the DLS. The motor cortex is known for innervating the DLS (Yin and Knowlton, 2006) and Andreska et al. recently reported that BDNF in this corticostriatal circuit is essential for motor learning (Andreska et al., 2020). We show herein that OFC BDNF neurons project to the dorsal striatum. Interestingly these projections were organized in a mediolateral manner. Specifically, the DMS receives

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

projections from BDNF neurons located in the mOFC whereas the BDNF neurons projecting to the DLS are located in the vlOFC.

It is well known that the OFC projects to the dorsal striatum (Gremel and Costa, 2013; Gremel et al., 2016; Zimmermann et al., 2017). Previous studies showed that the DMS receives input from the mOFC (Gourley et al., 2016; Green et al., 2020). The DMS plays an important role for goal directed behavior (Vandaele et al., 2019) and BDNF in the mOFC is essential to sustain goal-sensitive action in mice (Gourley et al., 2016). Thus, it is likely that this BDNF circuitry regulates goal-directed action control.

Surprisingly, we found that the majority of BDNF vlOFC neurons project selectively to the DLS. The functional implication of this circuitry is unknow. Gourley and colleagues reported that BDNF in the both vlOFC and mOFC participates in goal-directed behaviors (Gourley et al., 2013; Gourley et al., 2016; Zimmermann et al., 2017), and Pitts et al. reported that inhibiting TrkB signaling in the DLS blocks habit formation (Pitts et al., 2018). In addition, we previously reported that the mechanistic target of rapamycin (mTORC1) in the vlOFC is involved in habitual alcohol seeking (Morisot et al., 2019). Putting these studies together, it is plausible that BDNF in the vlOFC neurons projecting to the DLS plays a role in inflexible behavior and habit formation.

Another possible role for BDNF in this corticostriatal circuit is to shape and modulate the synaptic output of striatal neurons. Using a two-neuron microcircuit approach in primary cortico-striatal neurons, Paraskevopoulou and colleagues showed that BDNF and glutamate are co-released from cortical projections are required to modulate inhibitory synaptic transmission of striatal neurons (Paraskevopoulou et al., 2019). Therefore, co-release of BDNF with glutamate in the DLS by vlOFC neurons may modulate inhibitory sensitivity and dendritic morphology of striatal neurons in this vlOFC-DLS circuit.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

As mentioned above, a novel finding is that BDNF overexpression in neurons located in the vlOFC leads to the activation of TrkB signaling in the DLS. However, BDNF is also released postsynaptically at dendrites and act in an autocrine manner (Harward et al., 2016; Song et al., 2017). Therefore, the contribution of TrkB signaling within the vlOFC merits further investigation.

BDNF-TrkB signaling in the DLS

We report that BDNF overexpression in the vlOFC activates TrkB signaling specifically in the DLS but not in the DMS confirming our abovementioned findings that vlOFC neurons project selectively to the DLS. It is unclear however whether vlOFC BDNF activates TrkB signaling in specific subpopulations of DLS neurons. Most neurons in the striatum are the medium spiny neurons (MSN) which are divided into two subpopulations, dopamine D1 receptor expressing MSNs (D1) and dopamine D2 receptor expressing MSNs (D2) (Gerfen et al., 1990). In the adult brain, both D1 and D2 MSN express TrkB receptors (Lobo et al., 2010). Recently, Engeln and colleagues showed that a subset of mice congenitally lacking TrkB receptor in D1-MSN in the dorsal striatum exhibit repetitive circling behavior, suggesting a role of D1-MSN BDNF signaling in this type of behavior (Engeln et al., 2020). In the ventral striatum, Nestler and colleagues provided data to suggest that activation of BDNF-TrkB signaling in D1 versus D2 MSNs triggers opposite effects on cocaine and morphine-dependent rewarding behaviors (Lobo et al., 2010; Koo et al., 2014). The dorsal striatum is also organized into patch and matrix compartments which have distinct connectivity and genetic signature (Martin et al., 2019). As both of patch and matrix neuron express TrkB (Costantini et al., 1999), more studies are required to decipher the contribution of BDNF/TrkB signaling in subpopulation of DLS neurons.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

While our findings indicate a postsynaptic activation of TrkB signaling in the DLS, we cannot rule out a potential autocrine effect of BDNF onto cortical projections. In the hippocampus, BDNF can act on TrkB localized presynaptically, thereby inducing LTP and strengthening potentiation in a local and synapse-specific manner (Zakharenko et al., 2003; Harward et al., 2016; Lin et al., 2018). Since TrkB can also be found on cortical axons (Gomes et al., 2006; Yeh et al., 2017), BDNF released by vlOFC projecting neurons could act pre- and/or postsynaptically within the DLS.

In summary, in this study we mapped out BDNF-expressing neurons in the prefrontal cortex and deciphered BDNF-specific corticostriatal circuits. Furthermore, the discovery of vlOFC-DLS BDNF microcircuitry highlights the important for deciphering the function of BDNF in the context of microcircuits composed by very localized neuronal ensembles.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

References

Altar CA, Cai N, Bliven T, Juhasz M, Conner JM, Acheson AL, Lindsay RM, Wiegand SJ (1997) Anterograde transport of brain-derived neurotrophic factor and its role in the brain. Nature 389:856-860.

Andreska T, Rauskolb S, Schukraft N, Luningschror P, Sasi M, Signoret-Genest J, Behringer M, Blum R, Sauer M, Tovote P, Sendtner M (2020) Induction of BDNF Expression in Layer II/III and Layer V Neurons of the Motor Cortex Is Essential for Motor Learning. J Neurosci 40:6289-6308.

Back S et al. (2019) Neuron-Specific Genome Modification in the Adult Rat Brain Using CRISPR-Cas9 Transgenic Rats. Neuron 102:105-119 e108.

Baquet ZC, Gorski JA, Jones KR (2004) Early striatal dendrite deficits followed by neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. J Neurosci 24:4250-4258.

Baydyuk M, Xu B (2014) BDNF signaling and survival of striatal neurons. Front Cell Neurosci 8:254.

Bekinschtein P, Cammarota M, Medina JH (2014) BDNF and memory processing. Neuropharmacology 76 Pt C:677-683.

Besusso D, Geibel M, Kramer D, Schneider T, Pendolino V, Picconi B, Calabresi P, Bannerman DM, Minichiello L (2013) BDNF-TrkB signaling in striatopallidal neurons controls inhibition of locomotor behavior. Nat Commun 4:2031.

Choi DC, Maguschak KA, Ye K, Jang S-W, Myers KM, Ressler KJ (2010) Prelimbic cortical BDNF is required for memory of learned fear but not extinction or innate fear. Proceedings of the National Academy of Sciences 107:2675-2680.

Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S (1997) Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J Neurosci 17:2295-2313.

Costa RO, Martins H, Martins LF, Cwetsch AW, Mele M, Pedro JR, Tome D, Jeon NL, Cancedda L, Jaffrey SR, Almeida RD (2019) Synaptogenesis Stimulates a Proteasome-Mediated Ribosome Reduction in Axons. Cell Rep 28:864-876 e866.

Costantini LC, Feinstein SC, Radeke MJ, Snyder-Keller A (1999) Compartmental expression of trkB receptor protein in the developing striatum. Neuroscience 89:505-513.

Darcq E, Warnault V, Phamluong K, Besserer GM, Liu F, Ron D (2015) MicroRNA-30a-5p in the prefrontal cortex controls the transition from moderate to excessive alcohol consumption. Mol Psychiatry 20:1219-1231.

Darcq E, Morisot N, Phamluong K, Warnault V, Jeanblanc J, Longo FM, Massa SM, Ron D (2016) The Neurotrophic Factor Receptor p75 in the Rat Dorsolateral Striatum Drives Excessive Alcohol Drinking. J Neurosci 36:10116-10127.

De Vincenti AP, Rios AS, Paratcha G, Ledda F (2019) Mechanisms That Modulate and Diversify BDNF Functions: Implications for Hippocampal Synaptic Plasticity. Front Cell Neurosci 13:135.

Dieni S, Matsumoto T, Dekkers M, Rauskolb S, Ionescu MS, Deogracias R, Gundelfinger ED, Kojima M, Nestel S, Frotscher M, Barde YA (2012) BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. J Cell Biol 196:775-788.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Ehinger Y, Morisot N, Phamluong K, Sakhai SA, Soneja D, Adrover MF, Alvarez VA, Ron D (2020) cAMP-Fyn signaling in the dorsomedial striatum direct pathway drives excessive alcohol use. Neuropsychopharmacology.

Engeln M, Song Y, Chandra R, La A, Fox ME, Evans B, Turner MD, Thomas S, Francis TC, Hertzano R, Lobo MK (2020) Individual differences in stereotypy and neuron subtype translatome with TrkB deletion. Mol Psychiatry.

Friedman A, Homma D, Gibb LG, Amemori K, Rubin SJ, Hood AS, Riad MH, Graybiel AM (2015) A Corticostriatal Path Targeting Striosomes Controls Decision-Making under Conflict. Cell 161:1320-1333.

Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ, Jr., Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429-1432.

Gharami K, Xie Y, An JJ, Tonegawa S, Xu B (2008) Brain-derived neurotrophic factor over-expression in the forebrain ameliorates Huntington's disease phenotypes in mice. J Neurochem 105:369-379.

Gomes RA, Hampton C, El-Sabeawy F, Sabo SL, McAllister AK (2006) The dynamic distribution of TrkB receptors before, during, and after synapse formation between cortical neurons. J Neurosci 26:11487-11500.

Gourley SL, Zimmermann KS, Allen AG, Taylor JR (2016) The Medial Orbitofrontal Cortex Regulates Sensitivity to Outcome Value. J Neurosci 36:4600-4613.

Gourley SL, Olevska A, Zimmermann KS, Ressler KJ, Dileone RJ, Taylor JR (2013) The orbitofrontal cortex regulates outcome-based decision-making via the lateral striatum. Eur J Neurosci 38:2382-2388.

Green SM, Nathani S, Zimmerman J, Fireman D, Urs NM (2020) Retrograde Labeling Illuminates Distinct Topographical Organization of D1 and D2 Receptor-Positive Pyramidal Neurons in the Prefrontal Cortex of Mice. eNeuro 7.

Gremel CM, Costa RM (2013) Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. Nat Commun 4:2264.

Gremel CM, Chancey JH, Atwood BK, Luo G, Neve R, Ramakrishnan C, Deisseroth K, Lovinger DM, Costa RM (2016) Endocannabinoid Modulation of Orbitostriatal Circuits Gates Habit Formation. Neuron 90:1312-1324.

Hafner AS, Donlin-Asp PG, Leitch B, Herzog E, Schuman EM (2019) Local protein synthesis is a ubiquitous feature of neuronal pre- and postsynaptic compartments. Science 364.

Hart G, Bradfield LA, Balleine BW (2018) Prefrontal Corticostriatal Disconnection Blocks the Acquisition of Goal-Directed Action. J Neurosci 38:1311-1322.

Harvey E, Blurton-Jones M, Kennedy PJ (2019) Hippocampal BDNF regulates a shift from flexible, goal-directed to habit memory system function following cocaine abstinence. Hippocampus 29:1101-1113.

Harward SC, Hedrick NG, Hall CE, Parra-Bueno P, Milner TA, Pan E, Laviv T, Hempstead BL, Yasuda R, McNamara JO (2016) Autocrine BDNF-TrkB signalling within a single dendritic spine. Nature 538:99-103.

Hofer M, Pagliusi SR, Hohn A, Leibrock J, Barde YA (1990) Regional distribution of brain-derived neurotrophic factor mRNA in the adult mouse brain. EMBO J 9:2459-2464.

Huang EJ, Reichardt LF (2003) Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 72:609-642.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Hunnicutt BJ, Jongbloets BC, Birdsong WT, Gertz KJ, Zhong H, Mao T (2016) A comprehensive excitatory input map of the striatum reveals novel functional organization. Elife 5.

Izquierdo A (2017) Functional Heterogeneity within Rat Orbitofrontal Cortex in Reward Learning and Decision Making. J Neurosci 37:10529-10540.

Jeanblanc J, Logrip ML, Janak PH, Ron D (2013) BDNF-mediated regulation of ethanol consumption requires the activation of the MAP kinase pathway and protein synthesis. Eur J Neurosci 37:607-612.

Jeanblanc J, He DY, Carnicella S, Kharazia V, Janak PH, Ron D (2009) Endogenous BDNF in the dorsolateral striatum gates alcohol drinking. J Neurosci 29:13494-13502.

Jeanblanc J, He DY, McGough NN, Logrip ML, Phamluong K, Janak PH, Ron D (2006) The dopamine D3 receptor is part of a homeostatic pathway regulating ethanol consumption. J Neurosci 26:1457-1464.

Koo JW, Lobo MK, Chaudhury D, Labonte B, Friedman A, Heller E, Pena CJ, Han MH, Nestler EJ (2014) Loss of BDNF signaling in D1R-expressing NAc neurons enhances morphine reward by reducing GABA inhibition. Neuropsychopharmacology 39:2646-2653.

Kowianski P, Lietzau G, Czuba E, Waskow M, Steliga A, Morys J (2018) BDNF: A Key Factor with Multipotent Impact on Brain Signaling and Synaptic Plasticity. Cell Mol Neurobiol 38:579-593.

Lau AG, Irier HA, Gu J, Tian D, Ku L, Liu G, Xia M, Fritsch B, Zheng JQ, Dingledine R, Xu B, Lu B, Feng Y (2010) Distinct 3'UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF). Proc Natl Acad Sci U S A 107:15945-15950.

Leal G, Comprido D, Duarte CB (2014) BDNF-induced local protein synthesis and synaptic plasticity. Neuropharmacology 76 Pt C:639-656.

Leschik J, Eckenstaler R, Endres T, Munsch T, Edelmann E, Richter K, Kobler O, Fischer KD, Zuschratter W, Brigadski T, Lutz B, Lessmann V (2019) Prominent Postsynaptic and Dendritic Exocytosis of Endogenous BDNF Vesicles in BDNF-GFP Knock-in Mice. Mol Neurobiol 56:6833-6855.

Lin PY, Kavalali ET, Monteggia LM (2018) Genetic Dissection of Presynaptic and Postsynaptic BDNF-TrkB Signaling in Synaptic Efficacy of CA3-CA1 Synapses. Cell Rep 24:1550-1561.

Lipton DM, Gonzales BJ, Citri A (2019) Dorsal Striatal Circuits for Habits, Compulsions and Addictions. Front Syst Neurosci 13:28.

Liu QR, Lu L, Zhu XG, Gong JP, Shaham Y, Uhl GR (2006) Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. Brain Res 1067:1-12.

Lobo MK, Covington HE, 3rd, Chaudhury D, Friedman AK, Sun H, Damez-Werno D, Dietz DM, Zaman S, Koo JW, Kennedy PJ, Mouzon E, Mogri M, Neve RL, Deisseroth K, Han MH, Nestler EJ (2010) Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. Science 330:385-390.

Logrip ML, Janak PH, Ron D (2009) Escalating ethanol intake is associated with altered corticostriatal BDNF expression. J Neurochem 109:1459-1468.

Lu H, Cheng PL, Lim BK, Khoshnevisrad N, Poo MM (2010) Elevated BDNF after cocaine withdrawal facilitates LTP in medial prefrontal cortex by suppressing GABA inhibition. Neuron 67:821-833.

Marais L, Hattingh SM, Stein DJ, Daniels WMU (2009) A proteomic analysis of the ventral hippocampus of rats subjected to maternal separation and escitalopram treatment. Metab Brain Dis 24:569-586.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Martin A, Calvigioni D, Tzortzi O, Fuzik J, Warnberg E, Meletis K (2019) A Spatiomolecular Map of the Striatum. Cell Rep 29:4320-4333 e4325.

McGough NN, He DY, Logrip ML, Jeanblanc J, Phamluong K, Luong K, Kharazia V, Janak PH, Ron D (2004) RACK1 and brain-derived neurotrophic factor: a homeostatic pathway that regulates alcohol addiction. J Neurosci 24:10542-10552.

Miranda M, Morici JF, Zanoni MB, Bekinschtein P (2019) Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. Front Cell Neurosci 13:363.

Morisot N, Phamluong K, Ehinger Y, Berger AL, Moffat JJ, Ron D (2019) mTORC1 in the orbitofrontal cortex promotes habitual alcohol seeking. Elife 8.

Panja D, Bramham CR (2014) BDNF mechanisms in late LTP formation: A synthesis and breakdown. Neuropharmacology 76 Pt C:664-676.

Paraskevopoulou F, Herman MA, Rosenmund C (2019) Glutamatergic Innervation onto Striatal Neurons Potentiates GABAergic Synaptic Output. J Neurosci 39:4448-4460.

Pitts EG, Taylor JR, Gourley SL (2016) Prefrontal cortical BDNF: A regulatory key in cocaine- and food-reinforced behaviors. Neurobiol Dis 91:326-335.

Pitts EG, Li DC, Gourley SL (2018) Bidirectional coordination of actions and habits by TrkB in mice. Sci Rep 8:4495.

Shipman ML, Johnson GC, Bouton ME, Green JT (2019) Chemogenetic Silencing of Prelimbic Cortex to Anterior Dorsomedial Striatum Projection Attenuates Operant Responding. eNeuro 6.

Singer W et al. (2018) BDNF-Live-Exon-Visualization (BLEV) Allows Differential Detection of BDNF Transcripts in vitro and in vivo. Front Mol Neurosci 11:325.

Song M, Martinowich K, Lee FS (2017) BDNF at the synapse: why location matters. Mol Psychiatry 22:1370-1375.

Spires TL, Grote HE, Varshney NK, Cordery PM, van Dellen A, Blakemore C, Hannan AJ (2004) Environmental enrichment rescues protein deficits in a mouse model of Huntington's disease, indicating a possible disease mechanism. J Neurosci 24:2270-2276.

Strand AD, Baquet ZC, Aragaki AK, Holmans P, Yang L, Cleren C, Beal MF, Jones L, Kooperberg C, Olson JM, Jones KR (2007) Expression profiling of Huntington's disease models suggests that brain-derived neurotrophic factor depletion plays a major role in striatal degeneration. J Neurosci 27:11758-11768.

Tan CL, Cooke EK, Leib DE, Lin YC, Daly GE, Zimmerman CA, Knight ZA (2016) Warm-Sensitive Neurons that Control Body Temperature. Cell 167:47-59 e15.

Timmusk T, Palm K, Metsis M, Reintam T, Paalme V, Saarma M, Persson H (1993) Multiple promoters direct tissue-specific expression of the rat BDNF gene. Neuron 10:475-489.

Vandaele Y, Mahajan NR, Ottenheimer DJ, Richard JM, Mysore SP, Janak PH (2019) Distinct recruitment of dorsomedial and dorsolateral striatum erodes with extended training. Elife 8.

Vicente AM, Martins GJ, Costa RM (2020) Cortico-basal ganglia circuits underlying dysfunctional control of motor behaviors in neuropsychiatric disorders. Curr Opin Genet Dev 65:151-159.

Warnault V, Darcq E, Morisot N, Phamluong K, Wilbrecht L, Massa SM, Longo FM, Ron D (2016) The BDNF Valine 68 to Methionine Polymorphism Increases Compulsive Alcohol Drinking in Mice That Is Reversed by Tropomyosin Receptor Kinase B Activation. Biol Psychiatry 79:463-473.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Wosnitzka E, Nan X, Nan J, Chacon-Fernandez P, Kussmaul L, Schuler M, Hengerer B, Barde YA (2020) A New Mouse Line Reporting the Translation of Brain-Derived Neurotrophic Factor Using Green Fluorescent Protein. eNeuro 7.

Yan Q, Rosenfeld RD, Matheson CR, Hawkins N, Lopez OT, Bennett L, Welcher AA (1997) Expression of brain-derived neurotrophic factor protein in the adult rat central nervous system. Neuroscience 78:431-448.

Yeh ML, Selvam R, Levine ES (2017) BDNF-induced endocannabinoid release modulates neocortical glutamatergic neurotransmission. Synapse 71.

Yin HH, Knowlton BJ (2006) The role of the basal ganglia in habit formation. Nat Rev Neurosci 7:464-476.

Zagrebelsky M, Tacke C, Korte M (2020) BDNF signaling during the lifetime of dendritic spines. Cell Tissue Res 382:185-199.

Zakharenko SS, Patterson SL, Dragatsis I, Zeitlin SO, Siegelbaum SA, Kandel ER, Morozov A (2003) Presynaptic BDNF required for a presynaptic but not postsynaptic component of LTP at hippocampal CA1-CA3 synapses. Neuron 39:975-990.

Zhang Y, Zhu X, Huang C, Zhang X (2015) Molecular changes in the medial prefrontal cortex and nucleus accumbens are associated with blocking the behavioral sensitization to cocaine. Sci Rep 5:16172.

Zhou P, Zhang Y, Ma Q, Gu F, Day DS, He A, Zhou B, Li J, Stevens SM, Romo D, Pu WT (2013) Interrogating translational efficiency and lineage-specific transcriptomes using ribosome affinity purification. Proc Natl Acad Sci U S A 110:15395-15400.

Zimmermann KS, Yamin JA, Rainnie DG, Ressler KJ, Gourley SL (2017) Connections of the Mouse Orbitofrontal Cortex and Regulation of Goal-Directed Action Selection by Brain-Derived Neurotrophic Factor. Biol Psychiatry 81:366-377.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 1: BDNF positive neurons are detected in medial prefrontal cortex, motor cortex and orbitofrontal cortex

(**a**) Experimental strategy for detection of BDNF expressing neuron in the cortex. Bdnf-Cre are crossed with Ribotag mice, in which GFP-fused ribosomal subunit RPL10 is expressed in the presence of Cre recombinase, enabling the detection of BDNF expressing neurons. (**b**) Representative images of cortical nuclei. NeuN (red) and GFP (green) co-localization shows a high number of BDNF expressing neurons in the medial prefrontal cortex (mPFC), motor cortex (MC) and the orbitofrontal cortex (OFC). (**c**) Representative images of the striatum. Neurons in the dorsomedial (DMS) and dorsolateral (DLS) striatum do not express BDNF. Scale bar is indicated on each panel.

Figure 2: BDNF positive neurons in the mPFC and mOFC project to the DMS

(**a**) Retrograde viral strategy to map BDNF-expressing cortical neurons projecting to the dorsomedial striatum (DMS). The DMS of Bdnf-Cre mice was injected with a retrograde AAV EF1a Nuc-flox(mCherry)-EGFP viral construct enabling nuclear-localized mCherry to be expressed by default and to switch to nuclear-localized EGFP expression in the presence of Cre. (**b**) Retro AAV EF1a Nuc-flox(mCherry)-EGFP was infused in the DMS and BDNF positive neurons distribution was examined in the cortex. (**c**) Representative images of the DMS and the frontal cortex. BDNF expressing neurons can be identified by EGFP labeled nuclei and BDNF negative neurons by mCherry nuclei. Upper panel shows BDNF negative neurons (red) in the DMS. Lower panel shows BDNF expressing neurons (green) in the medial prefrontal cortex (mPFC) and medial orbitofrontal cortex (mOFC) projecting specifically to the DMS. BDNF

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

expressing neurons located in the ventrolateral orbitofrontal cortex (vlOFC) and motor cortex (MC) do not project to the DMS. Scale bar is indicated on each panel**.**

Figure 3: BDNF positive neurons in the motor cortex and vlOFC project to the DLS

(**a**) The dorsolateral striatum (DLS) of Bdnf-Cre mice was injected with a retrograde AAV EF1a Nuc-flox(mCherry)-EGFP viral construct and BDNF expressing neurons in the cortex were examined. (**b**) Representative images of DLS and frontal cortex. BDNF expressing neurons can be identified by EGFP labeled nuclei and BDNF negative neurons by mCherry nuclei. Upper panel shows BDNF negative neurons (red) in the DLS. Lower panel shows BDNF expressing neurons (green) in the motor cortex (MC), medial prefrontal cortex (mPFC) and ventrolateral orbitofrontal cortex (vlOFC) projecting specifically to the DLS. Few BDNF expressing neurons located in the medial orbitofrontal cortex (mOFC) project to the DMS. Scale bar is indicated on each panel.

Figure 4: The DLS receives projections from BDNF-positive vlOFC neurons

(**a**) AAV-Flex-GFP virus was injected in the ventrolateral orbitofrontal cortex (vlOFC) of Bdnf-Cre mice and BDNF expression in the dorsal striatum was examined. (**b**) Representative image of the prefrontal cortex (PFC) showing BDNF expressing neurons infected by the AAV-Flex-GFP localized in the vlOFC (**c-d**) Representative images of the vlOFC, dorsolateral striatum (DLS) and dorsomedial striatum (DMS), showing dense GFP-positive projections localized in the DLS. GFP is shown in green and DAPI in purple. Scale bar is indicated on each panel.

Figure 5: BDNF-positive vlOFC neurons extend excitatory presynaptic terminals within the DLS

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

AAV-Flex-GFP virus was injected in the ventrolateral orbitofrontal cortex (vlOFC) of Bdnf-Cre mice allowing visualization of projections in the dorsolateral striatum (DLS). GFP-positive projections in the DLS were stained for VGLUT1, a specific marker of cortical glutamatergic presynaptic compartment. Representative images of the DLS labeled with GFP (green) and VGLUT1 (red). White arrows indicate BDNF-positive presynaptic terminals. Scale bar is indicated.

Figure 6: Overexpression of BDNF in the vlOFC activates ERK1/2 in the DLS but not in the DMS

(**a**) Cartoon depicting BDNF binding to TrkB resulting in ERK1/2 phosphorylation. (**b**) The ventrolateral orbitofrontal cortex (vlOFC) of C57BL/6 mice were infected with an AAV expressing BDNF (AAV-BDNF) and an empty vector control (AAV-GFP), and the level of ERK1/2 phosphorylation (pERK1/2) was measured in the dorsal striatum. (**c**) Representative images of pERK1/2 (red) in the dorsomedial striatum (DMS) and dorsolateral striatum (DLS) following BDNF overexpression in the vlOFC. (**d**) Animals which received BDNF overexpression in the OFC had increased activation of pERK1/2 in the DLS compared to empty vector GFP controls (unpaired t-test, $t(7) = 3.4$, $^*p = 0.01$). As a control, NeuN was also quantified and did not differ significantly between conditions (unpaired t-test, $t(7) = 0.2$, $p = 0.82$). AAV-BDNF n = 4 mice, AAV-GFP n = 5 mice.

Figure 7: BDNF-specific circuits between the OFC and the DS

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

BDNF-positive neurons in the prefrontal cortex (PFC) projecting to the dorsal striatum (DS). BDNF-positive neurons in the medial prefrontal cortex (mPFC) project to the dorsomedial striatum (DMS) and dorsolateral striatum (DLS) whereas BDNF-positive neurons in the motor cortex (MC) project to the DLS only. BDNF neurons located in the orbitofrontal cortex (OFC) exhibit a mediolateral organization based on their dorsostriatal target. Specifically, BDNF-positive neurons in the ventrolateral orbitofrontal cortex (vlOFC) project to the DLS while BDNF-positive neurons in the medial orbitofrontal cortex (mOFC) project to the DMS.

Supplementary Figures

Supplementary Figure 1: The majority of OFC neurons project to the dorsal striatum ipsilaterally

(**a,c**) The dorsolateral striatum (DLS) (**a**) or dorsomedial striatum (DMS) (**c**) of Bdnf-Cre mice were injected with a retrograde AAV EF1a Nuc-flox(mCherry)-EGFP viral construct. (**b**) Representative image of contralateral prefrontal regions of mice injected with virus in the DLS. (**d**) Representative image of contralateral prefrontal regions of mice injected with virus in the DMS.

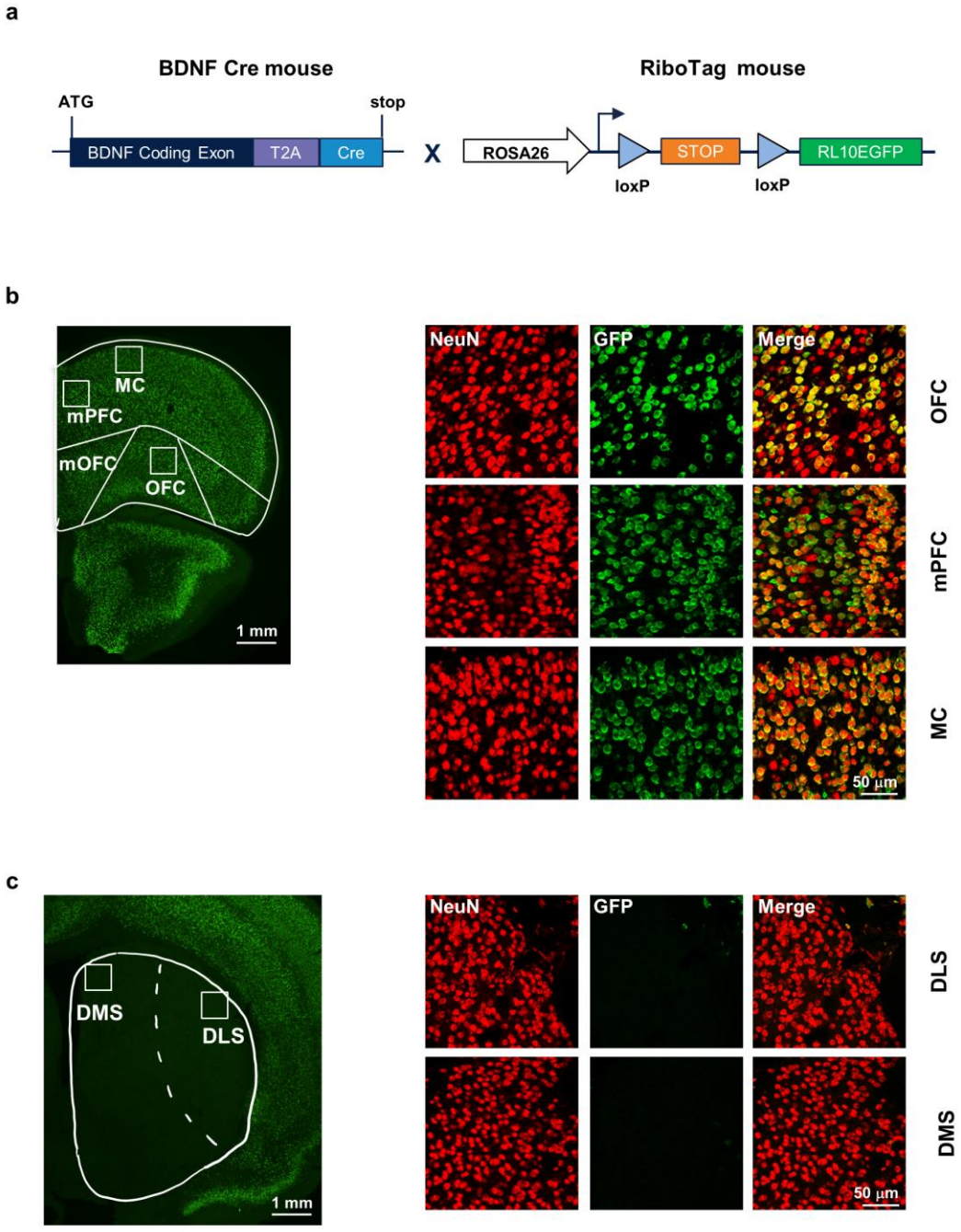
Supplementary Figure 2: Overexpression of BDNF in the vlOFC

(**a**) The ventrolateral orbitofrontal cortex (vlOFC) of C57BL/6 mice were infected with an AAV expressing BDNF (AAV-BDNF) and an empty vector control (AAV-GFP). (**b**) Representative images of vlOFC infection by the AAV-BDNF, NeuN (red) and GFP (in green). (c) RT-PCR analysis of *BDNF* expression in the vlOFC of mice infected with AAV-BDNF or AAV-GFP. *GAPDH* was used as a control.

bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 1

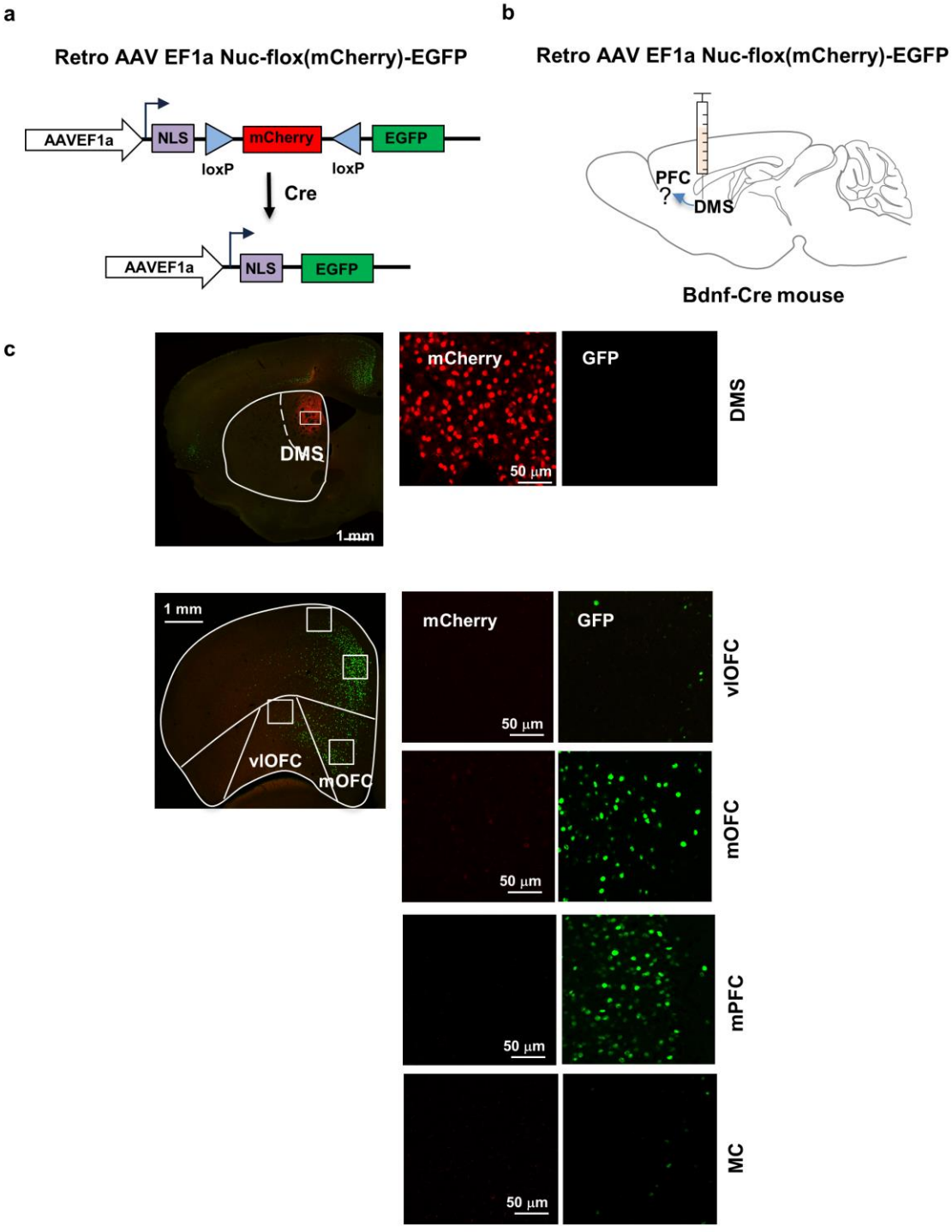
BDNF positive neurons are detected in medial prefrontal cortex, motor cortex and orbitofrontal cortex



bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

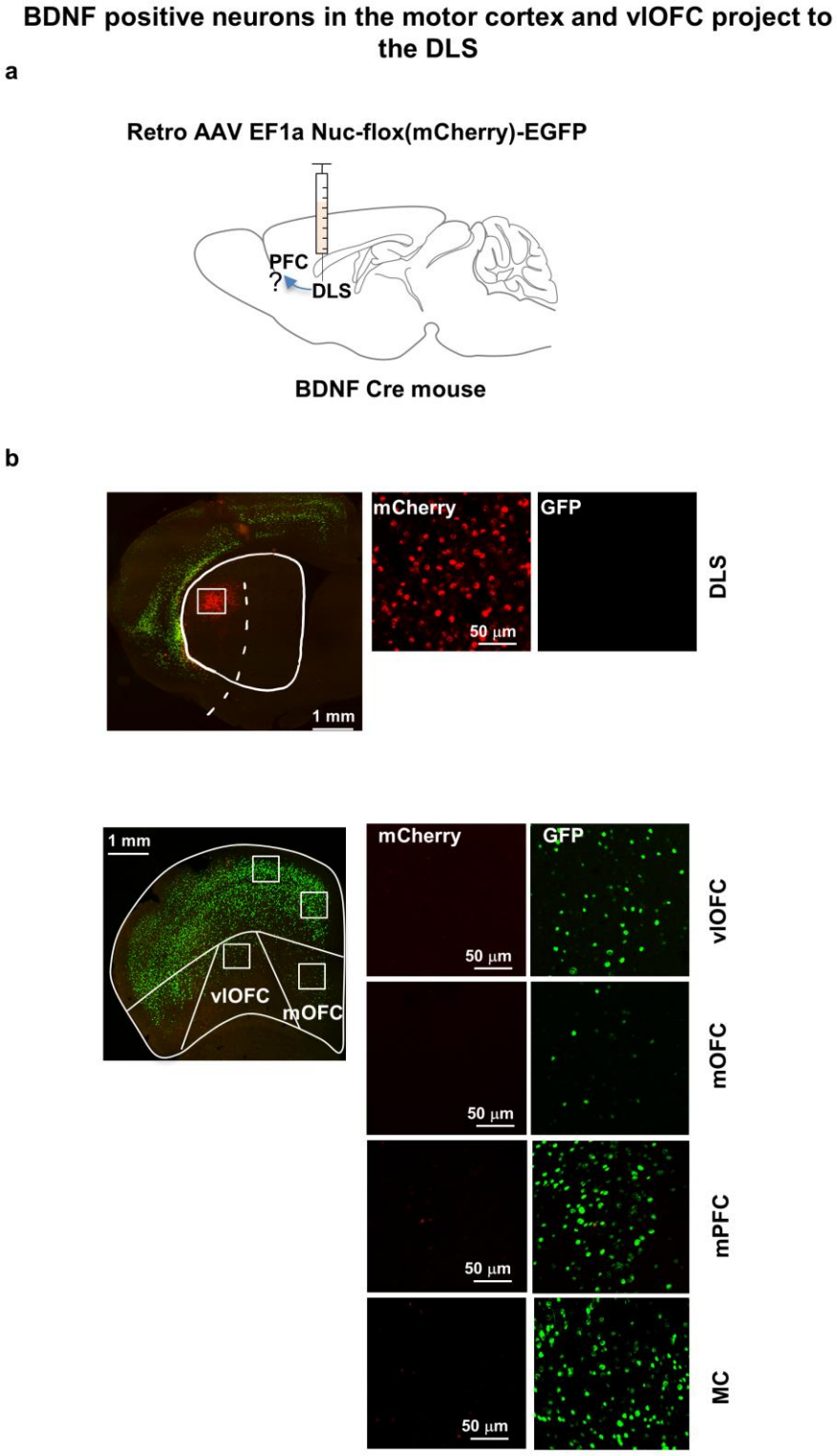
Figure 2

BDNF positive neurons in the mPFC and mOFC project to the DMS



bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

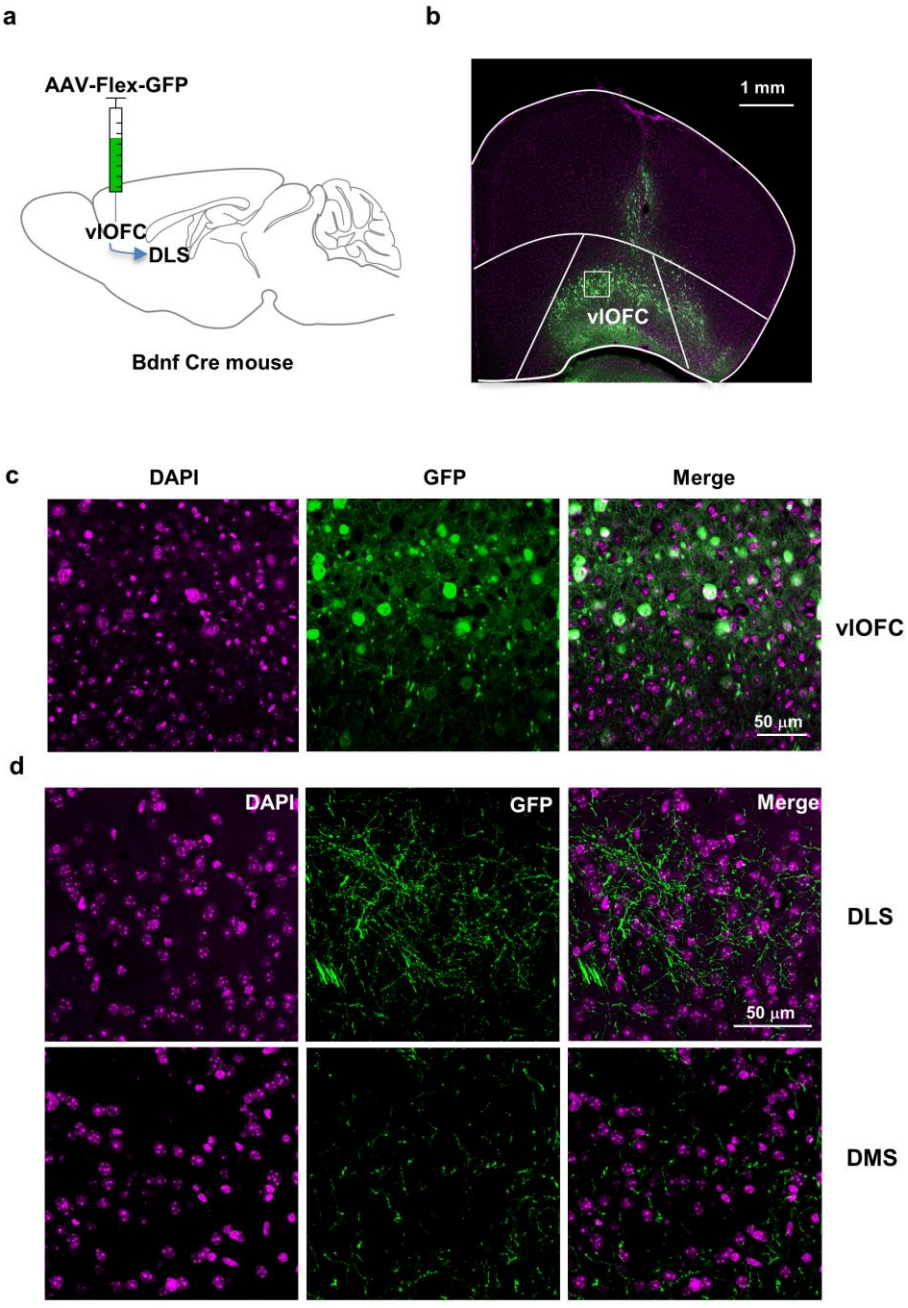
Figure 3



bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 4

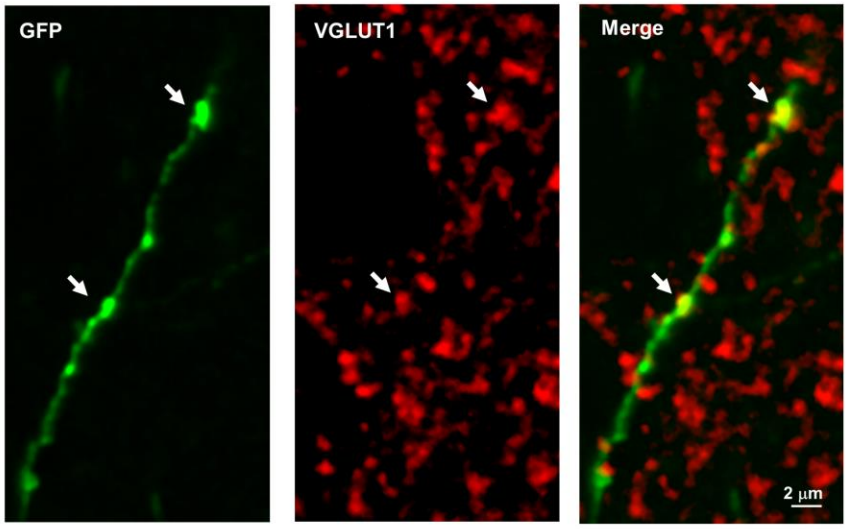
The dorsolateral striatum receives projections from BDNF-positive vlOFC neurons



bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 5

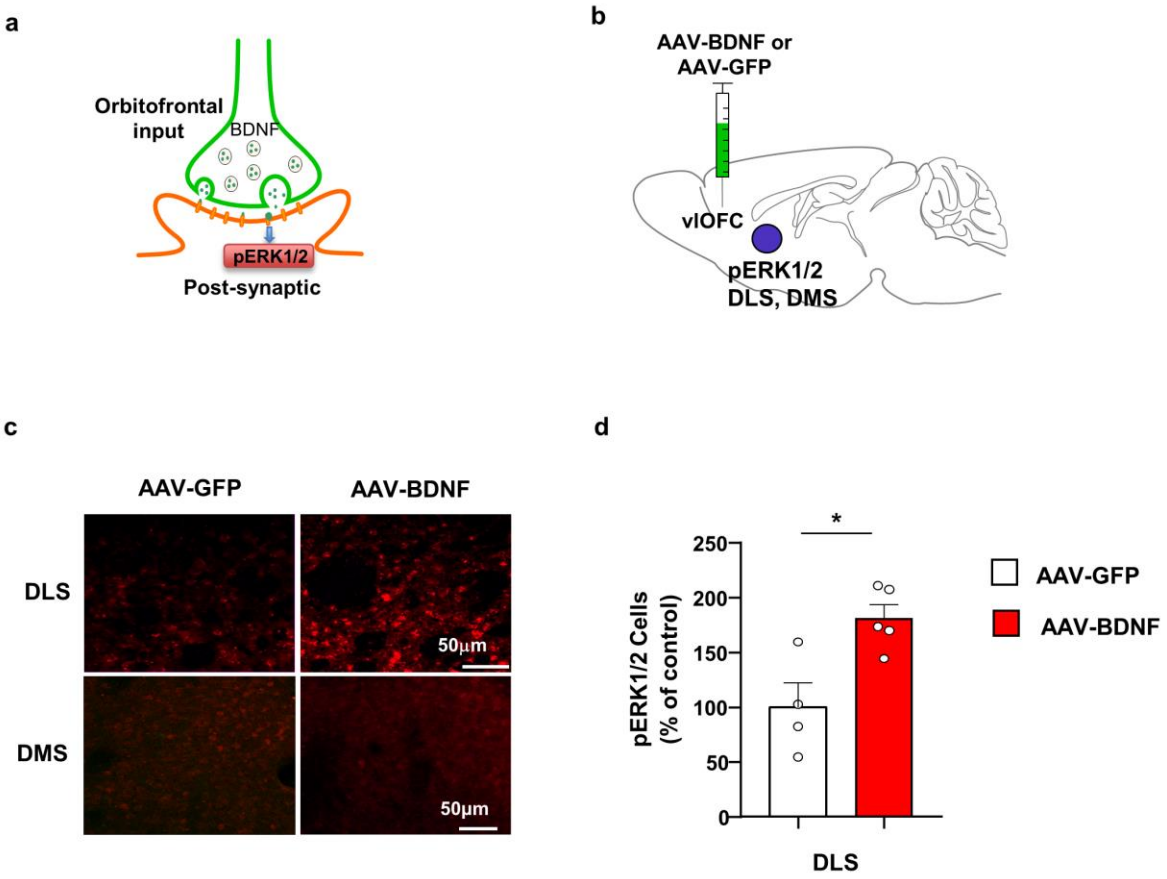
BDNF-positive vlOFC neurons extend excitatory presynaptic terminals within the DLS



bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 6

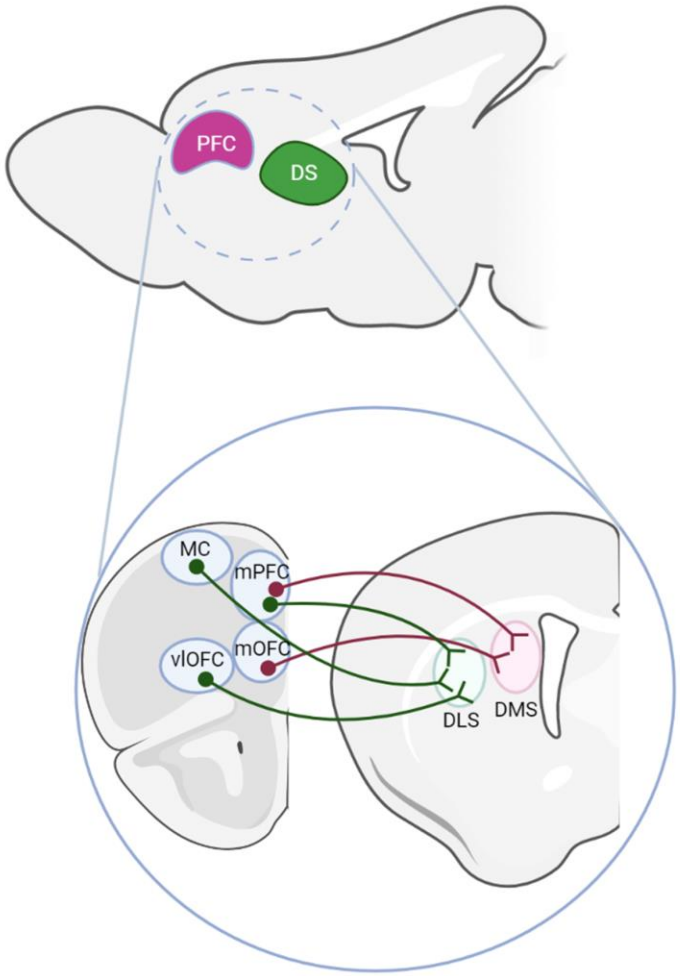
Overexpression of BDNF in the vlOFC activates ERK1/2 in the DLS but not in the DMS



bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure 7

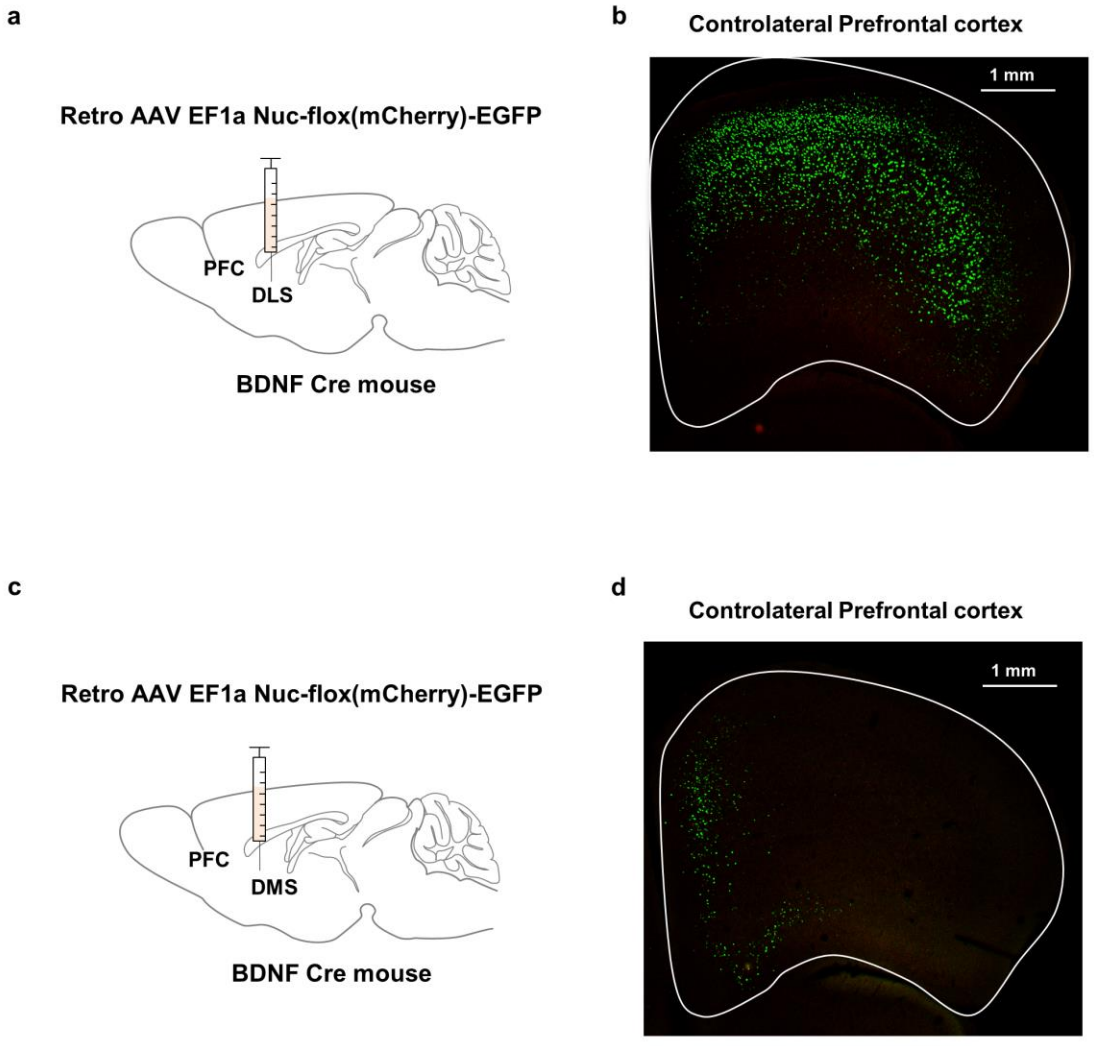
BDNF-specific circuits between the OFC and the DS



bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Supplementary Figure 1

The majority of OFC neurons project to the dorsal striatum ipsilaterally



bioRxiv preprint doi: https://doi.org/10.1101/2021.08.26.457842; this version posted August 26, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Supplementary Figure 2

Overexpression of BDNF in the vlOFC