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RESEARCH REPORT

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Administration of BDNF in the ventral tegmental area produces a switch from a nicotine-non-dependent D1R-mediated motivational state to a nicotine-dependent-like D2R-mediated motivational state

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Abstract

Brain-derived neurotrophic factor (BDNF) has been implicated in the transition from a non-dependent motivational state to a drug-dependent and drug-withdrawn motivational state. Chronic nicotine can increase BDNF in the rodent brain and is associated with smoking severity in humans; however, it is unknown whether this increased BDNF is linked functionally to the switch from a nicotine-non-dependent to a nicotine-dependent state. We used a place conditioning paradigm to measure the conditioned responses to nicotine, showing that a dose of acute nicotine that non-dependent male mice find aversive is found rewarding in chronic nicotine-treated mice experiencing withdrawal. A single BDNF injection in the ventral tegmental area (in the absence of chronic nicotine treatment) caused mice to behave as if they were nicotine dependent and in withdrawal, switching the neurobiological substrate mediating the conditioned motivational effects from dopamine D1 receptors to D2 receptors. Quantification of gene expression of BDNF and its receptor, tropomyosin-receptor-kinase B (TrkB), revealed an increase in TrkB mRNA but not BDNF mRNA in the VTA in nicotine-dependent and nicotine-withdrawn mice. These results suggest that BDNF signalling in the VTA is a critical neurobiological substrate for the transition to nicotine dependence.

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; CRF, corticotropin-releasing factor; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; DWD, dependent and withdrawn; KO, knockout; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; s.c., subcutaneous; siRNA, short-interference ribonucleic acid; TPP, tegmental pedunculopontine nucleus; TrkB, tropomyosin-receptor-kinase type B; VTA, ventral tegmental area; WT, wild type.

Significance statement: An increase in BDNF signalling induces a molecular switch from a previously nicotine-naive motivational state to a nicotine-dependent and nicotine-withdrawn motivational state in mice. This switch involves a change from a dopamine D1 receptor (D1R)-mediated motivational state to a dopamine D2 receptor (D2R)-mediated motivational state. The increase in brain-derived neurotrophic factor signalling in animals chronically treated with nicotine and in withdrawal results from an increase in the gene expression of the BDNF receptor (TrkB) and not from an increase in BDNF itself.

The modulation of BDNF signalling may be a promising new pharmacological avenue for the treatment of addictive behaviour.

1 | INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a molecular regulator of cell growth, survival and differentiation during neural development that has been implicated recently in the motivation to seek abused drugs. During motivated drug-taking and drug-seeking behaviour, a neurobiological and motivational switch occurs in the ventral tegmental area (VTA) of the brain reward system that produces a transition from non-dependence to drug dependence and withdrawal (Bechara et al., 1992; Grieder et al., 2014; Lepack et al., 2020; Mahler et al., 2019; Vargas-Perez et al., 2009). A single injection of BDNF in the VTA causes this switch from non-dependent to a morphine-dependent motivational state in the absence of chronic opiate treatment (Vargas-Perez et al., 2009, 2014) and potentiates the conditioned effects of cocaine (Bahi et al., 2008; Lu et al., 2004; Verheij et al., 2016), suggesting an important role for BDNF in the transition to opioid and cocaine dependence. Previous results have shown that chronic nicotine increases BDNF mRNA levels in the rat hippocampus (Kenny et al., 2000) and mouse medial prefrontal cortex (Cole et al., 2020) but decreases BDNF protein in the dorsal striatum of mice (Cole et al., 2020; Ortega et al., 2013) and that BDNF is elevated in the nucleus accumbens and VTA in mice after extended abstinence from chronic nicotine (Kivinummi et al., 2011). These distinct results suggest that the effects of nicotine treatment on BDNF expression may be region specific and vary depending on the dose of nicotine and duration of treatment and withdrawal. In humans, smoking severity and years smoked is positively correlated with BDNF levels (Galle et al., 2021; Jamal et al., 2015; Zhang et al., 2016); however, it is unknown whether an increase in BDNF signalling modulates the motivation to seek nicotine by causing the switch from a nicotine-non-dependent state to a nicotine-dependent and nicotine-withdrawn (DWD) state.

BDNF exerts its biological effects through binding to both high-affinity tropomyosin-receptor-kinase type B (TrkB) receptors and low-affinity p75 neurotrophin receptors, although its main functions are mediated by TrkB receptors (Carvalho et al., 2008). TrkB receptor knockdown prevents conditioned aversions to withdrawal from chronic opioids as well as withdrawal-induced vocalizations (Vargas-Perez et al., 2014), and TrkB receptors have been implicated in withdrawal from alcohol (Olsen et al., 2019) and methamphetamine (Ren et al., 2015). Recent research has suggested that nicotine may modulate neural TrkB levels (Park et al., 2019; Wei et al., 2018); however, the relationship between nicotine withdrawal and TrkB levels in the VTA is unknown.

A drug injection given to an animal in withdrawal from chronic drug administration has been hypothesized to be negatively reinforcing by overcoming an aversive opponent motivational process elicited in response to the rewarding initial effect of the drug (Grieder et al., 2010; Koob & Le Moal, 1997; Radke et al., 2011). Solomon and Corbit (1974) suggested that the initial affective response to a stimulus is the a-process, which is followed by a b-process that is opposite in direction and serves to maintain homeostatic balance in the system. We previously have used a place conditioning paradigm to show that the conditioned motivational response to nicotine is dopamine D1 receptor (D1R)-mediated in non-dependent mice and dopamine D2 receptor (D2R)-mediated in DWD mice (Grieder et al., 2012). Further, non-dependent mice given acute nicotine show a conditioned initial aversive a-process followed by a rewarding opponent b-process (Grieder et al., 2012). In contrast, DWD mice show a rewarding a-process to chronic nicotine followed by an aversive b-process during withdrawal (Grieder et al., 2010). It therefore appears that a switch occurs during the transition from a non-dependent state to a DWD motivational state. Here, we tested the hypothesis that increased BDNF signalling in the VTA causes the switch from D1R-mediated non-dependent nicotine motivation to a D2R-mediated nicotine-dependent motivational state.

2 | MATERIALS AND METHODS

All animal use procedures were approved by the University of Toronto Animal Care Committee, in accordance with the guidelines of the Canadian Council on Animal Care. Adult male C57BL/6 mice ($n = 183$) were purchased from Charles River (Montreal, Canada). Heterozygous D2R breeder mice were received as a gift from D. K. Grandy and M. J. Low (Oregon Health and Science University, Portland, OR), and homozygous D1R knockout (KO) and heterozygous D1R breeder mice from S. George (University of Toronto). Both strains were backcrossed a

minimum of 10 times on to a C57BL/6 background. Heterozygous mice were bred at the University of Toronto to obtain homozygous D1R and D2R KO mice and their wild-type (WT) controls that were on the same background as the control mice, who were C57BL/6 WT mice. Mice were at least 10 weeks old at the beginning of experiments. All mice were housed in a temperature-controlled room with lights on from 7 AM to 7 PM.

2.1 | Drugs

Nicotine hydrogen tartrate salt (Sigma-Aldrich, Ontario) was dissolved in saline at pH 7.0 ± 0.4 and administered via subcutaneous (s.c.) injection (1.75 mg/kg) or osmotic minipumps (chronic nicotine, 7 mg/kg/day for 12 days, minipump model 1002, Alzet, Cupertino, California). This dose and schedule of nicotine administration through minipumps leads to blood levels in mice that are similar to heavy human smokers (Grieder et al., 2010). DWD mice had their minipumps removed 8 h prior to conditioning at a time that our previous research has shown corresponds to peak physical withdrawal after chronic nicotine (measured by somatic abstinence signs; Grieder et al., 2010) and motivational withdrawal (measured using the place conditioning procedure; Grieder et al., 2010). All doses of drugs are expressed as mg of free base/kg of body weight. Doses and timing of nicotine injections were selected based on previous studies (Grieder et al., 2010, 2012). Recombinant human BDNF (Sigma) was dissolved in PBS, pH adjusted to 7.4, and infused bilaterally in the VTA (0.025 μg BDNF; 0.5 μl per hemisphere).

2.2 | Place conditioning

The place conditioning apparatus was obtained from Med Associates Inc. (SOF-700RA-25 Two Chamber Place Preference Apparatus, VT, USA). One environment was black with a metal rod floor, and the other was white with a wire mesh floor. An intermediate grey area housed a removable partition. Each cage was cleaned between animals, and each group was fully counterbalanced. During preference testing, the dividing partition was removed, and mice were given free access to both environments. All place conditioning and testing was performed between 10 AM and 6 PM. Each group of mice was conditioned and tested in the same order and at approximately the same time each day.

To study conditioned approach and avoidance behaviour, a unbiased place conditioning procedure was used that has been described previously, where a control group given saline will not show a baseline preference for either conditioning environment (Grieder et al., 2010). All groups were initially given a 10-min habituation session where they had free access to the conditioning environments. Their behaviour in this session did not influence their group assignment. For acute nicotine conditioning sessions in non-dependent mice (defined as those with less than five lifetime administrations of acute nicotine at the end of the experiments), mice were given a subcutaneous injection of nicotine (1.75 mg/kg) or saline immediately prior to conditioning. Each group underwent eight conditioning trials (four alternating drug and vehicle pairings) in one of the conditioning environments for 15 min. All conditioning was unbiased and fully counterbalanced for treatment compartment and order of drug presentation. Conditioning of nicotine-dependent mice (defined as those mice that had been given chronic nicotine for 12 days) occurred 8 h after minipump removal, during peak withdrawal from chronic nicotine. This conditioning protocol enables the pairing of the conditioned motivational effects of withdrawal, but not the direct effects of chronic nicotine, with the place conditioning environment (Grieder et al., 2010). Eight hours after minipump removal, when the DWD mouse was experiencing peak withdrawal from chronic nicotine (Grieder et al., 2010), it was given an s.c. injection of nicotine (1.75 mg/kg) or saline and was confined to one of the conditioning environments for 50 min. A single 10-min preference testing session was performed 5 days after the last conditioning day, at a time when subjects were drug-free and somatic withdrawal symptom-free. The difference score for each animal was calculated by subtracting the time spent in the saline-paired environment from the time spent in the nicotine- or withdrawal-paired environment.

2.3 | Intracranial infusions

For VTA infusions, animals were anaesthetized with inhaled isoflurane (3%) and placed in a stereotaxic device. BDNF of 0.025 μg was infused bilaterally in the VTA (0.5 μl per hemisphere) with a 33-gauge Hamilton syringe over 10 min, plus an additional 10 min to allow for adequate diffusion from the injector tip. The VTA injection coordinates, from bregma, were: AP, −3.0 mm; ML, −0.6 mm; and DV, −4.1 mm from the dural surface. These are the volumes and coordinates used in previous research studies using VTA BDNF infusions in mice (Ting-A-Kee et al., 2013; Vargas-Perez et al., 2014). Animals were given 1 week of postsurgical recovery preceding behavioural training.

2.4 | Quantitative polymerase chain reaction for endogenous BDNF and TrkB gene expression in the VTA of DWD mice

Mice that had undergone the DWD conditioning procedure (12 days of chronic nicotine via minipump, followed by minipump removal) were used for quantitative polymerase chain reaction (qPCR) and compared with nicotine-naive control mice. Eight hours after the minipump removal when mice were in their peak motivational withdrawal state, these experimental mice were given an injection of saline (as in the place conditioning procedure). This saline injection was given so that the levels of RNA measured would reflect the same conditions as the behavioural experiments. The mice were placed in a chamber with Isoflurane USP (Fresenius Kabi, #CP0406V2) for anaesthetization. The brains were extracted, and the VTA tissue was quickly dissected from each brain using tweezers, a pair of curved Noyes micro scissors and a scalpel under a stereo microscope. The VTA tissue samples were submerged in RNA*later* Stabilization Solution (Invitrogen, #AM7020) during dissection. Then, the samples were stored at 4°C as they were used within the month per the RNAlater manufacturer's instructions. RNA from these tissue samples was extracted using Norgen Biotek's Total RNA Purification Micro Kit (#35300) with the additional RNase-Free DNase I Set (Qiagen, #79254) to further remove residual DNA contamination. RNA quality and quantity were assessed using Nanodrop. From the RNA template, cDNA was reverse transcribed using the Superscript VILO cDNA Synthesis Kit (Invitrogen, #11754). Polymerase chain reaction (PCR) was performed using standardized TaqMan Gene Expression Assays (#4331182) in a QuantStudio 6 Flex System (Applied Biosystems). Each gene, *BDNF* (Mm01334047_ml), *TRKB* (Mm00435422_ml), *GAPDH* (Mm99999915_gl), *ACTB* (Mm02319580_gl) and *HPRT* (Mm03024075_ml) were run in triplicates. The ΔΔCt method was used to quantify the relative amounts of endogenous *BDNF* and *TrkB* using *HPRT*, *GAPDH* and *ACTB* as housekeeping genes. The relative fold changes of *BDNF* and *TRKB* in DWD mice compared with nicotine-naive mice are shown in Figure 3 as the log base 2 value of 2^-(ΔΔCt).

2.5 | Statistical analysis

Results were analysed using a one- or two-way analysis of variance (ANOVA) or Student's *t*-test with significance level of 0.05 (two-tailed). In all cases, a normality test and equal variance test were performed before an ANOVA to ensure its validity. Post hoc Bonferroni tests were used where appropriate.

3 | RESULTS

3.1 | Acute nicotine induces opposite motivational effects in nicotine-non-dependent versus nicotine-dependent and nicotine-withdrawn mice

Previously drug-naive mice given an acute injection of nicotine (1.75 mg/kg, s.c.) and conditioned in a place conditioning paradigm will show an initial conditioned aversive response (the a-process) to the environment paired with nicotine, followed 8 h later by a rewarding motivational response (the opponent b-process) (Grieder et al., 2010). In nicotine-dependent mice (7 mg/kg/d for 12 days by osmotic minipumps), 8 h of withdrawal from chronic nicotine also elicits a conditioned aversive response (Grieder et al., 2010), which nicotine overcomes through negative reinforcement (George et al., 2007; Grieder et al., 2014). To test whether the same dose of nicotine would have different conditioned motivational effects in non-dependent versus DWD mice, we administered nicotine (1.75 mg/kg, s.c.) or saline to previously drug-naive mice that were conditioned immediately after drug administration and to DWD groups of mice that were similarly conditioned with 1.75 mg/kg nicotine but at 8 h after the removal of the chronic nicotine pump during peak withdrawal. A two-way ANOVA revealed a significant motivational state (DWD or non-dependent) × process (a-process or b-process) interaction ($F_{(1,57)} = 49.97$, $P < 0.0001$; Figure 1). Similar to our previous reports (Grieder et al., 2010, 2012), groups of non-dependent mice given nicotine and conditioned immediately showed an aversive a-process ($P < 0.05$) and a rewarding b-process when conditioned 8 h after nicotine administration ($P < 0.05$). However, groups of DWD mice that were conditioned immediately after the same acute dose of nicotine (but were in withdrawal 8 h after chronic nicotine pump removal) showed a rewarding conditioned response (a-process) to nicotine in the withdrawal-paired environment ($P < 0.05$). These results suggest that acute nicotine at a dose that was aversive in non-dependent mice is instead negatively reinforcing and reinstates the a-process in mice that are in withdrawal from chronic nicotine. This negative reinforcement leads to a conditioned rewarding response to nicotine administered during withdrawal. Indeed, nicotine-dependent mice that were conditioned during peak withdrawal 8 h after chronic nicotine pump removal showed a conditioned aversive response to the withdrawal-paired environment (the b-process; $P < 0.05$). These results suggest that nicotine elicits opponent conditioned motivational responses that are in the opposite directions in non-dependent versus DWD mice and that it is possible to

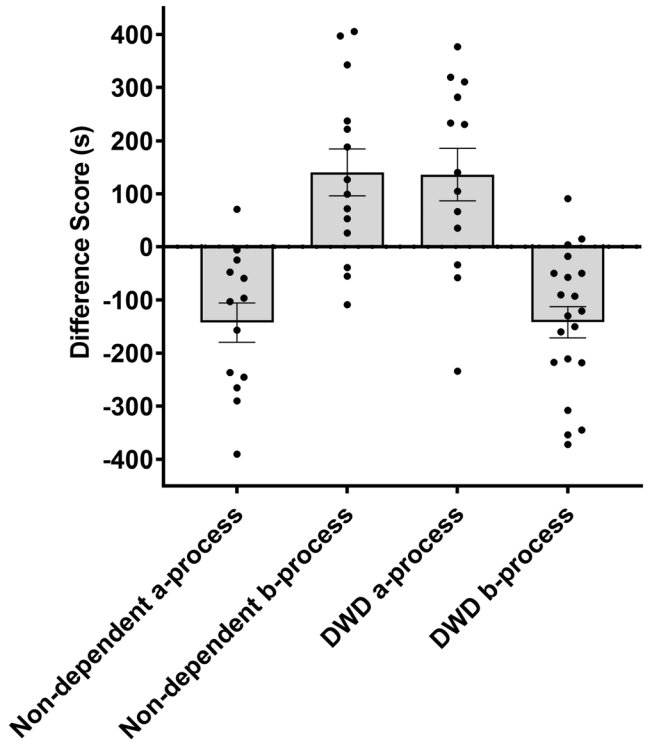

FIGURE 1 Acute nicotine administration leads to opposite opponent conditioned responses in non-dependent and nicotine-dependent and nicotine-withdrawn (DWD) mice. Previously drug-naive mice given nicotine (1.75 mg/kg; $n = 13$) showed an initial conditioned aversive response to the nicotine-paired environment (non-dependent a-process) when conditioned immediately after nicotine administration. Previously drug-naive mice that were conditioned 8 h after nicotine administration ($n = 14$) showed a conditioned opponent rewarding response to the nicotine-paired environment (non-dependent b-process). DWD mice given the same dose of acute nicotine (1.75 mg/kg, s.c. at 8 h after chronic nicotine pump removal; $n = 13$) and then conditioned immediately showed an initial rewarding response (DWD a-process) to the withdrawal-paired environment, presumably due to nicotine's ability to relieve withdrawal. Nicotine-dependent mice conditioned 8 h after nicotine administration ($n = 20$) showed a conditioned aversive response (DWD b-process) to the withdrawal-paired environment. Data boxes represent means ± SEMs

assess whether mice are in a non-dependent or DWD motivational state by observing their conditioned response to a single dose of systemic nicotine.

3.2 | VTA BDNF injection induces nicotine dependence without chronic nicotine treatment

We next investigated whether previously drug-naive mice could be made to behave as if they were in withdrawal from chronic nicotine by giving them a single injection of BDNF into the VTA. Groups of previously drug-naive mice were injected with saline or BDNF (0.5 μl/hemisphere) into the VTA 1 week before an acute dose of nicotine (1.75 mg/kg, s.c.). A two-way ANOVA showed a significant BDNF treatment x motivational process (a-process or b-process) interaction ($F_{(1,63)} = 39.11$, $P < 0.0001$; Figure 2a). Mice given saline in the VTA and conditioned immediately after receiving acute nicotine showed avoidance of the previously nicotine-paired environment, demonstrating an aversive a-process ($P < 0.05$). Another VTA saline group of mice conditioned 8 h after receiving acute nicotine preferred the nicotine-paired environment on testing ($P < 0.05$), showing an opponent rewarding b-process. Both groups replicated the data in nicotine-non-dependent mice in Figure 1. Most interestingly, mice injected once with BDNF in the VTA showed the opposite conditioned responses and reacted to acute nicotine as if they were nicotine dependent: The VTA BDNF groups showed a rewarding response when conditioned immediately after acute nicotine treatment ($P < 0.05$) and an opponent aversive b-process when they were conditioned 8 h after nicotine administration ($P < 0.05$). These conditioned responses in the BDNF groups (rewarding a-process and aversive b-process) phenocopied those shown by nicotine-dependent and nicotine-withdrawn mice (as shown in Figure 1). Additionally, mice whose cannula missed the VTA (determined by blinded histological analyses) during BDNF infusion ($n = 13$; six dorsolateral, four ventromedial, three anterior misses) showed an aversive a-process when conditioned immediately after acute nicotine administration that mimicked the response shown by non-dependent mice ($t_{30} = 0.2659$, $P = 0.7951$ comparing VTA saline a-process versus VTA misses a-process). These data suggest that BDNF signalling in the VTA can cause a switch from a non-dependent to a DWD motivational state and make a previously aversive dose of nicotine rewarding.

3.3 | VTA BDNF injection switches the substrate mediating the conditioned motivational response to acute nicotine from D1R to D2R

Functional D1Rs, but not D2Rs, are required for the expression of the aversive response to acute nicotine in non-dependent mice (Grieder et al., 2012). On the other hand, the D2R but not the D1R mediates the conditioned aversive response to nicotine withdrawal in DWD mice (Grieder et al., 2012). To examine whether BDNF injection in the VTA could itself cause the motivational switch from a D1R-mediated non-dependent state to a D2R-mediated DWD state, we injected saline or BDNF (0.5 μl/hemisphere) in the VTA of D1R KO and D2R KO mice

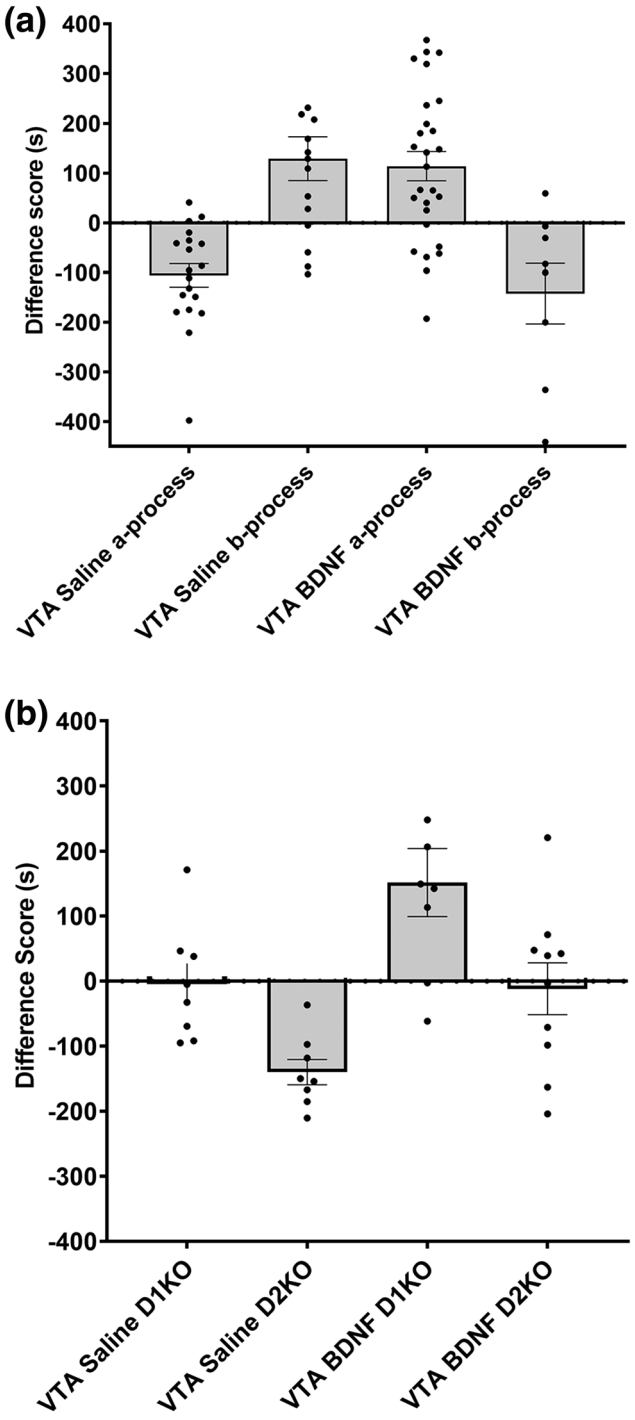

FIGURE 2 VTA BDNF injections make non-dependent mice behave as if they are nicotine dependent and withdrawn and switch the substrate mediating the conditioned motivational response to acute nicotine from D1R-mediated to D2R-mediated. (a) Mice given saline in the VTA and conditioned immediately after receiving acute nicotine (1.75 mg/kg; $n = 19$) were non-dependent and showed an immediate aversive a-process, whereas a group given VTA saline and conditioned 8 h after acute nicotine administration ($n = 15$) showed an opponent rewarding b-process. Mice injected with BDNF in the VTA responded to the same dose of acute nicotine (1.75 mg/kg; $n = 27$) as if they were nicotine dependent, showing a conditioned rewarding response to acute nicotine. Mice that received BDNF in the VTA that were conditioned 8 h after acute nicotine ($n = 8$) showed an opponent aversive b-process, as if they were experiencing withdrawal. Data boxes represent means ± SEMs. (b) In previously drug-naive mice given VTA saline injections, global genetic knockout of the dopamine D1 receptor (D1KO; $n = 8$) but not the D2 receptor (D2KO; $n = 8$) prevents the conditioned aversive response to acute nicotine (the VTA saline a-process observed in wild-type mice in (a)). After VTA BDNF injection, a conditioned rewarding response to acute nicotine is observed in C57BL/6 wild-type mice (the VTA BDNF a-process shown in (a)) that is prevented in D2KOs ($n = 10$) but not in D1KOs (n = 8). Data boxes represent means ± SEMs

and then 1 week later gave them acute nicotine (1.75 mg/kg, s.c.). A two-way ANOVA showed significant main effects of VTA BDNF treatment ($F_{1,31} = 7.417$, $P = 0.0105$; Figure 2b) and genotype ($F_{2,65} = 8.536$, $P = 0.0064$; Figure 2b). In mice given VTA saline injections, the conditioned aversive response to acute nicotine that was observed in WT control mice (the non-dependent a-process in Figure 1 and the VTA saline non-dependent a-process in Figure 2a) was demonstrated by D2R KO mice ($P < 0.05$) but was blocked in D1R KO mice ($P > 0.05$). These data confirm that the conditioned aversive responses to acute nicotine in non-dependent animals are D1R-mediated. In the groups given VTA BDNF injections, the conditioned rewarding response to an acute injection of nicotine that was observed in WT mice (VTA BDNF a-process in Figure 2a) was shown by D1KO mice ($P < 0.05$), but not D2KO mice ($P > 0.05$). The double dissociation shown in these data suggests that BDNF in the VTA caused a motivational switch that made acute nicotine rewarding and changed the neurobiological substrate from a D1R- to D2R-mediated system.

3.4 | TrkB receptor RNA is upregulated in the VTA of DWD mice

We next used qPCR to assess whether the transition from a non-dependent to a nicotine-dependent and nicotine-withdrawn motivational state corresponds to an increase in gene expression of BDNF or its receptor, TrkB, in the VTA. DWD groups of mice showed no significant change in VTA BDNF RNA ($t_3 = 1.9779$, $P = 0.1423$; Figure 3), but a significant increase in VTA TrkB RNA ($t_3 = 5.5035$, $P = 0.0118$; Figure 3) compared with nicotine-naive controls. These data suggest that the transition to a nicotine DWD state leads to an increase in TrkB, but not BDNF, gene expression in the VTA.

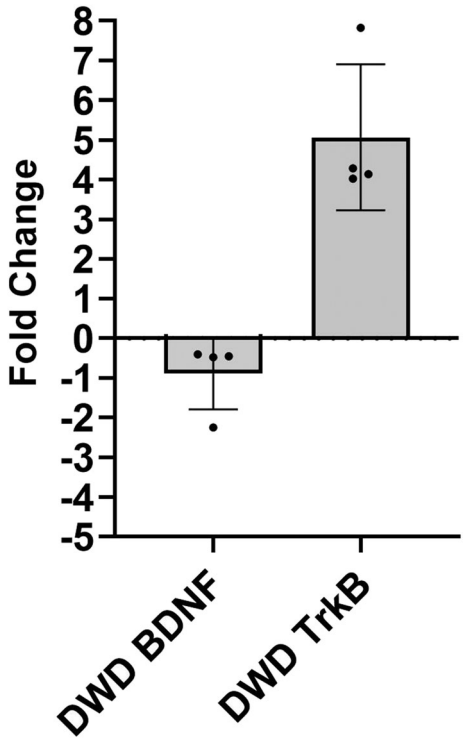

FIGURE 3 qPCR for BDNF and TrkB receptor RNA expression in nicotine-dependent and -withdrawn mice shows upregulation of TrkB RNA in the VTA. Fold change refers to the log base 2 value of 2^-(ΔΔCt) compared with the levels in nicotine-naive mice. Nicotine-dependent mice in withdrawal from chronic nicotine ($n = 4$) showed no significant change in BDNF RNA in the VTA, but a significant increase in TrkB RNA levels. Data boxes represent means ± SEMs

4 | DISCUSSION

A single injection of BDNF in the VTA causes a switch from a non-dependent D1R-mediated motivational state, where acute nicotine causes an aversive a-process followed by an opponent rewarding b-process, to a state that phenocopies the nicotine-dependent and nicotine-withdrawn D2R-mediated motivational state where acute nicotine causes a rewarding a-process and an aversive b-process. These results suggest that a single injection of BDNF in the VTA causes a change in the neurobiological substrate mediating the conditioned motivational response to acute nicotine. Thus, from a motivational point of view, the non-dependent animals' conditioned motivational response to nicotine matched the responses of animals that are nicotine dependent and withdrawn, although the non-dependent animals did not receive chronic nicotine treatment or experience withdrawal. In the absence of exogenous BDNF, this same motivational switch occurs after chronic nicotine treatment and withdrawal, where an increase in BDNF signalling appears to be mediated by an increase in TrkB receptor RNA (but not BDNF RNA) in the VTA.

We used a place conditioning paradigm to examine the motivational effects of nicotine both immediately and hours after nicotine administration. The animals approach conditioned cues in the nicotine-paired environment that they have associated with nicotine administration, similar to approaching a bar to get nicotine during self-administration. Although a self-administration paradigm may more closely resemble human nicotine intake (Rose & Corrigall, 1997), separating drug motivation due to the rewarding or aversive effects of acute nicotine or the alleviation of withdrawal is more easily performed using a place conditioning procedure (Mucha et al., 1982).

The ability of BDNF to induce a drug-dependent motivational state and its associated behavioural phenotypes has been demonstrated previously for opioids (Vargas-Perez et al., 2009, 2014), where a switch from non-dependent tegmental pedunculopontine nucleus (TPP)-mediated state to an opioid-dependent VTA-mediated state was observed after VTA BDNF administration in rats. BDNF in the VTA can also switch the motivational state in ethanol dependence (Ting-A-Kee et al., 2013). The present data show that VTA BDNF administration switches the motivational state of the animal as well as the dopamine receptor subtype involved in mediating nicotine's conditioned motivational effects. We previously demonstrated that a non-dependent D1-mediated response to acute nicotine occurred with increased phasic VTA dopamine activity and that a DWD D2-mediated response to nicotine withdrawal occurred with decreased tonic VTA dopamine activity (Grieder et al., 2012). Considered with our present results, we hypothesize that BDNF signalling in the VTA modifies the firing pattern of VTA dopamine neurons to switch the motivational processes after acute nicotine administration to those usually observed only after chronic nicotine and withdrawal. The present qPCR results further suggest that the nicotine DWD motivational state leads to an increase in TrkB receptors in the VTA, which may underlie the increase in BDNF signalling in these animals.

Under basal conditions, BDNF is highly expressed in microglia (Coull et al., 2005), and in cells in the VTA, amygdala, hippocampus and frontal cortex, with less expression in dorsal and ventral striatum (Conner et al., 1997), and its major signalling functions are mediated by TrkB receptors (Carvalho et al., 2008). For cocaine motivation, BDNF signalling at TrkB receptors has been implicated in cocaine self-administration (Lu et al., 2004; Verheij et al., 2016), and it has been hypothesized that even a single cocaine exposure leads to enhanced BDNF protein synthesis (Li & Wolf, 2015). Further, withdrawal from repeated cocaine or repeated opioid injections lead to elevated levels of BDNF protein in the VTA (Pu et al., 2006; Vargas-Perez et al., 2009). We suggest that chronic nicotine administration has a similar effect, leading to increased VTA BDNF signalling through an increase in TrkB receptor expression, which causes the molecular switch to a drug-dependent-like motivational state. Our results support this idea, because injection of BDNF directly into the VTA caused previously drug-naive mice to respond to nicotine as if they were in a DWD motivational state. Although they had not been treated with chronic nicotine, the mice showed a rewarding a-process followed by an aversive b-process, which are conditioned behaviours that phenocopy nicotine-dependent mice experiencing withdrawal. Further, mice that received BDNF injections outside of the VTA behaved as if they were non-dependent, suggesting that the VTA is the specific neural area where increased BDNF signalling causes the motivational switch.

The present results show that TrkB receptor RNA (but not BDNF RNA) is increased in the VTA in nicotine DWD animals. Though we have measured RNA and not protein, our functional behavioural data combined with an increase in TrkB receptor RNA suggest that the putative increase in TrkB receptor protein may persist in the absence of nicotine and lead to some of the motivational effects experienced during withdrawal. Although these results contrast with opiate dependence, where BDNF increases in the VTA but there is no significant change in TrkB (Vargas-Perez et al., 2009), it appears there are multiple ways of increasing BDNF signalling in drug-dependent and drug-withdrawn animals. We suggest that the switch to a DWD motivational state increases either the ligand or receptor concentration, which is involved in the aversive experience of withdrawal after chronic drug administration. A limitation of the present research is that male mice were utilized throughout the experiments. A recent study found sex differences in the VTA and nucleus accumbens proteome both at baseline and following nicotine exposure (Lee et al., 2021), which suggests that female mice may show different neurobehavioural responses to nicotine, and thus, further investigation of sex differences is warranted.

The aversiveness of withdrawal from nicotine and other abused drugs is a strong stimulus to relapse to drug use, making nicotine a powerful negative reinforcer. Multiple neural substrates interact to lead to a drug-dependent neuroadapted state where the aversive b-process dominates behaviour, including the brain corticotropin-releasing factor (CRF) system (George et al., 2007; Grieder et al., 2014), which is known to interact with BDNF to control neuroplasticity (Bennett & Lagopoulos, 2014). Previously nicotine-naive mice can be made to behave as if they are nicotine dependent and in withdrawal with an injection of BDNF in the VTA, suggesting that a switch can be thrown to lead to a drug-dependent motivational state without having experience with the drug. The hypothesis thus follows that blocking TrkB signalling in the brain, particularly in the VTA, could prevent the transition to nicotine dependence and the conditioned withdrawal effects. Indeed, brain-wide TrkB antagonism reduces cocaine intake and reinstatement (Verheij et al., 2016), and lentiviral siRNA knockdown of TrkB receptors prevents the aversive motivational state associated with opiate withdrawal and

the development of an opioid-dependent state (Vargas-Perez et al., 2014). Our present results suggest that blocking BDNF or TrkB activity in the VTA might prevent the transition to a nicotine-dependent state and thus the negatively reinforcing effects of acute nicotine to overcome the aversiveness of withdrawal from chronic nicotine. If a switch back to a non-dependent state could be induced pharmacologically, acute nicotine administration would elicit an aversive a-process rather than reward, in a similar way to disulfiram treatment for alcoholism (Jacobsen & Martensen-Larsen, 1949). In this sense, modulation of neural BDNF or TrkB may be or lead to promising new pharmacological treatments for nicotine addiction.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

TEG and DVDK designed the experiments. TEG, HVP, MY and GMB performed minipump and BDNF infusion surgeries. TEG, HVP, MY and RTAK performed place conditioning. MY performed qPCR. SG supplied knockout mice. TEG, MY and OG analysed the data. TEG, OG and DVDK wrote the paper. All of the authors discussed the results and read the paper.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. The data that support the findings of this study are available from the corresponding author, TEG, upon reasonable request.

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