EFFECTS OF INTRANASAL INFUSION OF GLIAL DERIVED NEUROTROPHIC FACTOR (GDNF) NANOPARTICLES ON ACTIVATION OF DOPAMINE NEURONS IN OPIOID DEPENDENT RATS.

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Abstract

Treatments for opioid use disorder (OUD) include the opioids methadone and buprenorphine, however, both have abuse potential. Thus, the development of non-opioid based treatments to effectively reduce craving and decrease the risk of relapse is still needed. Intranasal administration of glial derived neurotrophic factor (GDNF) plasmid DNA nanoparticles (NPs) can decrease cued reinstatement in abstinent oxycodone self-administering rats when examined 28 days after intranasal delivery of pGDNF DNA NPs. The current study addresses the following questions: 1) Do levels of Fos mRNA remain elevated several weeks after intranasal infusion in rats, 2) Are the effects of intranasal GDNF NPs on cued reinstatement in rats associated with changes in Fos activity in tyrosine hydroxylase (TH) expressing dopamine neurons in the ventral tegmental area (VTA) and substantia nigra. Adult male and female rats were implanted with jugular catheters and trained to lever press for OXY using a fixed ratio 1 (FR1) schedule for 9 days followed by FR5 for 3 days. On Day 13, all animals were tested for OXY motivated responding using a progressive ratio (PR) schedule. The next day rats were administered either intranasal saline vehicle or pGDNF NPs, with groups counterbalanced based on both their total drug intake (comparable OXY exposure) and their responding during PR (comparable motivated responding). All animals then underwent forced abstinence for 28 days and were then tested for cue-induced reinstatement. All rats were immediately euthanized at the end of reinstatement and were used to quantify Fos positive TH neurons using immunohistochemistry and measure Fos mRNA using qPCR. These studies are ongoing but will be discussed in the context of effects of intranasal pGDNF NPs as a potential therapy for relapse prevention.

1. Introduction

Opioid use disorder (OUD) has no effective non-opioid-based drug treatments (Chalhoub et al., 2020). Current therapies do not correct the underlying brain changes caused by opioids leading to compulsive drug use (Chalhoub et al., 2020). Opioids inhibit GABAergic neurons in the ventral tegmental area (VTA) which sends dopamine projections to the nucleus accumbens core (NAc), making both regions critically important in relapse (Adinoff, 2004). GABA inhibits the release of dopamine, thus a decreased level of GABA due to opioid use results in a dopamine surge (Pergolizzi et al., 2020). However, with chronic use, opioids begin to decrease the release of dopamine, diminishing the sensitivity of the reward system and causing dependence and OUD (Pergolizzi et al., 2020). GDNF is a protein that promotes the function of dopamine neurons. It is thought that correcting the dopamine imbalance can normalize the brain changes induced by opioids, preventing craving and relapse (Leu-Fen H. Lin et al., 1993).

The goal of the current study was to determine whether intranasal treatment with GDNF in oxycodone self-administering male and female rats increases activation of dopamine neurons in the VTA. To do this we examined the activation of the immediate early gene c-fos by measuring expression of the c-fos gene in the NAc using qPCR and expression of the FOS protein using immunohistochemistry in the VTA and substantia nigra. FOS expression is widely used as a marker of neuronal activity. These studies were conducted on brains collected immediately following behavioral testing to measure drug-seeking following 28 days of abstinence. The behavioral data demonstrating the efficacy of intranasal GDNF treatment are presented for completeness, however, the main focus of this study was on underlying mechanisms associated with decreased drug seeking following treatment with GDNF.

2. Materials and Methods

2.1 Animals and housing

Nulliparous male and female Sprague-Dawley rats (225-275g) were purchased from Charles River Breeding Laboratories (Kingston, NY, USA) and housed in a light (0700–1900 hours) and temperature (21–24°C) controlled room. Food and water were provided ad libitum.

2.2 Self-Administration

Oxycodone self-administration (SA) was conducted in Med Associates operant chambers with two retractable levers (i.e., one active lever triggering drug infusion and one inactive lever producing no response) following catheterization of animals. A cue light above the active lever was illuminated following a correct (i.e., active) response. Following one week of post-surgical recovery, animals were trained to lever press for oxycodone (0.1 mg/kg/infusion; 6h/day; 12 training days) using a fixed ratio 1 (FR1) schedule for 9 days and switching to a fixed ratio 5 (FR5) schedule for the last 3 days of training. On Day 13, all animals were tested for oxycodone SA under a progressive ratio schedule (PR) where presses required to initiate drug infusion increased exponentially after each correct response. Based on PR responding, animals were assigned to their treatment group. The next day animals were intranasally infused with either pGDNF NPs or vehicle. After 28 days of forced abstinence, groups were either returned to operant chambers and performed a 90-minute reinstatement SA session with only saline available in the syringe as a measure of drug seeking.

2.3 pGDNF NPs

pGDNF DNA NPs were supplied by Copernicus Therapeutics, Inc. These 10 kDa polyethylene glycol-substituted lysine 30-mer compacted DNA NPs unimolecularly compact a single molecule of expression plasmid, have a minimum diameter of 10 nm, and can be formulated in

a rod-like shape. They are non-immunogenic, non-inflammatory, small enough to enter the cell's nucleus via the nuclear membrane pore, and able to transfect post-mitotic cells in the brain, lung, and retina. Copernicus' hGDNF_1b plasmid has a high transfection efficiency and yields long term (>1 year) expression in mouse and rat striatum. As in preliminary studies, we used a dose of 90 µg.

2.4 Intranasal infusion

For intranasal treatment, all animals were anesthetized with ketamine- dexmedetomidine mixed solution (80 mg/kg and 2 mg/kg, respectively, IP) and placed in dorsal recumbency. A 10 µl Hamilton syringe was used to administer pGDNF NPs or saline in 2.5 µl increments per nare, alternating sides until the total volume of 20 µl was given. After administrating the intranasal infusion, animals remained in their supine position for 30 min.

2.5 Immunohistochemistry (IHC)

2.5.1. cFos IHC

After the rats were euthanized, the brains were removed and rapidly frozen in 2-methylbutane and were sectioned on a cryostat and mounted onto slides. The sections were fixed in 4% PFA at 4°C for 60 minutes followed by three washes in 1X PBS for five minutes, then allowed to air dry. The sections then incubated in 1:10 Tris-EDTA buffer in deionized water for 40 minutes at room temperature and again washed three times for five minutes in 1X PBS. The sections were then blocked with 5% normal goat serum and 5% normal donkey serum in PBS with 0.1% Triton X-100 for 60 minutes at room temperature. Tissue was then incubated in monoclonal primary rabbit anti-cFos antibody (1:1000, Encor, RPCA-c-Fos-AP) in blocking solution for two nights at 4°C. Sections underwent three five-minute washes in 0.1% PBST followed by incubation in donkey anti-rabbit Alexa Fluor 488 secondary antibody (1:500, Invitrogen, A-21206) for one hour at room temperature. Sections were washed 3 times for five minutes in 1X PBS and air dried. The slides were cover slipped with ProLong Gold antifade mounting medium with DAPI (Invitrogen, P36931) and air dried.

2.5.2. IHC Image Analysis

For quantification, each slide was imaged on an Echo Revolve microscope. Stained tissue was imaged bilaterally in each region (nucleus accumbens core and substantia nigra) with identical exposure levels for all of the images. Images were analyzed in ImageJ software. Images were thresholded and the "analyze particles" command was used to quantify the number of cFos+ cells in each image and averaged across each subject. Thresholding parameters and cell size

and circularity levels were optimized for each brain region and performed consistently across groups and within region

2.6 Quantitative real time polymerase chain reaction (qPCR)

2.6.1. qPCR

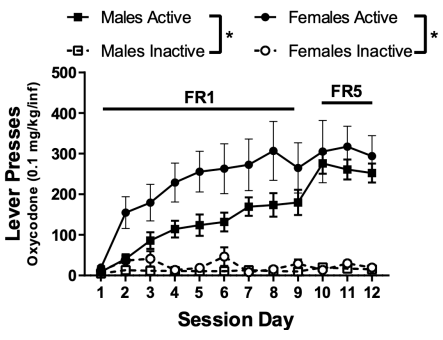
Tissue punches of the nucleus accumbens core were homogenized in lysis buffer and total RNA extracted using the RNeasy kit from Qiagen. Complementary DNA (cDNA) was synthesized from 100 ng of RNA using the RETROscript kit from Applied Biosystems. PCR was performed using an Applied Biosystems QuantStudio 3 under standard amplification conditions: 2 min at 50 °C, 10 min at 95 °C, 40 cycles of 15 s at 95 °C and 60 s at 60 °C. All PCR primers were TaqMan® Gene Expression Assays purchased from Applied Biosystems. The amplification efficiency of each of these assays has been validated by Applied Biosystems and averages 100% (±10). Assay ID and accession numbers were as follows: GapDH: Rn01775763_g1, Fos: Rn02396759_m1. All reactions were run in duplicate.

2.6.2. Quantification of gene transcription

Quantification of mRNA was obtained using the comparative cycle threshold (CT) method. The housekeeping gene, GAPDH, was used as an internal control against which each target signal was normalized (ΔCT). The ΔCT was then normalized against a calibrator (i.e. the mean of the control group for the target gene) to provide the ΔΔCT relative to the control group. Finally, data are transformed 2^ ΔΔCT and expressed as fold change.

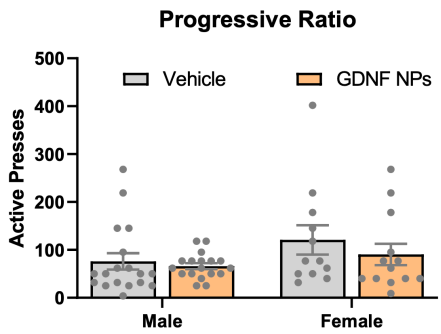
3. Results

3.1 Oxycodone Acquisition in Males and Females



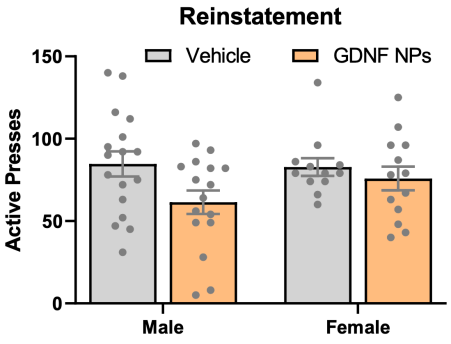
Mean (±SEM) active and inactive lever presses in male and female subjects across acquisition. There were significant main effects of lever (F [1, 1128] = 619.7, P<0.0001), session day (F [1, 1128] = 11.4, P<0.0001) and sex (F [1, 1128] = 47.7, P<0.0001) with active lever pressing increasing as compared to inactive lever pressing across session day in both males and females (F [1, 1128] = 10.9, P<0.001). Thus, in both males and females all subjects acquired oxycodone self-administration. *p<0.0001; Males N=32 and Females N=15

3.2 Treatment Group Assignment Based on PR



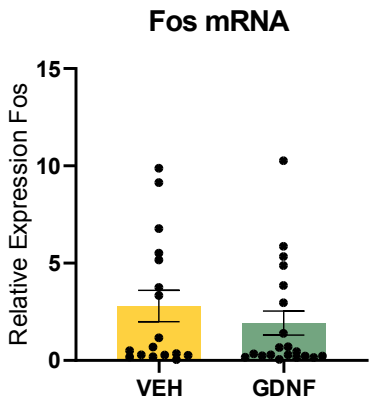
Mean (±SEM) active lever presses in male and female subjects under a PR schedule (30 min cutoff). There were no significant main effects based on treatment assignment (F [1, 48] = 0.48, P<=0.48) or sex (F [1, 48] = 2.95, P=0.09) and no significant interactions (F [1, 48] = 1.7, P=0.19).

3.3 Intranasal GDNF NPs Reduce reinstatement



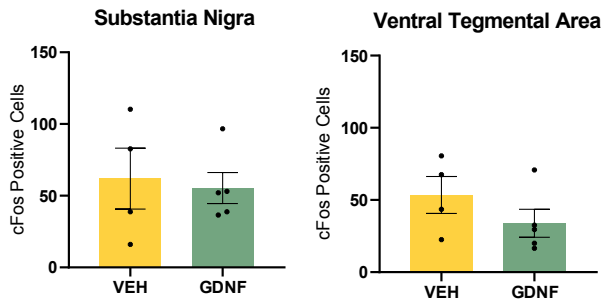
Mean (±SEM) active lever presses in male and female subjects during a 90-minute reinstatement session (FR1). There was a significant main effect of treatment (F [1, 43] = 5.78, P=0.02) with no main effect of sex (F [1, 43] = 0.06, P=0.8) and no significant interaction (F [1, 43] = 0.3, P=0.57). Post hoc analysis (Tukey's test) showed a significant effect of intranasal pGNDF NPs on drug-seeking during reinstatement in both males and females, * P<0.05. Overall, active lever pressing was reduced from 84.6 ± 5.7 to 60.5 ± 5.2 in vehicle and pGDNF NP-treated rats respectively (n=20-22/group; P<0.01), representing a 28% decrease in this measure.

3.4 qPCR of Nucleus Accumbens Core



mRNA for Fos in the NAc were not altered by intranasal pGDNF treatment. Mean (±SEM) relative expression of Fos in the nucleus accumbens core (NAcc) as measured 90 minutes after reinstatement testing. No significant effects were observed in Fos (t1,35=0.8653, p=0.3928).

3.5 Immunohistochemistry of VTA and substantial nigra



Mean (±SEM) of cFos positive cells within the substantia nigra with representative images per group. No significant effects of treatment ($t_{1,7}=0.237$, $p=0.7775$). Mean (±SEM) of cFos positive cells within the ventral tegmental area with representative images per group. No significant effects of treatment ($t_{1,7}=1.247$, $p=0.2524$).

4. Discussion

Intranasal administration of pGDNF NPs decrease drug seeking after 28 days of abstinence using a cued/context reinstatement protocol. This predicts efficacy in reducing relapse. There were no sex differences in GDNF efficacy demonstrating similar reductions in drug seeking. There were no significant effects of intranasal infusion of pGDNF NPs, however, these are preliminary findings and further testing and optimization is needed. There was no significant effect on Fos in the VTA and substantia nigra. There was no significant effect on relative expression of Fos mRNA in the core of the nucleus accumbens.

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References

Adinoff B. (2004). Neurobiologic processes in drug reward and addiction. Harvard review of psychiatry, 12(6), 305–320.

Chalhoub, R. M., & Kalivas, P. W. (2020). Non-Opioid Treatments for Opioid Use Disorder: Rationales and Data to Date. Drugs, 80(15), 1509–1524.

Leu-Fen H. Lin et al. ,GDNF: a Glial Cell Line-Derived Neurotrophic Factor for Midbrain Dopaminergic Neurons.Science260,1130-1132(1993).DOI:10.1126/science.8493557

Pergolizzi, J. V., Raffa, R. B., & Rosenblatt, M. H. (2020). Opioid withdrawal symptoms, a consequence of chronic opioid use and opioid use disorder: Current understanding and approaches to management. Journal of Clinical Pharmacy & Therapeutics, 45(5), 892–903