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Neuronal Activity Within the Ventral Tegmental Area Is Correlated with Cocaine-Seeking Behavior in Male, But Not Female, Rats

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Neuronal activity within the ventral tegmental area is correlated with cocaine-seeking behavior in male, but not female, rats

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Introduction

In 2020, approximately 1.3 million Americans suffered from Cocaine Use Disorder (CUD), and 19,447 died of cocaine-involved overdose. One of the greatest obstacles in treating CUD is its high rate of relapse, with social stressors being reported to induce cocaine craving and relapse. However, many animal models of drug relapse utilize physical (e.g. footshock) and/or pharmacologic stressors (e.g. yohimbine) as a means to trigger drug seeking. It is well known that social stressors engage distinct neurocircuitry from non-social stressors and as such, uncovering the mechanisms by which social stressors induce cocaine-seeking behavior may aid in the development of new pharmacotherapies for CUD. To date, the putative pharmacological treatments that arose from relapse models using nonsocial stressors have not yet yielded FDA-approved therapeutics for the treatment of CUD.

The objective of this study was to begin to determine the neurobiological underpinnings of psychosocial stress-induced cocaine seeking. Social defeat stress, achieved using the well-established resident-intruder procedure, is an ecologically-valid psychosocial stressor in rodents that may more closely recapitulate those psychosocial experiences that elicit cocaine craving and relapse in human cocaine users. Our laboratory has developed a model of psychosocial stress-induced relapse in rats in which extinguished cocaine seeking is reinstated by re-exposure to a discrete cue that signals impending social defeat stress. We previously reported that an individual rat's predilection towards the display of active coping behaviors during prior social defeat stress exposures was positively correlated with levels of psychosocial stress-induced cocaine seeking. The current study's goal was to expand upon these initial findings by assessing and comparing patterns of neural activation within the ventral tegmental area (VTA) during stress-induced cocaine seeking triggered by psychosocial stress-predictive or footshock stress-predictive cues. The VTA was selected for investigation in the present study because of its known role in the manifestation of stress-induced reinstatement of cocaine seeking. We postulated that neural activation in this brain region would be associated with the magnitude of observed psychosocial stress-induced cocaine seeking, thus providing important insights into the neurobiological underpinnings of this phenomenon.

Methods

SUBJECTS: Adult male and female Long-Evans rats (n=40).

COCAINE SELF-ADMINISTRATION: Rats were initially trained to lever-press for food reinforcement and were then implanted with intrajugular catheters. After recovery, rats self-administered cocaine (0.5 mg/kg/inf, 20-s cued timeout) in 2 h sessions 5-6 d/week under a FR1 schedule of reinforcement for a total of 20 sessions. Each session was terminated if 2 h elapsed or if the animal earned 60 reinforcers, whichever occurred first. During sessions 11, 14, 17, and 20, animals were placed in a perforated polycarbonate box that allowed for unrestricted access to levers and cue lights.

EXPERIMENTAL GROUPS:
On sessions **11, 14, 17, and 20**, animals in the **Social Defeat Stress group** (SDS, n=16) were removed from the self-administration chamber and immediately subjected to social defeat stress using the resident-intruder procedure. Briefly, the subject was placed into the home cage of a same-sex, aggressive conspecific rat until one of the following situations occurred: 1) The intruder was pinned in a submissive supine posture for 4 consecutive seconds, 2) the intruder was bitten twice, or 3) 4 min elapsed. The intruder rat was then placed back inside the perforated polycarbonate box and returned to the resident-aggressor's home cage for 5 additional mins of "protected' interaction, and subsequently returned to its home cage.

On sessions **11, 14, 17, and 20**, animals in the **Foot-shock group** (FS, n=12) were removed from the self-administration chamber and polycarbonate box and immediately replaced into the self-administration chamber for 15 mins during which the rat received 20 foot-shocks (0.5 mA, 0.5 s) presented at variable intervals (range 3-80 s), after which they were returned to their home cage

On sessions **11, 14, 17, and 20**, animals in the **Empty Cage control group** (EC, n=12) underwent the same procedure as the social defeat stress group with the exception that they were placed into an uninhabited resident-aggressor home cage.

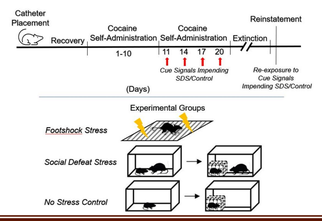
Self-administration on days **12, 13, 15, 16, 18, and 19** took place identical to days 1-10, i.e. with no polycarbonate box or stress/no-stress conditions or exposures.

EXTINCTION: Extinction sessions began for all animals on session 21. Extinction sessions lasted 2 h and were carried out 5-6 d/week, during which lever presses had no scheduled consequences. For each subject, lever-pressing was deemed extinguished when ≤ 15 active lever presses were emitted in 3 out of 4 consecutive sessions.

STRESS-INDUCED REINSTATEMENT: On the session immediately following satisfaction of extinction criteria, animals were re-exposed to the perforated polycarbonate box which originally signaled their assigned impending stress or no-stress control condition and were allowed to lever-press for 2 h under extinction conditions.

TISSUE PROCESSING & IMMUNOHISTOCHEMISTRY: Immediately at the end of the 2 h reinstatement test session, rats were deeply anesthetized, transcardially perfused with ice cold PBS followed by 4% PFA, and brains were extracted and post-fixed overnight in 4% PFA followed by submersion in 30% sucrose and rapid freezing. Tissue was sliced in the coronal plane at 30 µm using a cryostat . 2-3 sections from each subject containing the VTA were processed for c-Fos and tyrosine hydroxylase (TH) via immunohistochemistry and subsequently mounted and cover slipped with DAPI-containing mounting medium.

IMAGE ACQUISITION AND PROCESSING: VTA images were acquired at 20x using z-stack and x-y stitching capabilities on a Keyence BZ-X710 fluorescence microscope. The boundaries of the VTA in each image were identified using TH as a marker and a standard rat brain atlas. In each section, the number of Fos-positive cells within the VTA were manually counted using Keyence's Analyzer software, ImageJ, and Adobe Illustrator, normalized to the area of the VTA, and averaged first across left and right hemispheres and then averaged across multiple sections for each individual subject.



Cocaine self-administration is similar across SDS, FS, and EC groups of rats, but cocaine seeking is greater in SDS and FS rats

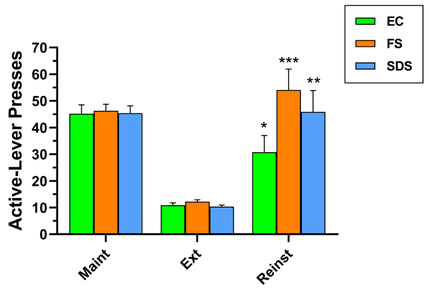


Figure 1: Cocaine self-administration and stress-induced reinstatement. Active-lever presses during sessions 8-10 of cocaine self-administration (Maint), final 3 days of extinction (Ext), and reinstatement test session (Reinst). Data represent mean ± SEM lever presses. * p<0.05, **p<0.01, ***P<0.001. n=12-16/group

Immunohistochemical detection of Fos expression within the VTA during cocaine seeking test sessions

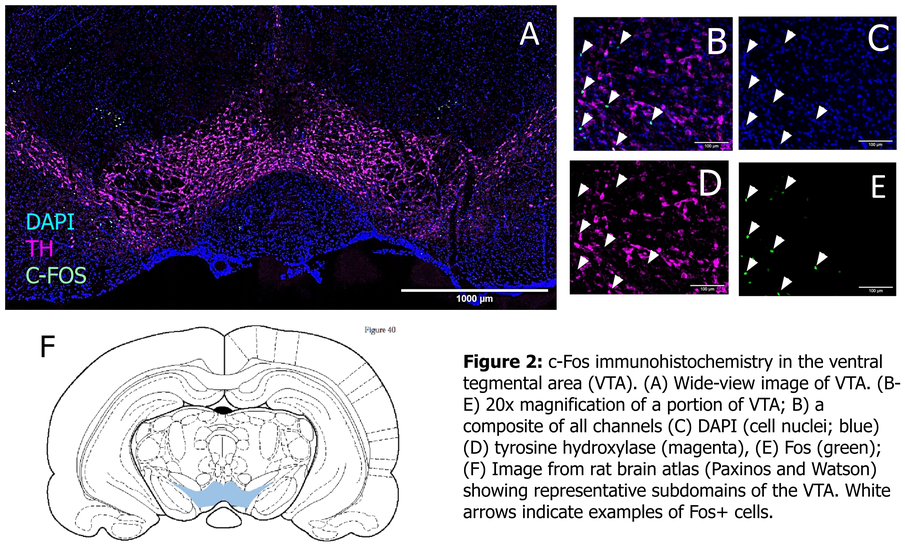


Figure 2: c-Fos immunohistochemistry in the ventral tegmental area (VTA). (A) Wide-view image of VTA. (B-E) 20x magnification of a portion of VTA; B) a composite of all channels (C) DAPI (cell nuclei; blue) (D) tyrosine hydroxylase (magenta), (E) Fos (green); (F) Image from rat brain atlas (Paxinos and Watson) showing representative subdomains of the VTA. White arrows indicate examples of Fos+ cells.

VTA neural activity during cocaine-seeking test sessions is greater following re-exposure to a footshock-predictive cue as compared to a cue signaling impending psychosocial stress or no-stress control condition

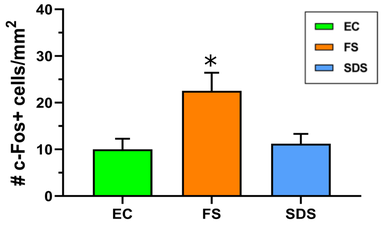


Figure 3: Quantification of Fos+ cells in VTA across groups. Data represent mean ± SEM Fos+ cells in VTA during reinstatement test sessions. * p<0.05, compared to all other groups. n=12-16/group.

VTA neural activity is significantly correlated with cocaine seeking in male, but not female, rats

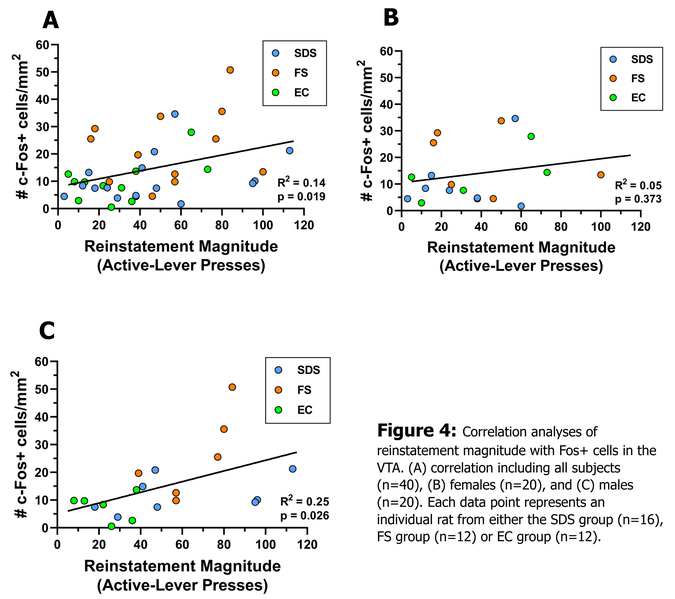


Figure 4: Correlation analyses of reinstatement magnitude with Fos+ cells in the VTA. (A) correlation including all subjects (n=40), (B) females (n=20), and (C) males (n=20). Each data point represents an individual rat from either the SDS group (n=16), FS group (n=12) or EC group (n=12).

Summary

- **Re-Exposure to a cue signaling impending social defeat stress (SDS), foot-shock (FS), or empty cage no-stress (EC) produces cocaine-seeking behavior, but the greatest responses are elicited by stress-predictive cues**
- **A greater VTA response was observed in the FS group as compared to the SDS or EC groups**
- **Cocaine seeking magnitude is positively correlated with Fos activation in the VTA, but appears to be primarily driven by male subjects**
- **We aim to follow-up our analysis by examining colocalization of Fos with TH to determine whether the detected neural activity is specific to dopamine neurons**

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