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Cortical-striatal integration of cocaine history and prefrontal dysfunction in animal modeling of dual diagnosis

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Abstract

Background—High rates of substance disorders in schizophrenia and other mental illnesses may reflect biological vulnerabilities to the addiction process. Interactions between addictive drug effects and mental illness involving circuits that generate motivated behavior may underpin this vulnerability.

Methods—We examined separate and combined effects of neonatal ventral hippocampal lesions (NVHL)—a neurodevelopmental model of schizophrenia (vs. SHAM-operated controls)—and a behaviorally sensitizing cocaine history (15 mg/kg/day × 5 days vs. saline injections), on acute cocaine-induced neural activation signaled by c-Fos expression. Stereological assessment of activation densities spanned 6 ventral to dorsal cortical-striatal compartments.

Results—Cortically, NVHLs showed hypo-activation and decreased volume of the ventral-medial prefrontal cortex. In contrast, cocaine history was only expressed sub-cortically, and as a hyper-activating effect in the dorsal striatum where significant NVHL-induced hyper-activation also emerged. Across all subjects and brain regions, only dorsal striatal activation was correlated with differences in sensitized locomotion. However, this activation was tightly correlated to a simple multiplicative function of ventral medial prefrontal hypo-activation and cocaine history-related increases striatal activation.

Conclusions—These findings suggest drug history and developmental temporal limbic abnormalities associated with prefrontal dysfunction produce compounding effects within cortical-striatal circuits as mechanistic basis for dual diagnosis.

Keywords

Prefrontal cortex; striatum; c-Fos; stereology; dual diagnosis; hippocampus

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Introduction

Drug addictions are mainstream in mentally ill populations, and 'dual diagnosis' is associated with greater medical and psychiatric illness and death (1). Growing evidence suggests mental illnesses and addiction vulnerability co-emerge involuntarily from a unified etiology, definable on the neurocircuit level, and contributed to by shared environmental and genetic factors (2, 3). Abnormalities of prefrontal cortex (PFC) and/or temporal-limbic regions (e.g. hippocampus) span mental disorders frequently co-occurring with addictions (4). These regions provide glutamatergic projections to ventral striatal targets (e.g. nucleus accumbens (NAC)), where in conjunction with mesolimbic dopamine, they modulate information processing and neuroplasticity underlying alterations in motivated behavior (5,6).

In animals, developmental temporal-limbic disruptions produce complex neuropsychiatric syndromes including signs of addiction vulnerability (4). Neonatal ventral hippocampal lesion (NVHL) rats demonstrate multiple schizophrenia features including positive-like neuroleptic-responsive symptoms of peri-adolescent onset, cognitive impairments, negative symptom-traits, and neurobiological makers of PFC dysfunction (7). NVHLs also show accentuated long-term behavioral sensitization to cocaine (8), heightened baseline and cocaine history-induced impulsivity (9) and enhanced drug-taking and drug-seeking in cocaine self-administration (10).

Characterization of a neural integration of addictive drug history and developmental temporal-limbic alterations underlying psychiatric syndromes has been lacking. We investigated how a behaviorally-sensitizing cocaine history alters, two weeks later, cocaine-induced c-Fos early gene expression in NVHL vs. SHAM-controls. Applying stereology to measuring c-Fos activation as a non-specific marker of regional neural activation, we mapped six cortical-striatal compartments: three cortical (ventral-medial prefrontal, dorsal-medial prefrontal, secondary motor cortices (vmPFC, dmPFC, sMOC)), and three striatal (ventral-medial NAC, dorsal-lateral NAC, dorsal-lateral caudate-putamen (vmNAC, dlNAC, dlCAPU)). These regions were chosen as key components of parallel cortical-striato-thalamo-cortical loops thought to interactively generate motivated behavior via a ventral-dorsal directionality of information processing (11-13). We sought to i) define separate and combined effects of NVHLs and cocaine history on cortical-striatal activation; ii) determine which regional activations correspond to behavior; and iii) explore quantitative relationships between regional activations as brought to light by NVHL and cocaine history effects.

Methods and Materials

Subjects/Surgeries/Behavior

Post-natal Day-7 (PD-7), male Sprague Dawley pups underwent NVHL or SHAM surgery as described previously (10). Pups were raised under standard conditions through weaning (PD-21) then pair-housed (PD-35). On PD-70, rats were randomized to daily injections (i.p.) of saline (1ml/kg), or Cocaine HCl (15 mg/kg in saline (1ml/kg)) for 5 days in home cages. Two weeks after completing injections (PD-89), a single cocaine injection (15 mg/kg) was delivered to all rats after 1-hour of a 2-hour of locomotor arena-session. All procedures, (Section 1 in Supplement 1) accorded with *NIH Guide for Care and Use of Laboratory Animals*.

Histology

Within 25-35 minutes after locomotor sessions (~90 minutes post-injections), rats were transcardially perfused (4% paraformaldehyde). Brains cut coronally at ~0.5mm AP (bregma), gave anterior halves for immunohistochemistry and posterior for lesion verification (Fig 1).

Anterior halves were freezing microtome-cut (35 μm), providing every fourth section for c-Fos staining (Section 2 in Supplement 1). Posterior halves were vibratome-cut (40 μm) through the hippocampus with every tenth section fixed and thionin stained.

Brain Mapping/Stereology

Investigators blind to treatment groups conducted regional mapping or stereology. Mapping utilized reliable anatomical landmarks to optimize stereological rigor (Fig. 1C). Cortical regions were mapped together in same sections bounded by pre-assigned rostral-caudal markers. Striatal regions were treated similarly (Section 3 in Supplement 1).

Immunohistochemistry reduced section thickness from 35 to 14 μm ; bottom/top guard zones were 2 μm , allowing 10 μm for optical fractionator-counting. c-Fos nuclei were identified (600X) following predetermined criteria. As prescribed by stereology, systematic-random-sampling (SRS) and counting frame (CF) grids were varied (SRS:200², 250² μm^2 ; CF: 50², 75², 100² μm^2) between, but not within, regions and brains (Section 4 in Supplement 1). Counting protocols and number of hemi-sections per region were evenly distributed across groups. The overall Coefficient of Error for all rats and regions included for analysis was 0.105 and did not differ by group in regional comparisons.

Results

Behavioral Sensitization

Cocaine increased locomotion over habituated levels (10-minute pre-injection bin) that were similar among groups (Fig. 2A). NVHLs and cocaine history increased cocaine-induced locomotion independently and additively (two-way ANOVA: lesion: $F(1,27)=15.1$, $p<0.01$; drug history: $F(1,27)=12.5$, $p<0.01$; lesion x drug history: $F(1,27)=0.6$, $p=0.5$)(Fig. 2B).

Stereological Analysis of c-Fos Activation

Densities of c-Fos activation by region and treatment group (Fig. 3A), were analyzed as two-way ANOVAs at each region. Cortically, NVHLs produced decreased activation exclusively in vmPFC ($F(1,27)=4.4$, $p<0.05$). Cocaine history had no cortical effects. In contrast, sub-cortically, both NVHLs and cocaine history produced increased activation only in the dlCAPU (lesion: $F(1,27)=5.0$, $p<0.05$); drug history: $F(1,27)=4.8$, $p<0.05$); lesion x drug history: $F(1,27)=0.37$, $p=0.55$). NVHLs and cocaine history had no statistical interactions in any brain region. As further suggested by simple post-hoc, same region comparisons between each of the three treatment groups and the SHAM-SAL group (Fig. 3B), and representative micrographs (Fig. 4), NVHLs decreased vmPFC activation regardless of cocaine history, but compounded with cocaine history-increases in dlCAPU.

Regional volumes were not affected by cocaine history or interactions with NVHLs. However, NVHLs produced generalized volume loss spanning all regions (Table 1), reaching significance in vmPFC ($F(1,27)=5.6$, $p<0.05$), dmPFC ($F(1,27)=4.6$, $p<0.05$) and dlCAPU ($F(1,27)=5.0$, $p<0.05$). A separate stereological analysis of general cell population densities (see Section 4 in Supplement 1) examined regions where significant c-Fos differences emerged (vmPFC and dlCAPU). NVHLs tended to increase cell packing densities in both regions, most prominently in the vmPFC (NVHL: $11.8(\pm 0.7) \times 10^{-5}$ cells/ μm^3 ; SHAM: $10.7(\pm 0.7) \times 10^{-5}$ cells/ μm^3), although not significantly. Cocaine history also had no effects on cell densities.

Neural Activation and Behavior

Across 6 separate Pearson correlations, each including all subjects, only dlCAPU activation was highly correlated with post-injection locomotion (Table 2). To explore whether dlCAPU activation might represent a function of regional activations within PFC and NAC, we defined

measures of 'vmPFC hypo-activation' and drug history effects on striatal activation (d), which could be quantified for each rat.

We defined ($\{sMOC\}/\{vmPFC\}$) as a measure of 'vmPFC hypo-activation', since from cortical regions, sMOC was least affected by lesion (i.e. $\{sMOC\}$ was the best cortical standard). Calculating $\{sMOC\}/\{vmPFC\}$ for each rat provides a unit-less measure that increases with greater vmPFC hypo-activation. As shown in Fig. 5A, $\{sMOC\}/\{vmPFC\}$ was >1 for NVHLs, and was significantly greater than for SHAMs (two-tailed $t(29)=-3.6$, $p<0.01$) which was <1 .

With respect to cocaine history effects, activation levels declined dorsally and with regularity across striatal compartments, but cocaine history appeared to diminish the rate of decline, leading to parametrically significant drug history increases in dlCAPU (Fig. 5B). Quantifying this effect, in terms of how drug history increases the ratio of dorsal striatal to accumbens activation, we defined $d=\{dlCAPU\}/\{dlNAC\}$ on a per rat basis, giving means of $d_c=0.74$ for cocaine history rats, and $d_s=0.56$ for saline history rats. As d algebraically rearranges to $\{dlCAPU\}=d(\{dlNAC\})$, we set a relationship predictive of dlCAPU activation: $\{dlCAPU\}_p=d\{dlNAC\}$. Incorporating the vmPFC hypo-activation argument into this expression as a multiplicative modifier of $d\{dlNAC\}$ gives:

$$\{dlCAPU\}_p = d \{dlNAC\} (\{sMOC\} / \{vmPFC\})$$

$$\text{where } d = \begin{cases} 0.74 & (\text{cocaine history } (d_c)) \\ 0.56 & (\text{saline history } (d_s)) \end{cases}$$

Computing $\{dlCAPU\}_p$ for each rat and plotting it vs. their actual dlCAPU activations (Fig. 5C), produces a linear regression line $\{dlCAPU\}=(0.98\pm0.11)\{dlCAPU\}_p - 1.4$ that closely approximates an exact relationship expecting $\{dlCAPU\}=1\{dlCAPU\}_p+0$. This correlation was significant ($F(1,30)=86.0<0.001$ ($R=.865$)), explaining 75% of variation in the data. Although none of vmPFC, sMOC or dlNAC activations alone correlated with post-injection locomotion, their integration in $\{dlCAPU\}_p$ correlated with it significantly (Pearson (0.57, $p<0.01$)).

Discussion

This study reveals compounding effects of a mental illness model and addictive drug history on cortical-striatal circuit function and behavioral output. The vmPFC hypo-activation and volume atrophy in NVHL rats generally agrees with evidence for abnormalities in PFC neuronal function, excitatory and inhibitory neurotransmission, and deficits in neuroplasticity, and dendritic complexity in the NVHL model, and PFC atrophy and dysfunction in human schizophrenia (7,14,15). Meanwhile, sub-cortical cocaine history effects we observed likely reflect enduring alterations in striatal network activation incurred by prior cocaine history (5), since all rats received the same cocaine dose prior to sacrifice.

Across all subjects and brain regions, dlCAPU activation was exclusively and highly correlated with degree of behavioral sensitization consistent with (16), suggesting a prominent role for dorsal striatal activation in motoric effects of psychostimulants. Unlike the NAC, which receives cortical projections predominantly from PFC (11), the CAPU receives input from cortical motor neurons, firing in registry with them, corresponding to motor performance (17,18). Consistent with this data, and evidence that ventral cortical-striatal circuits process

motivational information that influences dorsal-cortical-striatal motor control and learning (4,12,13), our data suggests the dlCAPU operated as an output station where activations depend on representational states within PFC and NAC. Specifically, we found quantitative evidence that vmPFC hypo-activation operates like a multiplicative amplifier of drug history effects on the ratio of dlCAPU to dlNAC activation, correlating with behavioral output.

Further studies using differential lesions and drug regimens are needed to better understand our findings, and to define neuronal sub-populations where c-Fos expression changes occurred. Nevertheless, our results provide initial animal modeling data suggesting that PFC dysfunction and/or atrophy often identified in non-mentally ill-addicted populations (3,19,20), represents a key substrate-mechanism in the generation of dual diagnoses. These findings impart novel clinical/translational significance to basic research characterizing relationships between ventral and dorsal cortical-striatal circuits in the generation and adaptability of motivated behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

NVHL histology and cortical-striatal regions of interest for c-Fos analysis. **(A)** Representative micrographs of appropriate NVHL damage vs. SHAM control. **(B)** Location and sizes of NVHLs for rats included in the study; red depicts largest, and blue, smallest lesion extent. From initial sets of 24 NVHL and 20 SHAM rats, 5 NVHL-SAL and 4 NVHL-COC rats were excluded due to extra-hippocampal or unilateral damage; 1 SHAM-COC rat was excluded due to pronounced needle track damage. **(C)** Cortical and striatal regions of interest. Red lines indicate anatomical landmarks used to define rostral and caudal extents of sections of interest, and within sections, local boundaries of regions of interest. Cortical regions vmPFC, dmPFC and sMOC approximated infralimbic, anterior cingulate and supplementary motor areas respectively, while vmNAC, and dINAC approximated the shell and core regions of the NAC.

Sections of interest included those ranging from +3.20 to +1.70 cortically, and +2.80 and +1.45 sub-cortically (maps and coordinates relative to bregma are based on Swanson, Brian Maps, 2004). These selections yielded means of 11.5 cortical and 10.6 striatal hemi-sections. Brains yielding ≤ 6 cortical or ≤ 5 striatal sections (one each from SHAM-SAL, NVHL-SAL, and NVHL-COC groups) were excluded to optimize tissue-yield homogeneity. Only rats bearing appropriate histology and providing adequate tissue yield for stereological analysis (SHAM-SAL, N=9; NVHL-SAL, N=6; SHAM-COC, N=9; and NVHL-COC, N=7) were subsequently analyzed.

Figure 2.

Locomotor activation in the cocaine challenge session. **(A)** Across the 6×10 -minute pre-injection bins, all groups habituated to a similar low baseline, while cocaine injections (at arrow) produced sustained activations that differed by group over the 60-minute post-injection period. **(B)** Total post-injection distance revealed main effects (** $p < 0.01$) of lesion and drug history. SHAM-SAL, $N=9$; NVHL-SAL, $N=6$; SHAM-COC, $N=9$; and NVHL-COC, $N=7$ groups. Data are expressed as mean $\pm$ S.E.M.

Figure 3.

Regional neural activations as measured by c-Fos staining nuclear densities. **(A)** Cortically, main lesion effects in decreasing activation emerged in the vmPFC (* $p<0.05$). Drug history had no cortical effects but increased activation sub-cortically in the dlCAPU (* $p<0.05$) where a lesion main effect also emerged (* $p<0.05$). SHAM-SAL, $N=9$; NVHL-SAL, $N=6$; SHAM-COC, $N=9$; and NVHL-COC, $N=7$. Data are expressed as mean $\pm$ S.E.M. **(B)** Circuit diagrams show region-specific statistical activation trends of the drug and/or NVHL groups relative to those of the SHAM-SAL group (assigned as the overall control group), in terms of color-coded t-scores (from simple independent t-tests) comparisons (scale at right). Blue shades indicate hypo-activation while red shades indicate relative hyper-activation of c-Fos expression in same-region comparisons with the SHAM-SAL group. Small dark arrows represent direct glutamatergic projections while large open arrows/spirals represent parallel cortical-striatal-thalamo-cortical loops that propagate and transform motivational to motor information in a ventral to dorsal orientation.

Figure 4.

Representative micrographs (100x magnification) of cortical and striatal effects of lesion and drug history on c-Fos nuclear staining densities. Regions of interest are bounded by green lines for vmPFC and blue lines for dICAPU. A paucity of c-Fos nuclei in the vmPFC of NVHL compared to SHAM groups was evident for rats with either saline or cocaine histories. In dICAPU, NVHL-COC rats had the greatest c-Fos densities, SHAM-SAL rats the least, with NVHL-SAL and SHAM-COC falling intermediately.

Figure 5.

Quantitative relationships between degree of vmPFC hypo-activation, cocaine history-related effects, and dlCAPU activation, inclusive of all rats in the study (SHAM-SAL, N=9; NVHL-SAL, N=6; SHAM-COC, N=9; and NVHL-COC, N=7). Data are expressed as mean $\pm$ S.E.M. (A) Degree of vmPFC hypo-activation calculated as the ratio of sMOC to vmPFC activation on a per rat basis, was significantly increased in NVHLs compared to SHAMs (t-tailed t-test, $P < 0.05$) (lesion groupings are irrespective of drug history). (B) c-Fos activation densities of drug history groups across striatal compartments (drug groupings are irrespective of lesion status) suggests a progressively increasing separation following a dorsal orientation reaching significance at the dlCAPU (drug history: $P < 0.05$). Calculating $d =$ ratio of dlCAPU to dlNAC

activation densities on a per rat basis provides means of $d_c=0.74$ for cocaine history rats and $d_s=0.56$ for saline history rats. (C) Plot of actual dlCAPU activation vs. predicted CAPU activation ($\{CAPU\}_p$) for each rat calculated as the multiplicative product of their corresponding drug history effect value (d_c or d_s), dlNAC activation, and degree of vmPFC hypo-activation ($\{sMOC\}/\{vmPFC\}$), suggests differential levels of dlCAPU activation, as generated by cocaine vs. saline history, are further split into sub-levels by a multiplicative operation of vmPFC hypo-activation. Actual $\{dlCAPU\}$ and $\{dlCAPU\}_p$ were highly correlated ($***p<0.001$) and the linear regression line of $\{dlCAPU\} = 0.98\{dlCAPU\}_p - 1.39$ (solid line) closely approximates a line that demarks a perfect relationship between $\{CAPU\}_p$ and actual CAPU activation (dotted line). Units are c-Fos counts $\times 10^{-7}/\mu m^3$.

Table 1

Planimetric volumes ($\times 10^9 \mu\text{m}^3$) ($\pm$ SEM) of regions of interest by lesion status, and % volume declines due to NVHLs
All rats were included irrespective of drug history. Bolded comparisons were significant. % volume decline calculated per region as (((mean NVHL vol) - (mean SHAM vol))/mean SHAM vol) x 100%.

Group	VMPPFC	DMPFC	SMOC	VMNAC	DLNAC	DLCAPU
SHAM (N=18)	1.2$\pm$.04	1.9$\pm$.07	3.6 $\pm$.11	2.1 $\pm$.12	1.5 $\pm$.80	2.1$\pm$.12
NVHL (N=13)	1.0$\pm$.06	1.6$\pm$.10	3.4 $\pm$.20	1.8 $\pm$.15	1.4 $\pm$.12	1.7$\pm$.16
% volume decline	-16.7%	-15.8%	-5.5%	-14.3%	-6.7%	-19.0%

Table 2
Pearson correlations between post-injection locomotion and regional c-Fos activation density

All rats were included (SHAM-SAL, N=9; NVHL-SAL, N=6; SHAM-COC, N=9; and NVHL-COC, N=7) for each analysis, irrespective of lesion or drug history status.

Brain region	R	P
vmPFC	-0.247	0.18
dmPFC	-0.125	0.50
sMOC	0.195	0.29
vmNAC	-0.016	0.93
dINAC	0.124	0.51
dICAPU	0.649	<0.001