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Research Report

Cocaine reduces cytochrome oxidase activity in the prefrontal cortex and modifies its functional connectivity with brainstem nuclei

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ABSTRACT

Cocaine-induced psychomotor stimulation may be mediated by metabolic hypofrontality and modification of brain functional connectivity. Functional connectivity refers to the pattern of relationships among brain regions, and one way to evaluate this pattern is using interactivity correlations of the metabolic marker cytochrome oxidase among different regions. This is the first study of how repeated cocaine modifies: (1) mean cytochrome oxidase activity in neural areas using quantitative enzyme histochemistry, and (2) functional connectivity among brain regions using inter-correlations of cytochrome oxidase activity. Rats were injected with 15 mg/kg i.p. cocaine or saline for 5 days, which lead to cocaine-enhanced total locomotion. Mean cytochrome oxidase activity was significantly decreased in cocaine-treated animals in the superficial dorsal and lateral frontal cortical association areas Fr2 and Fr3 when compared to saline-treated animals. Functional connectivity showed that the cytochrome oxidase activity of the noradrenergic locus coeruleus and the infralimbic cortex were positively inter-correlated in cocaine but not in control rats. Positive cytochrome oxidase activity inter-correlations were also observed between the dopaminergic substantia nigra compacta and Fr2 and Fr3 areas and the lateral orbital cortex in cocaine-treated animals. In contrast, cytochrome oxidase activity in the interpeduncular nucleus was negatively correlated with that of Fr2, anterior insular cortex, and lateral orbital cortex in saline but not in cocaine groups. After repeated cocaine specific prefrontal areas became hypometabolic and their functional connectivity changed in networks involving noradrenergic and dopaminergic brainstem nuclei. We suggest that this pattern of hypofrontality and altered functional connectivity may contribute to cocaine-induced psychomotor stimulation.

1. Introduction

Cocaine is a powerful psychomotor stimulant and its abuse and subsequent addiction are persistent public health problems. Human studies have shown a hypofrontality produced

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by chronic use of cocaine (Volkow et al., 1988; London et al., 1990; Matochik et al., 2003; Bolla et al., 2004). In animal models, repeated exposure to cocaine results in a progressive and enduring enhancement in locomotion (Post, 1980; Wise and Bozarth, 1987; Stewart and Badiani, 1993) and changes in various brain circuits, especially lower metabolic activity in frontal cortical areas and ventral striatum (Robinson and Berridge, 1993; Porrino et al., 2007). This study was conducted to investigate whether cocaine-enhanced locomotion may involve systems-level alterations in the interactivity or functional connectivity of specific prefrontal areas. While anatomical connectivity refers to patterns of structural relationships among brain regions, functional connectivity refers to patterns of relationships in metabolic activity among brain regions (McIntosh and Gonzalez-Lima, 1994a,b; Nair et al., 1999). If the enhancement of locomotion by repeated cocaine exposure is an emergent property of affected prefrontal areas interacting with subcortical regions, understanding it requires a network analysis of the patterns of interaction between brain regions.

Network functional connectivity uses covariance analyses that cannot determine directionality but can describe the patterns of interaction between brain regions, as has been evaluated by inter-regional correlation changes in cytochrome oxidase activity (Sakata et al., 2000; Padilla et al., 2011). This particular functional connectivity method using inter-correlations of cytochrome oxidase activity describes stable metabolic relationships among areas, and it also describes how the regions are modified across sustained behavioral paradigms (Puga et al., 2007; Conejo et al., 2010; Fidalgo et al., 2012) or drug treatments (Padilla et al., 2011; Riha et al., 2011). Characterizing which specific neural systems modify their metabolic capacity and functional connectivity as a result of repeated cocaine exposure may advance our understanding of cocaine-enhanced locomotion.

Cytochrome oxidase (also called cytochrome *c* oxidase, ferrocytochrome *c*: O_2 oxidoreductase, EC 1.9.3.1, cytochrome aa3, or the respiratory enzyme) is a ubiquitous mitochondrial membrane integral protein responsible for the last step of the electron transport chain that catalyzes the transfer of electrons to oxygen, which serves to generate ATP via oxidative phosphorylation (Wong-Riley, 1989; Gonzalez-Lima and Garrosa, 1991). Neurons depend mostly on oxidative metabolism as an energy source. For this reason, the enzymatic activity of cytochrome oxidase is used as a metabolic marker for neuronal activity (Wong-Riley, 1989) and cytochrome oxidase enzyme histochemistry serves to map sustained changes in brain energy metabolism (Wong-Riley, 1989; Gonzalez-Lima and Garrosa, 1991; Hevner et al., 1993; Sakata et al., 2005). In particular, we have not seen acute effects on cytochrome oxidase histochemistry one hour after a single drug injection, but the longer-term oxidative capacity for energy metabolism (protein-synthesis-dependent enzyme induction over hours or days) of brain regions can be investigated using quantitative cytochrome oxidase histochemistry (Gonzalez-Lima and Cada, 1994; Padilla et al., 2011; Riha et al., 2011). However, to date, there has not been any cytochrome oxidase study in animals exposed to cocaine. Therefore, we were interested in using cytochrome oxidase to investigate altered relationships between neural areas after 5 days of cocaine exposure, rather than monitoring acute effects of cocaine exposure.

Quantitative enzyme histochemistry of cytochrome oxidase (Gonzalez-Lima and Cada, 1994; Gonzalez-Lima, 1998) has been used successfully in over a hundred previous studies to map alterations in brain oxidative metabolism in numerous learning tasks and drug treatments (Poremba et al., 1997, 1998; Villarreal et al., 2002; Hu et al., 2006; Gonzalez-Pardo et al., 2008; O'Reilly et al., 2009; Conejo et al., 2010; Padilla et al., 2011; Rojas et al., 2012). Analysis of inter-regional correlations of cytochrome oxidase activity (Sakata et al., 2000; Padilla et al., 2011) between cortical and subcortical regions after cocaine administration, especially between the prefrontal cortex and monoaminergic nuclei, may also identify underlying initial brain effects of repeated cocaine.

2. Results

2.1. *Cocaine enhanced locomotion from days 1–5*

The behavioral protocol showed that rats treated with cocaine (15 mg/kg i.p.) for five days had an increase in total locomotion relative to saline-injected rats, two way ANOVA $F_{(9,110)}=3.20$ ($p<0.001$). Additionally on day 5, subjects injected with cocaine had a significantly ($p<0.05$) increased total locomotor activity (5621±533 pcc) when compared to day 1 (2966±552 pcc), two-way ANOVA $F_{(9,110)}=3.20$ ($p<0.05$). There was no significant change in total locomotor activity between day 1 (655±88) and day 5 (542±82) in saline-treated rats (Fig. 1).

2.2. *Prefrontal regions became hypometabolic after repeated cocaine*

Mean regional cytochrome oxidase effects of cocaine were focused on the prefrontal cortex. Cytochrome oxidase activity was significantly ($p<0.05$) decreased in cocaine-treated animals in the superficial layers of dorsal (Fr2) and lateral (Fr3) frontal cortex regions (DFS mean=217±8 and LFS mean=242±9) when compared to saline-treated animals (DFS mean=244±8 and LFS mean=265±6) (Fig. 2). Means and standard errors for all regions measured are reported in Table 1, which showed that the hypometabolic effect of repeated cocaine (15 mg/kg i.p. for 5 days) was specific to prefrontal cortical areas.

2.3. *Prefrontal regions increased their functional connectivity with noradrenergic and dopaminergic subcortical nuclei after repeated cocaine*

Specific prefrontal-subcortical nuclei inter-regional cytochrome oxidase correlations were significantly different between cocaine- and vehicle-treated animals (absolute value of $Z_{abs}>1.96$, $p<0.05$), indicating that cocaine had significant effects on the functional connectivity of these regions, as illustrated in Fig. 3. Inter-regional correlations of cytochrome oxidase activity showed significant cocaine effects focused on prefrontal regions and noradrenergic and dopaminergic nuclei