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Research Report

Developmental but not adult cannabinoid treatments persistently alter axonal and dendritic morphology within brain regions important for zebra finch vocal learning

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ABSTRACT

Prior work shows developmental cannabinoid exposure alters zebra finch vocal development in a manner associated with altered CNS physiology, including changes in patterns of CB_1 receptor immunoreactivity, endocannabinoid concentrations and dendritic spine densities. These results raise questions about the selectivity of developmental cannabinoid effects: are they a consequence of a generalized developmental disruption, or are effects produced through more selective and distinct interactions with biochemical pathways that control receptor, endogenous ligand and dendritic spine dynamics? To begin to address this question we have examined effects of developmental cannabinoid exposure on the pattern and density of expression of proteins critical to dendritic (MAP2) and axonal (Nf-200) structure to determine the extent to which dendritic vs. axonal neuronal morphology may be altered. Results demonstrate developmental, but not adult cannabinoid treatments produce generalized changes in expression of both dendritic and axonal cytoskeletal proteins within brain regions and cells known to express CB_1 cannabinoid receptors. Results clearly demonstrate that cannabinoid exposure during a period of sensorimotor development, but not adulthood, produce profound effects upon both dendritic and axonal morphology that persist through at least early adulthood. These findings suggest an ability of exogenous cannabinoids to alter general processes responsible for normal brain development. Results also further implicate the importance of endocannabinoid signaling to peri-pubertal periods of adolescence, and underscore potential consequences of cannabinoid abuse during periods of late-postnatal CNS development.

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1. Introduction

It has become clear that cannabinoid signaling plays an important modulatory role in establishing neuronal connectivity and morphology both during development and as a function of experience (reviewed by Soderstrom and Gilbert, 2012). This includes regulation of axonal migration (Berghuis et al., 2007) and dendritic structure (Hill et al., 2012). A distinct behavioral sensitivity to cannabinoid exposure during limited periods of peri-adolescent development is also now well-documented (reviewed by Schneider, 2008; Soderstrom and Gilbert, 2012) but physiological mechanisms responsible for these persistent, developmental effects on behavior remain poorly understood.

Zebra finch vocal learning is controlled by a well-characterized set of discrete, interconnected brain regions (reviewed by Mooney, 2009) that distinctly and densely express CB_1 cannabinoid receptors (Soderstrom et al., 2004; Soderstrom and Tian, 2006). As vocal development in these animals depends upon both successful progress through a sensitive period of development, and also sensorimotor practice and experience, we have hypothesized that previously-established cannabinoid-altered vocal learning (Soderstrom and Johnson, 2003; Soderstrom and Tian, 2004) may be attributable to disruption of endocannabinoid control of associated changes in neuronal morphology. This hypothesis is supported by evidence that developmental cannabinoid exposure persistently alters CB_1 receptor staining patterns and endocannabinoid levels in the CNS, and densities of dendritic spines within at least some brain regions important to song learning and control (Gilbert and Soderstrom, 2011; Soderstrom and Tian, 2008; Soderstrom et al., 2011). Importantly, all of these effects are restricted to developmental exposure, and are not produced by similar treatment of adults. Altered neuronal morphology following developmental cannabinoid treatment may be due to selective pharmacological disruption of biochemical processes, such as those controlling dendritic spine dynamics (Frost et al., 2010), or may be due to interaction with more general, non-selective or activity-dependent developmental mechanisms. Experiments described herein were designed to begin to address these possibilities.

Using antibodies against structural, cytoskeleton-associated proteins with expression largely restricted to dendrites (MAP2, Huber and Matus, 1984) or axons (Nf-200, Marszalek et al., 1996) we studied expression patterns and densities as a function of developmental vs. adult treatments. This permitted generalized changes in dendritic and/or axonal neuronal structures to be appreciated. Treatment differences were observed within several song control regions of zebra finch telencephalon important to vocal learning and production.

2. Results

2.1. Western blotting

Western blotting was performed to assess the selectivity of the monoclonal antibodies directed against the two neurocytoskeletal proteins used in immunohistochemistry experiments. SDS-PAGE separation of 30 μg of brain cytoskeletal fractions revealed the presence of a single predominant band of approximately 200 kDa labeled by the anti-phosphorylated Nf-200 antibody (Fig. 1) Two predominant protein bands consistent with high-molecular weight isoforms of MAP2 (MAP2a and MAP2b, at approximately 230 kDa), and the lower molecular weight isoforms of MAP2 (MAP2c and MAP2d, at approximately 80 kDa) were labeled by the anti-MAP2 antibody used. The sizes of these selectively labeled proteins are similar to those reported from other mammalian species, including mouse, rat, horse and human (Alexanian et al., 2008; Russo et al., 2012; Saraceno et al., 2012).

2.2. Patterns of Nf-200 staining

Regions of telencephalon (HVC used as a proper name, robust nucleus of arcopallium [RA], lateral magnocellular nucleus of anterior nidopallium [lMAN], Area X of striatum [Area X]); thalamus (nucleus ovoidalis [Ov], medial portion of the dorsolateral thalamus [DLM]); and cerebellum (CER) were studied. General Nf-200 staining patterns, and relative anatomical positions of the telencephalic song regions studied (HVC, RA, lMAN, Area X) are illustrated as a function of developmental treatments in Fig. 2.

2.2.1. Song regions within caudal telencephalon

Distinct, dense Nf-200 staining patterns were observed within HVC, relative to that seen within surrounding regions of

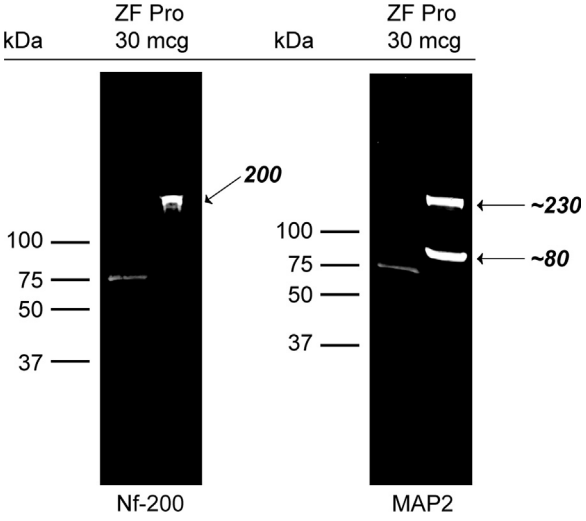

Fig. 1 – Western blotting demonstrates selectivity of Nf-200 and MAP2 antibody interactions with zebra finch brain proteins. Following separation and transfer of 30 μg zebra finch protein to membranes and exposure to NF-200 and MAP2 antibodies, near IR-labeling of bands of expected sizes were observed with very little background. Molecular weight markers were run in left lanes, and 75 kDa bands are apparent. Arrows indicate bands of expected size for both phosphorylated Nf-200 (~200 kDa) and MAP2 that consists of two alternative splicing isoforms of ~80 and 230 kDa (Loveland et al., 1999).