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Research report

Chronic CB1 cannabinoid receptor antagonism persistently increases dendritic spine densities in brain regions important to zebra finch vocal learning and production in an antidepressant-sensitive manner

Tessa L. Holland, Ken Soderstrom *

Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC 27834, USA

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ABSTRACT

During typical late-postnatal CNS development, net reductions in dendritic spine densities are associated with activity-dependent learning. Prior results showed agonist exposure in young animals increased spine densities in a subset of song regions while adult exposures did not, suggesting endocannabinoid signaling regulates dendritic spine dynamics important to vocal development. Here we addressed this question using the CB1 receptor-selective antagonist SR141716A (SR) to disrupt endocannabinoid signaling both during and after vocal learning. We hypothesized antagonist exposure during vocal development, but not adulthood, would alter spine densities. Following 25 days of exposure and a 25 day maturation period, 3D reconstructions of Golgi-Cox stained neurons were used to measure spine densities. We found antagonist treatments during both age periods increased densities within Area X (basal ganglia) and following adult treatments within HVC (premotor cortical-like). Results suggest both inappropriate cannabinoid receptor stimulation and inhibition are capable of similar disregulatory effects during establishment of circuits important to vocal learning, with antagonism extending these effects through adulthood. Given clinical evidence of depressant effects of SR, we tested the ability of the antidepressant monoamine oxidase inhibitor (MAOI) phenelzine to mitigate SR-induced spine density increases. This was confirmed implicating interaction between monoamine and endocannabinoid systems. Finally, we evaluated acute effects of these drugs to alter ability of novel song exposure to increase spine densities in auditory NCM and other regions, finding when combined, SR and phenelzine increased densities within Area X. These results contribute to understanding relevance of dendritic spine dynamics in neuronal development, drug abuse, and depression.

1. Introduction

Zebra finches are useful for studying vocal development because they learn a complex song, through sensorimotor integration and auditory feedback, in a process that shares features with acquisition of human language (Doupe and Kuhl, 1999). Prior work has shown that CB1 cannabinoid receptor expression is distinctly dense within brain regions that control learning and production of song, implying a role in vocal development (Soderstrom and Johnson, 2001). Developmental treatment with cannabinoid agonist alters zebra finch vocal learning by reducing the stereotypy of song motifs and reducing the number of distinct note types produced (Soderstrom and Johnson, 2003). Reduced note types are associated with fewer notes derived from tutors and increased production of improvised types (Soderstrom and Tian, 2004). Dramatic changes in CB1 receptor expression levels occur over normal zebra finch development: low densities are observed during the auditory learning stage (25 day olds); peak dense expression occurs during sensorimotor learning (50 and 75 days) and; levels wane to approximately that of the auditory stage in adulthood (>100 days, Soderstrom and Tian, 2006). Agonist treatment during this period also results in persistent changes to increase dendritic spine densities and expression of synaptic markers, suggesting that these changes are involved in the mechanism of cannabinoid-altered vocal learning (Gilbert and Soderstrom, 2014, 2011).

Abbreviations: CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; SR, SR141716A; MAOI, monoamine oxidase inhibitor; HVC, used as a proper name; Area X, Area X of striatum; DLM, nucleus dorsolateralis anterior, pars medialis; IMAN, lateral magnocellular nucleus of the anterior nidopallium; NCM, caudal medial nidopallium; RA, robust nucleus of the arcopallium.

* Corresponding author.
E-mail address: soderstromk@ecu.edu (K. Soderstrom).

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Processes important to CNS development during late-postnatal development include activity- and experience-dependent establishment of synaptic networks during brain maturation. In cortical regions of rodents (Blue and Parnavelas, 1983; De Felipe et al., 1997) and primates (Bourgeois and Rakic, 1993; Huttenlocher, 1990) development is associated with a general profusion of synaptic contacts followed by a reduction of spine densities to adulthood. In songbirds, similar processes occur in at least one cortical-like region necessary for zebra finch vocal learning (lMAN, Nixdorf-Bergweiler et al., 1995a). Importantly, these developmental spine density reductions are inhibited by manipulations that alter normal vocal learning, including rearing in social isolation (Wallhäusser-Franke et al., 1995) and exposure to cannabinoid agonists (Gilbert and Soderstrom, 2011).

This ability of cannabinoid agonist to alter learning, behavior and neuronal morphology within song regions suggests that endocannabinoid signaling is an important regulator of vocal development. Such a role is further supported by clear mammalian evidence of endocannabinoid-mediated control over establishment of neural circuits (reviewed by Keimpema et al., 2011; Lee et al., 2016). To the extent that endocannabinoid signaling is important to development of neural circuits related to vocal development, we hypothesized that interfering with this system by antagonizing endocannabinoid receptor activity would alter dendritic spine densities in brain regions important to vocal learning and production. The experiments reported herein test this hypothesis by evaluating effects of the CB1-selective antagonist/inverse agonist SR141716A (SR) to alter dendritic spine densities in brain regions relevant to song learning (Area X, Sohrabji et al., 1990), auditory perception and memory (NCM, Yanagihara and Yazaki-Sugiyama, 2016), and production (HVC, Nottebohm et al., 1976, see Fig. 1).

As clinical use of SR to treat obesity revealed that chronic cannabinoid antagonist exposure is associated with increased incidence of depression (Christensen et al., 2007) we also evaluated the ability of the MAOI antidepressant, phenelzine (a monoamine oxidase inhibitor [MAOI] class of antidepressant that enhances dopamine, norepinephrine and serotonin signaling) to block persistent spine density effects of cannabinoid antagonism in our system. In addition to comparing persistent effects of chronic treatments administered during development and in adulthood, we also evaluated acute effects of the drugs to alter responses following novel song exposure. We have used this approach previously to determine that CB1 activation interferes with the ability of novel song to rapidly increase dendritic spine densities within the auditory region, NCM (Gilbert and Soderstrom, 2013). Thus, the novel song paradigm provides a model system within which to study acute drug effects on dendritic spine dynamics.

2. Results

2.1. Chronic exposure experiments during development and adulthood

Prior chronic exposure experiments with the cannabinoid agonist WIN demonstrated that treatments during development, but not in adulthood, significantly increased dendritic spine densities in the vocal motor-associated region HVC and the region of basal ganglia, Area X (Gilbert and Soderstrom, 2011). This led us to test the hypothesis that treatments with the CB1-selective antagonist, SR, during similar treatment periods would alter, and perhaps lower, spine densities in these regions.

As described in the Methods section (4.7 below) we used a mixed-effects modeling statistical approach to analyze percent control spine density data. For chronic experiments, individual animals were treated as random subjects and individual neurons as

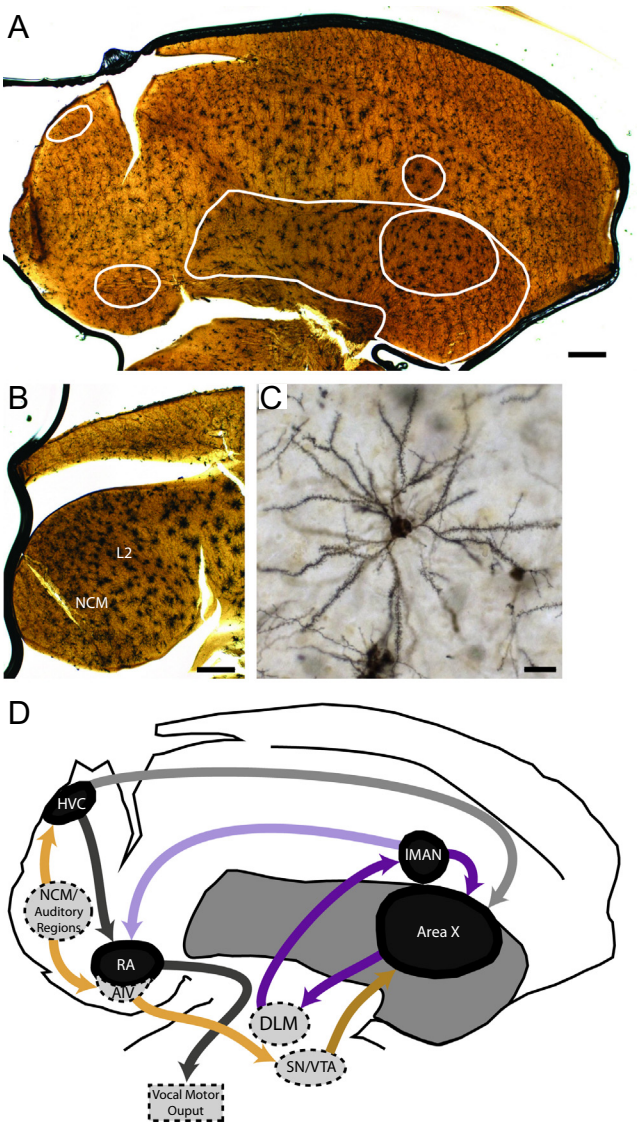

Fig. 1. Illustration of Golgi-Cox staining quality and locations of brain regions studied. **A**, Parasagittal section (~1 mm lateral of the midline) of a Golgi-Cox stained zebra finch brain (25X magnification) that contains song regions HVC, RA, Area X, and lMAN. Borders of song regions and striatum are traced in white (see labeling in panel D). **B**, A more medial parasagittal section (~0.15 mm lateral of the midline) imaged at 25X contains auditory regions NCM and L2. **C**, Higher power image (200X) illustrates a Golgi-Cox impregnated spiny neuron of the type used for dendritic spine density measurements. **D**, Camera lucida-type tracing of the section presented in panel A illustrates relative locations of, and a subset of relevant interconnections between, regions studied. **Black shading** corresponds to song regions traced in panel A (HVC, RA, lMAN, Area X) and **dark grey** shading indicates striatum. **Light grey** areas with dashed borders indicate relevant regions not present in the section from panel A. **Dark purple** arrows indicate connections of the anterior forebrain pathway (AFP), a cortico-basal ganglia-thalamic loop critical for sensorimotor vocal learning (reviewed by Perkel, 2004). **Light purple** arrow illustrates AFP output from lMAN to the vocal motor output region, RA (Bottjer et al., 1989). **Dark grey** indicates vocal motor pathways, **light grey** illustrates the output from pre-motor HVC to the basal ganglia region, Area X. **Light gold** arrows indicate relevant auditory input to the motor system (Kelley and Nottebohm, 1979; Vates et al., 1996) and from the ventral portion of the intermediate arcopallium (AIV) to dopaminergic neurons within substantia nigra (SN)/ventral tegmental area (VTA, Mandelblat-Cerf et al., 2014). **Dark gold** indicates SN/VTA dopaminergic projections to spiny interneurons within Area X (Ding and Perkel, 2002). In panels A and B rostral is right, dorsal is up and bars = 470 μm. In panel C bar = 30 μm. Abbreviations: DLM (nucleus dorsolateralis anterior, pars medialis), HVC (proper name), lMAN (lateral magnocellular nucleus of the anterior nidopallium), NCM (caudal medial nidopallium), RA (robust nucleus of the arcopallium). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)