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Neurochemical profiling of dopaminergic neurons in the forebrain of a cichlid fish, *Astatotilapia burtoni*☆

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ABSTRACT

Across vertebrates, the mesolimbic reward system is a highly conserved neural network that serves to evaluate the salience of environmental stimuli, with dopamine as the neurotransmitter most relevant to its function. Although brain regions in the dopaminergic reward system have been well characterized in mammals, homologizing these brain areas with structures in teleosts has been controversial, especially for the mesencephalo-diencephalic dopaminergic cell populations. Here we examine the neurochemical profile of five dopaminergic cell groups (Vc, POA, PPr, TPp, pTn) in the model cichlid *Astatotilapia burtoni* to better understand putative homology relationships between teleosts and mammals. We characterized in the adult brain the expression patterns of three genes (*etv5*, *nr4a2*, and *pitx3*) that either specify dopaminergic cell fate or maintain dopaminergic cell populations. We then determined whether these genes are expressed in dopaminergic cells. We find many striking similarities in these gene expression profiles between dopaminergic cell populations in teleosts and their putative mammalian homologs. Our results suggest that many of these dopaminergic cell groups are indeed evolutionarily ancient and conserved across vertebrates.

1. Introduction

Dopamine is an evolutionarily ancient neurotransmitter present in many eukaryotes and serves as a neuromodulator of many behavioral processes, such as learning and memory (Wise, 2004; Hyman et al., 2006), social behavior (Young et al., 2011; O'Connell and Hofmann, 2011a), and the selection of motor programs (Joshua et al., 2009; Vidal-Gadea et al., 2011). The functional contributions of various dopaminergic cell groups to behavioral patterns are well studied in laboratory rodents due to their established utility for understanding nervous system disorders (Koob and Volkow, 2010; Lodge and Grace, 2011; Plowman and Kleim, 2011). However, the evolutionary relationships of dopaminergic cell groups across vertebrates are not well understood as their location significantly varies across vertebrate lineages (Wullimann and Mueller, 2004; Butler and Hodos, 1996;

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Abbreviations: aTn, anterior tuberal nucleus; CP, central posterior thalamic nucleus; D, dorsal (pallial) part of the telencephalon; Dc, central part of D; Dc-2, subdivision of Dc; Dd, dorsal part of D; Dl, lateral part of D; Dld, dorsal region of Dl; Dlg, granular region of Dl; Dlv, ventral region of Dl; Dlvv, ventral zone of Dlv; Dm, medial part of D; Dm-1,3, subdivisions of Dm; Dm2c, caudal part of Dm-2; Dn, diffuse nucleus of the inferior lobe; Dp, posterior part of D; E, entopeduncular nucleus; Gn, glomerular nucleus; H, habenula; HC, horizontal commissure; LHn, lateral hypothalamic nucleus; LR, lateral recess; LT, longitudinal torus; mPGn, medial preglomerular nucleus; nLT, nucleus of the lateral torus; OB, olfactory bulb; OPT, optic tract; OT, optic tectum; PN, prethalamic nucleus; POA, preoptic area; PPd, dorsal periventricular pretectal nucleus; PPr, rostral periventricular pretectal nucleus; pTn, posterior tuberal nucleus; PVO, paraventricular organ; ST, semicircular torus; TPp, periventricular posterior tuberculum; V, ventral (subpallial) division of the telencephalon; Vc, central part of V; Vd, dorsal nucleus of V; Vdc, caudal region of Vd; VH, ventral hypothalamus; Vi, intermediate part of V; VM, ventromedial thalamic nucleus; Vp, postcommissural nucleus of V; Vs, supracommissural nucleus of V; Vsl, lateral region of Vs; Vsm, medial region of Vs; vTn, ventral tuberal nucleus; Vv, ventral part of V.

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Yamamoto and Vernier, 2011; O'Connell and Hofmann, 2011b, 2012). This is of particular relevance as non-mammalian model systems are becoming increasingly useful for studying the role of dopamine and other biogenic amines, especially with regard to questions that are not very tractable in rodent model systems, such as functional analyses of social networks (Winberg et al., 1997) and vocal communication (Sasaki et al., 2006; Huang and Hessler, 2008; Heimovics et al., 2009). Here we examine the neurochemical profiles of several teleost dopaminergic cell groups in order to better understand their putative homology relationships with mammalian dopaminergic cell groups.

The substantia nigra (SNc) and ventral tegmental area (VTA) are particularly well studied in mammals due to their central roles in regulating motor and behavioral decision-making, respectively (Mogenson et al., 1980; Berridge and Robinson, 1998; Platt, 2002; Sugrue et al., 2005; Balleine et al., 2007). The SNc regulates motor output and its dopaminergic cell population has been studied in much detail due to its role in motor deficits in disease phenotypes such as Parkinson's disease (Fearnley and Lees, 1991; Shulman et al., 2011; Wirdefeldt et al., 2011). On the other hand, the VTA is involved in risk-taking and reward evaluation (Schultz, 1998; Tobler et al., 2005; Preuschoff and Bossaerts, 2007) as well as the processing of nociception (Sotres-Bayón et al., 2001). In mammals, both of these cell groups are located in the mesencephalon and also extend into the basal diencephalon, have a common developmental origin, and thus have remarkably similar neurochemical and gene expression profiles (Grimm et al., 2004; Chung et al., 2005). In contrast, dopaminergic cell groups are located throughout the forebrain and hindbrain in teleosts, but not in the midbrain, which makes establishing functionally similar cell groups between the mammalian mesencephalic dopaminergic neurons and dopaminergic cell groups in teleosts exceedingly difficult (Wullimann and Mueller, 2004; O'Connell and Hofmann, 2011b; Yamamoto and Vernier, 2011). Nevertheless, several research groups have recently made significant strides toward elucidating the functionally analogous dopaminergic cell groups in teleosts and mammals (Yamamoto and Vernier, 2011; Schweitzer et al., 2011). Yet despite this progress it is still unclear which cell groups in the teleost brain are functionally analogous to the mammalian SNn and VTA. To investigate the putative relationships between mammalian and teleost dopaminergic cell populations, we examined the expression profiles of three transcription factors (*etv5*, *pitx3*, and *nr4a2*) involved in differentiation and maintenance of dopaminergic cells.

Much effort has been made to characterize the genomic contributions to dopamine neuron specification by searching for conserved motifs of dopamine pathway genes across species (Hobert et al., 2010; Fujimoto et al., 2011). Flames and Hobert (2009) first proposed a regulatory logic and conserved "DA motif" in *C. elegans*, which appeared to be conserved in mammals. Specifically, the "Ets-related" family of transcription factors was found to determine dopaminergic cell fate in *C. elegans* (*via ast-1*) and mouse olfactory dopamine neurons (*via etv1*). Following this regulatory logic, the Etv variant *etv5* expressed in the mammalian midbrain dopaminergic neurons (Gray et al., 2004) may be involved in regulating midbrain dopaminergic cell fate in vertebrates (although this may not be the case in *Mus musculus*; see Wang and Turner, 2010). The brain distribution of *etv5* has not been determined outside of rodents and thus it is unknown whether *etv5* is expressed in the SNc/VTA populations of other taxa.

In mammals, *nr4a2* (also known as *nurr1*) and *pitx3* are two important transcription factors that play crucial roles in the maintenance of midbrain dopaminergic neurons. Although not required for midbrain dopaminergic neuron development in mammals, *nr4a2* is essential for maintenance and transmitter synthesis and release (Smits et al., 2003; Kadkhodaei et al., 2009). Importantly, knockdown of *nr4a2* disrupts dopamine neuron maturation in zebrafish (*Danio rerio*; Luo et al., 2008). Nr4a2 directly regulates the expression of *pitx3* (Volpicelli et al., 2012), which is specifically required for terminal differentiation and maintenance of SNc neurons (Smidt et al., 1997, 2004) as *pitx3* knockout in mice results in ablation of dopaminergic SNc neurons, but VTA dopaminergic neurons are less affected (Smidt and Burbach, 2007). In comparison, the neural distribution, colocalization in dopaminergic cells, and functional relevance of *nr4a2* and *pitx3* are poorly understood in teleosts.

The cichlid family of fishes offers unique opportunities for comparative studies of the complex and plastic behavior patterns involved in behavioral decision-making and motor output, as rapid radiation of species with diverse social phenotypes allows comparison across closely related species (Hofmann, 2003; Kocher, 2004). Here we use the cichlid *Astatotilapia burtoni*, a model system in social neuroscience (Robinson et al., 2008), which is ideally suited for studying how the social environment influences phenotypic plasticity. A better understanding of the dopaminergic systems in this cichlid will allow us to not only gain insights into the neural substrates of social decision-making, but also provide an excellent model for determining how manipulations of specific dopaminergic cell populations alter social behavior, risk-taking, and motor output within ecologically relevant contexts.

We have previously described the distribution of dopamine-associated genes (tyrosine hydroxylase and dopamine D1 and D2 receptors) in *A. burtoni* (O'Connell et al., 2011), and here we expand on that work by examining the neurochemical profiles of five dopaminergic cell groups that are hypothesized to regulate behavior and motor output in teleosts (Schweitzer et al., 2011). Specifically, we examined dopaminergic populations in the central part of the ventral telencephalon (Vc), the preoptic area (POA), the rostral periventricular pretectal nucleus (PPr), the periventricular nucleus of the posterior tuberculum (TPp), and the posterior tuberal nucleus (pTn). Of particular interest are the TPp and pTn, which are candidate dopaminergic cell groups in teleosts that may be functionally analogous to the mammalian SNc/VTA or other mammalian diencephalic dopamine groups (Rink and Wullimann, 2001; Filippi et al., 2010; Yamamoto and Vernier, 2011; Tay et al., 2011). Importantly, the mammalian VTA and SNc do indeed extend into the basal diencephalon, up to the ventral part of the third prosomere, supporting their putative homology to the TPp in teleosts, which is also located mostly in the third prosomere (Wullimann and Rink, 2002). Our overall objective is to gain insight into which dopaminergic cell groups in the teleost forebrain may be neurochemically homologous to the mammalian midbrain dopaminergic cell groups by examining expression patterns of *etv5*, *nr4a2*, and *pitx3*, which encode transcription factors of known importance in dopaminergic cell specification or maintenance.

2. Methods

2.1. Animals

Male and female *A. burtoni* descended from a wild-caught stock population were kept in aquaria under conditions mimicking their natural environment as previously described (O'Connell et al., 2011). All work was carried out in compliance with the Institutional Animal Care and Use Committee at The University of Texas at Austin.

2.2. Cloning of A. burtoni etv5, nr4a2, and pitx3 cDNA

To obtain the *A. burtoni etv5*, *nr4a2*, and *pitx3* sequences, degenerate primers were designed using CODEHOP (http://blocks.fhcrc.org/codehop.html) based on the zebrafish protein sequences (GenBank accession numbers: Etv5, AAT68296; Nr4a2, NP_001106956.1; Pitx3, NP_991238.1) and homologous sequences from stickleback (*Gasterosteus aculeatus*), medaka (*Oryzias latipes*), and fugu (*Takifugu rubripes*) obtained using the UCSC Genome Browser (http://genome.ucsc.edu/).