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The use of fish models to study human neurological disorders

Hideaki Matsui[a,b,*]

[a] Department of Neuroscience of disease, Center for Transdisciplinary Research, Niigata University, 757, Ichibancho, Asahimachidori, Chuo-ku, Niigata-shi, Niigata, 951-8585, Japan
[b] Brain Research Institute, Niigata University, 757, Ichibancho, Asahimachidori, Chuo-ku, Niigata-shi, Niigata, 951-8585, Japan

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ABSTRACT

Small teleost fish including zebrafish and medaka have been used as animal models in basic science research due to the relative ease of handling and transparency during embryogenesis. Current advances in genetic engineering and progress in disease genetics allowed utilization of these fish to study neurological diseases and psychiatric disorders. This review summarizes the advantages and disadvantages of using fish for neuropsychiatric research using primarily our own studies as examples. We discuss how fish belong to a class of vertebrates, are feasible for imaging, and include diverse species with multiple research possibilities yet to be discovered.

1. Introduction

Small teleost fish have been used as animal models in developmental biology, comparative anatomy, and physiology due to the relative ease of handling and transparency during embryogenesis (Aida, 1921; Streisinger et al., 1981; Wittbrodt et al., 2002). In the field of neuroscience, various studies have been intensively pursued including studies of the motor system, the visual system, the cerebellum, neuroendocrinology, learning and memory, and emotions (Friedrich et al., 2010; Rinkwitz et al., 2011). Among the small teleost fish, zebrafish (*Danio rerio*) are commonly used in studies worldwide and medaka fish (*Oryzias latipes*) are commonly used in Japan and Europe.

Our group has used small teleost fish since 2006 to develop new models of neurological disorders. Back then, attempts to use fish in the research of neuroscience of diseases were not very popular. Currently, advances in genetic engineering, progress in disease genetics, and popularization of confocal microscopy and behavioral assays have enabled many researchers to use zebrafish models, not only for modeling neurological diseases, but also for elucidating mechanisms of psychiatric disorders (Kabashi et al., 2010; Norton, 2013).

This review summarizes the advantages and disadvantages of fish for use in neuropsychiatric research, mainly taking examples from our previous work, and discusses the future prospects of fish research in the study of human neurological disorders.

2. Fish belong to the vertebrate class

Zebrafish and medaka are types of fish and are therefore vertebrates. Although there are many small invertebrate animal models including flies (*Drosophila melanogaster*) and worms (*Caenorhabditis elegans*), small teleost models are especially useful. *Homo sapiens* are also vertebrates and possess many similar structures and functions to fish. Thus, basic neuronal structures and functions are well preserved from fish to humans (Northcutt, 2002). When discussing molecular functions or cellular phenotypes, differences between vertebrates and invertebrates may not matter in some cases, but when studying neuronal networks, the fact that fish is a vertebrate becomes a large advantage. It is especially important for a disease model to have similar neuronal structures to human, considering that human neurological and psychiatric disorders display phenotypes and pathologies based on neurophysiology and neuroanatomy that consist of neuronal and glial networks (Braak et al., 2003).

Our group first focused on Parkinson's disease models partly because it was not easy to model this disease using mice, the most common animal model. Parkinson's disease patients display movement disorders, dopamine neurodegeneration, and inclusion bodies named Lewy bodies, and the pathogenesis is largely

* Corresponding author at: Department of Neuroscience of disease, Center for Transdisciplinary Research, Niigata University, 757, Ichibancho, Asahimachidori, Chuo-ku, Niigata-shi, Niigata, 951-8585, Japan.
E-mail address: hide0729@kuhp.kyoto-u.ac.jp

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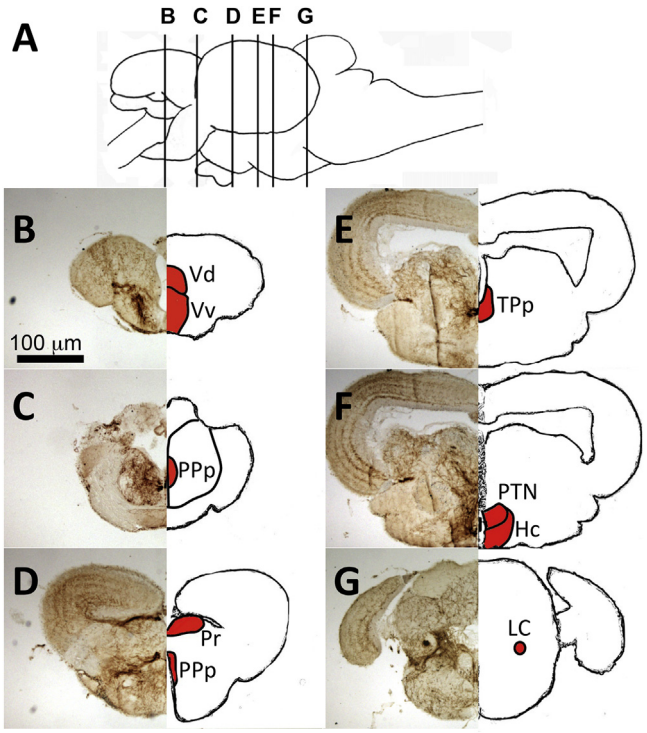

Fig. 1. Atlas of the medaka dopamine and noradrenaline neurons. (A) Sectioning positions of (B–G), the left is rostral and the right is caudal. (B) Dopamine neurons in the telencephalon (C–F) Dopamine neurons in the diencephalon The dopamine neurons in (E) show large cell body and are vulnerable to various toxins or genetic manipulations. (G) Noradrenaline neurons in the locus coeruleus. These neurons are also large and vulnerable. Figure reproduced from Matsui et al. (2009) with modification. Hc: caudal zone of periventricular hypothalamus, LC: locus coeruleus, PPp: parvocellular preoptic nucleus, posterior part, Pr: pretectum, PTN: posterior tuberal nucleus, TPp: periventricular nucleus of posterior tuberculum, Vd: dorsal nucleus of ventral telencephalic area, Vv: ventral nucleus of ventral telencephalic area.

unknown (Fahn, 1995). Mice deficient in three causative genes for familial forms of Parkinson's disease (*PINK1*, *Parkin*, *DJ-1*) do not demonstrate dopaminergic neurodegeneration (Kitada et al., 2009). The network of zebrafish dopaminergic neurons was previously reported (Rink and Wullimann, 2004), and our group examined medaka dopamine neurons, which illustrated a similar network of dopaminergic neurons (Matsui et al., 2009) (Fig. 1). The next important question to be asked was whether small teleost fish possess structures equivalent with human nigral dopamine neurons.

We used 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-hydroxydopamine (6-OHDA) to examine whether fish would indeed suffer from Parkinson's disease because these toxins have been well studied and are reportedly toxic to dopaminergic neurons in many animal models (Jenner and Marsden, 1986; Przedborski et al., 2001; Ungerstedt, 1968). The human substantia nigra, which is prone to neurodegeneration in Parkinson's disease among dopaminergic neuronal clusters, is located in the midbrain; however, dopaminergic neurons are not present in the midbrain of fish, rather they are abundant in the diencephalon and form several clusters. Among these dopaminergic clusters, neurons have relatively large cell bodies in the posterior tuberculum and are markedly vulnerable to MPTP or 6-OHDA (Matsui et al., 2009; Matsui et al., 2010a). Similarly, noradrenergic neurons in the locus coeruleus are prone to cell death caused by these toxins. Medaka fish exposed to these toxins show reduced spontaneous swimming movements similar to human Parkinson's disease patients (Matsui et al., 2009; Matsui et al., 2010a). Thus the dopamine cluster in the teleost posterior tuberculum may correspond to the substantia nigra from the aspect of cellular vulnerability (Matsui et al., 2012; Matsui et al., 2014c). Furthermore, this diencephalic cluster was demonstrated to project long axons into the striatum in fish (Tay et al., 2011). Recently, laser ablation of the same cluster was reported to induce reduced spontaneous swimming movements (Jay et al., 2015), indicating that this dopamine cluster has similar physiological roles compared to those in the human substantia nigra.

Genetically modified animals offer an alternative to neurotoxins for the development of models for Parkinson's disease. When we began using small teleost fish, well-designed gene modifying technologies such as transcription activator-like effector nuclease (TALEN), clustered regularly interspaced short palindromic repeats-associated proteins 9 (CRISPR-Cas9), and zinc finger nuclease (ZFN) did not exist. Therefore, we utilized targeting induced local lesions in genomes (TILLING) library of medaka developed by Dr. Taniguchi et al. (Taniguchi et al., 2006). Briefly, medaka males were exposed to N-ethyl-N-nitrosourea (ENU), and then crossed with wild type females to make F1 generation. The genome DNA and sperms were preserved in a corresponding manner from male F1 fish, and we were able to retrieve sperms with desirable mutations by genome sequencing. Through artificial fertilization, we are able to obtain the necessary heterozygous mutants. We reported the phenotypes of *pink1*, *parkin*, *atp13a2*, or *gba* mutant medaka fish; each gene is linked to familial forms of Parkinson's disease or risk alleles for idiopathic Parkinson's disease (Uemura et al., 2015; Matsui et al., 2013a; Matsui et al., 2013b; Matsui et al., 2010b). Dr. Bandmann's group at the University of Sheffield reported *pnik1* or *gba* mutants in zebrafish phenotype created using TILLING (Flinn et al., 2013; Keatinge et al., 2015). We emphasize the fact that dopaminergic neurons located in the posterior tuberculum and noradrenergic neurons located in the locus coeruleus are prominently affected in all the mutants.

In summary, compared to human neurons, teleost dopaminergic and noradreneric neurons not only show similar anatomical networks, but also display similar vulnerabilities and exhibit corresponding physiological roles. These are very important advantages when using fish to model Parkinson's disease.

Next, we will discuss the cerebellum of teleost fish. There have been many reports regarding the anatomy and connections of the fish cerebellum, especially in small teleost fish. Reviews by Dr. Hibi's group in Nagoya University contain detailed and comprehensive descriptions (Hashimoto and Hibi, 2012; Bae et al., 2009). Fiber connections, which consist of climbing fibers and parallel fibers, are resembling between humans and fish; and subtypes of neurons are also mostly preserved. One difference is that there is no deep cerebellar nucleus in teleost fish. However, eurydendroid cells located in the Purkinje layer receive inputs from Purkinje neurons and are considered the functional equivalent of human deep cerebellar nuclei (Pouwels, 1978).

The human cerebellum can be divided into three parts based on both phylogenetic and functional criteria. These three parts are the vestibulocerebellum (archicerebellum), spinocerebellum (paleocerebellum), and cerebrocerebellum (neocerebellum, pontocerebellum). Most of the cerebellum in fish is regarded as the vestibulocerebellum. Our group investigated the efferent neurons of the teleost cerebellum by using various tracing methods. We found that many Purkinje neurons project to the vestibular system directly or via eurydendroid cells. However, there was a cluster of Purkinje–eurydendroid cells located in the rostro-medial part of the teleost cerebellum that sends long projections to the spinal cord, red nucleus, thalamus, and reticular formation. During swimming movements induced by the optomotor response, the same region in the rostro-medial part of the Purkinje layer showed increased neuronal activity. On the other hand, during eye movements induced by optokinetic response, the caudal part of