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Aquatic toxicology of fluoxetine: Understanding the knowns and the unknowns

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ABSTRACT

Fluoxetine is one of the most prescribed psychotropic medications, and is an agent of increasing interest for environmental toxicology. Fish and other aquatic organisms are excellent models to study neuroactive small molecules like fluoxetine. However, prone to variance due to experimental factors, data obtained in these models need to be interpreted with caution, using proper experimental protocols, study designs, validated endpoints as well as well-established models and tests. Choosing the treatment protocol and dose range for fluoxetine and other serotonergic drugs is critical for obtaining valid test results and correct data interpretation. Here we discuss the value of aquatic models to study fluoxetine effects, based on prior high-quality research, and outline the directions of future translational studies in the field. We review fluoxetine-evoked phenotypes in acute vs. chronic protocols, discussing them in the contact of complex role of serotonin in behavioral regulation. We conclude that zebrafish and other aquatic models represent a useful *in-vivo* tool for fluoxetine pharmacology and (eco)toxicology research.

1. Introduction

Fluoxetine (Prozac) is a potent psychotropic drug, acting as a selective serotonin reuptake inhibitor (SSRI) to block the plasma membrane serotonin transporter, SERT (Fabbri et al., 2014; Kalueff et al., 2010; Murphy and Lesch, 2008; Stewart et al., 2013). SSRIs are currently the most prescribed psychotropic medications, and fluoxetine is the most commonly used SSRI. Therefore, fluoxetine has rapidly become one of the most important drugs in biomedicine. With the growing number of disorders treated by fluoxetine/SSRIs (from anxiety to depression and obsessions), both the annual intake and the number of patients taking these drugs, are rapidly rising. In addition to desired antidepressant effects, this also results in increased incidence of serotonin toxicity—a potentially lethal toxidrome associated with an overdose and/or combination of serotonergic drugs (Bertorini, 1997; Haberzettl et al., 2013; Kalueff et al., 2008). Paralleling clinical data, multiple experimental animal models, ranging from rodents (Haberzettl et al., 2013; Kalueff et al., 2008, 2010) to aquatic species (Egan et al., 2009; Stewart et al., 2013), have been developed to address various aspects of SSRI antidepressant action and toxicity.

Because of their increasing usage globally, fluoxetine and other SSRIs also represent a growing concern for environmental biology and aquatic toxicology (Brooks, 2014; Clements and Schreck, 2007; Dzieweczynski and Hebert, 2012; Fent et al., 2006; Fernandes et al., 2011; Kohlert et al., 2012; Lajeunesse et al., 2011; Mennigen et al., 2010a,b, 2011; Morando et al., 2009; Ramirez et al., 2009; Schultz et al., 2010, 2011; Silva et al., 2014; Sumpter et al., 2014; Sumpter and Margiotta-Casaluci, 2014; Weinberger and Klaper, 2014; Winder et al., 2012).

We have read with interest a recent thoughtful paper in this Journal by Sumpter and colleagues, evaluating the potency of fluoxetine in various aquatic species (Sumpter et al., 2014). As we welcome in-depth analyses of pharmacology and toxicology of this drug (Sumpter and Margiotta-Casaluci, 2014), the field may benefit from further critical discussion of this topic.

Our present contribution to this discussion will be limited to zebrafish (*Danio rerio*), an aquatic vertebrate species we have worked with extensively, testing SSRIs. This species also represents an excellent model for translational neuroscience of complex human brain disorders (Kalueff et al., 2014a,b; Stewart et al., 2014), and is particularly useful for studying genetic (Griffiths et al., 2012; Ziv et al., 2012) and pharmacological mechanisms (Nguyen et al., 2014) of depression and antidepressant action. Finally, zebrafish have been suggested as a sensitive *in-vivo* neurotoxicological and ecotoxicological screen for various serotonergic drugs, including fluoxetine and other SSRIs (Grossman et al., 2010; Maximino et al., 2013a; Neelkantan et al., 2013; Sackerman et al., 2010; Stewart et al., 2011a, 2013).

2. Effects of fluoxetine: lessons from anxious fish

An important distinction should be made, when one tries to understand the effects of fluoxetine *in-vivo*, between acute and chronic treatments. Acute treatment is not of main interest for translational research, because fluoxetine does not produce therapeutic effects acutely, but may trigger toxicity at high doses (Kalueff et al., 2008; Murphy and Lesch, 2008; Stewart et al., 2013). At the same time, acute fluoxetine treatment can be used as an important

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neuropharmacological tool, to modulate brain neurotransmission and examine the role of serotonin in behavior (see further).

In contrast, chronic fluoxetine treatment is highly relevant to both aquatic toxicology (as environmental exposure tends to be chronic rather than acute) and translational neuroscience (because pharmacotherapy of psychiatric disorders is mainly chronic). Our studies were first to administer chronic fluoxetine to adult zebrafish, in order to mimic anxiolytic effects of this compound in humans and rodents (Egan et al., 2009; Maximino et al., 2011). For example, in the novel tank test, we reported distinct swimming patterns (generated by a video-tracking software) for fish treated with 0.1–0.15 mg/L fluoxetine for 2 weeks. The recorded locomotor traces revealed marked differences in overall exploration and swimming activity, as control zebrafish swam mostly at the bottom of the tanks, and fluoxetine evoked the opposite, anxiolytic-like 'top swimming', which was also accompanied by lowered cortisol (Cachat et al., 2010b; Egan et al., 2009). In the light–dark test, treatment with fluoxetine for 2 weeks also decreases dark preference, consistent with an anxiolytic profile of this drug (Maximino et al., 2014a). Paralleling well-known clinical anti-stress effects of fluoxetine, this response was corroborated by several laboratories using fluoxetine treatment to reduce stress in zebrafish (Abreu et al., 2014; Griffiths et al., 2012; Maximino et al., 2011, 2014b, 2013a,b; Wong et al., 2013; Ziv et al., 2013). Subsequent sophisticated behavioral analyses also found marked similarities between fluoxetine and other serotonergic drugs on zebrafish behavior (Cachat et al., 2011; Neelkantan et al., 2013; Stewart et al., 2013).

Various behavioral tasks have been validated for assessing anxiolytic-like responses in zebrafish (Blaser and Rosemberg, 2012; Echevarria et al., 2011b; Rosemberg et al., 2011; Stewart et al., 2011b, 2014). For example, as already mentioned, the novel tank test is useful for testing zebrafish vertical exploratory activity, where the increase in some endpoints (e.g., time spent at top, number of entries to top, the latency to enter the top) is usually attributed to decreased anxiety. The light–dark test is another task that has been used to measure anxiety-like behaviors, in which the time spent in lit area also correlates with decreased anxiety (Maximino et al., 2011, 2010, 2014b). Analyzing the role of serotonergic neurotransmission, the blockade of serotonin uptake acutely reduces bottom-dwelling, but increases white avoidance in adult zebrafish (Maximino et al., 2014b; Sackerman et al., 2010). These apparently inconsistent data suggest that different motivational states may drive fish behavior in both tasks. For instance, while the main aspect of zebrafish novel tank response is the escape away from the surface, fish in the light–dark test may be driven by their natural aversion to lit areas (Blaser and Rosemberg, 2012; Rosemberg et al., 2011). Similarly to mammals, a dual context-specific role of serotonin may exist for teleosts, in which acute treatment with SSRIs may both increase anxiety-like and decrease fear-like behaviors (Herculano and Maximino, 2014). Thus, the available data, albeit seemingly conflicting, support the idea that serotonergic neurotransmission is involved in regulating zebrafish behavior differently in various tasks (e.g., the novel tank vs. the light–dark tests). Because zebrafish acutely treated with fluoxetine may also show increased anxiety-like behavior in the light–dark test, whereas chronic SSRIs predictably decrease it, similar to the novel tank test (Maximino et al., 2013a), the time of exposure becomes a key factor that should be considered for assessing fluoxetine-mediated responses in fish (see further).

3. Know thy dose: more lessons from fish on Prozac

One of the favorite questions in pharmacology and toxicology research deals with doses, stemming from Paracelsius' famous notion that "poison is in everything, and nothing is without poison: the dose makes it either a poison or a remedy". Analyzing fluoxetine doses used in various studies, one can expect the effective concentrations of fluoxetine to be similar for different exposure protocols, species, age groups and treatment durations (Sumpter et al., 2014). In contrast, we think that these critical experimental factors may markedly affect the pharmacology and the effective concentrations of fluoxetine in-vivo. For example, acute vs. chronic fluoxetine can not only explain dose differences reported in various studies, but are highly likely to have distinct mechanisms of action. As already mentioned, while *acute* fluoxetine in humans and rodents acts 'neurochemically' by inhibiting SERT and enhancing serotonergic neurotransmission, its long-term anxiolytic/antidepressant effects are seen only chronically (several weeks later), and are associated mainly with altered expression of serotonin receptors. We expect that the same rationale, applied to aquatic species (e.g., fish), may contribute to explaining the dose differences noted in (Sumpter et al., 2014). Likewise, the existing sex and strain differences in fish behavior and drug responses are also likely to contribute to data variance (Chatterjee et al., 2014; Egan et al., 2009; Mahabir et al., 2014; Maximino et al., 2013b; Pan et al., 2012), and must be carefully considered side-by-side in meta-analyses, before concluding whether the available data shows a clear pattern. Finally, due to species differences in physiology and development, it is not surprising to us that doses may differ between various invertebrate, lower vertebrate (fish) and mammalian species (Sumpter et al., 2014; Sumpter and Margiotta-Casaluci, 2014).

The dose–response curve is another favorite question of pharmacological and toxicological analyses. It is true that many studies on fluoxetine in aquatic species lack multi-dose experimental designs (Sumpter et al., 2014). However, it is important to put such studies in the context of specific research questions they addressed. For example, our groups were clearly more interested in affective neurobiology than fluoxetine pharmacology *per se*. Thus, we used fluoxetine as an anxiolytic tool (i.e., applying it in a known effective dose), rather than examining its full dose–response profile. Similar reasons may justify the approaches used by other groups (Abreu et al., 2014; Griffiths et al., 2012; Pittman and Ichikawa, 2013; Pittman and Lott, 2014; Richendrfer et al., 2012; Ziv et al., 2013), which therefore by design were not intended to tackle dose-dependence. Nevertheless, we agree that multi-dose analyses are important (Sumpter et al., 2014), and will eventually be performed to fill this knowledge gap.

4. Understanding the knowns and the unknowns

Clearly, critical analyses of model's strengths and limitations are important (Bruni et al., 2014; Maximino et al., 2014a; Sumpter et al., 2014; Zakhary et al., 2011), but they must be fair to both Science and the models. For example, concerns regarding the lack of replication of fluoxetine effects (Sumpter et al., 2014) may not be that problematic, because one can expect that such studies, like in any other area of research, used multi-step validation and replication before publishing. For example, we observe a robust behavioral profile from fluoxetine treatment, which was consistent across many replications (as well as seen in various modifications) of our zebrafish studies (Cachat et al., 2010a, 2011; Egan et al., 2009; Stewart et al., 2013; Wong et al., 2010). The acute vs. chronic fluoxetine treatment data and protocols, of course, merits further comparative analyses and meta-analyses. We also do not know whether SSRI withdrawal syndrome exists in fish, similar to this condition reported in rodents and humans.

While other concerns have been expressed regarding the use of standard endpoints in fluoxetine studies (Sumpter et al., 2014), we do not view fish anxiety research or their behavioral endpoints as unclear. For example, in the last decade, major progress has been