Chapter 27

Utilizing the Zebrafish Neurophenome Project (ZNP) Database for Analyses of Complex Neurophenotypes in Zebrafish Models

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Abstract

As the rate of biomedical discovery is rising exponentially, electronic databases have become particularly effective in organizing and sharing scientific knowledge. Due to a well-characterized genome, robust behavioral responses and physiological similarity to humans, the zebrafish (*Danio rerio*) has emerged as a useful species for neurobehavioral research. The growing utility of this model organism requires the development of specialized databases of zebrafish neurophenotypes, such as the Zebrafish Neurophenome Project (ZNP) (http://www.tulane.edu/~znpindex/search). Representing a new bioinformatics-based tool, the ZNP interactive searchable database consolidates neurobehavioral and related physiological phenotypes obtained in various zebrafish models and tests. This chapter outlines the contribution of the ZNP to increased accessibility of current zebrafish neurobiological knowledge, and discusses how this database may be used for various research projects.

Key words: Neurophenotypes, Zebrafish, Bioinformatics, Database, Data sharing, Data repository

1. Introduction

Animal models are widely used to study mechanisms underlying brain pathogenesis (1–5). As described in previous chapters of this book, zebrafish is a popular model species in neurobehavioral research, and its utility in this field continues to grow. On one hand, this dynamic field needs novel methodological and conceptual

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approaches for generating more data. On the other hand, such mounting body of biobehavioral information requires innovative tools for analyses and data-mining. This chapter introduces a novel bioinformatics-based repository of zebrafish neurophenotypic data, and discusses how this tool can facilitate translational biopsychiatry research.

The Zebrafish Neurophenome Project (ZNP) (6) was established as a centralized open-access database for behavioral and related physiological phenotypes observed in zebrafish models. The ZNP web-site (http://www.tulane.edu/~znpindex/search) utilizes a simple searchable interface, allowing researchers to quickly access and compare data collected by multiple laboratories with various treatments and tests. For example, among many other applications, this database allows principal investigators (PIs) to calculate effective pharmacological dose ranges, evaluate the effects of various treatments in a particular test, and determine the behavioral effects of different stressors. The goal of ZNP is to assist in the development of the zebrafish as a useful animal model for behavioral, neuropharmacological and neurogenetic research by consolidating and organizing zebrafish neurophenotypic data into an easy-to-use medium available to the scientific community.

2. The Database Overview

The ZNP is a *My Structured Query Language* (MySQL) database hosted on a secure professional-grade "Pulse" web-server maintained by the Tulane University Technology Services (New Orleans, LA). The ZNP database was created using VFront (7), a free open-source tool for MySQL databases (see (6) for details of the ZNP).

Figure 1 illustrates the functioning of ZNP and its main contributors. In addition to regular data searches by the ZNP team, the PIs can submit their findings (and also correct existing data) to the database, ensuring its reliability and accuracy. The ZNP team also constantly networks with zebrafish investigators, encouraging them to review, update or clarify their data (currently available in the database), as well as to submit their recent findings, including both published and unpublished observations. As the ZNP team inputs the data to the database, the scientific community can provide ZNP or the PIs with useful feedback, suggesting changes or corrections (Fig. 1). In addition to peer-reviewed papers indexed in PubMed, ZNP contains other data, including papers in journals not indexed in PubMed, as well as books, book chapters, PhD dissertations, theses, websites, posters, conference abstracts, patents, personal communications, and other sources.

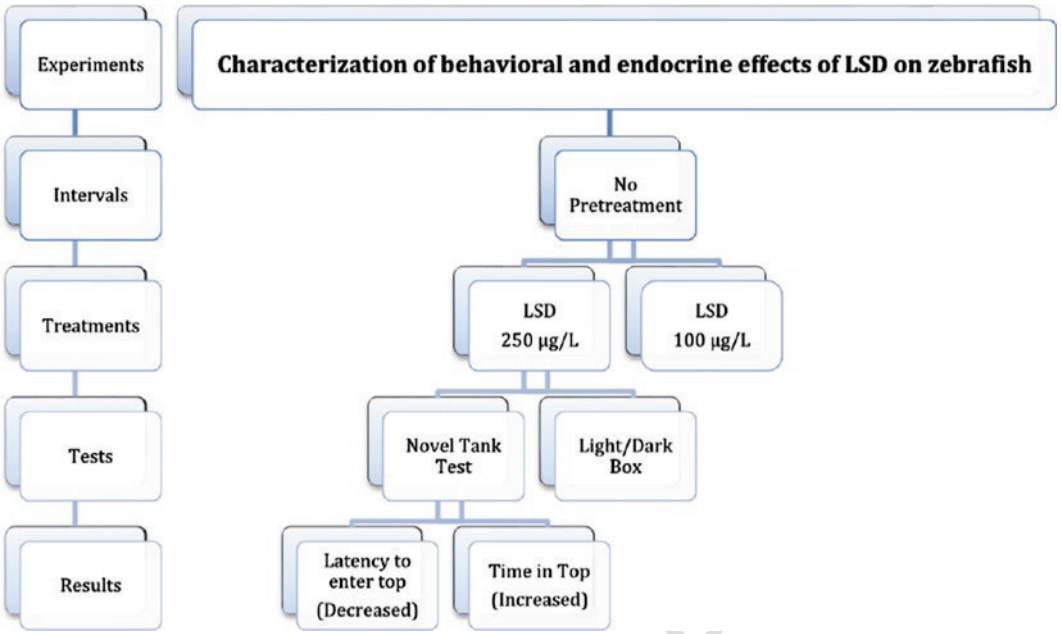

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Fig. 1. The Zebrafish Neurophenome Project (ZNP) database (http://www.kalueffl ab.com/znpindex.html) is organized in a parent/child format, and consists of multiple cross-reference tables. Each level of organization can have one or many subgroups, which allows the database to contain a large amount of data while remaining searchable and well-organized. In the given example, a hallucinogenic drug lysergic acid diethylamide (LSD) was tested in a recent published study (14) deposited into ZNP.

3. The Database Structure

The ZNP data is organized in a parent–child format that links multiple "children" modules to a "parent" module, and each "parent" to a separate group of "children" (Fig. 2). This format allows large amounts of data to be organized in a clear and logical manner within the current hierarchy of the ZNP database, including Experiments, Intervals, Treatments, Tests, and Results.

Since most scientific knowledge is currently presented in the form of manuscripts, a per-paper layout was chosen as the primary format for ZNP entries. The overarching experimental labels (for each study in the ZNP database) are the title and the PI of the paper, allowing for the treatments, manipulations, and tests to be traceable back to the lab that presented the data. Notably, the database was developed to serve as a reference guide for scientists (to evaluate prior research in the field and identify areas that remain novel or unexplored), rather than serving as a tool to help various labs to reproduce specific experiments. Therefore, in order to streamline the input process and to allow a user to efficiently use ZNP as a quick reference, methodological details of studies are described only briefly, and the original publication must be referred to for such

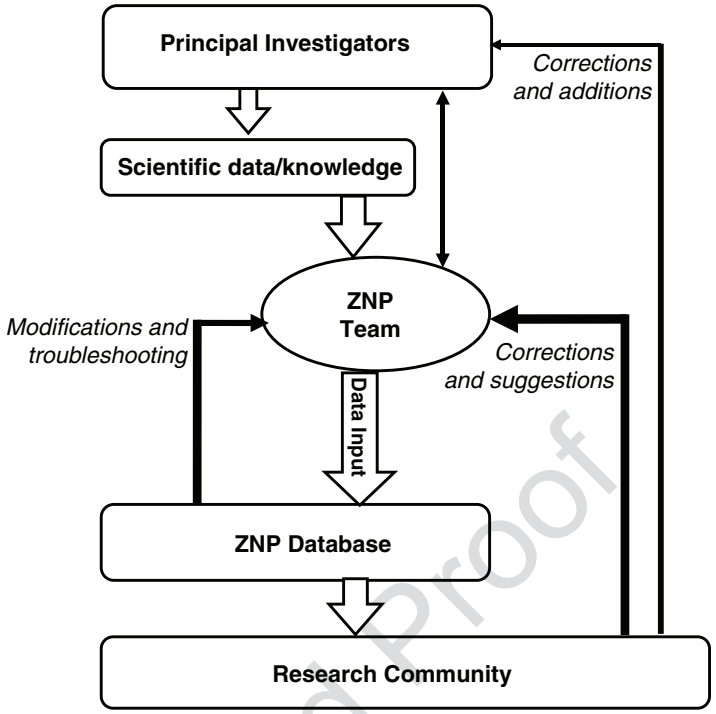

Fig. 2. A diagram summarizing the ZNP database information flow (see details in the text; the width of each *arrow* reflects relative frequency or importance of each interaction).

information. However, the experimental summary in ZNP lists all information necessary for correct interpretation of the results, such as age, strain of zebrafish used, observation software, euthanasia methods and husbandry parameters used by the reporting lab.

In addition to the Experiment level of organization (which has been used to refer to findings on a per-paper basis), ZNP also uses the Interval level. Intervals are important to characterizing the experiment as a whole, and must be included to the data description. For example, in a study exploring the effects of acute drug A following the chronic use of cocaine (known to evoked addiction-like phenotypes), the first interval will include chronic treatment with cocaine (e.g., 2 weeks, with the drug added to home tank water), and the second interval will reflect acute treatment with drug A (e.g., 30 min immersion in water bath prior to testing).

The Treatment level of organization contains the information about various manipulations performed within the Experiment. Treatment is the main qualifier of experimental data in ZNP, and its parameters are carefully detailed in the database (e.g., lysergic acid diethylamide [LSD] given at dose of 250 μg/L for 20 min via immersion, or forced light exposure at 2,000 lux for 5 s).

The child group to "Treatments" is the "Tests" module. Following a specific treatment, one or many tests may analyze the

fish behavioral response. The experimental tests (models) are listed separately in ZNP, each indicating the duration of the test, and the method of behavioral data collection used (e.g., manual registration by the observers, or video recording). If video recording is used, ZNP also mentions the program used for analysis, if provided in the original publication. Following this test information, an additional field ("Findings") is included, to briefly summarize the results of the study (e.g., "Anxiolytic effects in the novel tank test" for acute morphine or diazepam). This section helps the users to more easily review and interpret the results of the experiment of interest.

The lowest level of organization within the ZNP database is the "Results," since each test usually examines multiple behavioral endpoints. Within this module, the ZNP lists both statistically significant results and unaffected endpoints. Each endpoint is listed with its significance (*P* values), as well as the general direction of change (e.g., increased or decreased) as compared to the specified control group (e.g., male vs. female fish, drug-treated vs. unexposed controls, old vs. young zebrafish, etc.). The purpose of listing all endpoints (including those that were not significantly affected) is to provide a more complete picture of the experiment as a whole, especially since the importance of nonsignificant results is commonly underestimated in the literature when presenting behavioral phenotypes.

4. Searching the ZNP Database

In its current form, ZNP is a curated database maintained and regularly (weekly) updated by the ZNP team. Users do not need to register to be able to use and search the ZNP database. Figure 3 demonstrates the following easy, intuitive, and user-friendly procedure to access the ZNP data:

1. Access the main ZNP website at http://www.kaluefflab.com/znpindex.html and select *Search* from the main menu.
2. To find studies investigating a specific treatment (e.g., mutation or drug of interest), select the *Treatment* of interest (e.g., caffeine or ethanol) from the first drop-down menu.
3. Click *Search* to generate a list of study titles presented. Each of these studies (usually reflecting a paper published by a specific research group) contains at least one result using the specific treatment chosen.
4. To find a study of a specific drug, performed by a specific laboratory (Fig. 4), select the treatment of interest from the first drop-down menu; and a PI from the additional drop-down menu (listing all PIs contributing to the database).

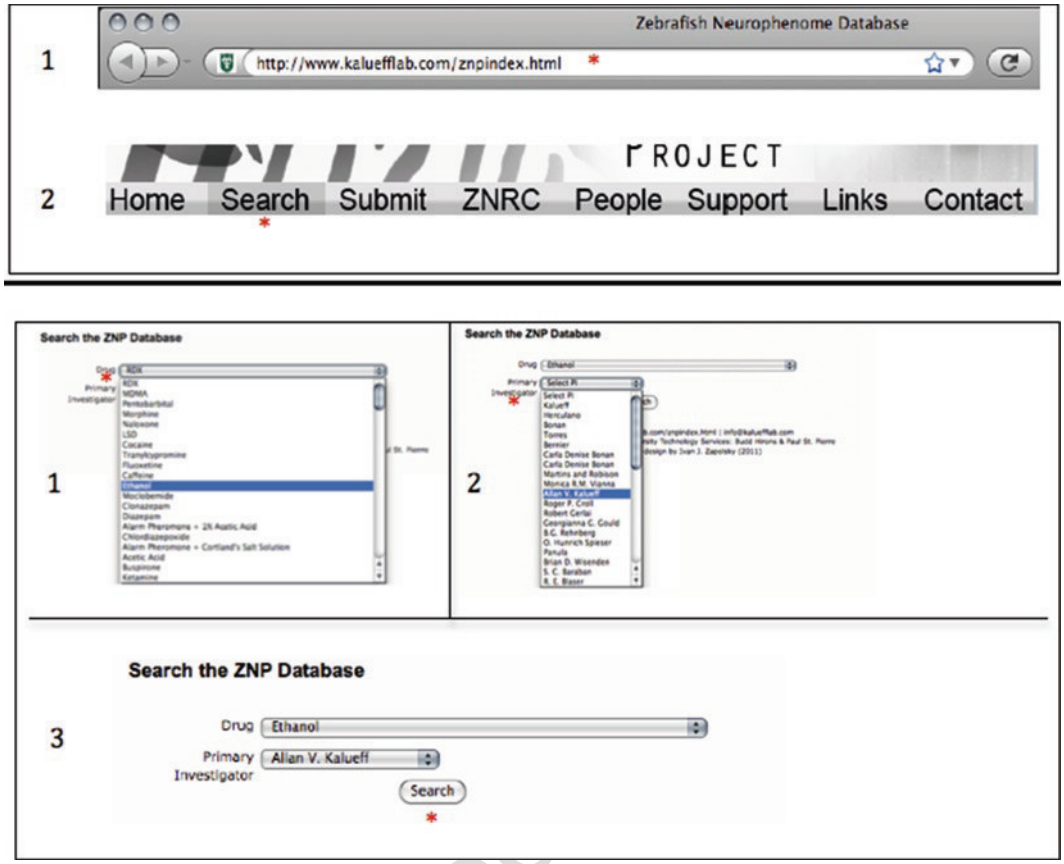

Fig. 3. A brief tutorial on searching the ZNP database.

Treatment ID	Dosage	Duration	Delivery	Test ID	Method	Software	Duration	Finding	Result ID	Endpoint Type	DrugEffect	PValue
99	0.3 % v.v.	6 min	Immersion	101	Both	TopScan	6 min	NTT - anxiolysis	355	Latency to top	Decreased	<0.01
									356	Transitions to top	Increased	<0.05
									357	Time in top	Increased	<0.05
									358	Unaffected endpoints	Erratic movements, freezing bouts, freezing duration	NS

Fig. 4. A representative example of the results view of the Zebrafish Neurophenome Database (ZND). This data is based on a published study (15) by the Kalueff laboratory (Tulane University, LA, USA) testing ethanol in the novel tank test, as in the example given in Fig. 3. In addition to detailed description of experimental/methodological parameters of the study, note the summary of positive findings with their statistical significance, as well as the summary of all *unaffected* endpoints (negative findings), collectively providing a comprehensive summary of the observed phenotypic data.

5. Click *Search* to generate a list of study titles, each of which will contain at least one treatment of the drug chosen, and completed by the selected PI.
6. If necessary, all ZNP data can be searched using the PI's name as a search term, extracting all studies from a specific lab within the database.

5. Typical Results and Potential Applications

Consider a situation when a laboratory plans to test cocaine and related compounds, but does not know the effective dose range for this drug in zebrafish. To use the ZNP for this study, access the ZNP web-site (Fig. 2), select *Search*, then select *cocaine* on the drop-down menu. The first dose listed in the database is 1 μM, given to adult zebrafish for 20 min via immersion in a study by (8), reporting no significant effects for this dose. The second dose listed online is 10 μM, tested by the same group by a 20-min immersion, and evoking mild anxiogenic effects. The third dose listed in the ZNP is 100 μM (20-min immersion), yielding a strong anxiogenic action with multiple erratic movements and freezing bouts. By using the ZNP database, the investigator will spend only several minutes to make an informed decision regarding the potential treatment for his own study (e.g., ~50 μM via a 10–20-min immersion) to produce a desired (e.g., mild anxiogenic) effect in zebrafish.

Likewise, while cocaine data can be found in ZNP, some related compounds, such as D-amphetamine, to the best of our knowledge, have not yet been tested in adult zebrafish behavioral/locomotor tests. Our understanding of the basic pharmacology of these drugs may be useful in conjunction with the ZNP. For example, clinical studies report higher sensitivity of patients to D-amphetamine compared to cocaine. Cocaine also has a shorter duration of behavioral action (within 1–1.5 h) compared to D-amphetamine (lasting up to 6 h), as well as a shorter half-life (1 vs. 12 h). At the same time, similar potency for the two drugs was reported in some animal (e.g., primate) studies (9, 10). Thus, if acute exposure to D-amphetamine is planned, it is logical to expect that slightly lower doses of this agent (than those of cocaine, used as a reference drug) can be used for pilot studies using zebrafish. As already mentioned, ZNP currently contains cocaine data with effective doses of 10–100 μM. Therefore, 5–50 μM may be a reasonable starting dose range for the pilot studies using D-amphetamine.

The ZNP database can also be used to study various domains affected by the same treatment. Thus, researchers interested in a particular aspect of behavior can refine their pilot dose range to study specific phenotypes of interest. For example, when viewing the data on caffeine exposure currently available in the ZNP database from a recent study (11), the first dose listed is 0.5 M

(by immersion for 20 min), yielding significant anxiogenesis in multiple testing paradigms. However, a larger dose of 1.25 M generated seizure-like behavior (including bursts of hyperactivity and spasms) in the same study listed in ZNP. Therefore, depending on the goal of his study, a researcher would conclude that a 0.25–0.75 M range would be sufficient for examining the effects of caffeine on anxiety, while doses of 1.25 M and higher may effectively model epilepsy and related behaviors.

The ZNP may also be useful for assisting with experimental design using treatments that have not yet been applied in zebrafish models. For example, if an investigator plans to use a drug like 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") for zebrafish research, but little research had yet been done to verify even a general dose range, a set of pilot experiments testing a range of dosages for efficacy will generally be required. In clinical research, it has been observed that the concentrations of MDMA required to elicit a psychotropic response are approximately three orders of magnitude greater than those of LSD (the canonical hallucinogenic drug), which produces a similar subjective experience in users. Keeping this in mind, an investigator may turn to ZNP and deduce the appropriate dose range for LSD in zebrafish (100–250 µg/L), multiply this by three orders of magnitude, and determine a good starting point (e.g., 100 mg/L) for his pilot studies using MDMA (as has been recently confirmed in (12)).

Using the previous examples, it is apparent that the ZNP Database addresses some of the 3R's of animal testing (Refinement, Replacement, and Reduction). First described by Russell and Burch in 1959 (13), the three R's are guiding principles for the use of animals in biomedical research, and can be further implemented by using the ZNP. There are several other applications for the ZNP database, briefly summarized in Table 1, that illustrate the developing utility of this tool for various research projects.

Table 1
Selected examples of applications of the zebrafish neurophenome project (ZNP) database in research and teaching

ZNP application	Detailed examples
Replacement (3R)	By searching ZNP for published or unpublished data on active doses of a specific drug of interest, the investigator eliminates the need to run pilot studies to determine an appropriate dose for his experiments
Refinement (3R)	A better refinement of research will be achieved by optimizing the research strategy, and therefore not exposing zebrafish through unnecessary pain or distress (e.g., due to an uninformed decision regarding an appropriate drug dosage)

(continued)

Table 1 (continued)

ZNP application	Detailed examples
Evaluation of scientific projects	A member of an IACUC (who is not an expert in zebrafish research) reviewing a protocol from another laboratory can easily check the safe dosages of certain drugs in ZNP, and compare them with the submitted protocol, to be verify their appropriateness. ZNP provides similar assistance to any other experts evaluating scientific projects, such as peer-reviewers of grant applications or journal articles
A reference for the experimental design	Before planning an experiment, especially if a novel drug will be used, it is recommended to search for other related studies using this drug or similar compounds. Finding these studies and reviewing their contents is empowered by the ZNP database
Preventing replication of an experiment that has already been performed by another laboratory	Before testing a drug at a certain dose, it is recommended to ensure that another laboratory has not already performed a similar experiment using the same dose or treatment. This will prevent any unnecessary zebrafish experimentation, as well as save time and funds for the experimenter
Optimizing scientific literature search	With the growing number of published zebrafish papers, it may be more time-efficient for a PI to quickly preview the results via the ZNP database before committing to reading the entire body of published literature
Fostering cross-species data analyses and comparisons	Researchers working with other popular fish species (e.g., guppies, goldfish or medaka fish), can benefit from using the ZNP database to compare their own results with those generated in zebrafish models
Improving data sharing and dissemination	The ZNP database serves as a free data repository for investigators working in the field of zebrafish neuroscience. This markedly increases their ability to share and disseminate zebrafish biomedical information
Promoting teaching and education using zebrafish models	The ZNP database is a useful tool in biomedical education and teaching. The high-school and college educators can utilize the database to identify sensitive reproducible experimental models, which can then be used in classroom demonstrations and independent research projects

6. Conclusion

We have developed a novel online tool to study zebrafish neurobehavioral phenotypes, and made it freely available to the scientific community. The prime application of the ZNP database is to search, compare, and analyze zebrafish responses to various experimental manipulations. Its simple and intuitive design makes it a useful reference tool for anyone interested in zebrafish behavior,

pharmacology, or genetics. The database summarizes the content of the studies it contains, thereby allowing its users to search and extract information in a much more time-efficient manner (compared to reading each study in its entirety). ZNP covers a wide range of studies in zebrafish neurobiology, which may be more comprehensive than PubMed (currently the prime source of research information), since data has been obtained from more sources than just published manuscripts. For example, data from conference presentations, books, chapters, and personal communications are all included within the ZNP database. Negative findings are also covered for every experiment listed in the ZNP database, collectively enabling the systematic organization and aggregation of zebrafish neurophenotypes data within a widely accessible data repository.

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