This is a case-study in high-throughput whole-organism chemical screening using zebrafish larvae presented by Andrew J. Rennekamp (arennekamp@mgh.harvard.edu)

Discloser Statement

Dr. Rennekamp is an inventor on Patent No. PCT/US2015/037755 for discovery of the Finazine compounds.

Some of his work has been conducted through academic-industry collaborations with Roche and Teleos Therapeutics.
Approximately 30% of adults in the United States suffer from some form of mental illness\(^1\). The economic burden caused by healthcare expenses and loss of productivity is estimated to be hundreds of billions of dollars each year.\(^2\)


Yet success rates for neuroactive small molecules in clinical trials are currently less than half those for non-CNS drugs.
One reason for this low success rate is the industry’s reliance on target-based approaches during the chemical discovery phase. These are likely too simplistic. Target-based approaches have the potential to miss systems-modulating compounds, which may be useful for the treatment of complex diseases like mental illnesses. Target-based approaches are also far less likely to yield polypharmacologic therapeutics.

In contrast, phenotype-based discovery approaches for CNS drug discovery have been 7 times more likely to produce successful 1st-in-class compounds than target-based programs.
We think whole-organism screens will be even better.

We think that, for CNS drug discovery, high-throughput whole-organism screens will be even better than the current phenotype and targets based screening approaches.

We use a whole-organism approach because we can... (read slide)
An ideal organism will be complex enough to resemble some human biology but small enough to fit in a 96-well plate.

7 mm Zebrafish larvae are tiny enough to fit in to 96-well plates, yet complex enough to share vertebrate characteristics with humans including the same neurotransmitters and many similar neural substrates. They also have intact blood-brain barriers. And furthermore, we now know of many human drugs that have similar effects on fish biology and vice versa.
An ideal phenotypic endpoint for a CNS drug screen is behavior modification. Zebrafish larvae can be used for whole-organism high-throughput discovery of behavior-modifying small molecules. And they are attractive for these reasons... (read slide) ... This presentation will demonstrate some of these points.

Here is an overview of our approach from a news-and-views article which accompanied our recent paper in Nature Chemical Biology.
The first whole-organism zebrafish chemical screen was conducted in 2000, by my postdoc mentor Randy Peterson (newly appointed Dean of the College of Pharmacy at the University of Utah) when he was in Stuart Schreiber’s lab.

In the past 15+ years, the phenotypic read-outs used in zebrafish chemical screens have become quite diverse. For this audience, it’s worth noting that some of these screens were based on High-Content imaging in optically transparent larvae.
To identify novel, complex neuroactive small molecules, we
developed an innovative and inexpensive, 7 min behavioral assay
using zebrafish larvae arrayed in 96-well plates, 10 fish per well. The
rate limiting step of is the manual pipping of fish into wells (which
takes about 8 min per plate). The story I will show you today was
featured on the cover of Nature Chemical Biology a few months ago.

We took advantage of an observation I made, which is that 7-day-old
fish rapidly become hypoactive in response to strobe light. We
believe this is a fear response for a few reasons: (1) the onset of
freezing behavior is quit fast, (2) strobe light increases levels of the
stress hormone cortisol, (3) adult fish respond with hyper-locomotion
(data published by others), and (4) (perhaps most importantly) the
behavior can be switched to escape.
Now before I get into the switch to escape, here is the normal fish behavior in our 7 min assay where we have alternating minutes of darkness and strobe light. The light you are seeing is the video is infrared light recored by an infrared camera. The fish cannot see this light, they only see the strobe light in the visible spectrum. You will not see the visible strobe light through because it’s no detected by the IR camera...

We found that freezing behavior is dependent on the frequency of strobe light flashing.
By screening libraries of known drugs, we found that specific classes of neurotherapeutics, notably neuroprotectives and nootropics, completely switch the strobe light response from freezing to hyperactive ‘escape’-like behavior.

Here we are using something we call the “freezing index” (average motion during strobe mine the average motion in the dark)...

This is an example of one such dose curve...
Of course the fish arrayed in the 96-well plates can’t escape the strobe light, so just to show you this is indeed and escape behavior, I’m now showing you fish in a 10 cm dish where half the plate is covered so that the fish can escape to the dark side and avoid the strobe light.

We’ve done this many times and here is a summary of that 10 cm dish place preference data.
Many of these drug can be reverse. For example the muscarinic antagonist, Atropine can cross the vertebrate blood-brain barrier and reverse the effects of the AHCE inhibitor donepezil. A close analogue, Methylatropine, which cannot cross the BBB does not rescue freezing behavior.

We then screened for novel compounds and identified structural families of small molecules able to phenocopy the known drugs. (800 compounds per day) Our screen identified more than 30 hits, some of which could be grouped into families based on their structural similarity. The data do not form a nice Gaussian bell curve; untreated fish never show escape behavior (i.e. cross the zero line). We did however, end up using a >3 std at the cut off when defining our hits.
One family of hit compounds identified consisted of 11 small molecules. We named these the ‘Finazines’ (in honor of the fish).

We performed dose curves on each of these Finazine compounds, and we calculated the concentration that causes the behavioral switch.
We synthesized several additional Finazine analogs with varying in vivo potencies in fish and screened them (along with the original 11) for binding to >40 candidate mammalian neuronal targets \textit{in vitro}. Our results implicated Sigma-1 receptor as an efficacy target with nanomolar affinity for our lead Finazine compounds.

Another way to look at the data is to plot the effective \textit{in vivo} concentration in the fish vs. the Sigma-1 Ki value determined from the \textit{in vitro} mammalian protein binding data. As you can see with the 11 original compounds we get a very nice correlation.
We found that well-known human Sigma-1 agonists can phenocopy the Finazines. Here is the sSigma-1 agonist SKF 10,0044 (for which sigma-1 is named) as well as another Sigma-1 agonist (cutamesine) currently in clinical trials as a neuroprotectant after ischemic stroke also as a neuroprotectant of motor neurons in ALS. We found that (-) enantiomer of SKF 10,0047, which has weaker affinity at mammalian Sigma-1 also has reduced activity in our fish experiment.

To genetically confirm sigma-1’s involvement, we created two knockout zebrafish lines using CRISPR-cas9 mediated frame-shift induction. This technology was first pioneered in fish by my colleagues in the Peterson lab.
In the presence of the anti-muscarinic compound scopolamine, our lead Finazine compound no longer has an effect in sigma-1 KO fish, but still has an effect in scopolamine-treated WT fish. (This indicates polypharmacology, as these particular compounds appear to be both sigmaergic and pro-muscarinic.)

We are now testing our lead Finazine in rodents. Here I show a well-established assay of behavioral freezing in mice, called contextual fear conditioning. Mice tend to freeze when re-exposed to a chamber where they previously received foot shocks. As you can see the vehicle-treated mice responded to the conditioning context by exhibiting a high level of freezing behavior. Finazine-treated mice, however, spent less than 40% of their time immobile.
These data suggest that the Finazine is able to penetrate the blood-brain barrier in mice as well as zebrafish and modulate the behavior of both species.

Dr. Andrew J. Rennekamp is currently on the job market, interested in tenure-track Assistant Professor Faculty positions. His email is arennekamp@mgh.harvard.edu. Please visit his website for more information (http://scholar.harvard.edu/rennekamp).