Little Fish in a Big Pond Reveal New Answers to Old Questions

By Shana R. Spindler, PhD

Studying the neural control of behavior is a challenge. Researchers must consider an animal’s environment, past experiences, and motivations. Work in relatively simple organisms, for example the invertebrate C. elegans, has teased apart the neural circuitry of highly stereotyped behaviors, like foraging. But in mammals, very little is known, “and that’s surprising given just how important behaviors like this are,” said Dr. Eric Horstick, who studies the molecular mechanisms underlying animal behavior.

Horstick, a postdoctoral fellow in the NICHD Section of Behavioral Neurogenetics, came to the NIH with a goal to characterize genes that control behavior in vertebrates—information that could advance therapies for behavior-related diseases in humans. He joined the lab of Dr. Harold Burgess, whose earlier work studying light-mediated behaviors in larval zebrafish would set the stage for Horstick’s studies.

For more than 20 years, labs have used a classic behavior paradigm in fish called the visual motor response (VMR). Investigators place individual larval zebrafish into separate wells of a multi-well plate and record larval movement in light and dark conditions. With the onset of darkness, the fish move rapidly for several minutes in a highly stereotyped response.

As Horstick considered ways to examine if the movement was random or spatially patterned, he realized that the multi-well set up restricted the type and resolution of collectable data. According to Horstick, he “blew it up.” He placed individual larva in chambers measuring 400 square millimeters, a roomy space for a 3mm creature. This time, when the lights went out, the zebrafish initiated a slow, descending spiral, not the rapid motion typical of VMR. Eventually, the

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Letter from the Editor

When you run an experiment with a well-established method, do you ever question if an alternative set up could provide new information? Based on a recent publication from the Burgess lab, maybe you should. In our “Hot Off the Press” column this month, we delve into the world of zebrafish behavior with Dr. Eric Horstick. His modified approach to studying visual motor response elicits unexpected swim patterns in the larval fish—driving him deep into the ecology literature where he makes fascinating discoveries.

To study fish swim patterns, the Burgess lab creates videos that help visualize movements. With increased computer storage capacity and the relative ease of recording on mobile devices, video has become a staple in many NICHD labs. In some cases, the video is produced as a time-lapse, squeezing hours of filming into minutes of presentation. A time stamp can help indicate how much time the video spans. In “The Arts” column, Jeremy Swan takes us through a ten-step tutorial for adding timecode to a time-lapse video.

On a final note, we are excited to introduce our new basic science representative for the institute, Dr. Suna Gulay. To learn more about Dr. Gulay and her role as the NICHD postdoc representative, check out her profile on page 10.

Enjoy the issue!

Your Editor in Chief,
Shana R. Spindler, PhD

Please send questions and comments for our editor to Shana.Spindler@gmail.com.
zebrafish transitioned from a spiral movement into an outward, exploratory pattern.

“As soon as we gave them more room, something much more complex started to emerge, a stereotyped behavior,” said Horstick. “Beautiful patterns are always an attractive component in research.” At this point, Horstick left his comfort zone of molecular neuroscience to delve into the ecology literature, where he found similar patterns throughout the animal kingdom. His results were consistent with search and foraging behaviors observed in mammals all the way down to flies and worms. They had stumbled upon a highly conserved behavioral paradigm that had been masked by the confined environment common to VMR studies.

Horstick paints an example of this behavior in humans. “The scenario I like to describe is when you walk out of your house and reach for your keys, but they’re not there. Take a moment and picture what you do. You normally stand there, move your head, scan the environment, and step around in a circle just like our fish do, just like what worms do, just like what flies will do when they’re searching for something that they can’t find immediately. So even us, when we’re not conscientiously aware of it, we perform these highly engrained patterns of behavior.”

Horstick now possessed an untapped treasure trove of behaviors with the upgraded VMR assay. Using the wealth of techniques available in zebrafish—including established mutants and CRISPR technology—Horstick found that zebrafish required eyesight for the restricted spiral pattern during VMR. But two systems stood out for their involvement in the transition to outward movement: somatostatin signaling and deep brain photoreceptors.

Somatostatin, in particular, piqued Horstick’s interests. The small peptide hormone is a potent neuromodulator in humans. And based on work in mammals, somatostatin serves as a regulator of growth hormone output and a modulator of behavior states, such as anxiety. Horstick ponders, “Is somatostatin correlated with feeding drives? This could have true relevance to human conditions in terms of obesity, drive to eat, drive to seek food—maybe with somatostatin we could start opening up some of those angles?”

In the near future, Horstick hopes to gain insight into the somatostatin neurons involved in this behavior to elucidate the circuitry further. He questions how these neurons tie into the input of sensory information to then modulate downstream motor output. But Horstick’s goals don’t stop there. His big question: “Is this peptide tuning not just this behavior, but behavioral states as a whole?”

Stay tuned.
3...2...1... Timecode!
By Jeremy Swan

In our last two articles of “The Arts” column, we created time-lapse videos in both Photoshop and After Effects. Now, let’s add a “time stamp” to show the precise time each image was captured. Spoiler: It involves keyframes and nesting. In this article, we present a ten-step process for adding timecode.

“Nesting—Not Just for the Birds, But Fish Too” is required reading before continuing with this article. Caught up? Great!

SETTING UP TO ADD TIMECODE TO A TIME-LAPSE VIDEO
First off, let’s grab our example video, which can be downloaded here: timelapse-after-effects.mp4

Drag the video into the “Project” window to add it to your project.

Right click on the video and choose “New Composition from Selection”

This makes a composition that automatically has the same frames per minute, length of time, height, and width of the video file.

It’s time to add a timecode label.

1. Launch and prepare an After Effects project
   On the top menubar, click: Layer > New > Text.
   Begin typing to place text on the layer.

2. Modifying text to make it readable
   To modify the type of font (A) and color (B), click “Command + a” to select the text and make your color and font selection.

   Ensure that your text is readable by scrubbing through the footage (grab the handle and drag to the left/right).

   To make the text stand out more, you can add a stroke by clicking the box next to the text color (C). Adjust the stroke width (D) as necessary.

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3. **Adding a solid block of color behind text**
If your text is still difficult to see, you can add a block of color behind the text by clicking Layer > New > Solid.

Position the layer in between the text and background layers. You can also change the opacity to make it more discreet.

You can click the box and drag it around to move the position and change the size by adjusting the handles (after you begin to change scale, press the shift key to maintain the original ratio). **Note:** You can use this as simple “text” or continue to the following step to create the timecode.

4. **Finding the right font for timecode-specific text**
For a timecode layer of text, I’d recommend using “Crystal” (available as freeware here: http://www.fontspace.com/allen-r-walden/crystal).

If you would like to use a different font for the time, be sure that the font is “fixed width.” This means that the characters are all the same width, so that the text will not jump around as the clock progresses. **(Note:** The “Crystal” font is for all-caps and numbers only, so if your text contains lowercase letters, they will be displayed with another font.)

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5. **Create the “stop watch” effect**
Open your “Effects and Presets” window and open “Expression Controls”

Drag “Slider Control” over your text, or onto the text layer in the timeline, to apply the effect. You should now be able to click the arrows to see “Slider Control” in your timeline.

Hold the *Option key* down while clicking on the stopwatch icon to display the expression editor on the right.

Paste the following code into the expression editor:

```javascript
function padZero(n)
{
   if (n < 10) return "0" + n else return "" + n
}

t = Math.floor(effect("Slider Control")("Slider"));
hr = Math.floor(t/3600);
min = Math.floor((t%3600)/60);
sec = Math.floor(t%60);
/
/*
delete the comment tags and put in one line to add hours
padZero(hr)+":"+
*/

padZero(min)+":"+padZero(sec)

/* Script written by Josh Vickerson | https://twitter.com/joshvickerson */
```

This code is shared here: [https://github.com/anbdesign/After_Effects_Expressions/blob/master/Keyframe_Controlled_Countdown_Timer.txt](https://github.com/anbdesign/After_Effects_Expressions/blob/master/Keyframe_Controlled_Countdown_Timer.txt)

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6. Set the time for the beginning of the time-lapse (in seconds)

In the sample, Dr. Fabio Fauz (Stratakis lab) captured the images every 10 minutes, starting at 90 minutes, until a total of 150 images had been collected. This means that we'll need to start at 1:30 and end at 24 hours. Converting to seconds, that is 5400 and 86400.

On the Timeline, move the playhead to the first frame and enter 5400.

You'll see that it says 30:00 because hours aren't calculated in the code. Let's fix that. Click in the “Expression Source Text” code window and delete the comment tags and the comment, shown in blue.

These comment symbols—/* and */—cause everything in between to be ignored so that programmers can “toggle” parts of the code on and off and leave comments within the actual code.

The result should look like this:

Once you've clicked off the code window, you'll see the extra set of numbers displaying 01:30:00.

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7. Setting the length of the timeline
We will set a “Keyframe” at the beginning and the end of the timeline. Keyframes are used to define the start and end points of a transition, so that the frames in between can be calculated automatically for a smooth transition between each keyframe. With the playhead still at the beginning of the timeline, click the stopwatch icon. This will add a keyframe at the playhead, which is represented by a blue diamond shape. Note: Clicking the Stopwatch icon a second time will remove all keyframes.

Now, let’s move the playhead to the end of the video (50 seconds) and change the slider value to 86400. A new keyframe will automatically be inserted in the timeline.

8. Setting the frames per second
We’re almost there... This would be perfect, except that each image in the time-lapse lasts for 10 frames, meaning that a new value is displayed for each of those 10 frames. Let’s fix this by reducing the Frames Per Second (FPS) to match the exact number of images.

The original movie was created from 150 images at a rate of 3 frames per second (FPS) for a total of 50 seconds. We exported at 30 frames per second (or 29.97, since that is the standard that works well on YouTube and is preferred by video editors). Doing that math, each image is displayed for 10 frames, but we can change this: Right-click on the composition in the project window, and change the FPS to 3 FPS.

When you scrub through the footage, the seconds (:00 at the end) should remain constant.

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9. Finishing touches
Now for some finishing touches. Let’s add an outer glow to make the text more readable against the background. Right-click the Timecode Layer and select Layer Styles > Outer Glow.

You can change the color (I like neutral colors like white and black) and other parameters, such as “spread” and “opacity” to find the perfect balance. It should be SUBTLE!

10. Hiding the last two digits
We can use a mask to cut off the last two digits by right-clicking on the timecode layer and choosing “New Mask.”

Click the handles and move them around to exclude the “seconds” column.

Your movie should be ready for export! You can follow the instructions from the previous article or click File > Export > Add to render queue. Your result should look something like this: timelapse-timecode-after-effects.mp4.
Meet Dr. Suna Gulay, Our New Basic Science Postdoc Representative

Our basic science representative is a postdoctoral fellow who serves on the NIH Fellows Committee (FelCom) on behalf of the institute’s fellow population. In general, most institutes have one basic science representative and one clinical representative. Representative appointments last for 12 months and can be renewed for an additional year.

Responsibilities of the institute’s representative include attending all scheduled FelCom meetings, participating on a subcommittee, disseminating information to the fellows in the institute communicating fellow's concerns to the Committee, and coordinating the distribution of information via subcommittees. In NICHD, our representatives also work closely with the Office of Education to plan events for all NICHD postdoctoral trainees.

If you would like more information about serving as a basic science or clinical postdoctoral representative, please contact Dr. Yvette Pittman (yvette.pittman@nih.gov), and for information about serving on a FelCom subcommittee, contact one of the committee chairs directly.

A BIT ABOUT DR. GULAY
I am originally from Turkey, where I received my bachelor’s and master’s degrees. I worked in a variety of fields, from biotechnology to cancer biology, before coming to the University of Maryland, College Park, for my doctoral studies. I conducted my dissertation research in Dr. Jonathan Dinman’s laboratory, probing the ribosomal RNA secondary structure throughout protein translation elongation cycle. I met my current NIH mentor, Dr. Alan Hinnebusch, at a conference and collaborated with him on a project before joining his laboratory as a postdoctoral fellow. Currently, I am interested in the functional interactions between a eukaryotic translation initiation factor complex, eIF4F, and an RNA helicase known to regulate translation initiation of a subset of mRNAs in yeast, Ded1.

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I am not all business and, in my spare time, love going on hikes with my husband and friends, visiting small towns, and trying different cuisines (looking into Uyghur cuisine next). At home, I enjoy cuddling with my cat (when he is in the mood 😊).

While I would like to advance into a combined teaching and research career in a primarily undergraduate institution, I believe one should keep their options open. The many resources NIH and NICHD offer have shown me the variety of career options available to biomedical scientists. As I assume the role of NICHD Basic Science Representative, I am excited to be in a position to pay it forward and help other fellows—working alongside Dr. Yvette Pittman to develop and improve tools that enrich postdoctoral training at NICHD.

I would like to encourage all NICHD postdoc fellows, both IRTAs and Visiting fellows, to consider joining the Fellows Advisory Committee (contact Dr. Yvette Pittman, yyvette.pittman@nih.gov, for more information). This is an excellent way to let us know about your training needs, as well as to be the first to hear about new tools, grant opportunities, and workshops. You get to have a voice in the process that produces training activities, from planning to execution. Please feel free to email me at suna.gulay@nih.gov, especially for issues and ideas regarding FelCom activities.
Left to right: People’s Choice winner Pranati Panuganti (Samosa Pie); Best Savory Pie winner Vincent Black (Crab Pie); Dave Songco, NICHD CIO; Second Place winner Erin Goldstein (Chai Strawberry-Blueberry Pie); Third Place winner Angela Wish (French Silk Chocolate Pie). Not pictured: First Place winner Debbie Brock (Samoa Pie).
April Announcements

A NEW GRANT OPPORTUNITY FOR NICHD POSTDOCS AND CLINICAL FELLOWS

DIR is launching a competitive research funding opportunity for NICHD postdoctoral, visiting, and clinical fellows—Intramural Research Fellowships (IRFs). Its main objective is to promote grant writing among our intramural trainees while enhancing awareness of various application components for an NIH grant.

These research fellowships are specifically for NICHD intramural fellows within their second and third year of training, preparing them for the entire process of applying to an NIH grant. We ask that the fellows prepare the applications, but mentors are expected to offer guidance and input throughout the writing process. Using the NIH scoring system, a panel of our Board of Scientific Counselors will review all of the applications.

For applications that result in an award, the appointed lab will receive $30,000 for one fiscal year (FY2018), to use toward the fellow's stipend, equipment, and/or travel to a scientific conference related to their proposed work.

The application deadline is August 1, 2017, and the required sections are listed below:

» Biographical sketch for the fellow
» Specific Aims for 1 year of training
» Budget Justification
» Significance and Approach
» Statement of Support from the NICHD mentor

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GRANT WRITING TRAINING SESSION FOR 2017 IRF APPLICANTS, MAY 15 AT 2PM
For all prospective applicants, the Office of Education will offer a training session on Monday, May 15, at 2 p.m. in Bldg. 31, conference room 2A48 (A-wing, 2nd floor). We will cover various components of an NIH grant, details about the application and review processes, and tips on preparing an IRF application.
*Attendance at this training session is a requirement for submission.*

FOR ALL NICHD FELLOWS: “RESUME HOUR” OFFERED
Every Wednesday, 1–2 p.m., Building 31, Room 1B44, or you can schedule an individual session for another day and time

Do you need some tips on putting together an excellent resume or CV for your ideal job? Learn how to perfect your application documents during our weekly office hours. Don't miss this unique opportunity to review your resume or CV in great detail with Dr. Pittman.

You will:
» Learn about keywords and tailoring for specific jobs
» Examine various samples
» Review formatting
» Establish what to include—and not to include
» Identify your target audience

And much more!

ANNUAL NICHD FELLOWS RETREAT IS NEAR: MAY 1, ALL-DAY EVENT
Speaker profiles, meeting agenda, and poster information can be found on the [retreat website](http://retreat.nichd.nih.gov). Registration is now closed.

If you are interested in covering any part of the meeting for *The NICHD Connection*, please contact our editor Shana Spindler at Shana.Spindler@gmail.com. See you there!

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SAVE THE DATE: POSTBAC FAREWELL, MAY 24 AT 3:30 PM
Please save the date of Wednesday, May 24, from 3:30 to 4:30 p.m. for our postbac farewell event. We will announce our three 2017 Postbac Poster Day winners for our institute, raffle off gifts from the R&W store, and enjoy some good desserts.

Mark your calendars! More information to follow.

WRITE WINNING NIH GRANT PROPOSALS, THIS SUMMER
Thursday, July 13, 2017
9:00 a.m. – 1:30 p.m.

This workshop will address both practical and conceptual aspects that are important to the proposal writing process. Attendees will receive the “Grant Writer’s Workbook”—an invaluable, up-to-date reference tool for those who intend to write NIH grant proposals. It includes topics such as how to prepare a compelling Specific Aims page—many reviewers believe this is the most important section of your grant application.

For more information or if you would like to register early, please contact Dr. Yvette Pittman at yvette.pittman@nih.gov.

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DON'T FORGET! FELLOWS INTRAMURAL GRANTS SUPPLEMENT
AWARD ONGOING
Grant writing is important for professional development during your
postdoctoral training.

The objective of the NICHD FIGS program is to encourage fellows in the
Division of Intramural Research to apply for competitive funding from
intramural NIH or outside organizations and agencies, in order to develop your
skills in grantsmanship and in support of your career development. Award
recipients are also contributing to the DIR by bringing in additional funding
and resources, and as such merit recognition from the institute.

ELIGIBILITY:
Postdoctoral, visiting, and clinical fellows of NICHD

TERMS:
The grant for which the fellow applies must be for a competitive award of
$30,000 or more. The fellow must be the primary applicant on the grant
application; or, in exceptional cases, the co-investigator.

AWARDS:
The Scientific Director must sign off on submissions prior to them going
forward (see NICHD Grants Administration Policy). The fellow must provide
confirmation of receipt of the application by the awarding agency to the Office
of Education.

A $250 one-time stipend increase (limited to 2 applications maximum per
year), is processed at the time of the fellow’s renewal. For applications that
result in an award, the fellow receives a one-time $1,000 stipend increase.

All stipend increases will be funded centrally through the Office of Education,
typically at the time of the fellow’s renewal. In the case of a trainee on an
FTE appointment, a one-time award will be made, in lieu of the stipend
increase, subject to the NIH Office of Human Resources approval of the award
justification.

Note: For the Japan Society for the Promotion of Science (JSPS) fellowship, FIGS
applies only when an application is submitted and not if it results in an award.

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MENTOR OF THE YEAR: ACCEPTING NOMINATIONS NOW!
The time has come for you to nominate your fellow or PI for the 2017 NICHD Mentor of the Year Awards. This is your chance to recognize an individual in the Division of Intramural Research (DIR) whose mentoring has made a difference in your life at the NIH!

Information about the NICHD’s two annual intramural Mentor of the Year Awards, one for a fellow and one for an investigator is located on the NICHD fellows wiki. Please submit your nomination form and a 500-word (maximum) narrative electronically to Dr. Yvette Pittman at yvette.pittman@nih.gov. The submission deadline is Monday, May 8th.

Contact the Office of Education, if you have any questions about the nomination instructions and selection process.

POSTER JUDGES NEEDED FOR POSTBAC POSTER DAY: MAY 4
Please contact Dr. Yvette Pittman at yvette.pittman@nih.gov if you would like to help judge the NICHD postbaccalaureate fellows' posters this spring. We would like to recruit seven postdoc and graduate student judges—to visit about five posters each and attend a meeting to select the three “best poster” winners for 2017. This is a great learning experience for both the judges and postbac trainees!

For more information about the Postbac Poster Day, please visit https://www.training.nih.gov/postbac_poster_day.
April Events

THURSDAY, APRIL 27, 9 AM – 4 PM
23rd Annual NIH Take Your Child to Work Day

Inspire the next generation of NIH daughters and sons in grades 1–12 to explore career paths in science and public service at our nation’s medical research agency!

For registration information, please visit http://takeyourchildtowork.nih.gov.