

The NICHD Connection

July 2014

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You're Stressing Them Out!

By Anthony Hickey, PhD

How scents from human males (and other animals) induce stress responses in laboratory mice, which can impact experimental results.

During my early years as a graduate student, I worked with a very meticulous scientist who paid extraordinary attention to detail. On more than one occasion, he would lecture me on the virtues of careful note taking and had even once stated (half) kiddingly that the cycle of the moon during an experiment should be noted in one's experimental journal. While it is common scientific practice to document every possible detail and variable of an experiment, a recent article published last April in *Nature Methods* by Sorge *et al.* discusses an experimental variable that is almost always overlooked: the experimenter.

According to the study conducted at McGill University and led by Dr. Jeffrey Mogil, experimental mice respond differently to painful stimuli depending on the gender of the observing experimenter. The researchers report that rodent pain responses to the injection of proinflammatory molecules, as measured by facial grimacing and wound licking, are significantly blunted in the presence of male experimental observers (or clothing worn by male observers), but not female observers. The authors of this study concluded that this phenomenon is due to a stress/fear response that is triggered in mice upon olfactory stimulation by male axillary secretions (as documented by increases in murine corticosterone levels), which results in a protective response known as stress-induced analgesia. The team further demonstrates that the gender of an experimenter influences baseline measurements in behavioral testing and suggests that experimenter gender be accounted for in standard laboratory protocols regarding studies involving animal stress.

"The results of these experiments are surprising, but important," said Dr. Thangavel Karuppudurai, a postdoctoral fellow studying synaptic transmission in *Drosophila* visual neurons and visual-based behavior in the laboratory of Dr. Chi-Hon Lee. Dr. Karuppudurai was quick to point out that *Drosophila* have an olfactory system as well, and although different from its mammalian

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Letter from the Editor

If there were a common theme among NIH fellows, I'd argue that it would be that they always try their best. People walk with a purpose on campus. Perhaps it boils down to the numerous coffee shops and overused coffee pots, but NIH fellows have a simple goal—to improve the health and wellness of our community through the advancement of scientific knowledge—and they are determined to reach it. They are, in other words, the best that they can be.

All fellows know that the success of their research depends on how well the experiments are controlled. The ideal experiment will take into account all possible variables to avoid misinterpretation of data. On this issue's front page, **Dr. Anthony Hickey covers a recent article published in *Nature Methods*** that questions how well current rodent-based publications record an important variable in the methods section. You might be surprised to learn what that variable is.

If you decide to establish your own lab some day, surrounding yourself with a good team of people will help your lab run at its optimum.

Drs. Yvette Pittman and Prasanna Satpute-Krishnan provide **a thorough recap of the recent workshop “Building an Effective Team: Interview and Hiring Practices in the Sciences.”** They provide valuable tips for the interview process during the formation of your own research group.

But not all of our fellows are about to start their own labs. Even though they are early in their research careers, some of our most junior trainees are proving that they are aiming for the top. Check out some of the latest award-winning research from our NICHD postbac fellows on **page 5**, followed by a long list of **NICHD Fellows Award for Research Excellence recipients!**

As always, don't forget to glance at this month's **announcements** and **events**, including a link to a video produced by the NICHD bioviz team in *Wired* magazine.

Your Editor in Chief,
Shana R. Spindler, PhD

Please send questions, comments, and ideas to **Shana.Spindler@gmail.com**.

You're Stressing Them Out!

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counterpart, much behavioral testing of this animal model is conducted using this system.

For Dr. Julia Rodiger, a postdoctoral fellow working in the laboratory of Dr. Yun-Bo Shi, the findings of this study go beyond just the gender of the experimenter. The authors of this work have also demonstrated that exposure of laboratory mice to other unfamiliar male mice (and their bedding), or to other male animals of different species, produces similar effects as does exposure to human males, which may have ramifications for animal husbandry practices. "Many other factors may also cause stress for the animals," said Dr. Rodiger, who studies intestinal epithelial stem cell development in mice, "including the noise level in the animal facility, the number of people in the room when animals receive treatment, or even the presence of other male animals. The findings of this article could provide an explanation why different laboratories can have trouble reproducing the results of other groups."

This study is the first to show that different experimenters can influence both the behavior and physiology of a laboratory animal in a non-technical manner. It also reminds us that even the most meticulously designed experiments can still have non-obvious variables that are unaccounted for. Although this may be alarming to some, it is important to remember that scientific progress is about moving forward by learning from observations made in the past. This new knowledge has not only already improved our understanding of experimental systems, but will also provide insight on how to design tighter and better controlled experiments in the future. At the very least, these findings validate the sentiments of my former mentor in that no experimental detail is too small to record.

» Sorge, R. E. *et al.* (2014) *Nature Methods* 11, 629–32.



Interview and Hiring Practices in the Sciences: Recap

By Yvette Pittman, PhD and Prasanna Satpute-Krishnan, PhD

If you are transitioning to academe or the private sector in the coming year, you may find information from NICHHD's recent workshop "Building an Effective Team: Interview and Hiring Practices in the Sciences" helpful as you staff your lab or office. The workshop highlighted the benefits of using behavior-based interview questions and an objective evaluation process to help identify the strongest candidates.

Behavior-based interviews prompt candidates to describe *specific* experiences for different categories, such as technical skills, interpersonal skills, organization and time management, and personal motivators. For example, an interviewer could ask: "Tell me about a time when your protocol failed to give you the expected outcome and how did you proceed?" or "Tell me about a time when you worked on multiple projects at once and how did you handle it?" Behavior-based questions often provide more insight into the candidate's work ethic and personality than traditional questions such as "Can you perform these (desired) set of tasks?" or "Do you work well in a team?" which typically yield socially desirable answers (i.e., "Yes").

With behavior-based questioning, the candidate's responses reveal true strengths and weaknesses while showing the candidate's thought processes. This allows the interviewer to identify the best individual for the job by using previous learning experiences and past responses to predict future behavior.

To maximize the benefit from these

interviews, the interviewer should utilize an evaluation form. This forces the interviewer to thoroughly consider the job requirements before beginning the interview process and provides a format to keep an account of every answer the candidate provides. Rate each response on a one to ten scale—a one corresponds to an illogical answer and a ten indicates a superb answer. An ideal candidate will share very logical and articulate responses, indicating his or her ability to work effectively in your position.

The evaluation form will prove to be invaluable for comparing and ranking a diverse pool of candidates for job placement. Standardization of forms and questions will help reduce the likelihood of personal bias or forming an overall favorable impression of a candidate too soon during an interview.

The workshop also focused on the intricacies of the interviewee-interviewer interaction. In a properly structured interview, the interviewer should spend roughly 80 percent of his or her time in listening mode. Occasionally, an interviewer may feel the need to "rescue" a candidate when a period of silence occurs; avoid that temptation! Moments of silence allow candidates to process information and to formulate a clear, concise response.

Overall, Drs. Diane Epperson and Elaine Brenner conducted an extremely effective workshop to help us better understand effective interviewing and hiring practices. We hope that this information can assist you as you build your scientific team.

From Brittle Bones to Transposons: Postbac Award-Winning Research

Each year, postbacs from across the NIH gather at the annual postbac poster day to share their research with the NIH community. We are excited to announce that a total of seven NICHD postbacs received a best poster award. To honor this achievement, *The NICHD Connection* invited the winning postbacs to publish a synopsis of their research. Read below to learn more about the award-winning studies from several of our winners!

Presenting this year's NICHD awardees and their work:

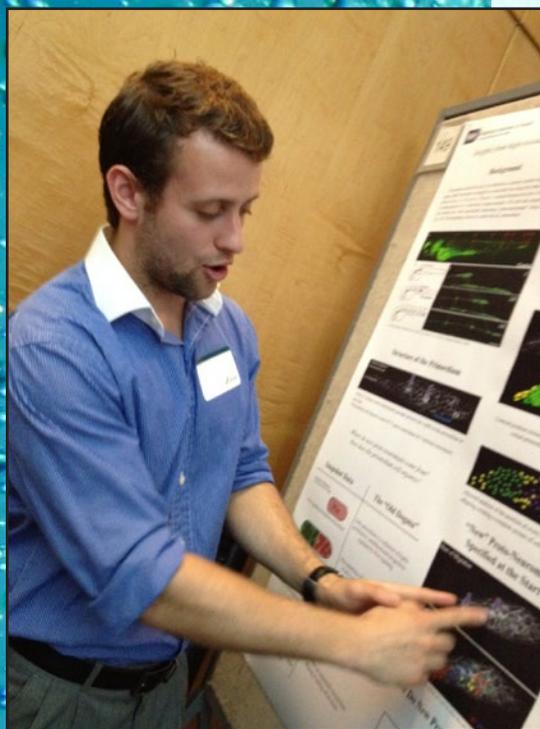
NICHD-Selected Best Poster Award (NICHD judges)

- » Andrea Attenasio, Marini lab
- » Garrett Cheung, Ahn lab
- » Alicia Johns, Albert lab
- » Maya Sangesland, H. Levin lab

Overall Best Poster Award (OITE judges)

- » Andrea Attenasio, Marini lab
- » Jeffery Head, Chitnis lab
- » Joan Nambuba, Pacak lab
- » Daniela Reyes-Capo, Han lab

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 Jeffery Head showing his poster on cell movement in the zebrafish lateral line

Postbac Award-Winning Research

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Calling on Collagen: Causative Mutations in Brittle Bone Disease

BY ANDREA ATTENASIO

ADVISOR: JOAN MARINI, M.D. PH.D.

Osteogenesis Imperfecta (OI), or brittle bone disease, is a genetic disease that is characterized by susceptibility to fractures, bone deformity, and short stature. Most cases are the result of autosomal dominant mutations in the type I collagen gene, meaning anyone who inherits a copy will have the disease. Less common cases of OI result from mutations in other genes that code for proteins that interact with collagen. These types are often recessively inherited, requiring two bad copies of the gene in order to have the disease. OI has a broad range of severity, ranging from mildly deforming to lethal, and a multitude of new genes have been recently discovered.

I sought to determine the genetic cause of OI in seven patients by first determining any abnormalities in collagen type one. Collagen is a protein comprised of three chains that spiral together to form a helix. When a mutation occurs in one of the genes coding for the chains, it disrupts the folding of the collagen helix. I performed a steady-state collagen assay for each patient to detect any abnormalities in the biochemistry of the collagen. Abnormal results indicate that the patient may have a mutation in the collagen gene, disrupting the structure of the collagen helix. Normal results suggest that the mutation is most likely not in the collagen gene.

After analyzing the collagen biochemistry of the seven patients, four were found to be abnormal. We sequenced the DNA of the four patients and found that each had an OI causing mutation in the collagen type one gene.

We found that the remaining three patients have normal collagen biochemistry. I performed tests to rule out some of the less common causes, but have yet to determine the OI causing mutation. In the future, techniques such whole exome and next generation sequencing will be useful to determine the genetic cause of these patients' OI.

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Postbac Award-Winning Research (continued from page 6)

Location, Location, Location...But Timing Too

BY GARRETT CHEUNG

ADVISOR: SOHYUN AHN, PH.D.

One often hears of dopamine in popular culture as being a mood-boosting chemical. But in addition to its emotional role, it is responsible for motor control and various cognitive functions. In the neurological disorder Parkinson's Disease (PD), midbrain dopaminergic (mDA) neurons are lost, resulting in impaired voluntary movement and a range of other symptoms. Thus, there is great interest in making mDA neurons in a dish, using advanced stem cell technologies, to replace the lost cells.

I study the early development of mDA neurons in the mouse model system. These neurons arise from a region of the developing brain known as the ventral mesencephalon (vMes), which requires a complex interplay of signaling pathways to properly generate the various cell types that eventually make up the adult midbrain.

One of those pathways is Sonic Hedgehog (Shh) signaling. During neural development, Shh acts in a concentration gradient to specify different neuronal identities. However, there is increasing evidence that the timing of Shh signaling is important as well. A former graduate student, Lindsay Hayes, found that Shh signaling is only transiently present in the vMes but is absolutely required for mDA neuron development.

We wanted to know what would happen if we sustained Shh signaling past its normal window. Initial attempts using existing genetic tools were unsuccessful. Thus, we generated a novel mouse line that conditionally produces a constitutively active form of Gli2, the main transcriptional activator of Shh signaling. Preliminary results confirm that this version of Gli2 can potently activate Shh target genes. We are currently analyzing the effects of prolonged Shh signaling on the development of the vMes and mDA neurons.

By unraveling the precise temporal requirements of Shh signaling, we can improve cell replacement strategies for PD and attain proper mDA neurons with greater efficiency. This would be a small but critical step in finding a cure for PD.



Garrett Cheung

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Postbac Award-Winning Research

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Fetal Weight Linked to Mom's Weight Gain

BY ALICIA JOHNS

ADVISOR: PAUL ALBERT, PH.D.

Pregnancy is a vital time for both mother and baby. Researchers have conducted numerous studies to determine what factors affect the result of having a healthy baby. Weight gain during pregnancy is one of those factors of interest. To date, studies have shown a positive association between the total weight that a woman gains during her pregnancy and infant birthweight, which is an important predictor of short- and long-term morbidities for the child. However, there have been few studies that have assessed how maternal weight gain throughout pregnancy corresponds to the growth of the fetus during gestation, which could be informative for planning weight gain interventions and understanding the etiology of the observed associations with birthweight.

In our study, we examined the association of weight gain across pregnancy with fetal growth. We also analyzed how changes in the rate of maternal weight gain impact the probability of a mother having a small-for-gestational-age (SGA) fetus (defined as an estimated fetal weight less than the 10th percentile of a reference population).

Our study consisted of 1,763 pregnant women from Norway and Sweden

from 1986 to 1988. The research team performed ultrasounds at four targeted study visits at 17, 25, 33, and 37 weeks of gestation. At each visit, we used biometric measurements of the fetus to calculate an estimated fetal weight. Maternal weight was abstracted from a woman's antenatal personal health record for a total of up to 21 instances.

In order to analyze the data collected, we used several novel statistical techniques to ascertain results. One of the most important statistical methods we used was the linear mixed model. The linear mixed model allows for us to precisely estimate each individual woman's weight trajectory as well as her estimated fetal weight trajectory. We then used traditional correlation analysis to examine for association between these mother-fetus trajectories.

Our results from the study demonstrated a strong positive relationship between maternal weight gain and estimated fetal weight. We also determined that maternal weight gain could be used to predict the probability of having an SGA fetus.



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Postbac Award-Winning Research (continued from page 8)

A Mutation, a Blood Disease, Two Cancers, and Many Questions

BY JOAN NAMBUBA

ADVISOR: KAREL PACAK, MD, PHD, DSC

Neuroendocrine tumors (NETs) are abnormal formations of tissue that originate from both the endocrine (hormone-secreting) and nervous systems. Except in cases where there is a known family history, it is very rare and unlikely that an individual will develop more than one type of NET. But that is not the case in a syndrome that Drs. Karel Pacak and Zhengping Zhuang, along with their inquisitive team of collaborators, recently identified.

In this syndrome, recently suggested as the Pacak-Zhuang syndrome, patients are found to have genetic mutations that overactivate hypoxia-inducible factor 2α (*HIF2A*), a transcription factor that turns on oxygen related genes. Beginning from an early age, patients are found to have secondary polycythemia and usually experience flushing and itching from abnormally high levels of a hormone that affects red blood cell production, requiring frequent phlebotomies. Patients then develop paragangliomas, NETs arising from the adrenal gland that affect multiple parts of the body. Patients next develop somatostatinomas—NETs arising from the lining of the digestive tract. While somatostatinomas are known to affect both the pancreas and gastrointestinal tract, individuals suffering from this syndrome only develop tumors in the second portion of the duodenum.

This syndrome was initially described in two patients in *The New England Journal of Medicine* in 2012 and was later expanded with newer findings reported on an additional two patients in the *Journal of Clinical Oncology*. Due to its novelty, many questions are still unanswered. With a larger cohort of patients, our group is currently looking into how to better characterize this syndrome. Some questions we are curious to answer are how mutations in *HIF2A* arise, how these tumors evolve over time, and if unique therapies exist that can be personalized to patients suffering from this particular set of disease entities.

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Joan Nambuba
Photo by Lauren Cowart

Postbac Award-Winning Research (continued from page 9)

Where Would You Be Without Transposons?

BY MAYA SANGESLAND

ADVISOR: HENRY LEVIN, PH.D.

How do cells regulate genetic diversity during times of environmental stress? The answer may be in transposable elements. Retrotransposons, a type of transposable element, are mobile portions of DNA that are able to move from one location to another in a host genome through an RNA intermediate. The retrotransposon of interest in our study is Tfl, which is unique to *Schizosaccharomyces pombe*, a species of yeast. The lifecycle of Tfl is of particular interest as it mimics the lifecycle of many retroviruses, such as HIV-1.

Genome-wide integration profiles conducted by our lab found that Tfl preferentially targets the promoters of RNA polymerase II transcribed stress response genes. Furthermore, these insertions are able to increase the expression of stress response genes. However, it is largely unknown which host factors are responsible for mediating this integration preference.

To identify host factors responsible for Tfl integration, we conducted a systematic screen of *S. pombe* lacking each non-essential gene. Through the use of genetic assays that measure levels of transposition (integration) and homologous recombination, we identified 116 candidate genes as contributing specifically to integration. The identified candidates have diverse functions relating to DNA repair (particularly post-replication repair), chromatin modification, nucleosome assembly, transcription factors, as well as cell cycle regulation.

I performed quantitative assays on the 116 candidate strains measuring levels of homologous recombination to determine whether cDNA is produced and delivered to the nucleus. I have found that the amount of cDNA present is near wild-type levels in these candidates that are defective for integration, demonstrating that the defects are at the integration step and not in cDNA production and delivery. I also conducted complementation rescue experiments to validate genes of interest, and have validated a specific histone variant as contributing to integration.

Understanding which host factors mediate integration is crucial as it may facilitate further understanding of retroviral propagation and targeting of integration sites in infection.



Maya Sangesland

FARE Recipients for 2015 Announced!

Congratulations to all 22 NICHD fellows who received a Fellows Award for Research Excellence (FARE) in the 2015 competition. FARE is a competition to recognize the noteworthy research completed by intramural fellows. Each winner of the FARE competition receives a \$1000 travel stipend to present his or her work at an upcoming scientific meeting, the chance to display a poster at the FARE awards presentation ceremony, and the opportunity to serve as a judge for the following year's FARE competition.

According to a letter from Dr. Stratakis to the NICHD community, NICHD placed third—behind NCI and NIAID—with a total of 22 winners out of 78 total NICHD submissions.

Meet your 2015 NICHD FARE recipients:

Ekaterini Nella (Merke lab)
Mark Ziats (Rennert lab)
Josefina Ocampo (Clark lab)
Yi-Han Lin (Machner lab)
Santosh Verma (Chernomordik lab)
Taylor Updegrove (Storz lab)
Celine Cluzeau (Porter lab)
Eva Szarek (Stratakis lab)
David Young (Hinnebusch lab)
Maria Bagh (Mukherjee lab)
Ginny Farias (Bonifacino lab)

Shaofei Zhang (Dasso lab)
Emily Mitchell (Perkins lab, DIPHR)
Kathryn Tabor (Burgess lab)
Yong Cheng (Loh lab)
Maeve Wallace (Mendola lab, DIPHR)
Julia Rodiger (Shi lab)
Jianxin Yu (Weinstein lab)
Parmit Singh (H. Levin lab)
Lori Griner (DePamphilis lab)
Yuheng Wei (Lilly lab)
Sandip De (Kassis lab)

July Announcements

NICHD VIDEO IN WIRED MAGAZINE!

The NICHD Bioviz team collaborated with *Wired Magazine* to produce a video of the new 3D Print Exchange. Check it out! <http://www.wired.com/2014/06/science-graphic-week-3d-print>

RETROELEMENTS: FRIENDS OR FOES?

A note from Dr. Parmit Singh:

Journal club is an excellent way to meet and discuss recent findings that are published in scientific journals. You learn not only about current happenings in your field, but also about other important areas of science.

I am organizing a summer intern journal club “Retroelements: Friends or Foes?” along with my colleague Dr. Caroline Esnault. It will be held **every Wednesday** from **June 25 to July 30, 2014**, in **building 18T/32, lecture hall A, from 2:00 to 3:00 p.m.** In each meeting, we will discuss a current research article from one of the top journals. The article will be related to retroelements, namely retrotransposons and retroviruses.

The last date for registration is already over, but if you are interested to know more about retrotransposons and retroviruses, you can still join our club and come directly to the meeting.

For the more details, please visit to the website: https://www.training.nih.gov/assets/Summer_Journal_Clubs_-_2014.pdf

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July Announcements (continued from page 12)

SAVE THE DATE! SUMMER POSTER DAY, AUGUST 7

The Summer Poster Day for NIH interns will be held on Thursday, August 7th at the Natcher Conference Center. The registration deadline for summer interns to submit poster titles is Tuesday, July 8, at 5:00 p.m. Learn more at https://www.training.nih.gov/summer_poster_day.

OITE ENGLISH COURSE

OITE is offering a two-day English language course for NIH trainees on July 11 and 14, from 8:30 a.m. to 1:30 p.m. This course is designed to help graduate students and postdoc fellows who have been in the U.S. for less than two years practice and feel more comfortable speaking English. Course activities include the following: practicing scientific discussions, exploring U.S. culture, and learning about additional English language resources.

If you feel this course will be valuable to your training while at NIH, you can register at https://www.training.nih.gov/events/view/2/1396/Improving_Spoken_English_Two-day_Class. Any questions about the class can be directed to Julie Gold at goldje@mail.nih.gov.

July Events

WEDNESDAY, JULY 2, 9, 16, 23, 30, 2-3 PM

Journal Club “Retroelements: Friends or Foes?”
Building 18T/32, lecture hall A

FRIDAY/MONDAY, JULY 11/14, 8:30 AM-1:30 PM

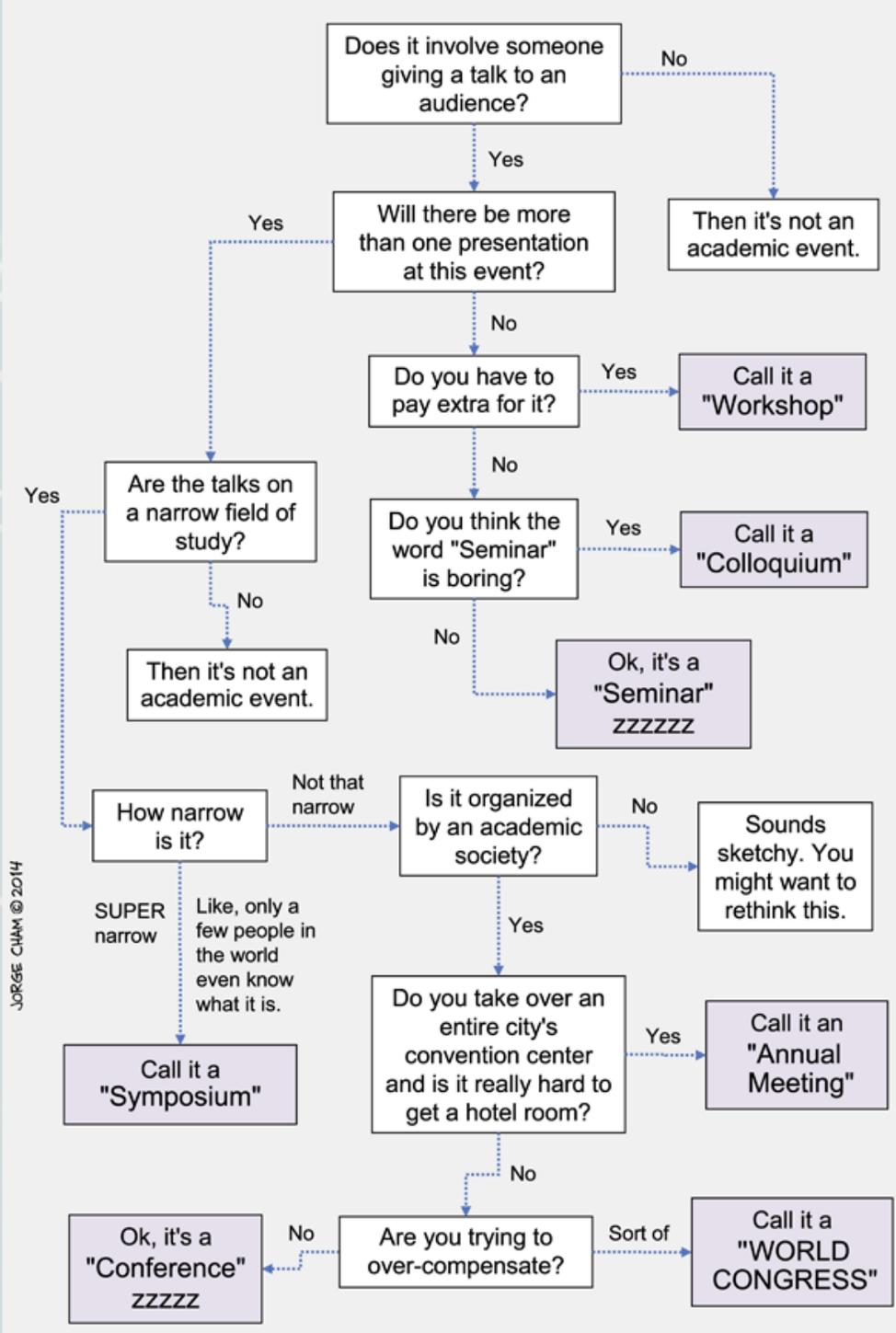
OITE-sponsored two-day English language course
Event registration is limited to Graduate Students, Postbacs, and
Postdocs/Fellows
Registration required

WEDNESDAY, JULY 16, 9 AM-3:30 PM

NIH Graduate and Professional School Fair
For more information and registration, go to https://www.training.nih.gov/gp_fair

PhD Comics

What to call your Academic Event:



JORGE CHAM © 2014