# INTERNATIONAL NEUROTOXICOLOGY ASSOCIATION

## INA News September 2016

### Message from the President on INA-16

INA 16 will be held May 20-24 in Florianopolis, Brazil. This will be will be a great opportunity to meet together with the Neurotoxicity Society (NTS), which has a variety of converging interests with INA concerning mechanistic research of toxic impacts on the nervous system. Florianopolis is a vibrant, culturally and intellectually rich city on the coast of south Brazil with a very high quality of life. It is the capital city of Santa Catarina state. Florianopolis is home to the Universidade Federal de Santa Catarina which has a considerable research effort including neurotoxicology. NTS like INA is a global society. It has a strong cadre of members in South America. The aim of NTS is to unite basic and clinical scientists in neurotoxicity to increase understanding of processes, mechanisms, and outcomes of neurodegeneration and regeneration to facilitate the advance of preclinical research discoveries to clinical and medical utility. There will be a variety of symposia by both societies from cellular/molecular through translational to clinical/epidemiological topics. There will also be ample opportunity for individual presentations at platform and poster sessions. Student and post-doc travel awards will be available. The Hooisma Lecturer will be Dr. Tim Greenamyre, a pioneer in research connecting pesticide exposure with Parkinson's disease. Check out the information at http://www.neurotoxicology.org/next-meeting-2/ and be looking for announcements soon about submissions of papers and registration for this important meeting in a wonderful locale.

See you there!
Ed Levin, INA President

### Message from the Past-President

One of the last tasks that a past-president of INA has to complete is the search for new officers. Now it is my turn and with the help of two other long-standing INA members as the nominating committee to search for candidates running for the various vacant officer positions. However, even such a distinguished committee might overlook eligible candidates. So please feel free to send me your suggestions. Self-nominations are also welcome. Thus, be warned, some of you might find my email in your inbox! We hope to complete the search for candidates by October and have the ballot later this year. In addition, the newsletter editorship is now open. Please contact Ed Levin or Remco Westerink if you are interested in volunteering for this activity.

Meanwhile all papers submitted for the special issue of INA-15 that will be published in *NeuroToxicology* have been reviewed, revised, and we are almost ready to publish the printed version. Since their final acceptance, all papers are available online and some of them have already been cited. As an international association I'm happy to say that this issue really reflects our worldwide membership. We have original papers from Brazil and Japan, as well as various contributions from Europe and the USA. The members of the scientific committee served as guest editors and they did a great job since convincing reviewers is sometimes hard. There are also some additional papers covering the INA-15 presentations that have been published in several issues of *Neurotoxicology and Teratology*. Thus, a vivid joint meeting with NBTS/ DNTS finally achieved a scientific output of high impact.

Christoph van Thriel, INA Past-president

## Message from the President-elect

In this newsletter we have again a 'Researcher in the spotlight'. This time it is Merle Paule for his article "Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys", which is the most cited article in *Neurotoxicology and Teratology* since 2010. Luckily, we also have two contributions for our new 'Young researcher series' as part of our new Postdoc/Grad Student Section. Johanna Nyffeler, winner of the David Ray Award at INA-15 in Montreal, and Jessica Plavicki provide us some insight in their daily scientific struggles and achievements. Merle, Jessica and Johanna, thanks for your insightful contributions! If you want to nominate a (young) researcher to be in the next newsletter's spotlight, let us know!

As mentioned in the previous newsletter, we need your help and input to create a vivid newsletter (and website)! Therefore, please don't hesitate to send us your photos from INA meetings, to announce jobs in your lab, or to write some interesting sentences about your last publication, etc. Also let us know if you offer a product or service yourself and want to be listed on our website or in our newsletter. Our advertisement fees are low and will be entirely used to **generate a student travel fund** that will help our young members to visit our biennial meetings.

All the best and enjoy our website and newsletter!

Remco Westerink, INA President-Elect

## Secretary's Report

For 2016 we have had 4 new members (1 full and 3 student). Currently we have 198 full members and 15 student members from 30 countries.

Sandra Allen, INA Secretary

## Treasurer's Report

It's been a year since Jan Lammers passed the treasurer's files on to me and urged me to make you all pay. The good news is that I still like it. Also, it turns out that I can apparently be convincing: so far 51 members paid their dues. Many thanks to these 51 people! However, considering the total number of members I have to conclude that only 25% of the members has paid its dues so far… There is clearly room for improvement! Those of you who forgot to pay until now have received a second reminder recently. Please keep in mind that paying your dues is required to be eligible for the reduced INA-meeting registration fee. There are two types of membership: full membership (paid) and student membership (free).

During the business meeting in Montreal we decided to pay dues for two years at once covering the time between two INA meetings. Therefore, for the period 2016-2017 full members pay 50 Euros or 60 Dollars membership fee. Payments can be made via wiring to the INA bank account (details in the dues payment document on the website) or the Paypal account of the INA treasury

(treaurerinternationalneurotox@gmail.com). This is also the email address to contact me with questions regarding dues payment.

With financial greetings,
Harm Heusinkveld, INA Treasurer

## 2016 INA Business Meeting

**Minutes for INA Business Meeting March 15, 2016 12.15- 1.15pm room Belle Chasse, Hilton New Orleans Riverside (Headquarters), New Orleans, USA**

Attendees: Ed Levin, Christoph van Thriel, Remco Westerink, Helena Hogberg, Will Boyes, Lucio G. Costa, Lilah Glazer, Yael Abreu-Villaça, Pam Lein, Diane Rohlman, Roberto Lucchini, Joan Cramner, Jean Harry

The meeting was hosted by INA president Ed Levin.

Welcome – Ed Levin

Minutes from the INA business meeting held in Montréal, June 2015 were distributed and approved. The handouts also included the secretary's report and the treasure's report.

1) Presidents report:
Ed Levin briefly summarized the facts (participants, symposia, student symposium, Hooisma lecture etc.) of the Montréal meeting and the fruitful collaboration with NBTS (now DNTS). A special issue will be published in Neurotoxicology soon. The session on flame retardants as already been published in NTT. In general, INA 15 was a wonderful event even though it was a little bit different from the "traditional" INA meetings. Finally, there will be a loss of approximately $800 (e.g. catering was more expensive) and Ed Levin will provide a financial report soon.

2) Secretary's report (given by Ed Levin)
Two of the new member Lilah Glazer and Yael Abreu-Villaça were present and introduced themselves to the participants. The update of the member list is ongoing and everyone is requested to help with email addresses etc. of members that are not contactable.

3) Treasurer's report (given by Remco Westerink)
The account detail could be found in the handout and due to the Montréal meeting and charges for the website maintenance the US account has dropped to $1,692 in 2015 and is even less now. The US account is still necessary for the next meetings as well as for the website and some transfer from the Dutch account might be needed during this year. With respect to the dues, 54 members paid already but the number of paying members needs to be improved (26% of the 204 full members). Harm and Sandra are working on this and ideas and support are welcome.

4) Update INA-16 in Florianopolis, Brazil (together with NTS)
The dates are May 22 to 26 and estimates of the costs will be given soon. Currently, the local organizers are negotiating with two different locations (city/ university or beach) and Mikki Aschner will visit Florianopolis next month. After that on-site visit, all meeting details will be send to the membership (before the summer). In collaboration with NTS a program/local committee will be assembled soon. The scientific committee, headed by Ed Levin, will be announced in due time and this committee will develop a time line or symposia schedule and a call for symposia proposal in agreement with NTS. The number of the pre-organized symposia should be reduced and adopted to the time schedule. Funding for symposia sponsors

should be included in all proposals. Clear rules for funding or reimbursement of speakers as well as the uncertainties about reimbursement due to the funding situation will be communicated in the call. It was suggested to have a finance committee supporting fund raising. Financial crises in Brazil needs to be taken into account but was suggested by Yael Abreu-Villaça that the Brazilian might be able to support invited speakers.

Ideas of further sponsoring/ financial support:

- International meeting sponsoring by SOT; formal procedure by Ed Levin
- NIH funding to NTS and US INA member must be checked and organized
- EU travel grants, US EPA might sponsor HTS and ToxCast initiatives
- Alternative models might be sponsored the JRC in Ispra, CAAT
  Everyone should look for sponsors (e.g. companies selling lab equipment) and the sponsor should be informed that they are mentioned!

The publication of papers in two journals will be without problems but have to be concerted in advance.

5) Website update:
Helena Hogberg will try to update the "front page" with new photos and student members should be activate to help/ contribute making the website more vivid and attractive (e.g. lab pictures stories etc.).

6) INA-17 (2019) update:
Düsseldorf was selected and Christoph van Thriel explained the plan of having a "traditional INA-only" meeting somewhere outside of Düsseldorf. Updates will be given continuously.

Other business:

Ed Levin proposed to have INA-18 in 2021 in North Carolina together with DNTS. Other proposals should be selected soon and send to the membership in the near future.

Pam Lein suggested to send a survey to the participants after the meeting to get more feedback. That was supported by the participants.

Remco Westerink asked for new newsletter contributions! The next newsletter should be sent out after the summer.

## Researchers in the Spotlight

Merle G. Paule (Director, Division of Neurotoxicology, National Center for Toxicological Research US FDA) - "Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys" Paule et al., *Neurotoxicol Teratol* (2011) 33, 220-230.

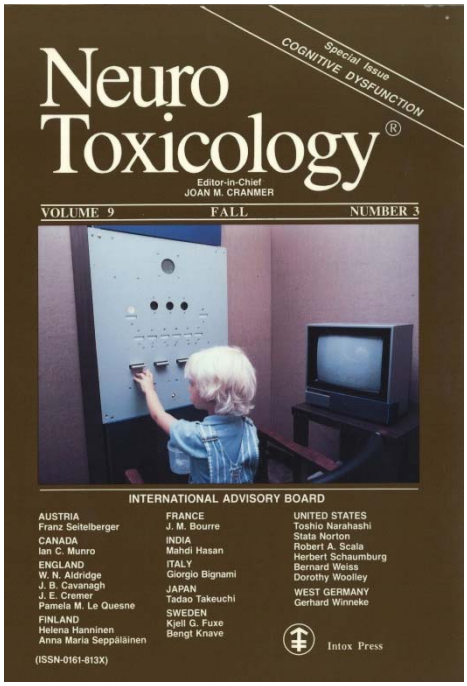
I grew up in the states as a California boy and received my B.S. in Biochemistry and Ph.D. in Pharmacology and Toxicology from the University of California at Davis after which I moved to Little Rock to conduct post-doctoral studies in Behavioral Pharmacology and Toxicology at the University of Arkansas for Medical Sciences. I then (early 1980s) began work at the FDA's National Center for Toxicological Research (NCTR) in Jefferson, Arkansas, where I was recruited to develop a nonhuman primate behavioral pharmacology/toxicology laboratory, initially to study the effects of chronic marijuana smoke exposure on cognitive function in 'teenage' monkeys. (Talk about an interesting experience……they get as lazy as human teens after a few months of toking.) The unique opportunities and great scientific and collegial atmosphere at NCTR have made it a joy for me to have remained there to this day. In 2000 I was honored

with certification as one of FDA's few Senior Biomedical Research Scientists and in 2005 I became the Director of the Division of Neurotoxicology at NCTR.

Throughout my career I have developed and utilized automated operant behavioral systems – including the NCTR Operant Test Battery (OTB) – for objectively monitoring multiple complex brain functions in nonhuman primates, children, and rodents (see photo). These functions include learning, short-term memory, motivation, color and position discrimination and time perception and are used as measures for determining the effects of drug and other chemical exposures. Utilization of similar or identical behavioral tasks across species serves to facilitate interspecies extrapolation and, thus, the risk assessment process. My main research interests involve behavioral pharmacology and toxicology emphasizing cognitive function deficiencies as indicators of toxicity; the pharmacology and toxicology of central nervous system drugs with an emphasis on general anesthetics; developmental neurotoxicology and pharmacology; and operant behavioral analyses to quantitate specific brain functions in children and laboratory animal models, particularly nonhuman primates, for animal-to-human extrapolation.

John Chelonis (left) and Merle Paule (right) with an early version of the NCTR OTB apparatus.

Much of my current research involves the preclinical assessment of the developmental neurotoxicity associated with general anesthetics typically used in the pediatric setting. A paper I published with colleagues in 2011[1] described seemingly permanent cognitive dysfunction in rhesus monkeys induced by a single, albeit prolonged (24hr), bout of ketamine-induced general anesthesia during early infancy. That paper provided proof-of-concept that general anesthesia, when effected during a sensitive developmental period, can lead to cognitive dysfunction observable into adulthood and was the most cited paper in the journal *Neurotoxicology and Teratology* from 2011-2014. Since then, we have demonstrated similar-if not worse- adverse effects with other agents (isoflurane plus nitrous oxide: and sevoflurane) given for much shorter durations (8 hr). The good news is that there appear to be several agents that are capable of greatly diminishing--if not completely preventing--the majority of the neurotoxicity associated with general anesthesia during periods of rapid brain development. Ongoing and future work will focus on identifying: specific developmental periods of susceptibility; general anesthesia duration thresholds for the induction of neurotoxicity; comparative toxicities among agents; and dose-response and toxicity profiles for protective agents. In association with this work, I am currently Co-Editing a Special Issue of *Neurotoxicology and Teratology* entitled "Developmental




Merle Paule's first human subject that underwent test at the humans subject laboratory in the Arkansas Children's Hospital and appeared on the cover of Neurotoxicology many years ago. *Reproduced with permission of Editor/Publisher Joan M Cranmer*

Neurotoxicity Associated with Pediatric General Anesthesia: Preclinical Findings.

In addition to maintaining an active research agenda, I have served as an elected officer or appointed committee member in several prestigious scientific societies including Past President of the Behavioral Toxicology Society, the Neurobehavioral Teratology Society and the Neurotoxicology Specialty Section of the Society of Toxicology. I am also a member of several other scientific societies including the Society for Neuroscience, the Society of Toxicology, the American Society for Pharmacology and Experimental Therapeutics, and INA, of course. I serve as a reviewer for several scientific journals and am Associate editor for the journals *Neurotoxicology* and *Neurotoxicology and Teratology.* I have published over 220 research articles and 35 book chapters and am currently co-editing the second edition of the book Developmental Neurotoxicology, first published in 1998. I maintain enjoyable academic interactions via Adjunct Professorships at the University of Arkansas for Medical Sciences in the Departments of Pharmacology and Toxicology and in Pediatrics. I am an elected Fellow in the Academy of Toxicological Sciences and in the International Behavioral Neuroscience Society. I continue to look forward to going to work every morning, where Hawaiian shirts are a must every Wednesday and the science continues to provide a never-ending source of intrigue, excitement, and achievement. May the luck continue!

[1]Paule, M.G., Li., M., Allen, R.R., Liu, F., Zou, X., Hotchkiss, C., Hanig, J.P., Patterson, T.A., Slikker, W., Jr., and Wang, C.: Ketamine anesthesia during the first week of life can cause long-lasting cognitive deficits in rhesus monkeys. Neurotoxicol. Teratol., 33: 220-230, 2011.

## Postdoc/Graduate Student Section
## Young researcher series

Johanna Nyffeler (University of Konstanz, Germany)

My name is Johanna Nyffeler and I am a PhD student at the Doerenkamp-Zbinden Chair of *in vitro* Toxicology and Biomedicine in Marcel Leist's lab at the University of Konstanz, Germany.

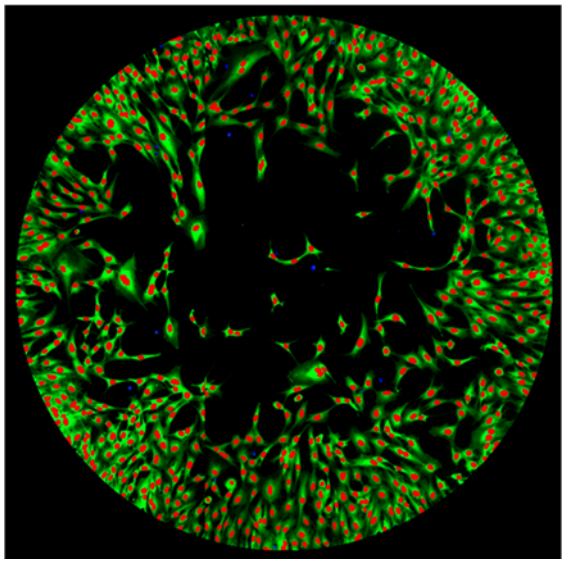
My first contact with INA was to participate last year at the INA-15 meeting in Montreal, where I presented my project in a poster and a talk. It was exciting for me to get insights into the field of neurotoxicology. As I work fully *in vitro*, it was challenging to understand projects with animal experiments and behavioral tests. But it was interesting to see how slight disturbance of the nervous system can have a tremendous effect on the individual or the population.

My path into neurotoxicology was not very straight forward. However, I already knew as a teenager, that I wanted to become a scientist. My interest was in medical plants and I wanted to cure diseases using natural occurring drugs. Therefore, I decided to study biomedicine and biochemistry. With time, my interest shifted and I thought instead of curing diseases, it would be good to know the causes so that we could avoid them. Hence, I did my Masters degree in genetics. For my Master thesis, I worked on the genetic causes of autism and became interested in neurobiology. I learned that some toxicants are suspected to cause/influence neurodevelopmental disorders. Once again, my interested shifted as I recognized that it would be good to avoid toxic compounds. I started to realize that part of the problem is

that we do not even know yet which compounds are hazardous. That is how I came to Marcel Leist's lab in Konstanz.

Our research focuses on alternatives to animal testing. We develop new assays to measure (developmental) neurotoxicity *in vitro*. We mainly work with human cells; thus we differentiate neural cells from human pluripotent stem cells. This is quite time-consuming and tricky, as the cells are sensitive to any kind of disturbance (different handling, different medium supplier, etc). I work with neural crest cells (NCC), a special cell type arising during early embryonic development and giving rise to many cell types. *In vivo*, these NCC arise at the neural tube border and migrate to different places in the embryo where they give rise - amongst others -to peripheral and enteric neurons, oligodendrocytes but also some bones and cartilages and teeth. In our lab, these cells take one month to differentiate and one month to expand, before I can start my experiments!

As migration is one of the key features of NCC, my goal was to develop an assay to measure whether toxicants disturb this migration process. As a starting point, we had a wound healing assay, but my first aim was to adapt the assay to higher throughput. I use an automated microscope to image 96-well cell culture plates and we developed a software to count the number of migrating cells. It took me about two years to establish this assay, with all positive and negative controls and I learned a lot about assay development.



Example of the migration assay

Having set up the assay, we screened around 80 compounds. We found several compounds that were not previously known to inhibit NCC migration. At the moment, I am looking more into the mechanism of selected NCC migration-inhibiting compounds. As cell migration is a very complex process, many molecular pathways are involved. This makes it very difficult to find which pathway(s) is/are affected by a certain toxicant.

I like my PhD project. It was interesting to develop an assay and challenging to find the mechanisms. I like data analysis, and therefore I enjoyed to analyze the data of all 80 tested compounds. However, to perform all the necessary experiments to obtain this data was probably my biggest struggle so far. The assay is well established and easy to execute and hence I struggled sometimes with the very repetitive work. When I started my PhD, it was also challenging for me to enter a completely new field. But I am happy about it, as I think that this field is important and that I can make a useful contribution with my work. We still know only very little about developmental neurotoxicity. Many chemicals have never been tested and if so, only very limited animal data is available. I think it is essential to gather more information to increase safety.

I am currently at the end of my PhD and I hope to hand in my thesis in a few months. I would like to continue as a PostDoc in toxicology. As I am experienced in data analysis and programming, I would like to move towards computational toxicology. Or alternatively, I would like to develop new cellular assays. I do not yet have a PostDoc position, but if you have any suggestions, feel free to contact me.

Best regards,
Johanna Nyffeler

Jessica S. Plavicki, Ph.D. (University of Wisconsin - MA, USA)

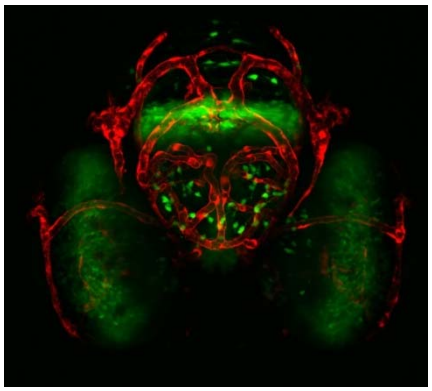
I received my Bachelors degree from the University of Texas at Austin. My Undergraduate Honors Thesis provided me with training in systems neuroscience, behavior, and endocrinology, and impressed upon me the importance of utilizing multiple levels of analysis in research in order to form an integrative understanding of biological processes. Consequently, as a graduate student I sought training at a different level of analysis and joined a developmental genetics lab working with Dr. Grace Boekhoff-Falk at the University of Wisconsin at Madison. For my PhD research, I was intrigued by the hypothesis that the ancestral function of the transcription factor *Distal-less (Dll)*, which has conserved functions in limb development, may have been in building a primitive nervous system. For my PhD thesis, I demonstrated that *Dll* is expressed during multiple stages of *Drosophila* nervous system development in both neurons and glia in the CNS and PNS. In work published in the *Proceedings of the National Academy of Sciences*, I demonstrated that *Dll* is necessary for the development of olfactory receptor neurons (ORN), ORN dendritic morphology, ORN axon pathfinding, and formation of the mushroom bodies, a brain region that mediates learning and memory in insects.

My graduate training established a passion for developmental biology and biological imaging. As I approached the end of graduate school, I knew I wanted to move to a vertebrate genetic model and make my research more applicable to human health. Given a longstanding interest in environmental issues, I sought training in toxicology. In April of 2009, I accepted a NIEHS-funded postdoctoral position with Dr. Richard Peterson studying dioxin-induced cardiotoxicity in a zebrafish model. I used my training in developmental biology and microscopy in conjunction with my new training in toxicology to determine that dioxin-induced activation of the zebrafish *aryl hydrocarbon receptor 2* (*Ahr2*) during embryogenesis prevents the formation of proepicardial progenitor cells that give rise to the outer most layer of the heart, the epicardium. Loss of the epicardium is lethal and can account for most if not all of the cardiotoxicity produced by TCDD exposure. This work was subsequently nominated for Paper of the Year by the Developmental and Reproductive Specialty Section of the Society of Toxicology (SOT) and was also the basis of a Midwest Regional SOT Young Investigator Award. As post-doctoral research, I successful competed for a NIH K99 Pathway Independence Award. I spent two additional years as an Assistant Scientist at UW-Madison completing K99 research aims. My K99 research integrates my training in neuroscience, genetics, cardiovascular development, and toxicology and set the stage for my current research.

In September of 2016, I will start my first faculty position as a tenure track Assistant Professor at Brown University in the Department of Pathology and Laboratory Medicine. My current research uses the zebrafish as a model for studying the molecular mechanisms that mediate CNS angiogenesis and blood brain barrier development, and how these mechanisms may be misregulated following chemical exposures.

The vasculature plays critical roles in brain development and function. It supplies oxygen and essential nutrients to the brain parenchyma as well as protects the brain from neurotoxic blood-borne substances. During embryonic development, endothelial cells coalesce and undergo morphogenetic movements to form vessels in a process known as vasculogenesis. These early steps in vessel formation are followed by CNS angiogenesis in which nascent blood vessels sprout off of pre-existing vessels and vascularize the developing brain parenchyma. The CNS vasculature is composed of specialized brain endothelial cells

(BECs). BECs along with their associated pericytes, astrocytes, neurons, and the extracellular matrix, form the “neurovascular unit”. Together, the components of the neurovascular unit create physical and chemical barriers that confer selectivity and determine which substances move from the blood into the brain parenchyma, thus creating the blood brain barrier (BBB). The BBB is crucial for maintaining CNS homeostasis and health. However, the selectivity of the BBB also prohibits the entry of biological therapeutics, providing a significant challenge for the development of new CNS drugs. Although the BBB was discovered over a century ago, many open questions exist regarding the mechanisms that regulate BBB development.



Immunostain of the zebrafish brain

The zebrafish BBB is phenotypically and functionally comparable to that of mammals and the signals known to induce CNS angiogenesis and barriergenesis are conserved between fish and mammals. Given that vasculogenesis, angiogenesis, and barriergenesis all occur in zebrafish during the first week of development, it is possible to image all of the major steps in neurovascular and BBB development *in vivo* and in real time. Despite the advantages conferred by the zebrafish model for studying CNS angiogenesis and BBB development, the system has yet to be fully exploited.

AHR signaling is necessary for proper vascular development during mammalian organogenesis and, in fish, Ahr2 is activated in the vascular endothelium following exposure to persistent environmental contaminants such as dioxin. Dioxin exposure has been reported to impair development of two cranial vessels on the dorsal surface on the brain, the prosencephalic artery and the mesencephalic vein. However, it is not known whether dioxin disrupts the vascularization of the underlying brain parenchyma. Using *in vivo* imaging of transgenic reporter lines, I have found that dioxin exposure impairs CNS angiogenesis and BBB formation. Using qPCR, I have determined that dioxin exposure alters the expression of efflux transporters normal expressed in BECs. Dioxin exposure also alters the development of other cell types within the neurovascular unit. In addition, I have identified novel functions for the transcription factor, *sox9b*, in neurovascular development. Loss of *sox9b* likely accounts for some the neurovascular changes observed following dioxin exposure. Using the Tol2 transgenesis system, I am generating transgenic fish lines to manipulate Ahr2 activity in a cell-type specific manner in order to determine which cell types mediate the dioxin-induced phenotypes.

Like most scientists, I have encountered a variety of challenges over the course of my research training. One thing I learned early on is that everything takes longer than you think it will take and you will not anticipate all the ways something can go wrong. Generating transgenic fish, in particular, has been a challenging experience. My advice to other young researchers is to have a clear plan for a manuscript when starting a project and to be persistent.

Thank you for the opportunity to share my research training experiences, my accomplishments, my current research, and the challenges I have encountered along the way.

Jessica S. Plavicki

## Current INA Officers

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|---|---|---|
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