**Prep for Peyman call**

**Friday March 27, 2015 11 am**

**Notes from his grand rounds talk:**

*Live imaging in behaving animals.*

*20 gabaergic interneuron types in cortex and hippocampus*

*excitatory neurons are not a homogeneous group. Cells that appear identical even in the same layer can have distinct long range…*

*Miller et al., 1981 – pruning of circuits thru development*

*In vivo 2 phonton imaging*

*Cells in the visual cortex are … selective depending on the light pattern.*

*McAdams and Maun…*

*Shuler and Bear, 1996*

*Ji and Wilson, 2007 - a particular cell fires at a certain place in a maze*

*Niell and Stryker, 2010*

*Kitamura et al., 2008. In vivo patch recording*

*Cholinergic blockade w/ antagonist*

*Noradrenergic antagonists flatlines the cortex, prevented depolarization seen with running*

*These recordings are of thevisual system, with light pattern input*

*Part3 attention*

*Licking*

*Part5 – SK potassium channelopathy PTEN model of autism*

*PTEN knockout 🡪 macroencephalopathy (big brain)*

*Neuron by neuron, saw that dendrites continue to grow in PTEN cre dependent KO mice*

*Blocking sk channels rescues the intrinsic physiology deficit that is defined by increased hyperpolarization in PTEN mutant mice.*

*SK channel abundance increases in PTEN het mice.*

**Emails between Peyman and Pulst**

Dear Stefan,

Happy to talk about the R37.

Thanks

Peyman

Peyman Golshani, MD/PhD

Associate Professor (Effective July 2015)

Department of Neurology

David Geffen School of Medicine. UCLA

Neurologist, WLAVA Medical Center

On Mar 24, 2015, at 8:18 AM, "Stefan M. Pulst" <[stefan.pulst@hsc.utah.edu](https://www.umail.utah.edu/owa/redir.aspx?C=1toe3ZvhHU-iMerar_3xsw2mvYkwPNIIB4r3RQnd9BbcduXp8-n7rB07uylBWf24AR6RnvXSaRc.&URL=mailto%3astefan.pulst%40hsc.utah.edu" \t "_blank)>

 wrote:

Dear Peyman,

I had a talk with Tom Otis yesterday about our ataxia program project and our existing R37 (Javits) grant for which Tom used to have a subcontract.

I would like to talk about these two exciting projects with the University of Utah at UCLA.

Let me know when you have time. Lauren will coordinate.

Stefan

*Stefan-M. Pulst, M.D., Dr. med*

*Professor and Chair*

*Department of Neurology*

*University of Utah*

[http://healthcare.utah.edu/fad/mddetail.php?physicianID=u0595302](https://www.umail.utah.edu/owa/redir.aspx?C=1toe3ZvhHU-iMerar_3xsw2mvYkwPNIIB4r3RQnd9BbcduXp8-n7rB07uylBWf24AR6RnvXSaRc.&URL=http%3a%2f%2fhealthcare.utah.edu%2ffad%2fmddetail.php%3fphysicianID%3du0595302" \t "_blank)
[http://pulstlab.genetics.utah.edu/](https://www.umail.utah.edu/owa/redir.aspx?C=1toe3ZvhHU-iMerar_3xsw2mvYkwPNIIB4r3RQnd9BbcduXp8-n7rB07uylBWf24AR6RnvXSaRc.&URL=http%3a%2f%2fpulstlab.genetics.utah.edu%2f" \t "_blank)

**Notes on Peyman conference call**

Tom at Roche

Several projects with tom, cerebellar projects

Tom went thru with him, and compounds from isis

Currenty have ro1, on which tom has subcontract, Meera on

Peyman in marion davies close to deans office, is supposed to get one in 1 yr close to Tom’s lab

How tom has handled is, to leave the sub at ucla, but at some point need to decide what to do w/ sub

Goes w/ . . . mglur signaling and ca homeostasis

Hansen paper, close correlation in firing frequency and behavior

 Transcr 4 wks, 6 wks see decreased pc firing, 6-8 wks first rotarod phenotype, all progressive

 Not shown if pc firing is causative for the behavior, but is correlated

Ro1 cal hyp for PC cells

Program Project

 Tom, Ilya, Stefan, Two Cores

Had hoped tom could do at Roche, but Roche not interested because 7 mil$ not enough, and legal details including roche and ASOs that they are developing themselves.

Tom talked to Peyman about being the project 3 PI

Key personnel and equipment would remain,

Enthusiasm because no other P01 on the cerebellum – Harry Orr’s ended

Havn’t yet discussed with Katrina Gwinn

Is something that interest Peyman

Would have re-submitted earlier but tom left – then talked to Katrina and thought doable but . . .

Peyman’s qualifications – good publ record, on things related, calcium and neuronal firing.

 We are dependent on physiology on the discovery side and in evaluating

May also have to look at rebudgeting the Javit award, tom can’t draw salary, salary option for Peyman.

We plan to submit in September, lots of changes, may then have to resubmit again.

Peyman’s interest in autism

**Prep for Tony Oro Call**

**Friday March 27, 2015 3:30 pm**

Dear Tony,
these are indeed intriguing and exciting results.

Rather than sending frozen stuff to you I would like to do  these analyses in our group as we have everything ready for not only the Pcp2 model, but also for our BAC model and a new flag-tagged model. For any transfer of materials we would have to set up an MTA, which we should do anyway.
We have pretty deep analyses on the transcriptome and protein level for these various  lines.

The behavior in the Pcp2 mice is very solid and has been seen in three continents now so it is pretty invariant to environment or GxE interactions.

We would need to set up breeding for behavior experiments, but could combine behavior with other experiments at the end of the rotarod experiments.

Let's set up a talk when convenient (can be this week). Lauren will coordinate.
best regards
Stefan

Stefan-M. Pulst, M.D., Dr. med
Professor and Chair
Department of Neurology
University of Utah
[http://healthcare.utah.edu/fad/mddetail.php?physicianID=u0595302](https://www.umail.utah.edu/owa/redir.aspx?C=1toe3ZvhHU-iMerar_3xsw2mvYkwPNIIB4r3RQnd9BbcduXp8-n7rB07uylBWf24AR6RnvXSaRc.&URL=http%3a%2f%2fhealthcare.utah.edu%2ffad%2fmddetail.php%3fphysicianID%3du0595302" \t "_blank)
[http://pulstlab.genetics.utah.edu/](https://www.umail.utah.edu/owa/redir.aspx?C=1toe3ZvhHU-iMerar_3xsw2mvYkwPNIIB4r3RQnd9BbcduXp8-n7rB07uylBWf24AR6RnvXSaRc.&URL=http%3a%2f%2fpulstlab.genetics.utah.edu%2f" \t "_blank)

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From: Anthony Oro [oro@stanford.edu]
Sent: Tuesday, March 24, 2015 12:18 AM
To: Stefan M. Pulst
Cc: Lauren Brown; Thomas Otis
Subject: SCA2/MIM collaboration

Dear Stefan,
I hope you are doing well. I believe Tom spoke with you today about our result that
Src family kinases apparently rescue the depressed basal firing rate of SCA2 purkinje cells
from their baseline rate of about 12-14 Hz to about 40 Hz.  We are excited about the result and would
love to work with you to explore the relationship between MIM, Src family kinases, and SCA2 disease proteins.

I'd like to arrange a time to talk about final experiments for the paper.  In particular, I think rescuing the
SCA2 behavior with Src kinases as measured by rotorod or activity tests would be top of the list. We have worked out
treatment protocols to optimize inhibition in the cerebellum.  This experiment we could perform today if you had enough
SCA2 mice.  Also, we'd love to get SCA2 tissue blocks and fresh frozen tissue extracts to check MIM levels by western or
localization in SCA2 mice, to ascertain whether MIM acts in a parallel  (more likely) or linear (less likely) pathway with SCA2.

Would love your input and availability for a skype call later this week or early next week.

Best wishes,

Tony

Anthony Oro MD/PhD
Professor
Program in Epithelial Biology
[http://orolab.stanford.edu](https://www.umail.utah.edu/owa/redir.aspx?C=1toe3ZvhHU-iMerar_3xsw2mvYkwPNIIB4r3RQnd9BbcduXp8-n7rB07uylBWf24AR6RnvXSaRc.&URL=http%3a%2f%2forolab.stanford.edu" \t "_blank)
oro@stanford.edu

Notes on the call:

Mtss1 or MIM is the gene. When you mutated in drosophila, embryonic phenotype, did a genetic screen for dominant suppressor for lof phenotype, genes when you mutate that restore phenotype. #1 thing got was everything in the src kinase pathway. When make mim double mutants, the fly embryonic phenotype is rescued. Then in veterbrates, whevere you ko mim you can rescue with src inhibitors, and saw cerebellar ataxia.

Sca1 and sca5 had similar pathways in terms of regulating endocytosis in normal function, so tried this with src inhibitors, questions was do acute slice cultures rather than treat the mice for days. Meera did timecorse adding the drug in solution, can see a time-dependant rescue from 12 hz up to 48 hz over 5 hours. Then leveled out at 35 hz. If treat wt mice it goes from 42 hz to 38 hz, not sure if this is an “unchanged” phenotype, and are using a high dose. Meera used a dose that has been published in the literature but not exhaustive dose exponse curve. Then did 3 doses looking PC degeneration, 40% rescue if use the drug for a month. No developmental phenotype. Stefan asked if they fell over less—he said they didn’t actually do this experiment until they fall, have ataxia phenotype at 1 month.

Stefan points out that fire frequency is correlated --

Tony says, looking in the lit there are lots of ataxic mice with suppressed firing rates w/o pc killing. There are 2 arms – the ataxia phenotype and reduced basal firing rate, and a similar process with reduced firing rate and purkinje cell loss. In the MIM mutant it looks like are seeing both.

Stefan says, we’ve thought about what we could do, so if you think the ff goes up fast in vivo, we could do a short experiment and see if the mice become less ataxic on the rotarod. Even if= it didn’t effect the underlying degenerative process its better than we have to offer to pts now. Need to do longer study for

Treated mice for 5 days, biomarkers for kinase in the cerebellum, can depress 90% of src activity with the dosing regiment. At that dose rate Meera can see firing freq changes, so argues that if you give the drug 3-5 days you should be able to see dramatic improvement in the rotarod experiment.

Stefan says ideally take older mice that have a clear phenotype and see if they get better on the rotarod. Tony agrees, that is the “killer” experiment. Meera used sca2 mice at 15 hz, perfused the src inhibitor, was just like the mim mice, 5 hrs firing went to 42hz, then leveled out at 6 hrs, then at 9-10 hrs the slices “poop out” and firing rate goes down, slices dying. So if do slice at 5-6 hrs can see the maximal effect of the drug.

How do you treat the src kinase inhibitor? He says are using IP injections are challenging, (up to now have only used IP injections). Plan gavage experiments to determine the dose.

Hugenart neural core director

What is the fastest way to do the behavior studies – tom and meera has enough mice to do it now, but would have to get the core to do it. 8-10 wks to breed then – but we have mice now – but Stefan says first do some biochem work and see that src genes are changed, or western blots done by Sharan. The problem with the mice we have now is that they are young and not good for evaluating acute . . .

Tony says is a little nervous about a paper in Cell last week published by Huda Zogni, is talking about MEK inhibitors and reduced firing rate, so just a matter of time before figures it out, might be seeing the same thing, src involved in the mapK path.

Wants to get enough data to submit the paper right away, not 6 months because might get scouped by the MEK group. Stefan injected might get junk data if use the UCLA core. What about shipping sca2 mice housed at UCLA to us? If they are older mice. The mice Meera has are same background as in the Hansen paper. So Stefna suggests we can use our mice for longer term treatment and use Meera’s mice for a quick motor testing. The lab tech will send the cocktail or can come out.

Stefan says that we first need an amendment of the IACUC, would first have to do a pilot so don’t – maybe can do an expedited

Pharmacokinetics toward moving this to phase I in other published studies, should be able to predict proper dose IP.

ASkes whether we have PC loss, suggests our PC loss is mild. Stefan mentions is late, In the MIM mouse they see the same thing except compressed in 2 months.

**Experimental design**

He will ask Meera how many mice

WT transgenic +/– placebo / drug

Rotarod

Treat 5 days

Stefan says make sure the animals learn the task, then measure performance.

Day 3 then do the trial

1 test all

take 3 mice out of each group, look at src targets, transcriptome things,

Carry on the experiment another 4 weeks

Did one exp where injected every day for 1 month, mice were fine, (did or didn’t ) see PC rescue.

Thinks Huda will be one of the reviewers.

Stress granules, for some genes the RNA is there but don’t see expression levels, the mRNA is reduced but the protein level reduced out of proportion.

MIM has 3kb UTR (what?) and something about regulation.

The pathogenic form of SCA2 is enhancing staufen function

WHAT? He said pumilio interacts with staufen, but I don’t see this in the Zogbi paper.

Need to look at MIM expression levels

Looking at Huda, Pumilio is involved

Some differences in MIM localization

Tony looked at slices that were het and they had the same effect. Also have a floxed allele, the phenotype is similar in the pcp2-cre mice as in the whole animal (knockout everywhere), focusing on the PC cells.

We will initiate shipping of mice,

Tony will send antibody - in mail by Monday, we will receive tue or wed

Want to use TBS not PBS can’t use phospho antibodies.

MIM antibody works in westerns and sections.

Will send a note to Meera, with copy to Stefan

Goal to submit the paper have figures ready up until the sca2 part, get it to the editor, do the other stuff and have this ready for the review.