Background items

Core Issues for Renewal

1. **Productivity**
   - **Overall low productivity**
   - **Very low productivity with regard to SAs of grant**
2. **New Aims for Renewal**
   - **Define very early molecular changes across multiple models**
   - **More detailed neurophys changes**
   - **? shaker**

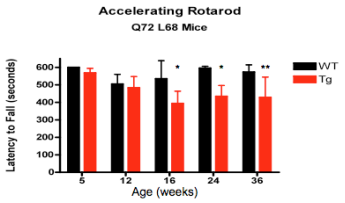
Completed papers

1. Steve's neurophysiology paper
2. Dan's ETS1 paper

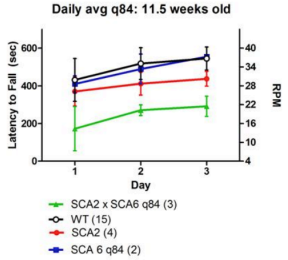
Ongoing papers

3. Lance's RAN translation paper
4. Dan's LncRNA paper
5. BAC mouse paper
   -what's needed?
6. Glutamate signaling in tgSCA2q127
7. Otis/Pulst Review

BAC Q72 Line 68 mice

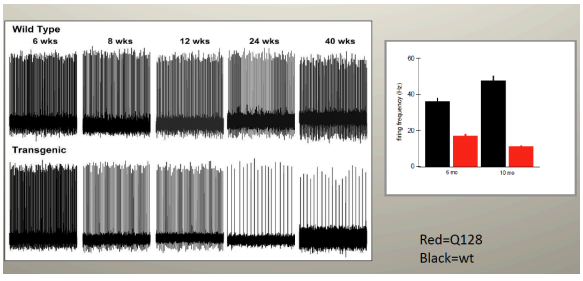


Papers relating to SAs
SA1: CACNA1A ongoing ; needs physiology
SA2: abandon aim as no effect



Rotarod

Q127, poor performance (DBA2j/B6 hybrid background, B6 have delayed onset)
BAC72 phenotype starts later and not as severe
BAC22 not different than WT



Phenotype modifiers

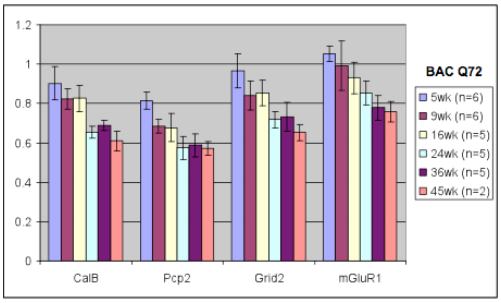
Q127 x SCA6 Q84
Shows phenotype worse than Q127
SCA6 Q84 not different than WT
Q127 x Parkin-Exon7
Nothing going on so halt

Neurophys

Q127, lower firing freq by 8 wks
KO mice, no phenotype difference vs WT

Endophenotype

- Q127 and BAC127 show changes like mimic rotarod
- Something done on KO mice?



RNA-Seq

- Q127 vs WT
- Discovered Rsg8 and other novel informative genes
- iPS dedifferentiation timecourse
- Do we have RNA seq data on iPS comparing patient vs normal?

Promoter analysis

- Advanced understanding on ATXN2 regulatory control
  - ETS1
  - Start codon
  - No RAN translation
  - LncRNA identification

Luciferase mouse

- Advanced our understanding on ATXN2 localization
  - Variable but high expression in cerebellum
  - Olfactory bulb
  - Epididymis

Q127 Characterization

- Calbindin / gross morphology
- Inclusions
- Rotarod
- Electrophys

Digigait

- Alcohol study
- Evaluation of the instrument's utility

Things we want to do

Laser Capture Microdissection (LCM) - Lance is doing
Capture PC layer and equal sized GC layer
Evaluate LncRNA expression
qPCR evaluate hATXN2 / Wasf, Actin, mATXN2

Rotarod

Endophenotype

Neurophys (Tom Otis)

Dan's Notes before meeting:

Turnover study?
TALEN modifications—to add a GFP on ATXN2 to study turnover?? (Duong's idea)
Anything we can do with optogenetics and perhaps calcium?

Notes on our conference call with Tom on 9-12-12

Hege6 (? What did he say) mouse for optogenetics being developed by tom
Rotary encoder on a pie-plate type treadmill
It's a device that's graded in 1000 angular increments when spins once
Can track speed
Video in Steve'a dropbox
Induce ataxia and dystonia optogenetically

Shaker rat
Recently lot of interest in calcium transporters
Pmca3 mutation in congenital x-linked ataxia
Usual ca imaging studies in vitro
Another paper, pmca2 mouse paper mutation shows ataxia, cerebellar and motor neuron degeneration
Two of our rats show dystonia-fixed posture
…mild PC dysfunction leading to dystonia

LCM
Mglur1 in shaker rat
Regions where you get most advanced cell loss then mglur1 likely high

RNA seq to look at early time points—pilot experiment worked well
RGS8 is regulator of g protein signaling but we don't know much more
Fam107b- unknown function
3 cerebellar extracts put together in 1 pool and did RNA-seq
we expect more interesting stuff if do more specimens
goes across different models

BAC transgenic and shaker
Physiological motor and genomic
Genes cells and disease -- need some genomic stuff

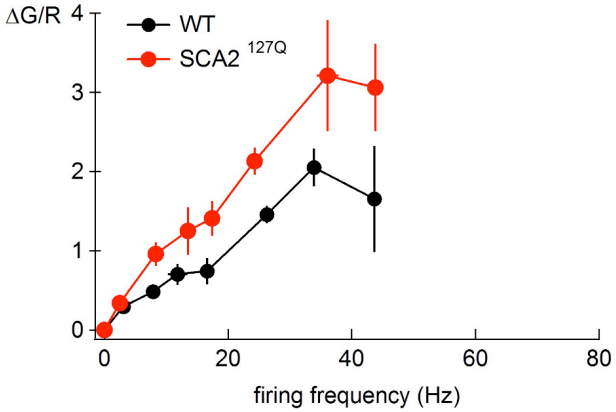
Myria's got a lot of data, Tom wants to put in a application for his-self sometime. We can use it

Continue in our renewal witih Tom with a subproject
Tom's pref for us to complementary behavioral stuff in his application on Q127 line
Helps to have molecular and behavioral stuff
Stef… get's around the prog project

Tom would steer his grant to a "special"

Genes cells and disease—look at rna transcriptome using rat as model, come together with 2 mouse models and rat to see what's similar/different.
Marc found SCA7 transcriptome paper…paper that scouped us

Tom—do molecular analysis in same animal w advanced pathology in one region, with LSM, could
Some of the experiments Myria's doing on calcium are highly relevant
Stefan—Lance grad student -- but new world to look at…
Tom—Jpg file he sent…



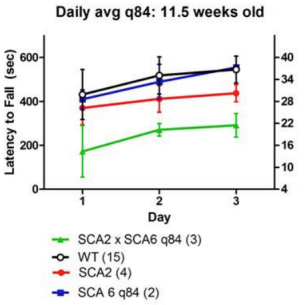
Bcroun I Ip3R hypothesis with Ilya
C yperactive
Ca feeds back into mglur
Runaway pos feedback loop
mGlur1 is solid… pdf file Tom sent.
Increase of mGlur1 agonist, in line w hypothesis
In this chart→
Myria's done experiment on 8 wk old animal
Used 2 photon microscopy to measure ca with orgeon green something… plotting change in fluorescence (dG) divided by red dye (alexa 594). Compare across cells and different regions
Further she's injected current. If firing hyperpolarize to shut them off to get basal ca then inj current to allow freq to vary, pulses 1-1.5 min long. 8 wt cells 15 sca2 cells. SCA2 cells have consistently higher calcium. Not shown, in basal cond, where cell silenced, is significantly more ca. This is a delta G plot so you don't really see that at zero point in this chart. Calcium is elevated and that when the cell firesa cross its range of firing… Ip3r receptor dysfunction related. MGlur1 antagonist may help out.

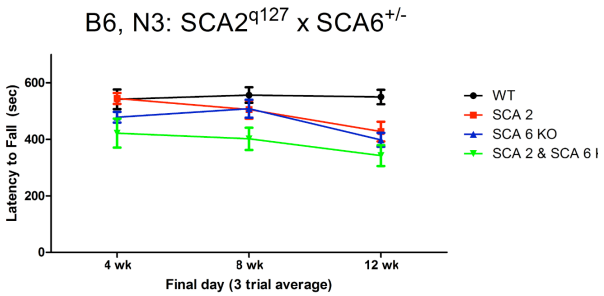
Tom thinks maybe the Pmc3 may support the same physiological defect.

Theme emerging, CALCIUM… with all three animal types

Tom says 2 photon not te bet wy, better to biocytin etc,
Tom—be sparing w/ sca2 and sca6 animals, because physiology in some of the genoyptes in some timepoints is necessary, but he dosn't want to do 6 times in 8 genotypes, b/c too long to do.
Pulst…maybe can have a paper on this, or data for the grant, b/c havent'' answered any specific aims.
Need to do some things on this genetic interaction. Steve is doing phenotype on rotarod.

Tom's looking at that now… →
Mira says has a complete dataset now… to complement this data





On knockouts… Steve has some rotarod and qPCR for knockouts… but wan't in the powerpoint
Tom asks what happens to the knockout… knockout worse, and …
Tom asks if crossed with sca2… Steve says yeah… will send… they are as bad as the SCA2

Tom on histopathology… no aggregates, no drop in calb1
Tom…do they look like humans, changes in other areas, motor neurons not PCs
Relevance of ALS like pathology

Can extend this grant by saying we'd start culturing motor neurons. There is not much physiology on motor neurons. … novel avenue… look at staining for calb1 in spinal cords.

Steve, before you came in…
Inhibitory opsin… turn on the light, inhibits purkinje cells

Grant ideas… prudent to do both

Ilya submitted grant w/o any collaboration from Tom.

Papers
RAN translation
ETS paper
Steve's neurophys HMG
LncRN pper
BAC paper
    What to do
    Antibodies to other proteins in BAC animals
    Tom thinks most reviewers will want more neurophysiology
        Can do this vs SCA6 – but we need progress on SA1
    Tom says is a ton of work in the HMG paper

Quick papers
    SCA6—behavior qPCR physiology mRNA transcriptome
        Tom can add images
            If morphometric analysis, biocytin is essential

Steve question on data on mGlur1 figure tom sent
    Tom wants to connect mGlur1 to calcium movement

Renewal of R01, try for the March deadline, then would go to council by the end of the year (start in October). Tom too would want to do R21 or R01 for the same submission date.

Tom want's the Q127 line

Stefan mentioned that when Q127 bred into the B6 background that the phenotype is milder… tom asks is the onset delayed… Steve says shifted 4-8 weeks, biochemistry as well as behavior. They are still progressive in phenotype. Tom says would be useful to have the most severe line (mixed background). Stefan notes that every 5 breedings we try to cross back to the well defined hybrid… The hybrid animals are B6/D2 from Jackson.

Tom is coming for a week in January… condo in deer valley with his sis. Will arrive Dec 28 or 29 and then will be here thru the Jan 5.

Towards closing…Plan to talk again in 2 weeks. We will try to put together preliminary aims, with lacking overlap with Tom's grant. We can share figures and data for background. Tom hopes to set up to do *in vivo* recording in rats by the end of the year. Will be thinking about rapid publication options on mglur idea.

SERCA pump global ca2+ dysregulation and link to physiology and PCs