Tel conf wed may 28, 12:30 mountain time

Tom Ilya Stefan Dan

Isis sent Ilya a panel and they screened in MEF cells.

50% knockdown after 48 hour incubation.

BAC model, will send to Plos Genetics, for quick turnaround.

Auberger paper, CAG42 knockin paper. What he found didn’t make sense.

Ilya said that short repeats in HTT didn’t form but little bit of inclusions.

Auberger paper, some tail clasping late, need to wait 2 years for phenotype

Our critique, behavior paridigms, wanted more, beam walk we could add, maybe we can show it for the PCP2 ATXN2-Q127 model.

The FVB BAC mice did not survive,

Never breed homozygous to prevent against integration events.

Spread sheet shows things critised multiply.

Off target effects and gliosis.

ASO spread, have more data on this now

Reviewer 5, pcp2 specific model does not reproduce the phenotype

Called the motor testing simplistic.

Any other tests?? Footprint analysis. Huynh 2000 paper and in Ilya’s paper

Animal core – diagram of how animals would be shipped.

Questions about the Iacuc:

Ilya used pumps for compounds directly in the brain.

Ilya’s LMWH, didn’t like, so Ilya will just remove it. Will do IP3R ASO validation.

Another mosue model (OPT model) with 50% reduction of IP3R, cossed with \_\_\_ mouse.

Stefay says: would target with ASOs and genetic model.

They probably disliked LMWH because they didn’t know they crossed the bbb. Ilya will make that aim more mechanistic, target validation, aso on ITPR.

Another reviewer said the ITPR model did not hold up in HD. Interaction between mutant HTT with ITPR has been validated.

Non-cell class specificity of these approaches. ITPR in multiple neuronal populations. For SK channels, they play a predominant role in PC and SN. ITPR and mGLUR 1 are primarly enriched in PCs.

State that ASOs are enriched in PCs. Pilot data on ITPR on ASO.

Tom says motor neurons probably express itpr1, but the physiol of the cells is not predominated by like in PCs. The intracellular calcium signals is not a big factor in function of motor neurons. If you would pick sm mol signaling mol that are specif to PC, you would pick ITPR and mGLUR1,a nd third would be the SK channels. The repetitively firing characteristic of the PC is why they are enriched in calcium signaling.

Tom’s insight: Our commitment to translational neuroscience, but some want more mechanistic. In the overview we will have to address this, that we are doing both. Concern that the cells are on the edge, and by stimulating them - parallel fiber (hardman and conner) 100hz 5 pulses, ampa gaba recp blocked, cal signals vs wt counterparts (5-6 mo old) calcium signals are enormous, timecourse prolonged. In PCP2-atxn2-q127 model. Don’t have expeirments implicating RGS proteins, but all are consistent with that if you lose any one of these things would make worse “perfect storm”… worry is watching cells die just by stimulating them.

RGS6 is also down, and RGS6 ko causes ataxia in the mouse. Provide ref for RGS6 . For RGS8, mut atxn2 inhibits translation of RGS8 but not other proteins, so is specific to subsets of mRNAs.

Tom will take out the optogenetic behavior.

Tom has old animals, but in humans we think we have a different array of PCs, so this mechanism is probably relevant, as the final step that puts a PC over the edge.

Control experiments, mglur1 antagonist, … will look at younger animals

For us, we have these mutant proteins, work in multiple pathways, atxn2 interacts with staufen, to change mRNA stability, atxn2 interacts w specific RNAs, converges on multiple pathways on Ca regulation in PCs. Final outcome on abnormal Ca and PC death.

Third model, transgenic with endogenous protein, atxn2 – FLAG tagged.

Asking Tom when they would be ready for young animals – 6-8 wk old Q127 animals Tom would like, work would be easier and faster to work on. Tom would like 6 mice, 6 tg. Dan will talk to Pattie.

Paper Katrina mentioned in March, on animals not replicated, 20 compounds, coQ10, riluzole,

Ilya continued

IPTR ASO target knockdown.

Slow down

Can you win the BATTLE by saving PCs but lose the WAR because the rescued PCs are less effective for inhibiting other neurons.

Tom mentioned: DCN recording most dramatic and direct,

Animal core conducting phenotype testing. -- -

Can say that each project does a primary evaluation, then a smaller set goes forth the be tested in the core. This way we ensure replication

Empphasize we are doing pathway mechanistic and are committed to doing translational

Tom:

Take out the optogenetic aim, they didn’t understand.

Expand the basic mechanistic inquiry of the calcium hypothesis. Will include new data for resubmissiono on q127, they asked about BAC model too, and Tom is grasping at whether the changes seen in PCs would be seen in other neurons in the BAC model. Discuss more specific what would be tested across models, did some of that re climbing fibers etc.

Rev 3 said aims required considerable mouse crossing efforts,,, but that was the optogenetics…

They aslo mentioned why not look at mGLUR1 ko, and didn’t mention because it was in the R01.

Tom says its easy for him, to “double down “ on calcium recordings. Will have a lot of DCN recording too.

PC firing slowing in the cortex, Cameron said that he didn’t think there would be slowing in the cortex *in vivo*. Tom thinks he is wrong. Slice vs in vivo. His perspective is from EA models. Tom did not do in vivo but Ilya says he is doing *in vivo* recordings in PC this summer, using Q58 mice (since this summer, suggests work will be done in St Petersburg) then he said they are there.

Ship Q127s to St Petersburg. –can send to Dallas and the Russian tech will work on them in Dallas, she will come in the spring, then they will ship Q127s to St Petersburg later.

Ilya would be able to call in from Russia this summer. He is leaving in 2 weeks in June.

Tom will be in Sweden until July 29. Then in Boston for a week.

Jan 25 PPG resubmission