Data set structure

This data set has 3 independent variables and 76 dependent variables.

The 3 independent variables are:

Genotype: mice are either wildtype or a genetically engineered model of Down Syndrome (DS)

Behavior: whether they were exposed to a learning task or a control experience

Treatment: whether they received memantine, a drug with activity in Alzheimer's Disease

The dependent variables are the levels of 76 proteins or protein modifications measured in cell nuclei in the mouse cerebral cortex. This is a subset of a larger data set which included membrane-bound and cytosolic fractions as well as the nuclear fraction and also included all three cellular fractions from the hippocampus.

The proteins were chosen for measurement based on known or hypothesized relationships to DS and/or learning and memory; they are:

- Genes on the human Chromosome 21 (and therefore with extra copies in DS)
- Proteins related to Alzheimer's Disease
- MTOR pathway
- MAPK pathway
- Glutamate receptors and proteins they interact with
- proteins involved in apoptosis or inflammation
- immediate early gene proteins (markers of neuronal activation)
- histone modifications

While I can speculate on the possible functional reasons for changes in specific protein/modification levels, many of these proteins are principally known for their function as membrane-bound or cytosolic proteins. Therefore, it will not always be obvious what changes in nuclear levels mean for the overall function of the cells.

Statistical Analysis

I performed 3-way ANOVAs for each protein to identify effects of each variable and their interactions. As was visible in the data story, many proteins were affected by the behavior paradigm (learning vs no learning), while few were affected by either genotype (down syndrome model) or the drug memantine. I am most interested in the interaction between genotype and behavior. That is, how does a mouse with Down Syndrome respond to a learning task differently than a wildtype mouse?

I therefore sorted the proteins by the p-value of the interaction between genotype and behavior. When corrected for multiple comparisons using the Benjamini-Hochberg procedure with a False Discovery Rate of 0.05, 11 proteins or modifications showed significant interaction between genotype and behavior:

Protein or modification	**Full name**	**Change**
pNUMB	phosphorylated NUMB endocytic adaptor protein	Increases with learning in wildtype from below mutant levels to above
RAPTOR	regulatory associated protein of MTOR complex 1	Decreases with learning in mutant
TIAM1	T-cell lymphoma invasion and metastasis-inducing protein 1	High baseline in mutant, decreases with learning in mutant
NR2B	glutamate ionotropic receptor NMDA type subunit 2B	Decreases with learning in mutant
AMPKA	5'-AMP-activated protein kinase catalytic subunit alpha	Decreases with learning in mutant
NR2A	glutamate ionotropic receptor NMDA type subunit 2A	Increases with learning in wildtype
BDNF	brain-derived neurotrophic factor	Increases with learning in wildtype
MTOR	mammalian target of rapamycin	Decreases with learning in mutant
ERK	mitogen-activated protein kinase 1	Increases with learning in wildtype
PKCA	protein kinase C alpha	Increases with learning in wildtype

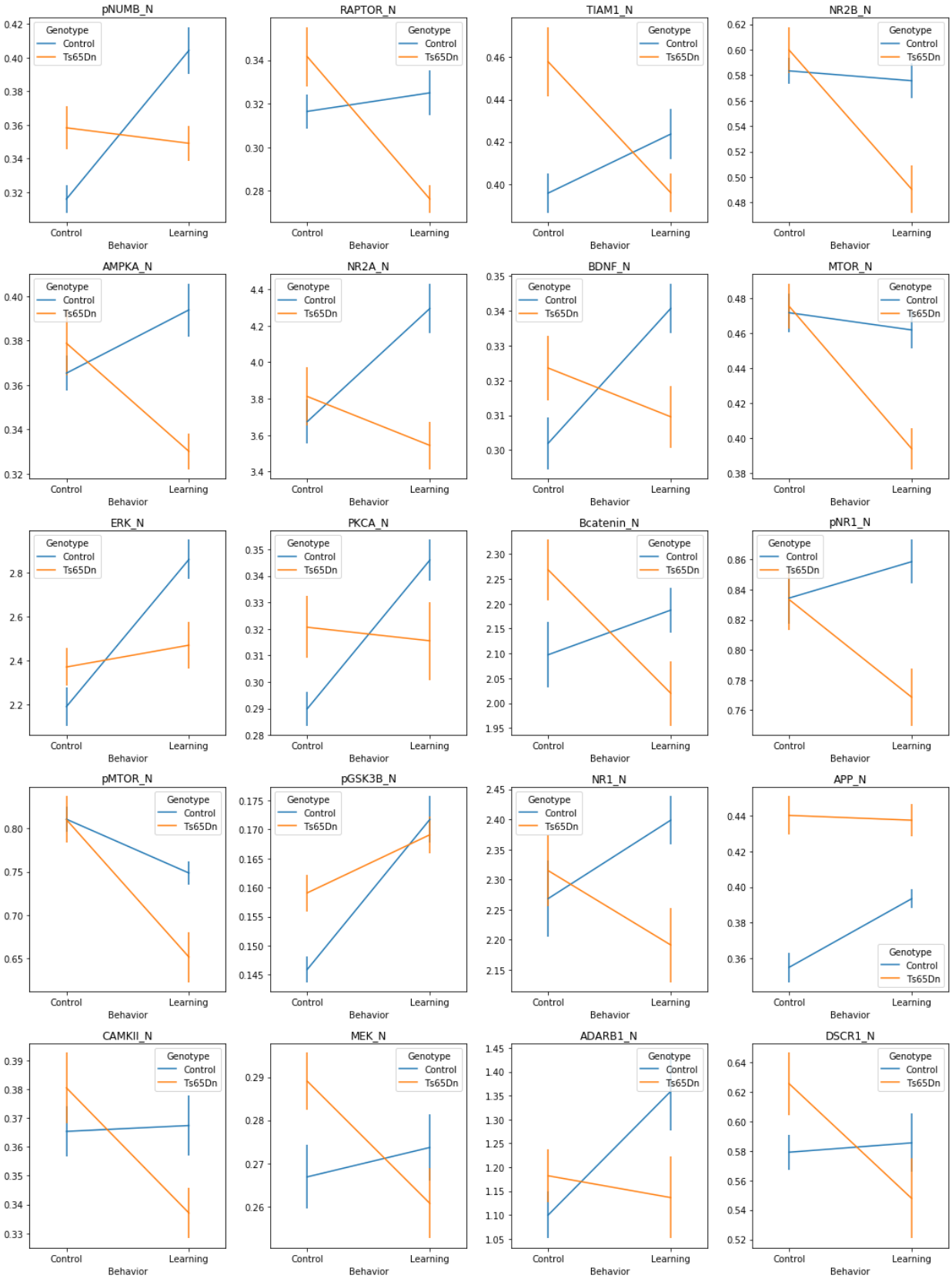
Bcatenin	catenin beta 1	Decreases with learning in mutant

Some notes on specific results

Interestingly, this list includes 2 subunits of the mTORC1 complex (mTOR and RAPTOR) and 2 subunits of NMDA receptors (NR2A and NR2B). Both mTOR and RAPTOR decreased in the mutant in the learning condition, while levels did not change much in the wildtype. The functional receptor consists of 2 NR1 subunits, 1 NR2, and 1 NR3. Each of these subunits have variants which determine the properties of the assembled receptor. Higher NR2B has been found to lead to better learning via long term potentiation (LTP), and NR2B was decreased with learning specifically in the DS mice. Meanwhile, NR2A increased with learning only in wildtype mice. NR1 and phosphorylated NR1 also showed interaction effects with less stringent correction of p-values.

The only protein in this subset that is on the human chromosome 21 is TIAM1, which was higher in the mutant than in the wildtype at baseline but decreased in the learning condition. TIAM1 is involved in response to BDNF (which increased with learning in the wildtype). BDNF signaling is important for NMDA receptor trafficking and LTP. At least in migrating neurons, the phosphorylation of NUMB (also increased with learning in the wildtype) is an important component of BDNF-mediated cell polarization.

Below are interaction plots for 20 proteins in ascending order of (uncorrected) p-values for the interaction between genotype and behavior (ignoring treatment conditions):


pNUMB_N
RAPTOR_N
TIAM1_N
NR2B_N
AMPKA_N
NR2A_N
BDNF_N
MTOR_N
ERK_N
PKCA_N
Bcatenin_N
pNR1_N
pMTOR_N
pGSK3B_N
NR1_N
APP_N
CAMKII_N
MEK_N
ADARB1_N
DSCR1_N
Genotype
Control
Ts65Dn
Behavior
Control
Learning