Couchii muscle results summary (very superficial)

IC50:

* Jessica already did this but I did it again (and I think the below protocol from Bobby’s manuscript matches but I need to double-check her paper to be sure)
  + “The transient muscular force assessment and EFS optimization was repeated with a new, freshly dissected muscle. The optimal transient was stimulated and recorded. Known quantities of TTX dissolved in citrate buffer pH 6.8 was added to the bath of known volume. Doses began as low as 1nM and increased until the transient force peak magnitude decayed. In many cases, [TTX] was increased until the absolute abolishment of muscular activity was achieved, though this was not possible for every experiment. The volume of TTX in citrate buffer added to the bath was kept below 0.01% of the total bath volume to minimize effects of diluting the Krebs buffer. However, in cases when exceedingly great [TTX] were needed to inhibit the contraction and stock concentrations of TTX dilutions were too low to keep the volume added below 0.01% of the volume, a separate experiment where an identical volume of citrate buffer (without TTX) was added to verify the absence of dilution-related effects. Dose response curves were generated from the peak transient contraction force magnitude. The responses were fit to a sigmoidal curve of the equation Force (N/g)= A2+ (A1-A2)/(1+e^((log([TTX] (nM))-log(x0))/dx)) where A1and A2are the upper-and lower-bounds, x0is the center of the curve (toxin concentration at half-maximal inhibition, IC50) and dx is the rate of decline in force with increasing [TTX]. Comparisons of IC50(nM) and dx (N/g nM-1) were restricted to animals within the same species, grouping animals by genotype. To abide by the limitations of the natural logarithm, the dose at baseline transient force magnitude (0 nM TTX) was revised to 0.1 nM. If the contraction recorded at 0 nM TTX was unusable or unavailable, the dose responses were normalized to the maximum force produced throughout the routine (frequently occurring within the first 10 nM TTX). Dose response data could not be fit with fewer than four concentration-force points collected. However, when three points were available, a fourth point was fabricated where the force would visually converge to zero N/g (i.e., 100μM which abolishes contractility in even the most resistant snake muscles)”
* Strongly correlated with whole animal resistance according to both pearson and kendall
  + 0.7087 for pearson, 0.5100 for kendall
* Linear regression stats look good

A red line with black dots

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C4P:

* Only just realized that because there are four observations per muscle/individual there may be some artificial correlations just because of reduced variation between observations of the same individual, need to look into that more
  + *Bobby dealt with this by only comparing results from the same numbered pulses*
  + “The resulting transient contractions were analyzed for peak force magnitude (N/g of tissue), contraction duration (from center of the stimulus to 50% relaxation), and latency (time from center of the stimulus to the peak force generated). The first derivative of each trace was analyzed for its peak rates of force development and relaxation (kN/g s-1). Of the train of four pulses, comparisons were only made between contractions of the same order”
* Slight positive correlation between LD50 and time to max force change according to both pearson and kendall, fairly certain that doesn’t mean anything in practice but it is a result
* Also between LD50 and time to 10 percent
* Other things have positive correlations based on different metrics
  + Max contraction amplitude (Pearson)
  + F max rate of change (Pearson)
  + F min rate of change (Pearson)
* Makes sense that these would be correlated
* Linear regression shows no relationships at all, highest r^2 is a whopping 0.03
  + Plots not exported for space purposes, can easily load them in R
* Comparisons with IC50:
  + some slightly better r2 values: 0.19 for contrampl, 0.2 for fmaxrate ngs, 0.2 for fminrate ngs, 0.19 for DiffFChgMaxtoMinms (but with bad RMSE)
  + ContrAmpl, To50pctms, X10to50pctms, ToFChgMinms, and DIffFChgMaxtoMinms all correlated under both tests
  + Fmax and FMinRateofChgNgs both only with Pearson

C4P re-analyzed with the different pulses treated separately:

* MAMU-C4P correlation analyses (22/88 obs) gave me so many results (88) and none of them were significant
* MAMU-C4p linear regressions:
  + R2 = 0.09 for ContrApl for all pulses
  + R2 = 0.09 for min and max rate of change F Ngs for all 4 pulses
    - I guess these two results are improvements in fit compared to the aggregated results
  + All other r2 = 0-0.02
* IC50-C4P correlation analyses (16/64 obs):
  + FMaxRate and FMinRate significantly correlated with IC50 for all 4 pulses, Kendall only
* IC50-C4P linear regressions (16 obs each)
  + DiffFChgMaxToMin r2 = 0.19 for all
  + ToFChgMin r2 = 0.1 for all
  + FMinRateOfChg r2 = 0.2 for all
  + FMaxRateOfChg r2 = 0.2 for all
  + X10to50pct r2 = 0.09-0.11 for all
  + ContrAmpl r2 = 0.19 for all
  + This actually improved the fits of all of these which implies there are inter-pulse differences, those differences are just likely to be consistent in individuals
    - Want to try to think of some figures to show this
* TLDR these results actually got more interesting when analyzed properly (which makes sense), but now I need to decide how to handle this sort of R2 value (ask Chris and also read about it)

C4P with outliers removed:

* Pulses grouped:
  + To10pctms and ToFChgMax both significantly correlated according to both Pearson and Kendall corrs
* Pulses split
  + MAMU correlations
    - None significant
  + MAMU linear regressions
    - All R^2 in 0-0.1 range (and higher R^2 differed from which ones were higher with outliers in)
  + IC50 correlations
    - DiffFChgMinToMaxms sig for all 4 pulses for Kendall, otherwise all non-significant
  + IC50 linear regressions
    - Base force ~0.02
    - ContrAmpl ~0.05
    - ToMaxF ~ 0.11
    - To10pct <0.1
    - To50pct ~0.14
    - 10to50pct ~0.15
    - FMaxRateOfChgNgs ~0.14
    - FMinRateOfChgNgs ~0.12
    - ToFChgMax = 0
    - ToFChgMin ~0.16
    - DiffFChgMaxToMin ~0.26

Outlier-no outlier comparison:

* MAMU-pulses grouped correlations have same results, significant correlations for To10pctms and ToFChgMax according to both metrics
* MAMU-split pulse correlation same (all non-significant)
* MAMU-split pulse linear regressions similar (all r^2 still sub-0.1, though dif ones are higher this time)
* IC50-split pulse correlations different, was significant for FMaxRate and FMinRate according to Kendall and now is for DiffFChgMinToMaxms according to Kendall (all others remained insignificant
* IC50-split pulse linear regressions:
  + DiffFChgMaxToMin got higher r^2, so did ToFChgMin and 10To50pctms; To50pct presumably did bc I didn’t record it previously
  + FMaxRateOfChg and FMinRateOfChg got lower r^2

Tetanus:

* Only just realized that each muscle has two identical obs in the tetanus sheet, need to look into that and redo analyses in light of that
  + Based on Bobby’s notes it seems like there should not be two obs per muscle, maybe an error in the data or analyzing code?
  + “Under the same optimized EFS conditions as the transient force assay, the muscle was stimulated at 1000 Hz for 2 seconds. The resulting tetanic contraction was analyzed for peak force magnitude (N/g of tissue), contraction duration (from stimulus onset to 50% relaxation), and latency (time from stimulus onset to the peak force generated). The first derivative of each trace was analyzed for its peak rates of force development and relaxation (kN/g s-1). The tetanus routine is so stressful that it is terminal. A new muscle was dissected and used following this protocol. Transient force, rheobase, and tetanic force protocols were run within a period of 15 minutes with 1 minute rest between protocols”
* Only one of these metrics correlates with MAMU according to the analyses, the base force (Kendall test)
* Linear regression also shows no relations, highest r^2 is 0.05
  + These plots also not exported for space purposes
* IC50 analyses:
  + Also improved R-squared fits some: 0.11 for To10pctms, 0.17 for To50pctms, 0.17 for X10to50pctms, 0.23 for toFChgMinms, 0.16 for FeeFChgMaxtoMinms (but again, all pretty bad RMSE)
  + BaseFNG correlated with both
  + To10pctms correlated with kendall
  + ToFChgMinms and FiiFChgMaxtoMinms with Pearson

Tetanus re-analyzed with single replicates (so not repetitive):

* (30 obs bc 1 missing MAMU?) Linear regressions mostly got worse, r-squared for BaseFNg increased to 0.06 but the next-highest is 0.02
* Correlation tests of MAMU to tetanus metrics, none are significantly associated by either the Pearson or Kendall tests (Base force Kendall test comes the closest, p = 0.0526)
* Comparing IC50 to tetanus results, still have some tentative correlations from linear regressions (still super loose):
  + To50pctms has r2 = 0.1
  + X10to50pctms has r2 = 0.1
  + ToFChgMinms has r2= 0.19
  + FiiFChgMaxToMinms has r2 = 0.1
* IC50-tetanus correlation results (15 obs):
  + No traits significantly associated with IC50 according to kendall or pearson tests
  + Base force again got the closest (p = 0.08 for Pearson and 0.06 for Kendall)
* TL;DR: re-analyzing the data like this got rid of the spurious correlations from before, which I’m not too upset about because now I don’t have to try to justify why that weird handful of traits are correlated according to select metrics
* Does seem maybe noteworthy that base force is the most correlated of everything (except when looking at the linear regressions of IC50 on tetanus results)

Tetanus with outliers removed:

* MAMU correlations
  + No significance
* MAMU linear regressions
  + R^2 all 0.05 or lower
* IC50 correlations
  + No significance
* IC50 linear regressions
  + To50pctms = 0.18
  + 10to50pctms = 0.18
  + ToFChgMinms = 0.28
  + FChgMaxToMinms = 0.35
  + Rest all <0.06

Outlier-no outlier comparison:

* MAMU correlations the same in that they’re all insignificant
* MAMU linear regressions all basically the same, different brands of no noise
* IC50 correlations also the same in that they’re all insignificant
* The 4 best r^2 values from the IC50 linear regressions all got higher, which is interesting
  + Need to read what the cutoff is for believing an r^2

Rheobase:

A close-up of a text

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A diagram of a function

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* Suspect I will need to go back to the raw data, not sure what to do with this rheobase output though because it must have been calculated for a reason?