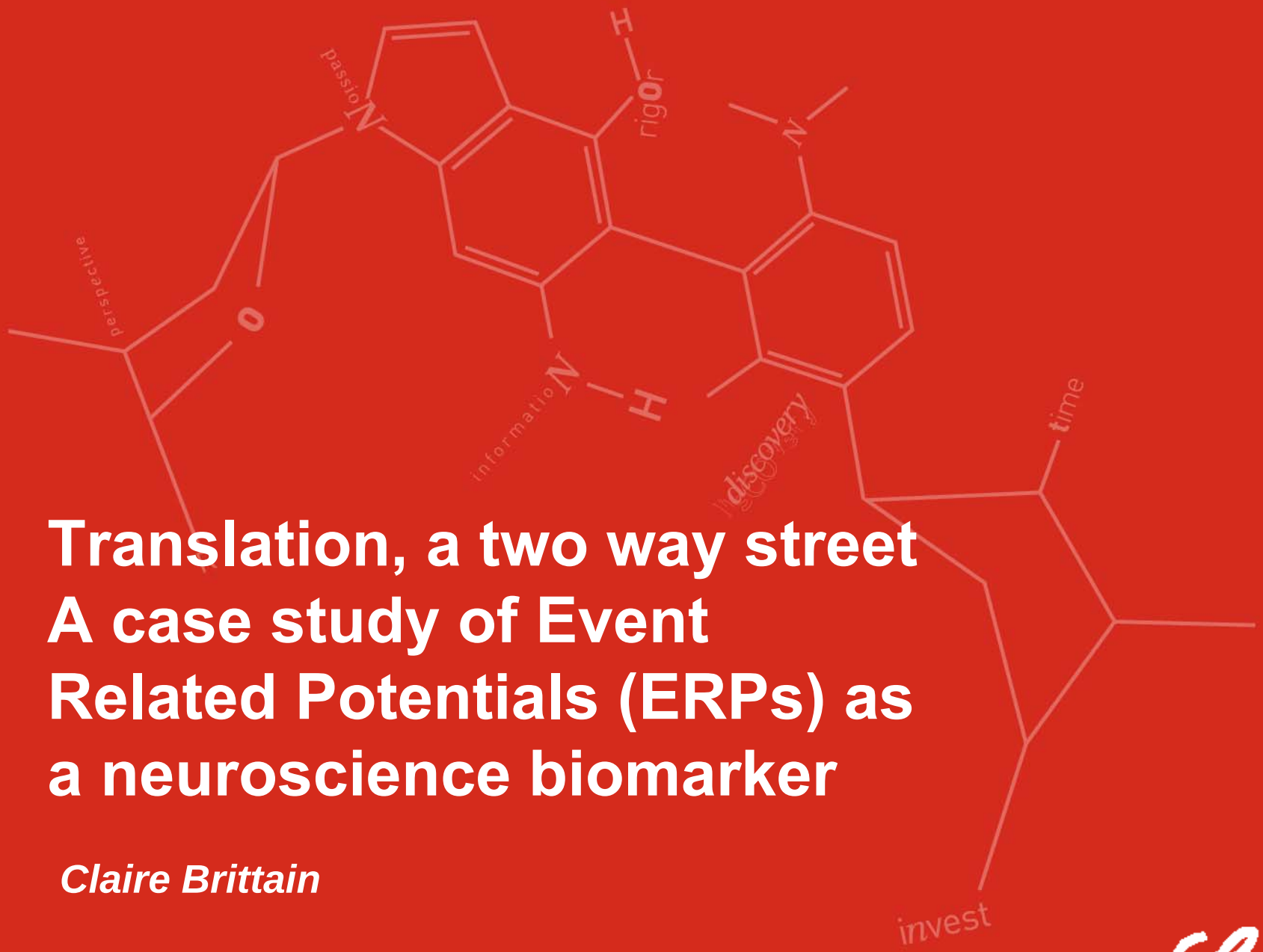


Translation, a two way street A case study of Event Related Potentials (ERPs) as a neuroscience biomarker

Claire Brittain

Background

- High failure rate in neuroscience drug development
  - 1-8% success rates through to Phase 3 for CNS drugs, 5-20% for cardiovascular drugs

- Biomarkers capable of providing proof of mechanism are considered critical tools
  - Carry the right drug/dose into clinic
    - Help reduce attrition during Phase II clinical trials
    - Set doses for Phase II clinical trials
  - Ability to demonstrate pharmacologically driven biological activity in the brain, as a result of the interaction of a drug with its intended target

Our goal:

To find a biomarker to use in Phase 1 for CNS active drugs that could be used in patients, healthy volunteers and animals

My prior experience of translation

1. Test biomarker in animals
2. Test different biomarker in humans
3. Once the human data is obtained this will supersede any animal data which is often discarded
4. Don’t attempt to feedback to discovery on if the methods/results matched or not
5. Begin cycle again

Goal and New plan

Goal: Find a marker of pharmacodynamic effect for CNS active drugs

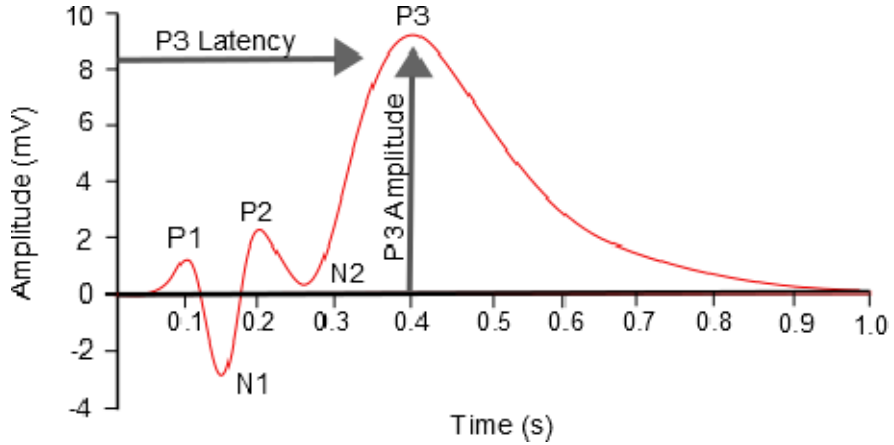
- Find a new measure that can used in both species and thus allows us to compare directly
- Work iteratively with discovery to match all parts of the process

Step 1- Finding your measure/endpoint

Endpoint	Preclincal	Clinical	Cost	Comments
PET Imaging	√	√	**£££**	Requires a PET Ligand
fMRI	√	√	**£££**	Requires animal to be restrained Very exploratory biomarker
CSF/Blood markers (AB1-42, tau proteins, inflammatory markers, CRP)	√	√	**££**	Much ongoing work across industry Can they cross blood brain barrier and relate to cognition?
Questionnaires/Interviews	**X**	√	**£**	
Planned histology	√	**X**	**££**	Terminal experiment (no longitudinal)
Psychomotor vigilance testing	√	√	**£**	Makes rats sleepy
Transcranial magnetic stimulation	√	√	**££**	Very exploratory biomarker. One animal lab in world
MEG Imaging	√	√	**££**	Requires animal to be restrained
Memory tests/computerised batteries	√	√	**£**	Motor and motivational confounding
Sleep/wake polysomnography	√	√	**££**	Hard to embed within a study
Event related potential	√	√	**£**	**Can add into a Phase 1 study** **Equipment portable to any CRU**
EEG	√	√	**£**	Can add into a Phase 1 study Equipment portable to any CRU

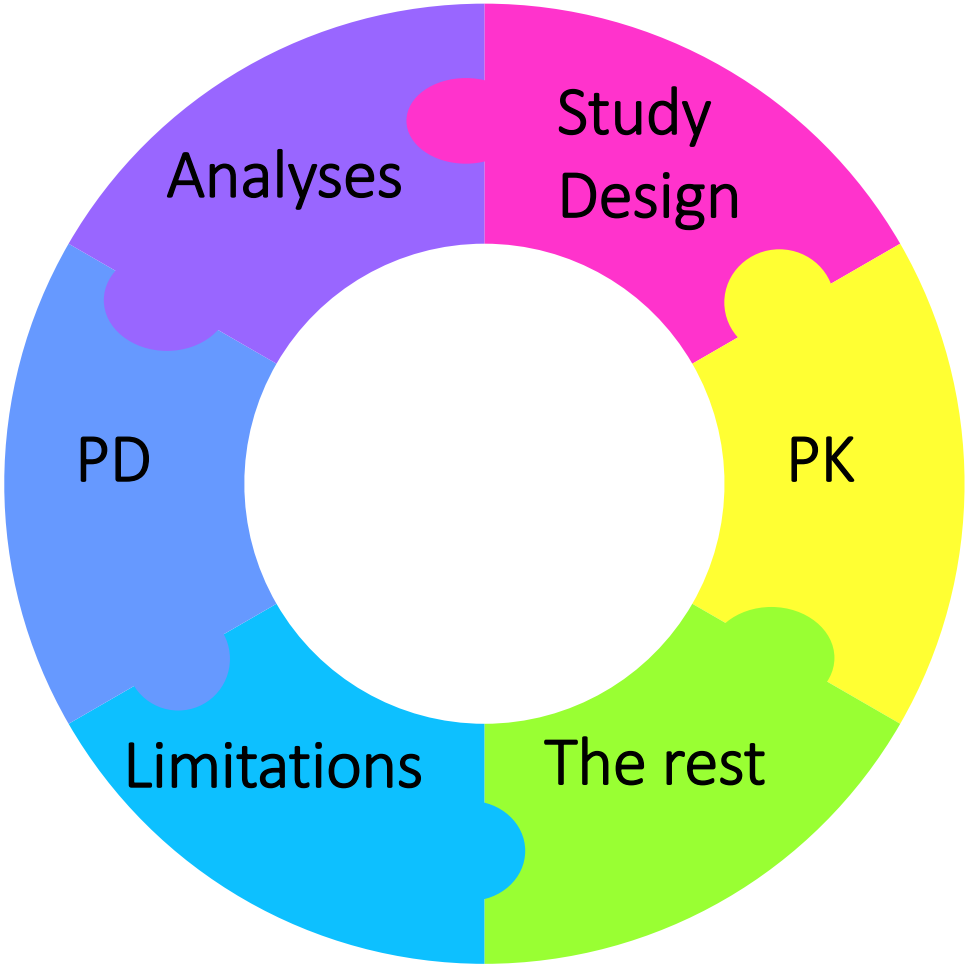
Step 2- Understand your measure/endpoint

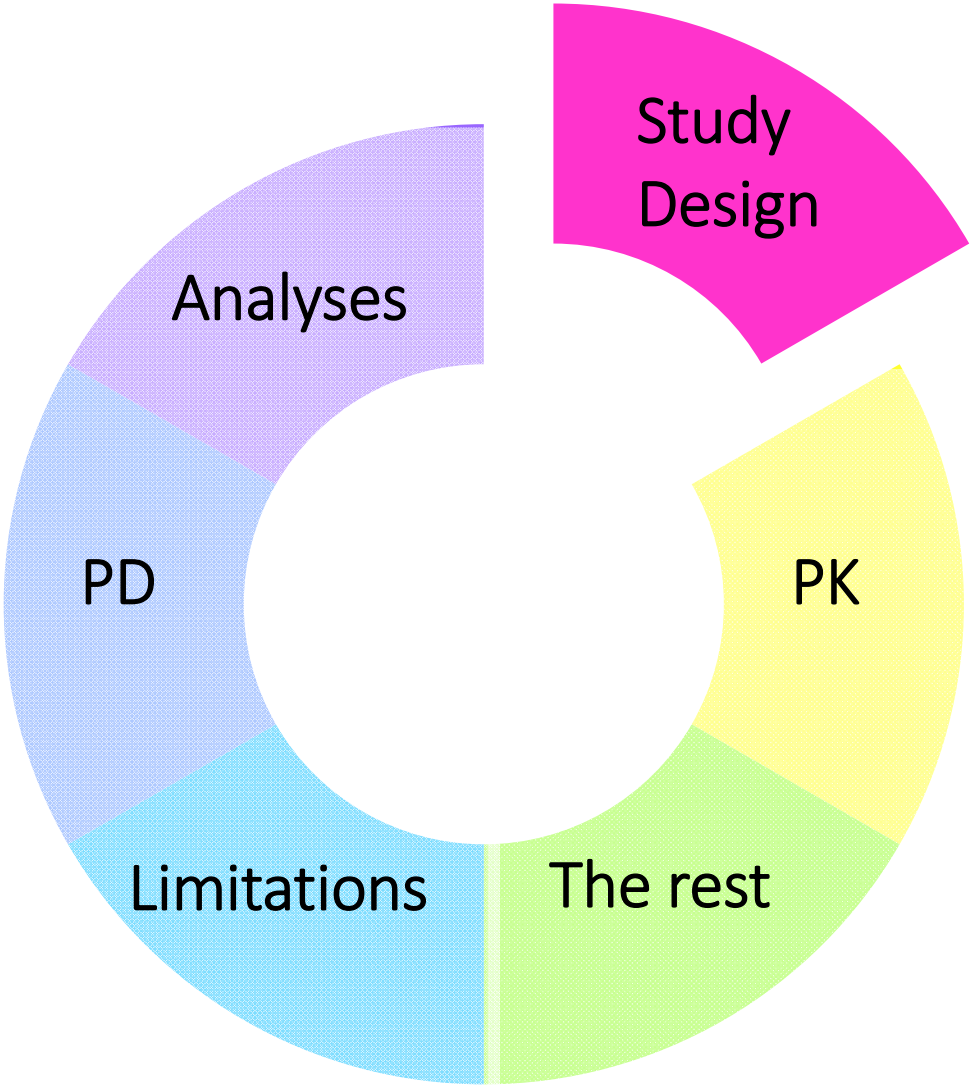
- Evoked (Related) Potentials (ERPs) have been extensively used for clinical research over the last 30 years, with >50,000 ERP-related publications
  - Patients and Healthy volunteers
- Evoked potentials are EEG patterns produced in response to specific stimuli, comprising primarily N1,P2,N2,P3.
- Auditory ERPs are most commonly studies in drug intervention studies, using an Oddball Paradigm



- P3 elicited by stimuli in attention demanding tasks – potential PD biomarker

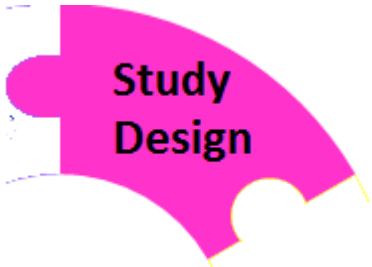
Step 3 – What aspects are translatable?





Study Design

Human – Within subject PK/PD



Example Treatment Allocation

Sequence	Period 1	Period 2	Period 3	Period 4	Period 5
1	P	A1	A2	B1	B2
2	A1	A2	B2	P	B1
3	A2	B2	B1	A1	P
4	B1	P	A1	B2	A2
5	B2	B1	P	A2	A1

Note: A1 = Treatment 1: low dose (0.5 mg) lorazepam; A2 = Treatment 2: high dose (2 mg) lorazepam; B1 = Treatment 3: low dose (10 mg) methylphenidate; B2 =Treatment 4: high dose (40 mg) methylphenidate; P = Treatment 5: Placebo.

Randomised, crossover, placebo controlled, single (subject) blind, double-dummy in older HVs

Sample size: 13 completers

Doses driven by doses administered in clinical practice (low and high dose)

ERP and EEG measured prior to dosing, then at 2 hr, 4 hr and 24 hr post dose (Cmax 2-4hrs for both drugs)

Primary measure P3 latency (ERP)

Secondary parameters other ERP components and EEG (power spectra)

Study Design

Rat

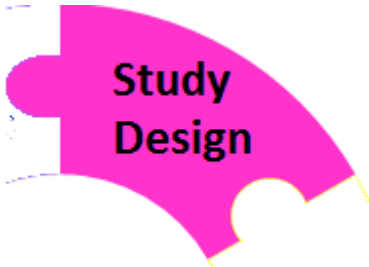
PK experiment 1 – Dose matching

Drug	Doses rat	N
Lorazepam	0.3mg/kg	5
	0.1mg/kg	5
	3mg/kg	5
Methylphenidate	1mg/kg	5
	3mg/kg	5
	10mg/kg	5

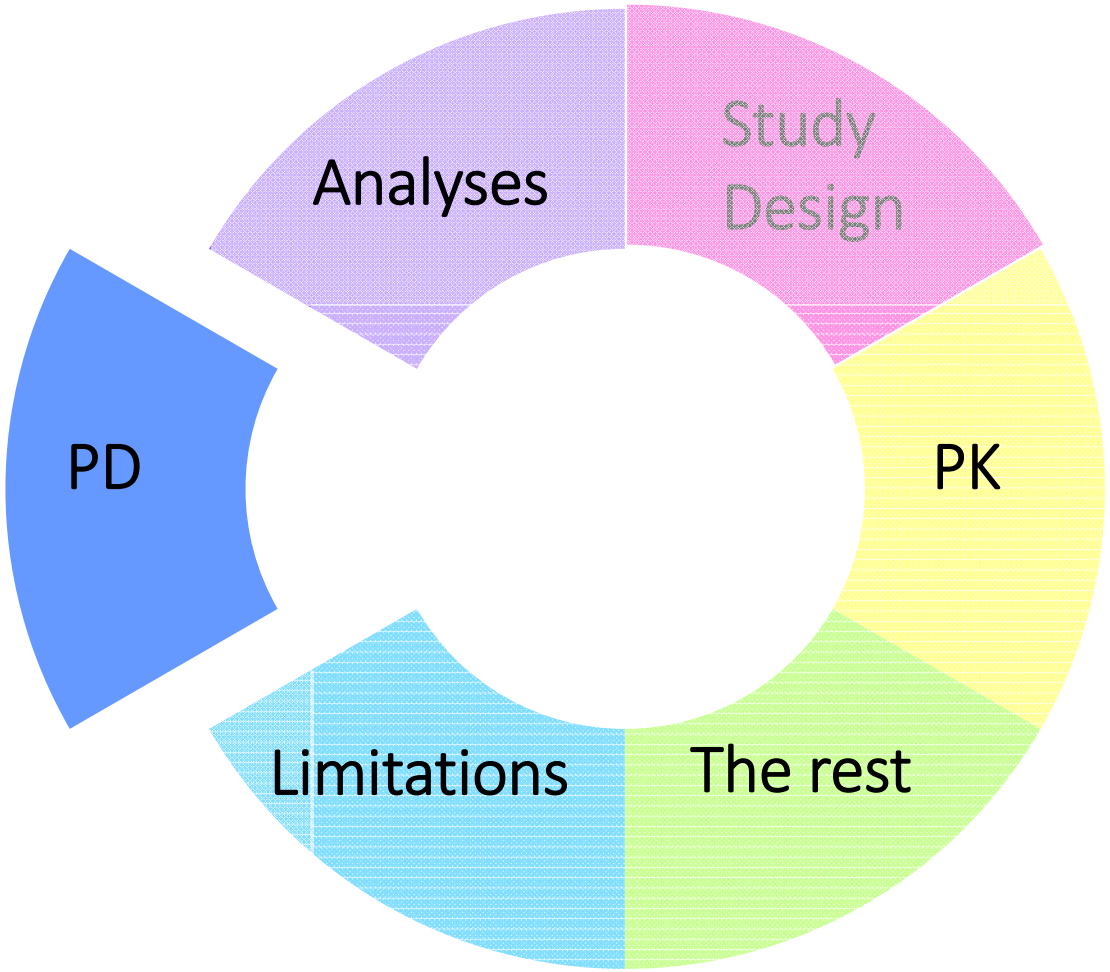
Human Dose Matching

Drug	Rat (mg/kg)	Human (mg)
Lorazepam	0.3	0.5
	1.2	2
MPH	10	10
	20	40

PK experiment 2 at matched doses
N=5 per dose

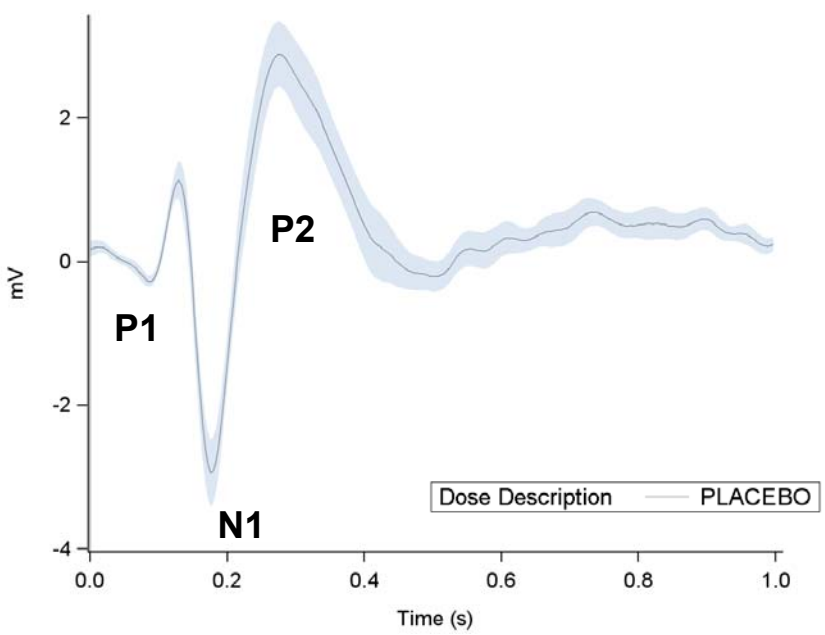


PD Study
Separate animals - unable to take blood from PD animals
Parallel design ~14 per dose
Historical Placebo/Vehicle used
Timepoints predose, 1, 2, and 4 hours postdose

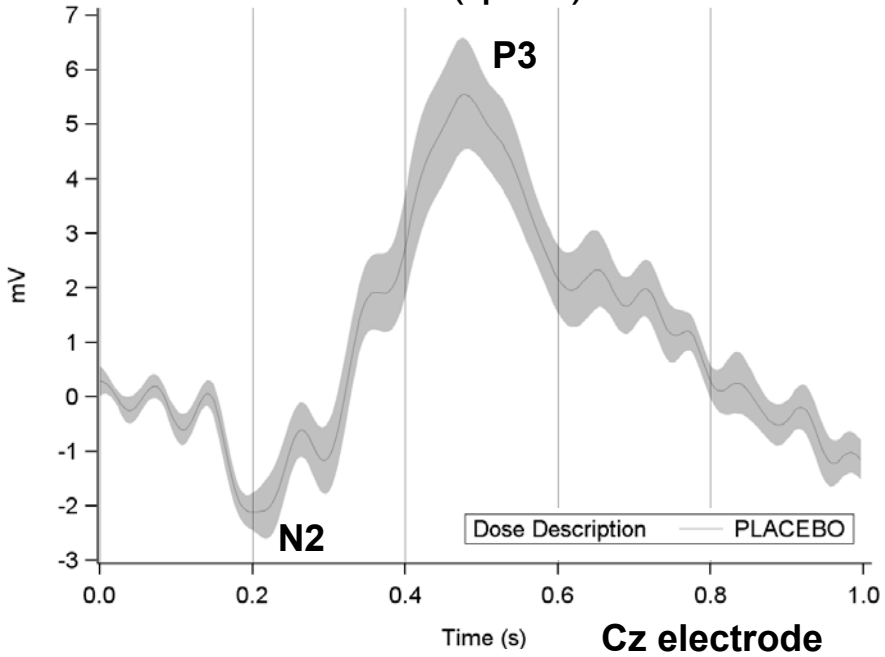


PD - Human ERPs

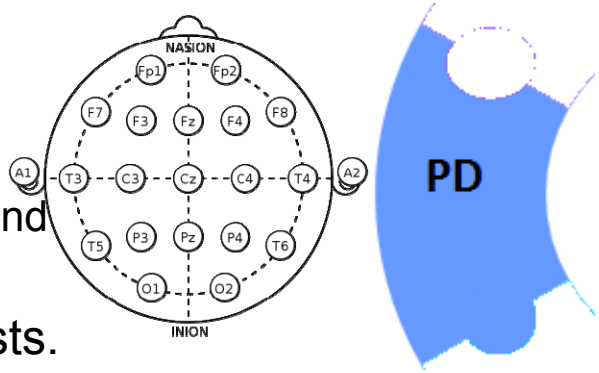
Standard Tones



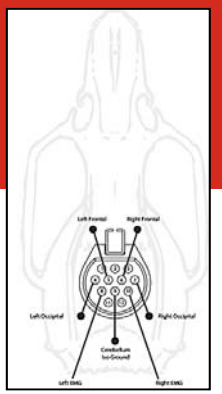
Oddball (quiet) Tones



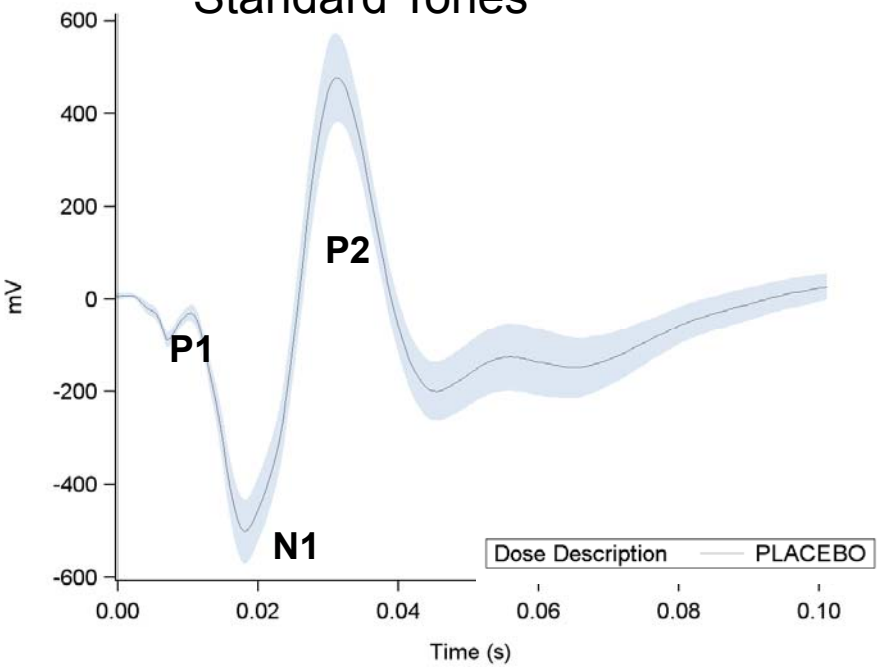
- Assessment 20 mins
- Loud tone bursts with a constant inter-stimulus interval of 1 second. Randomly interrupted by soft tone bursts.
  - 90% of the tones presented per trial were a standard (loud) and 10% were a deviant (soft).
- The subjects are asked to mentally count the soft tone bursts.



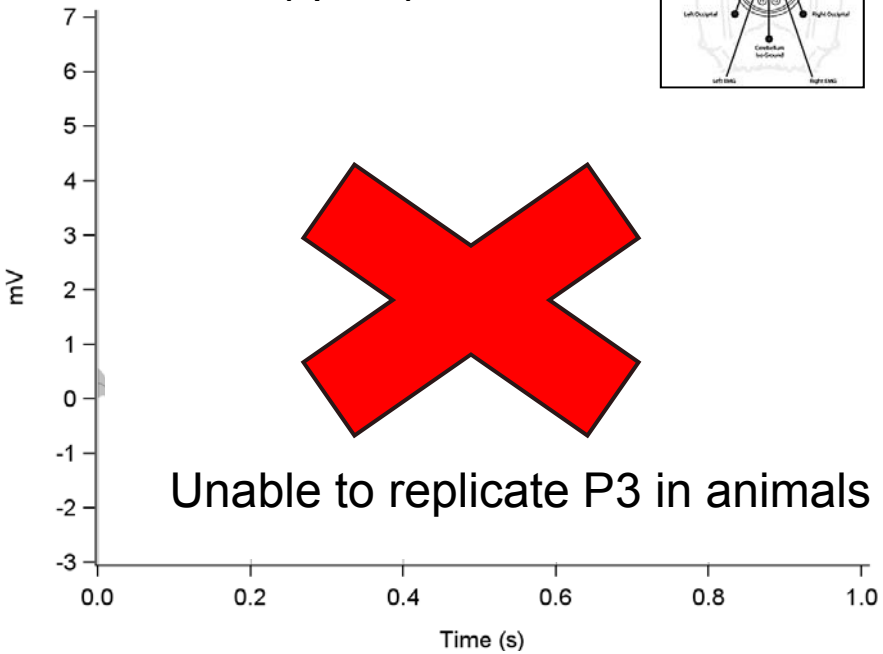
PD- Rat ERPs



Standard Tones



Oddball (quiet) Tones



Unable to replicate P3 in animals

ERP Assessment window 20mins

Speakers mounted to the cage presented a single tone every 1 second during the first 20 minutes of each hour from lights on for 24 hours.

- 90% of the tones presented per trial were a standard (low) and 10% were a deviant (high).
- No oddball produced regardless of stimulus type (pitch/volume etc)

PD - ERPs

Unable to elicit P3 oddball peak in animals, this was our primary endpoint in

Do you:

A: Give up

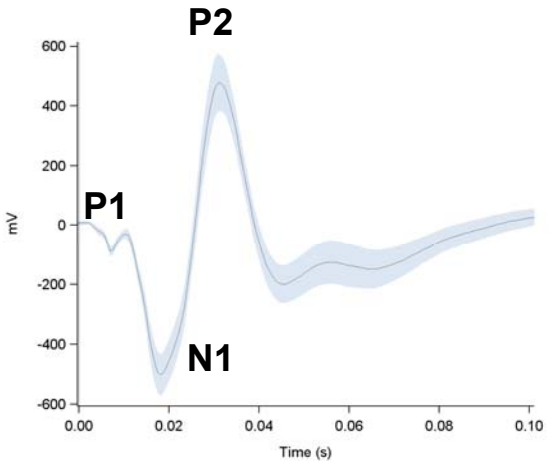
B: Focus on standard to

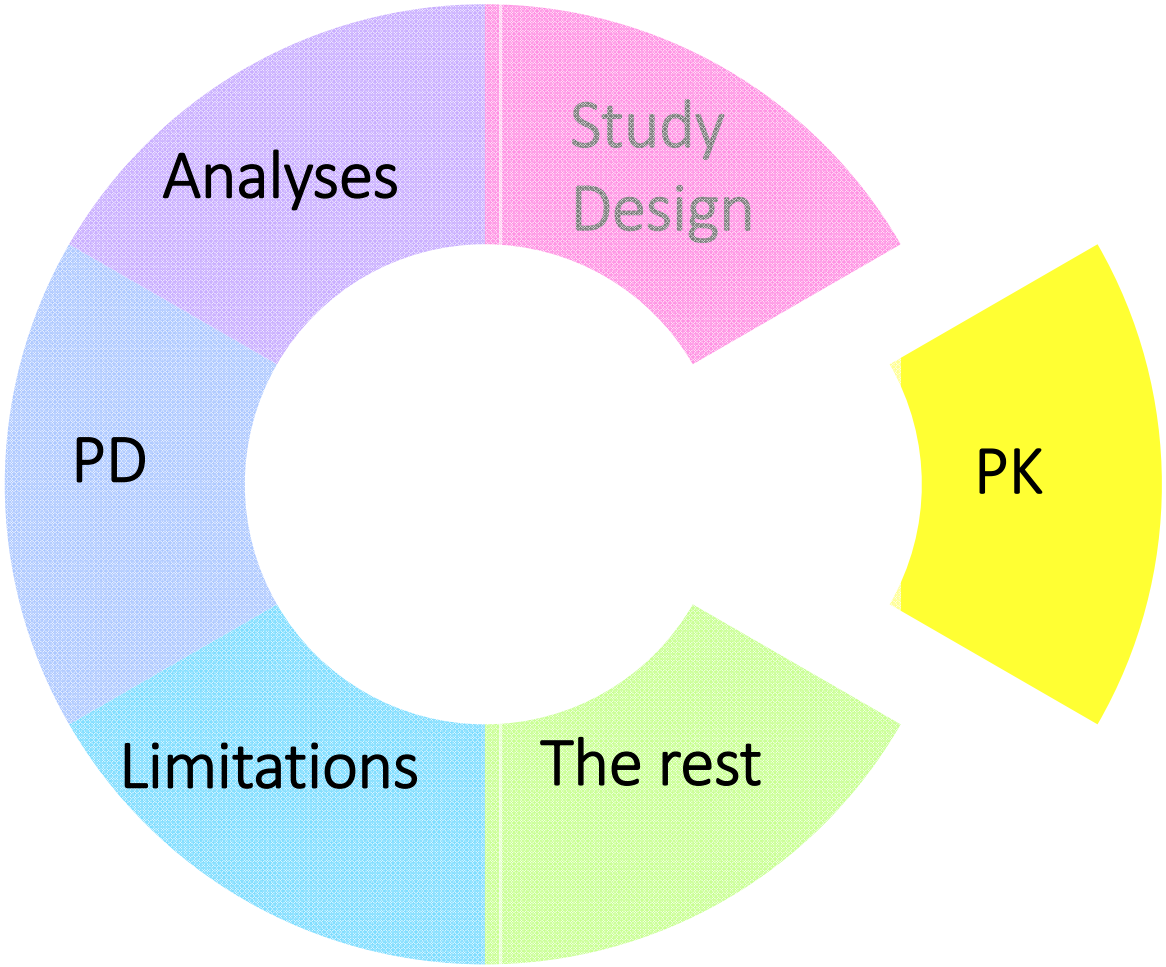
Decision to focus on standard tone

- Growing amount of literature to show this alone is modified in several neurological disease states a

Only problem is that P1 is not normally collected in humans

- Had to be re-derived individually from raw wave form

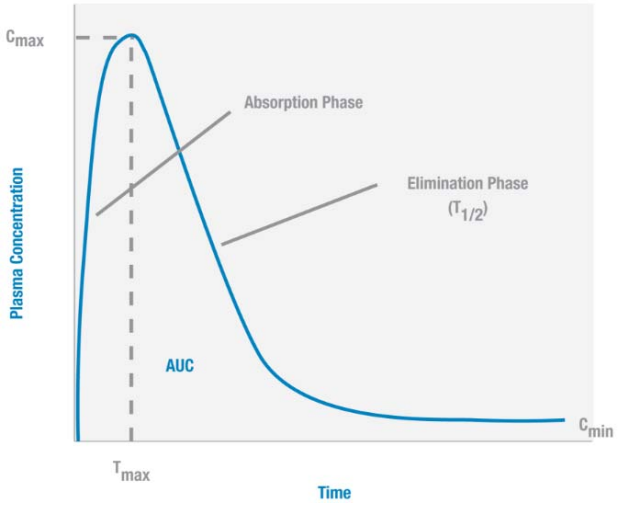




PK

How best to match PK to PD

- Different animals (PK and PD) at matched timepoints
- Same humans at matched timepoint



Focus on AUC

- Holistic measure
  - Best accounts for overall PK exposure
  - Cmax is more variable than AUC for translation between species

PK

Dry blood sampling – allows you to take a small blood spot from the animal's tail

- This study was the first instance of PK sampling within an animal EEG experiment.

For the first time ever we can take PK and PD samples at the same timepoints from the same animal

But at this stage both our human and rat experiments are cor
Do you:

A: Work with what you've gc

B: Go back to the start and repeat the animal experir

PK

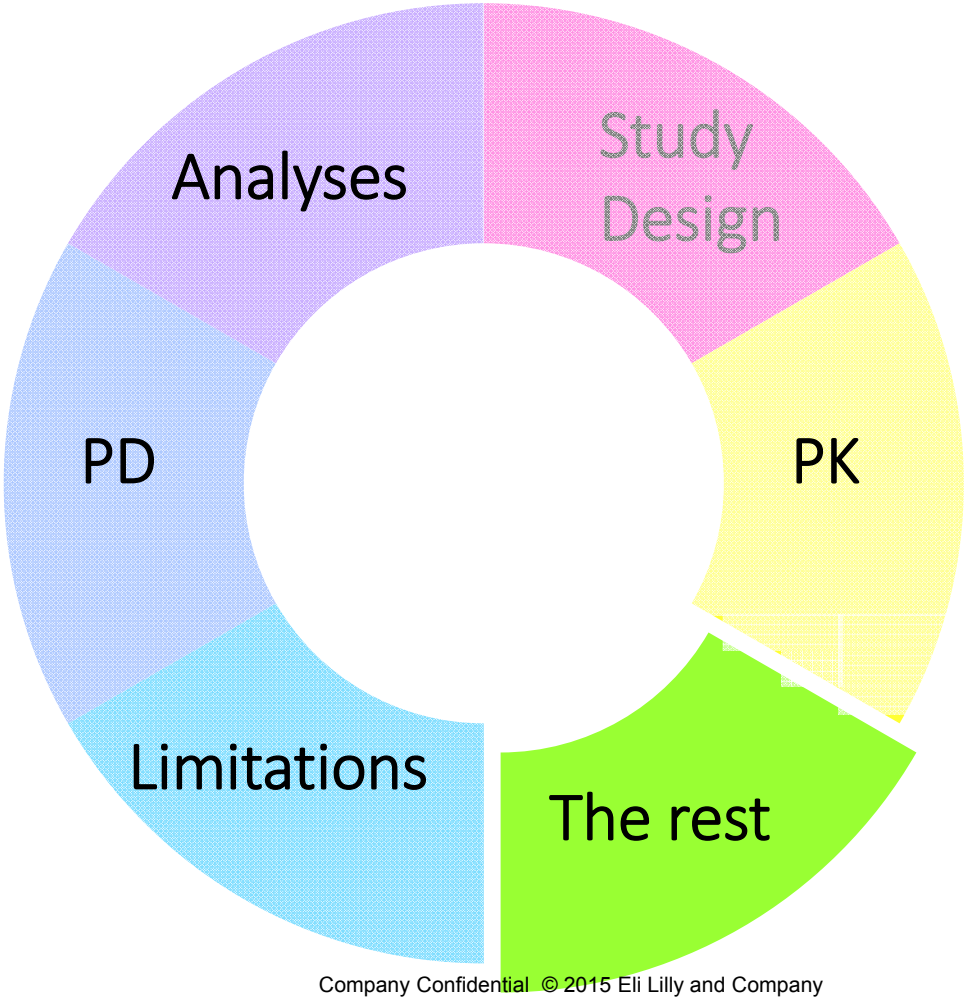
New rat experimental design

- Now a crossover!
- Vehicle is now within the same rats (not historical)
- Separate experiments for Lorazepam and Methylphenidate
- ERP measures taken at baseline and around Cmax

Limited PK blood spotting achievable during experiment

- Dry blood spots were taken at 3 time points (1,2 and 4 hours post treatment) from 5-6 unique animals each week from the high and low dose groups
- PK interpolation required

Cage	Week 1	Week 2	Week 3
1	Low Dose	High Dose	Vehicle
2	Low Dose	Vehicle	High Dose
3	High Dose	Low Dose	Vehicle
4	Vehicle	High Dose	Low Dose
5	Low Dose	High Dose	Vehicle
6	Low Dose	Vehicle	High Dose
7	Low Dose	Vehicle	High Dose
8	High Dose	Vehicle	Low Dose
9	Vehicle	Low Dose	High Dose
10	Vehicle	Low Dose	High Dose
11	High Dose	Vehicle	Low Dose
12	Low Dose	High Dose	Vehicle
13	Vehicle	High Dose	Low Dose
14	High Dose	Vehicle	Low Dose
15	High Dose	Low Dose	Vehicle
16	Vehicle	Low Dose	High Dose

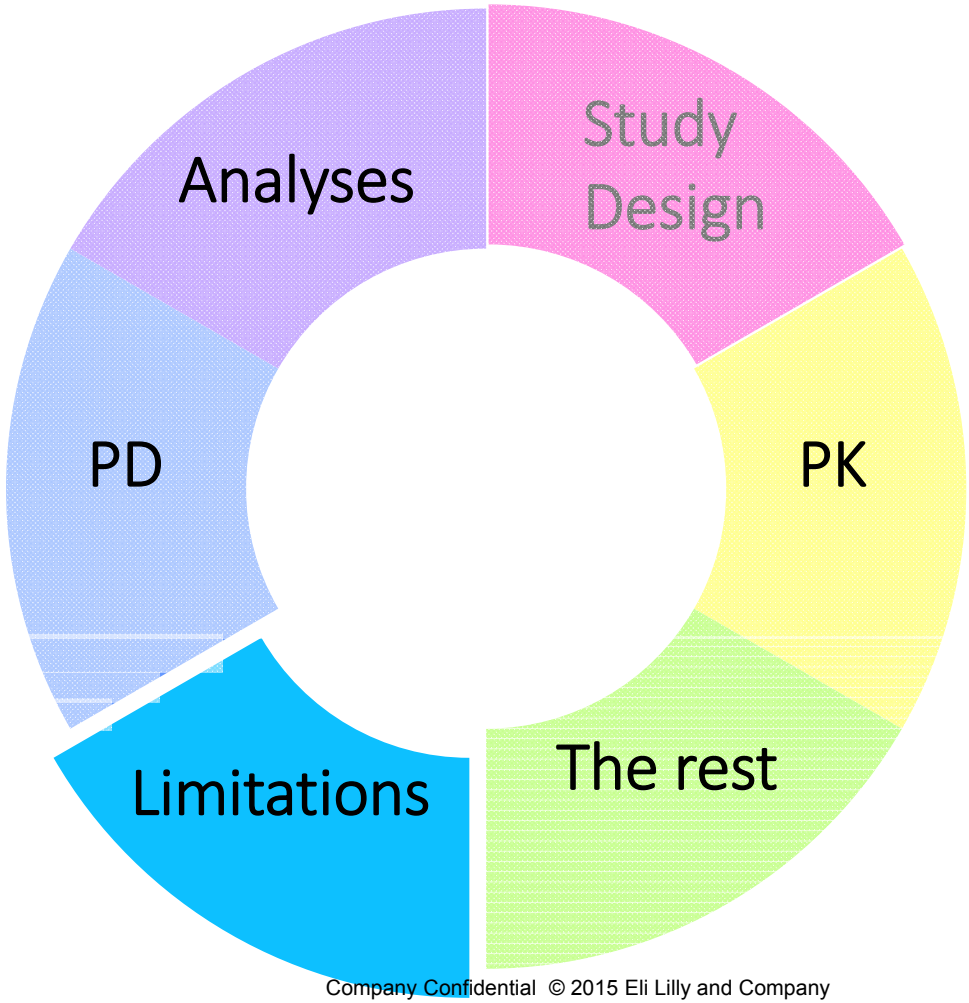


The Rest

Think about the little things:

- Technician, room and machine effects
  - Not a problem for rats – just one
  - Multiple for humans – ensure the data is collected
    - Observed a technician effect
    - One machine slightly higher calibrated than the other

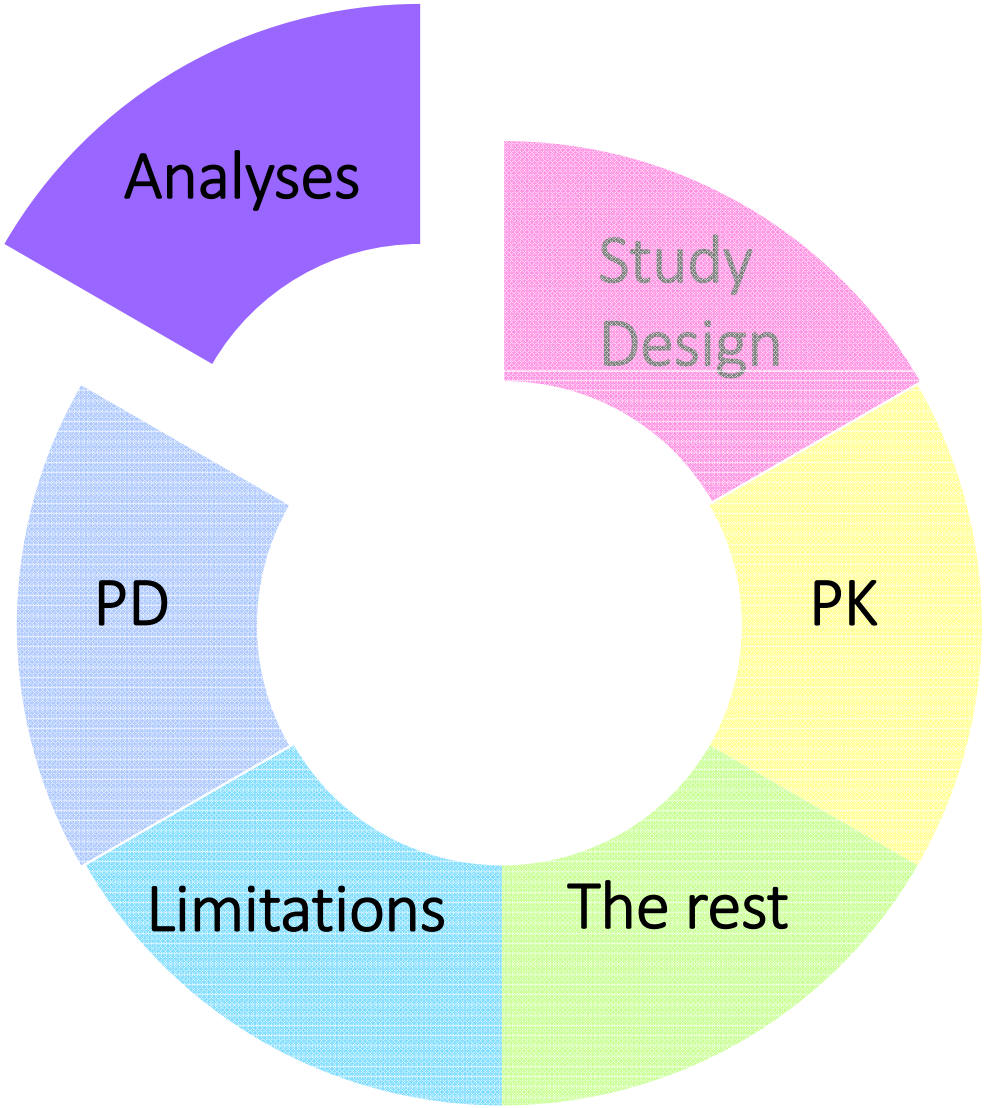
- Animals sleep during the day
  - Ensure you can monitor sleep and remove any segments where animals were asleep from the analyses



Limitations

- Accept what you can't match and try and equate
  - Units/ voltage (skull thickness)
    - Consider %increase and match scales
  - Leads 19 in humans – 7 in rats
    - Will cover more of the cortex in humans but will include regions we don't expect to affect

- Results for animal PK/PD experiment have more variable baselines
  - ERPs are not as clean
    - Rats hear the tones begin and then are aware that a big PK needle will appear
    - Currently using multiple baselines – rat sleep/wake cycle is short perhaps span too long of a period
  - Potential options
    - Consider a signal baseline close to predose
    - Move away from change from baseline –> absolute measures compared to placebo
    - Use the experiment with different PK/PD animals
    - Consider an experiment with PK on one day, PD another day
      - Across time rats have relatively low variability PK/PD
      - Longer experiment



Analyses

Primary analyses

Does treatment have an effect on change from baseline in ERP?

New rat study design -> same analyses for both species

```
PROC MIXED data=ERP_DATA;
     CLASS TREATMENT SUBJECT PERIOD TIMEPOINT;
           MODEL ERP1_CFB= ERP1_SUBJECT_BASELINE ERP1_PERIOD_ADJUSTED_BASELINE PERIOD
                           TIMEPOINT|TREATMENT /ddfm=kr;
           RANDOM intercept PERIOD /SUBJECT=SUBJECT;
           REPEATED TIMEPOINT/SUBJECT=SUBJECT*PEROID type=UN;
RUN;
```

Change from baseline in ERP measure (e.g. N1)
No account for multiple measures

Baseline:
- Subject average baseline is the average of the baselines across all periods for the subject.
- Adjusted baseline equals the baseline for that period minus the subject average baseline.

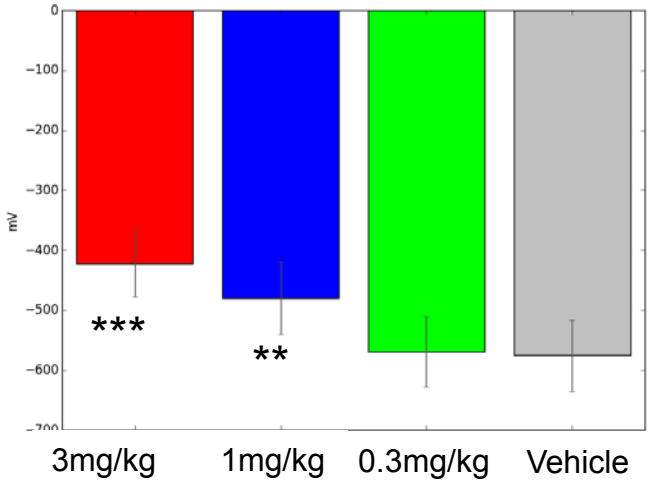
As per Kenward-Roger paper for fitting baseline in a crossover

Double repeated measures
- Time will be accounted for as a repeated effect within subject and period.
- Subject will be fitted as a random effect in the model

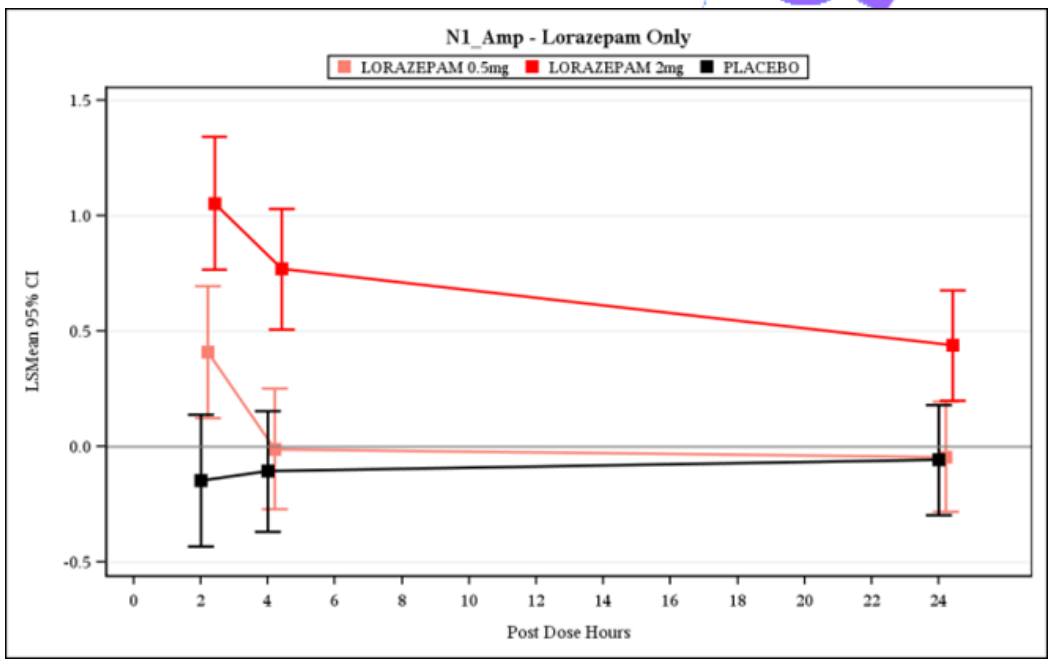
Consolidate how to report analyses

First attempt

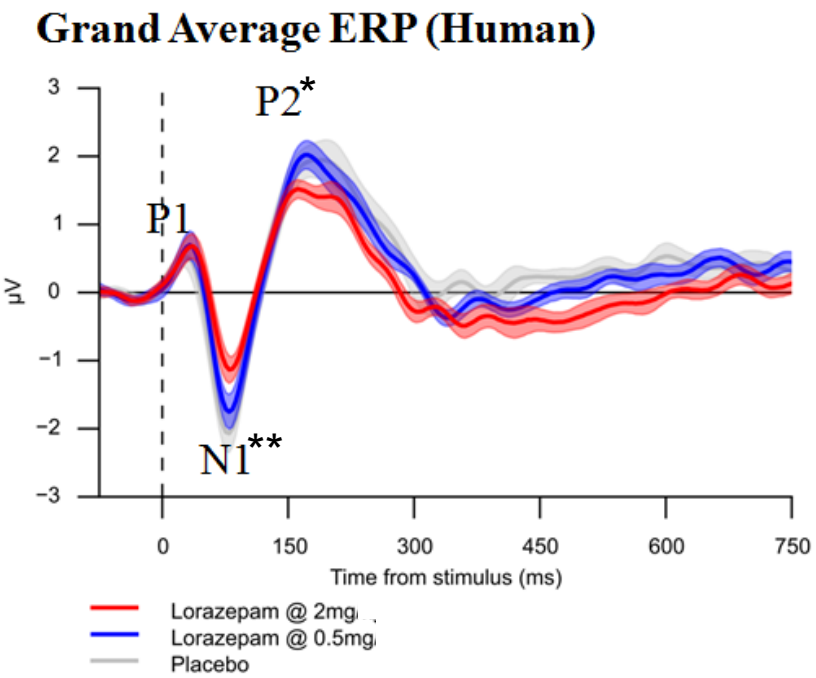
N1 – Rats – 2hrs

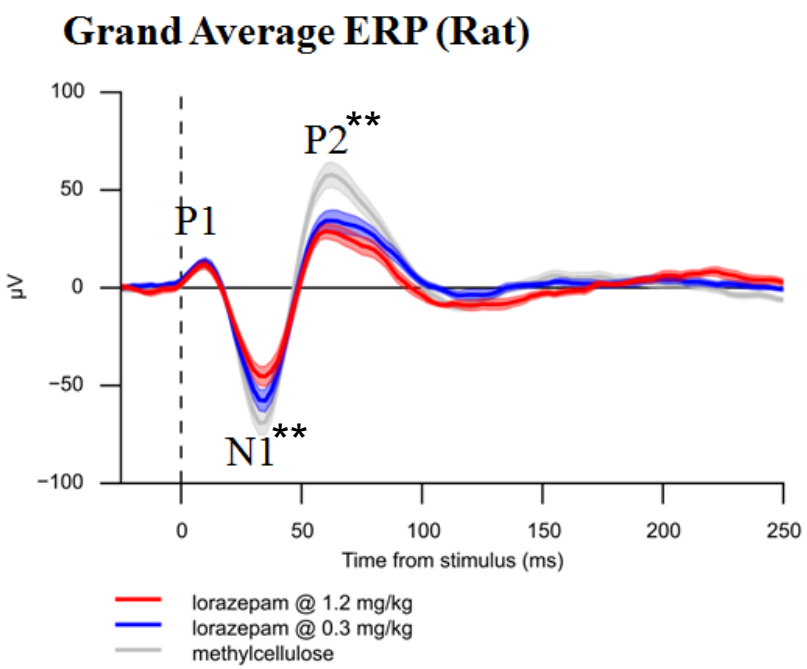


N1 – Humans – all timepoints



Results!





Use MMRM to find out which parts of waveform are significant.
*significant effect at high dose, **significant effect at both doses

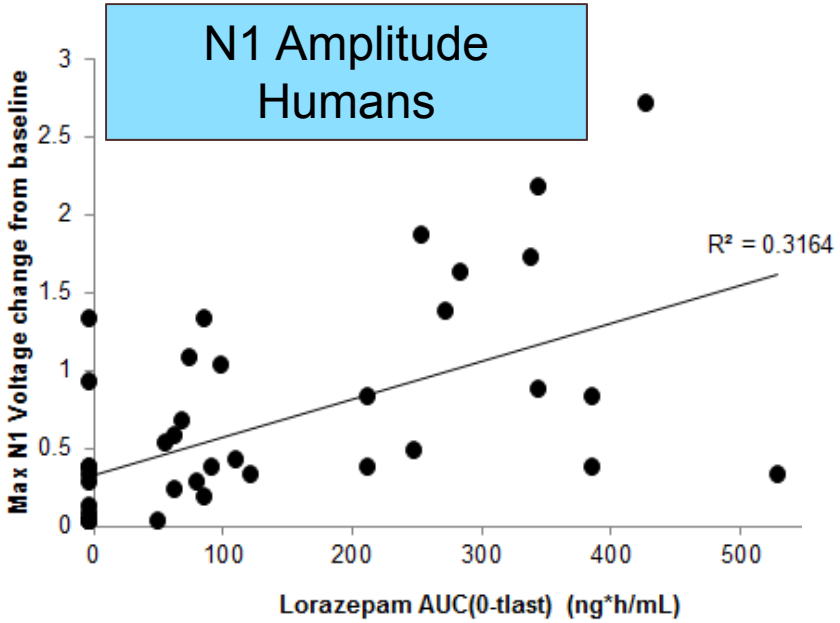
Consistent significant dose and exposure dependent effects of Lorazepam on N1 amplitude across species.

P2 amplitude effect seen in both species (stronger in rat)

No effects seen on any parameters for methylphenidate for either species

PK/PD

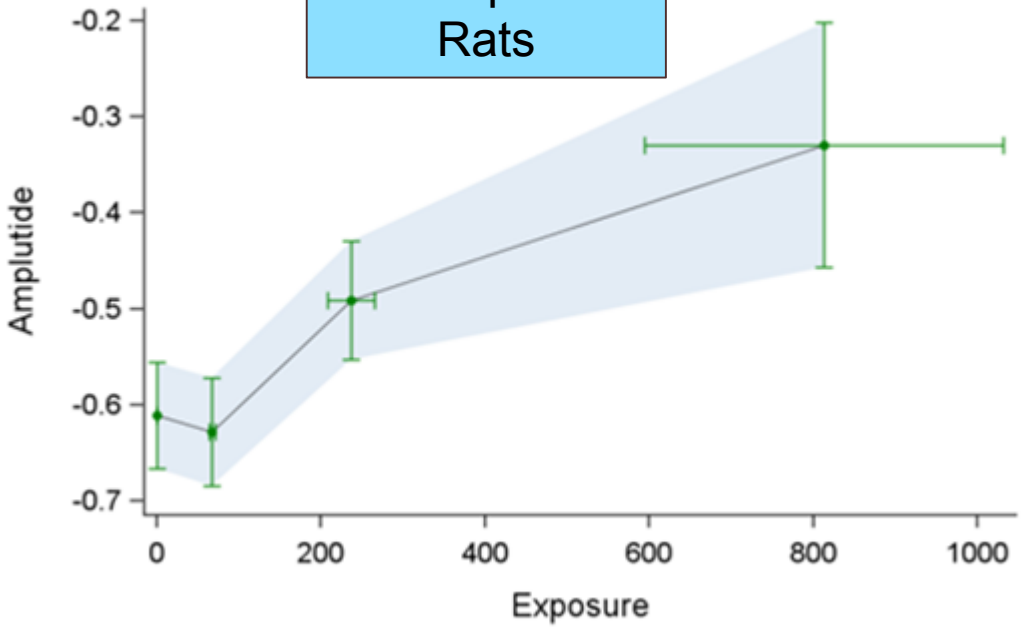
N1 Amplitude Humans

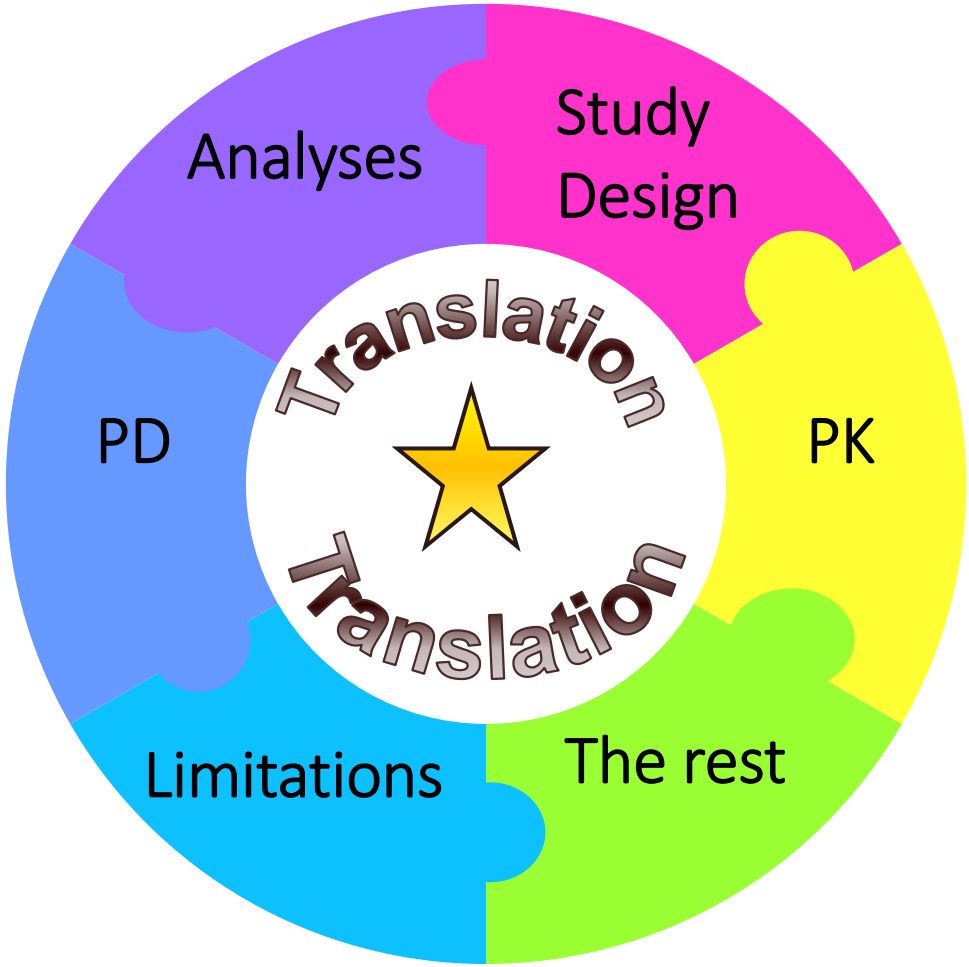


Early indications so a strong PK/PD relationship for N1

PK/PD Emax modelling for both species is ongoing

N1 Amplitude Rats




Analyses
Study Design
PD
PK
Translation
Translation
Limitations
The rest

Translation Conclusions

- Early indications of qualitative translation:
  - Lorazepam; matched directional responses in standard tone ERP and EEG across species
  - Methylphenidate; consistent lack of effect of ERP for standard tone ERP across both species, with EEG beta effect for both species
- The dose and exposure dependent responses of both ERP and EEG to Lorazepam within a therapeutically relevant dose range supports the use of these measures as PD biomarkers to support dose ranging in Phase 2 studies.
  - Attractive sample size for future studies, post hoc analyses suggest N<12
  - Efficient utilisation of PD biomarker as EEG can easily be added into ERP study design (after ERP measures)
    - Currently using in clinic with Lilly Phase 1 molecules

Learnings

- Work iteratively
  - In this case we established what would relate best to patients and worked backwards
  - Plan a course of action and work together!
  - Don’t be afraid to update as science updates
- Back to basics
  - Sometimes involves starting from scratch with a endpoint you have little experience of
- Don’t believe all you read in the literature – use as a guide
  - Methylphenidate did not replicate in humans
  - P3 could not be reproduced in rats
- Statistics **can** lead a translational team
  - Statistics is the same preclinically and clinically and you can driving making the studies/endpoints/measures match
  - **Caveat: If you take the lead on a translational project, ensure to surround yourself with the best scientific experts!**

Acknowledgements

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Song Fang Fang