

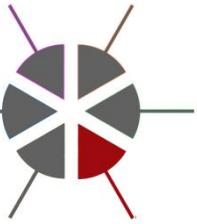


Multicentre Preclinical Animal Research Team

Multi-PART

Work Package 3 – Experimental Design

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WP 3

Purpose of WP 3 – Experimental design

- to develop strategies to maximise both the internal and external validity of the studies performed within Multi-PART or other multi-centre research consortia



WP 3

Topics to discuss

- **Randomisation and blinding:**

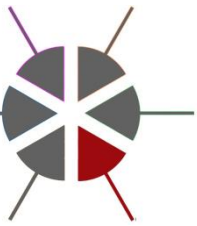
Month 12: Preliminary solutions for randomisation and blinding for further discussion and refinement

- **Database issues:**

Variation in husbandry variables among labs

- **External validity:**

Within-lab vs. between-lab variation



WP 3

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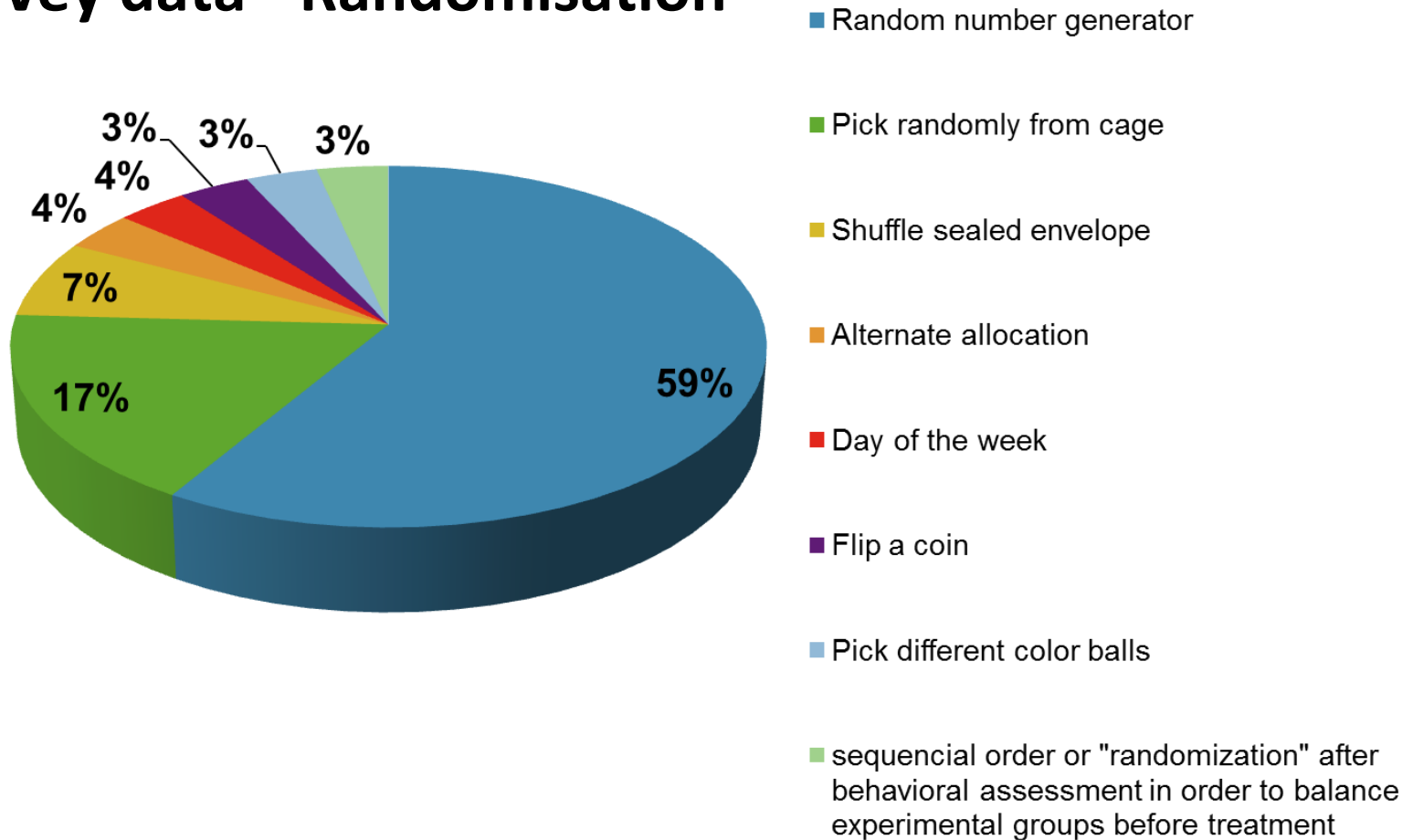
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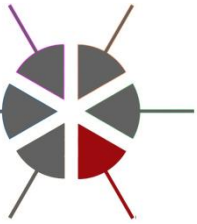
Within-lab vs. between-lab variation



WP 3 – Randomisation & Blinding

Survey data - Randomisation





WP 3 – Randomisation & Blinding

Types of randomisation

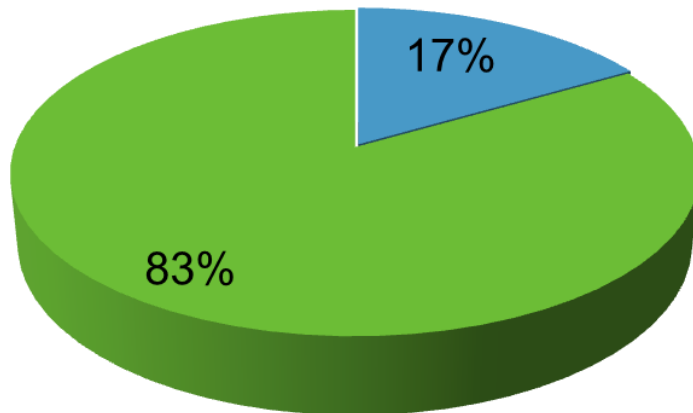
- Simple randomisation
- Block randomisation
factorial designs (labs, replicate cohorts, cages)
fixed or random block size
- Stratified randomisation
Inclusion of relevant covariates (sex, age groups, comorbidities)
- Covariate adaptive randomisation



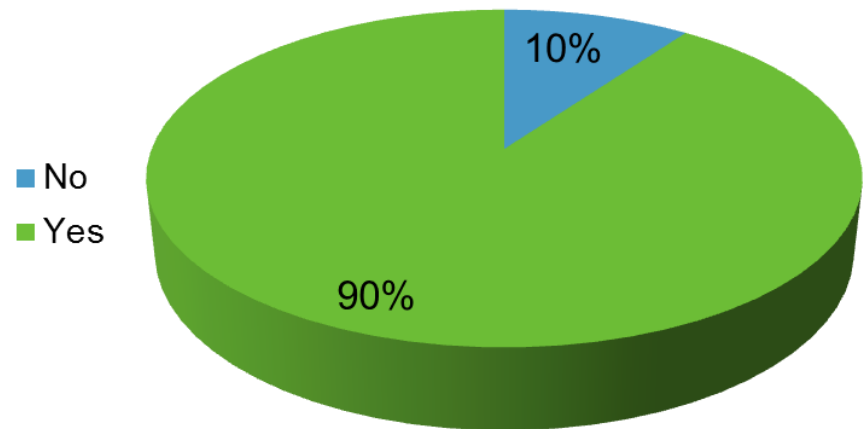
WP 3 – Randomisation & Blinding

Survey data – Allocation concealment & Blinding

During Surgery



During Experiment





WP 3 – Randomisation & Blinding

Survey data – Blinding

- 100% blinded outcome assessment
(infarct volume, behavioural and other outcomes)
- Common reason given for inability to blind:
Cage cards and records prevent allocation concealment during experiment



WP 3 – Blinding

Levels of blinding

- Level 0: Allocation concealment
- Level 1: Blinding experimenters
(e.g. care takers, surgeons, experimenters)
- Level 2: Blinding data collectors
(e.g. technicians, experimenters)
- Level 3: Blinding outcome assessors
(e.g. experimenters, external assessors)
- Level 4: Blinding data analysts
(e.g. experimenters, external analysts)



WP 3

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- **Randomisation and blinding:**
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- **Database issues:**
Variation in husbandry variables among labs
- **External validity:**
Within-lab vs. between-lab variation



WP 3 – Database issues

Survey data – Variation in husbandry across labs (n=30)

- **Biosecurity:** SPF: **13** OHC: **2** Conv: **10** n/a: **5**
- **Acclimatisation:** 0-3d: **1** 4-7d: **16** >7d: **8** n/a: **5**
- **Enrichment:** no: **9** nest: **5** shelter: **4** mult: **12**
- **Animals per cage**
 - Before surgery: **1 - 5** (median: 4)
 - After surgery: **1-11** (median: 3)
- **Cage changes:** **1 - 3** per week
- **Handling:** very variable, even within labs



WP 3 – External validity

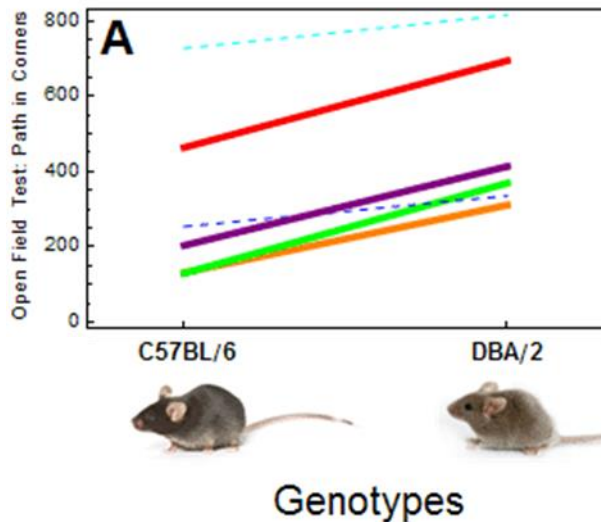
Within-lab vs. between-lab variation

- Analysis of 7 multi-lab studies on behavioural phenotyping of inbred and mutant mouse lines
- Comparison of single-lab effects of genotype vs. overall effects across labs
 - assessment of replicability of single-lab studies
- Adjustment of within-lab variation by an estimate of between-lab variation
 - assessment of a possible strategy to improve replicability of single-lab studies



WP 3 – External validity

Within-lab vs. between-lab variation



----- : $p > 0.05$ t-test

— : $p < 0.05$ t-test

— : $p < 0.05$ after GxL adjustment

($\sigma^2_{G \times L}$; derived from mixed model)

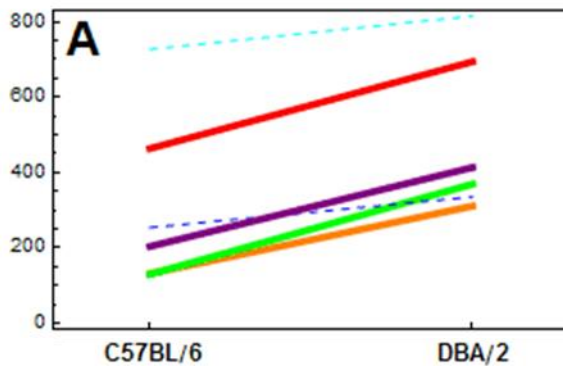
Overall effect: $p < 0.01$

Mixed model with genotype as fixed effect and lab as random effect



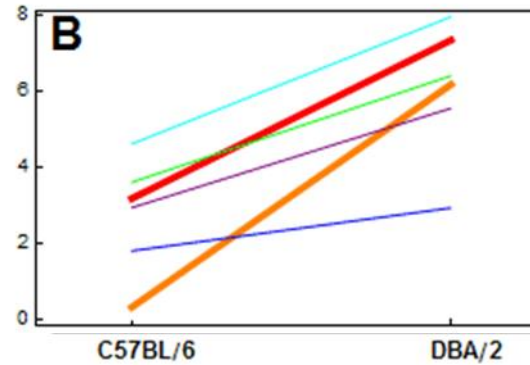
WP 3 – External validity

Within-lab vs. between-lab variation



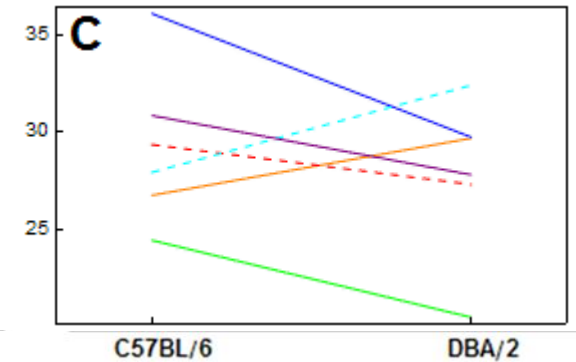
Genotypes

$p < 0.01$



Genotypes

$p < 0.01$



Genotypes

$p = 0.47$



WP 3 – External validity

Within-lab vs. between-lab variation

Study	A	B	C	D	E	F	G
No. of laboratories	6	6	3	3	2	2	3
No. of genotypes	2	2	3	3	14	16	2
No. of measures	29	29	20	20	36	36	11
Type-I Error Rate							
Standard method	38.1%	35.6%	40.7%	33.3%	22.0%	26.7%	43.3%
GxL-adjusted	8.3%	3.3%	6.2%	5.1%	9.0%	9.0%	5.0%
Power							
Standard method	74.4%	60.7%	90.9%	86.3%	76.8%	77.9%	58.3%
GxL-adjusted	45.6%	36.9%	69.7%	66.7%	68.9%	66.5%	58.3%



WP 3 – Experimental Design

Deliverables

- **Month 18:** Preliminary solutions for sample size calculation and systematic variation for further discussion and refinement
- **Month 24:** Final report describing the most appropriate design of future preclinical trials (randomisation, blinding, sample size calculations, systematic variation)