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‘Mouse models of motor neuron disease; scientific and ethical aspects"

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MLPM Summer School
21-25 September 2015
Museum of Science and Industry
Manchester, UK

Machine Learning for Personalized Medicine

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Overview

- Ethics of animal research
- UK law and regulations
- Background on MND
- Mouse models of MND

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Why do we need animals in biomedical research?

- Currently impossible to model intact physiological systems *in vitro* or *in silico*
  - e.g. Immune system, nervous system
- Even if it were possible – we would still need to confirm a model prediction at some point *in vivo*

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Three main types of research involving animals

- Advancing scientific knowledge
  - How animals behave, develop or function
- As models of human disease and to develop interventions
- In toxicity testing of pharmaceuticals and chemicals (including invironmental toxicity testing)
- (Teaching)

Ethical aspects

- Animal research causes harm to animals
  - Pain, suffering, death
- Is it justifiable for one species to cause harm to another, primarily for the benefit of the first species?
  - Speciesism
- (Does research in animals produce useful results?)

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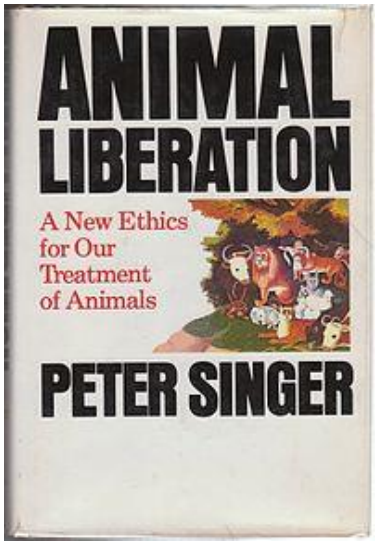
Are Human interests special?

- Humans are morally distinctive (clear-line view)
- Hierarchy of moral importance (sliding scale view)
- No categorical distinction (moral equality view)

Speciesism



- Popularised by Peter Singer in 'Animal Liberation' (1975)
  - Utilitarian approach
    The greatest good of the greatest number
  - The boundary between humans and other animals is arbitrary
  - Animals share the capacity to suffer with humans
  - Speciesism violates the principle of equal consideration of interests (as does racism, sexism)

The Law (UK)

- Animals (Scientific Procedure) Act 1986
- Revised 1 January 2013 to transpose European Directive 2010/63/EU
- Criminal Act -can be enforced by prosecution through the courts
- Implemented by the Home Office in the UK
  - Grant licenses (Project, Personal, Establishment)
  - Inspection regime

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Animals (Scientific Procedure) Act 1986

- Utilitarian approach (sliding scale view) to ethical justification of animal research
  - Benefits (to animals and humans) must outweigh the costs (harm) to animals
  - Ethical review of licenses required

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Animals (Scientific Procedure) Act 1986

5)The Secretary of State shall not grant a project licence unless he is satisfied—

(a)that the purpose of the programme to be specified in the licence cannot be achieved satisfactorily by any other reasonably practicable method not entailing the use of protected animals; and

(b)that the regulated procedures to be used are those which use the minimum number of animals, involve animals with the lowest degree of neurophysiological sensitivity, cause the least pain, suffering, distress or lasting harm, and are most likely to produce satisfactory results

The 3 ‘R’s

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The 3Rs

- **Replacement** – methods which avoid or replace animals (protected under the UK ASPA) in areas where they otherwise would have been used
- **Reduction** – methods which minimise the number of animals used, or maximise the information gained from a given number of animals
- **Refinement** – improvements to husbandry and procedures which minimise actual or potential pain, suffering, distress or lasting harm and/or improve animal welfare

Russel and Burch 1959

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BACKGROUND ON MOTOR NEURON DISEASE/AMYOTROPHIC LATERAL SCLEROSIS

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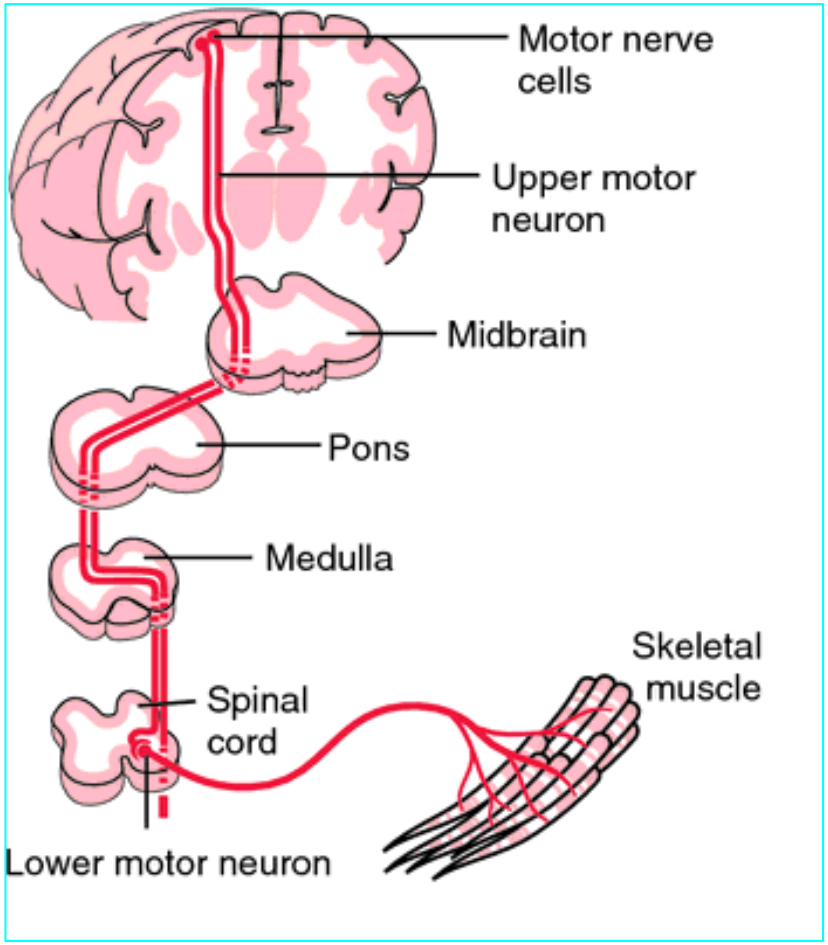
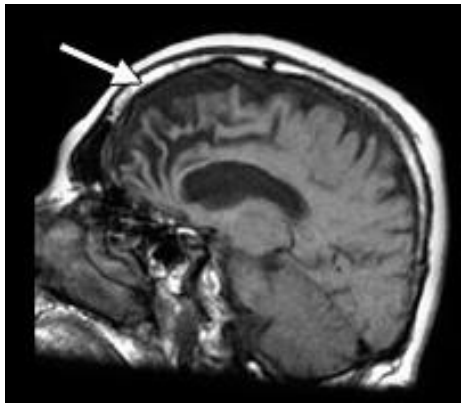
Motor neurone disease: Overview

- Most common form of MND
- Adult onset neurodegenerative disease
- Upper and lower motor neuron degeneration.
- Life expectancy following diagnosis is around 2.5 years.
- Riluzole prolongs life by up to 3 months.
- 5000 people with the disease at any one time in the UK
- Lifetime risk is <1/100000

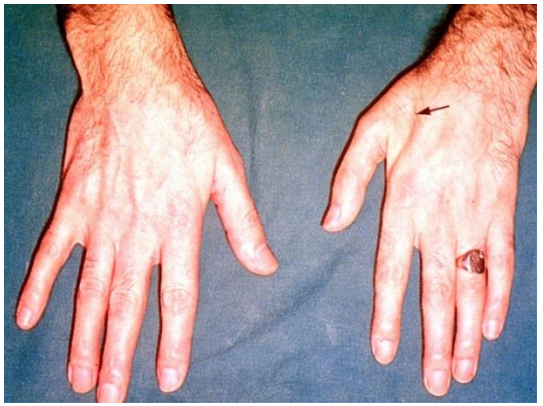


Motor neurone disease: Clinical consequences

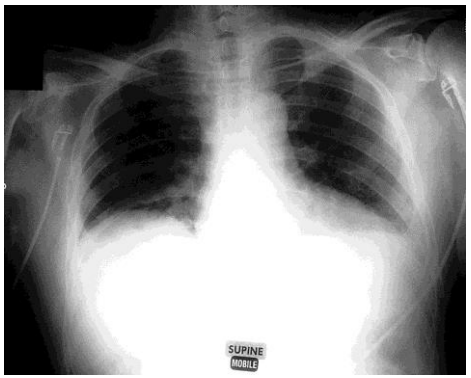
Cognitive changes



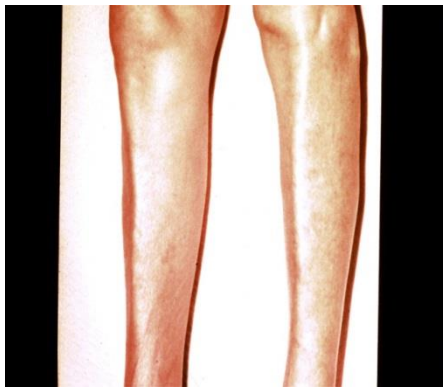
Upper limb



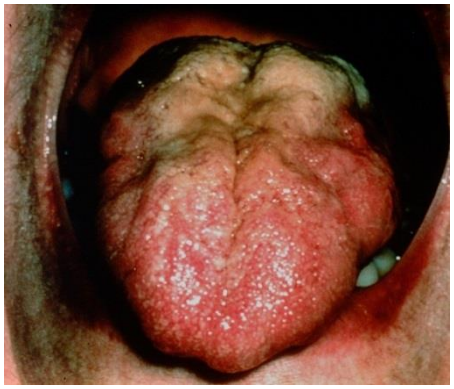
Respiratory



Lower limb



Bulbar



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Genetics of MND

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ALS Locus	Onset	Pattern	Locus	Gene	Protein
ALS1	J	AD/AR	21q22.1	SOD1	Cu/Zn Superoxide Dismutase
ALS2	A	AR	2q33.35	ALS2	Alsin
ALS3	A	AD	18q21	Unknown	Unknown
ALS4	J	AD	9q34	SETX	Senataxin
ALS5	J	AD	15q15-21	SPG11	Spatacsin
ALS6	A	AD	16p11.2	FUS	Fused in Sarcoma Protein
ALS7	A	AD	20p13	Unknown	Unknown
ALS8	A	AD	20q13.33	VAPB	VAMP-associated Protein B
					Protein
ALS15			Xp11.21	UBQLN2	Ubiquilin 2
ALS16			9p13.3	SIGMAR1	Sigma non-opioid Intracellular Receptor
ALS18			17p13.3	PFN1	Profilin 1
ALS/FTD1	A	AD	9q21-22	C9ORF72	C9ORF72
ALS/FTD2	J	AD	9p13.2-21.3	Unknown	Unknown

But… Only 5-10% of MND has a genetic cause (familial), the rest is apparently sporadic

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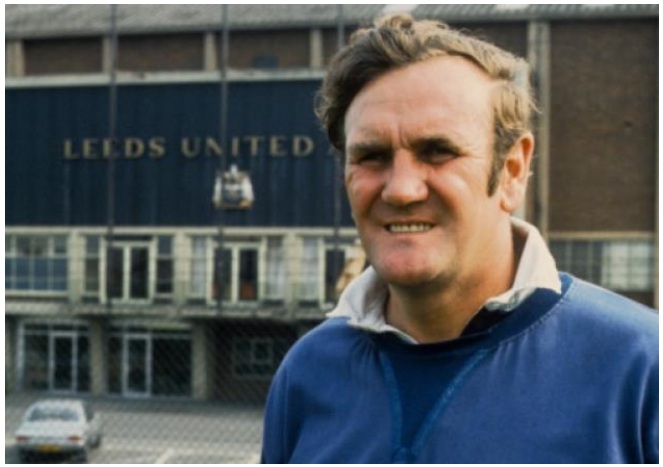
Risk factors

- Age
- Sex
- Physical activity
  - Being an Italian footballer (!)
- Trauma (fractures, head injury)



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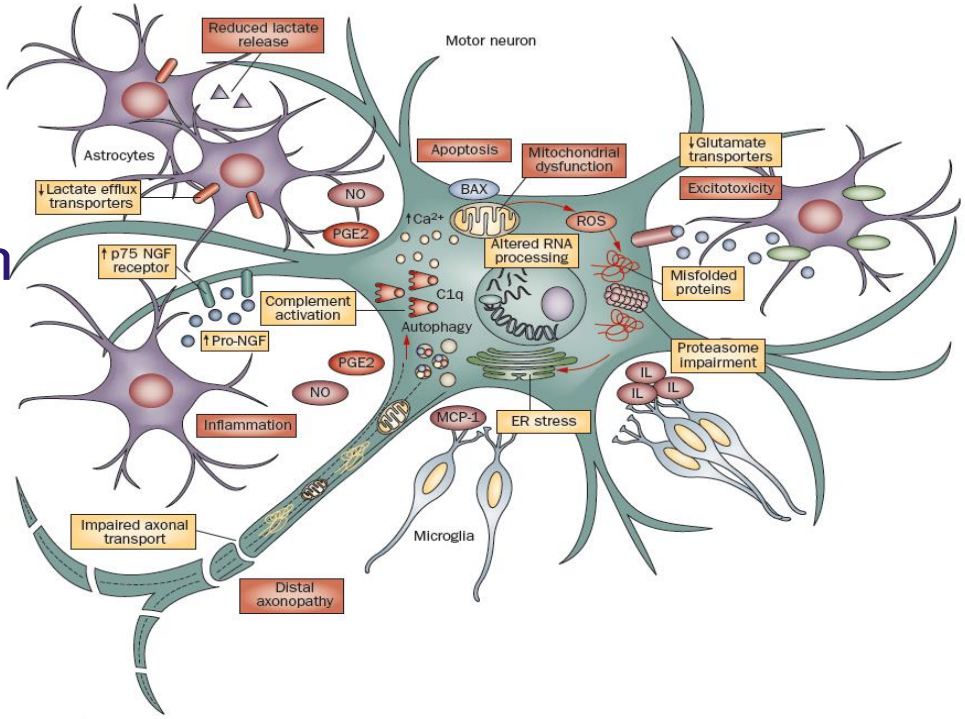
Disease mechanisms

- Excitotoxicity
- Oxidative stress
- Mitochondrial dysfunction
- Protein aggregation
- ER stress
- Axonal transport defects
- Glial toxicity
- Inflammation
- RNA processing



Ferraiuolo et al Nature Reviews Neurology 2011

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Pathology

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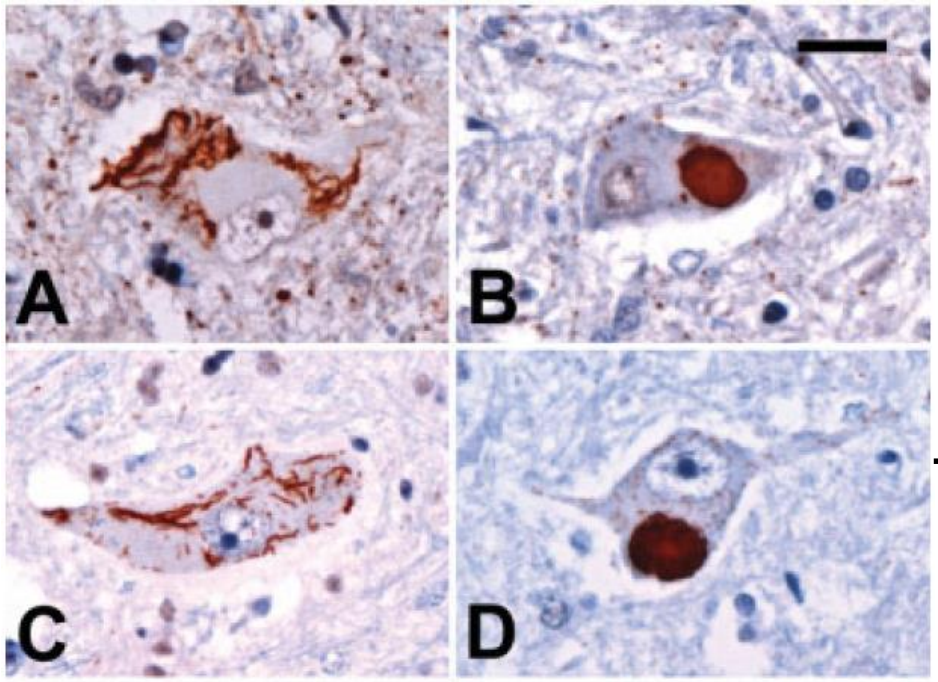
Sporadic

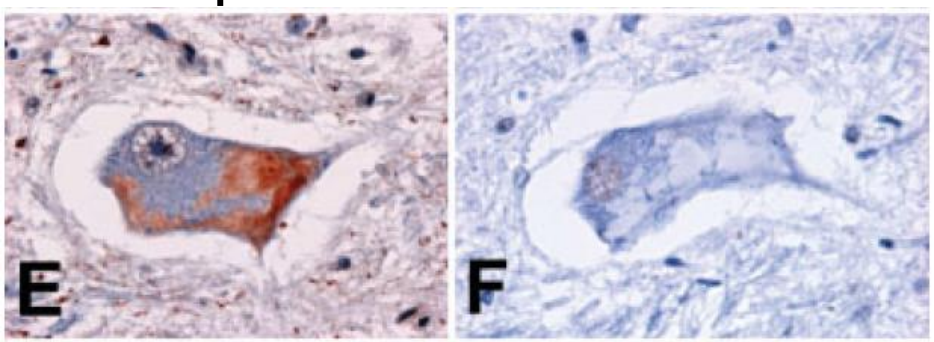


Ubiquitin

TDP-43

SOD1

Ubiquitin TDP-43



Mackenzie, I.R., et al., Ann Neurol, 2007. **61**(5): p. 427-34.

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Ideal preclinical MND model

- Replicate genetics of MND
- Replicate selective vulnerability of motor neurones
- Show progressive motor neuron degeneration and death with associated motor deficits (+/- cognitive deficits)
- Same pathology at end-stage (ubiquitinated inclusions, TDP43+ inclusions, loss of nuclear TDP-43)
- Robust and reproducible
- Respond to treatment with Riluzole

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Transgenic models

	SOD1	TDP43	C9ORF72
Genetics	5-10% familial 0.5-1% overall	5% familial 0.5% overall	40% familial 8-10% sporadic ~13% Overall?
Selective vulnerability	+++	++	Models being developed
MN degeneration	+++	++ (model dependent)	?
Pathology	+/-	++	?
Robust and reproducible	Yes (on right background)	?	?
Riluzole works?	+/-	?	?
Broadly representative of MND?	+	+++	++

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SOD1^{G93A} TRANSGENIC MOUSE MODEL OF MND

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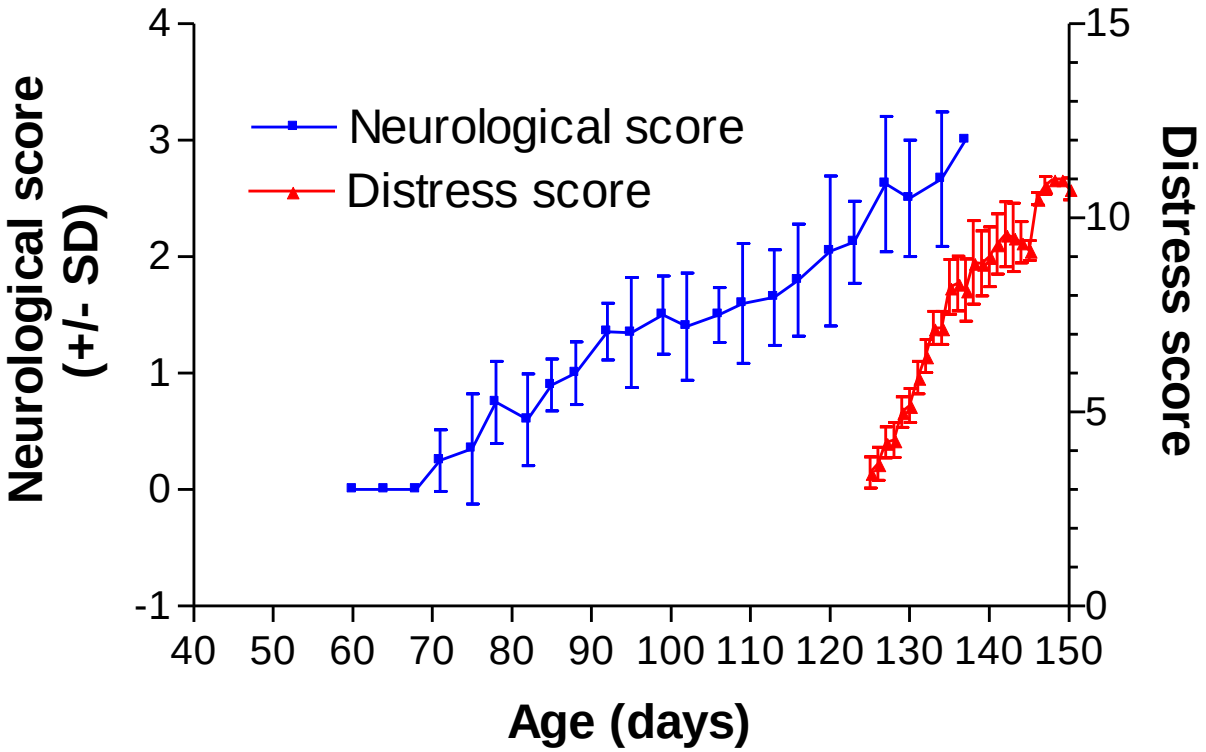
Disease progression in SOD1^{G93A} transgenic mice



Neurological Score (NS)

0.5	Onset (tremor, splay defect)
1	Abnormal gait
2	Dragging Hind-limb
3	Hind-limb paralysis
4	Humane end-point (loss of righting reflex >10s)

Distress Score (based on weight loss, provoked behaviour, appearance and NS)

0-5	Normal/mild
6-10	Moderate
11-15	Severe

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The problem

- "Results in the SOD1G93A transgenic mouse model do not translate to the clinic"
- Compounds taken forward from positive mouse studies to the clinic have failed e.g.
  - Minocycline (worsened progression), Ceftriaxone, Celecoxib, Gabapentin
- Mechanistic Issues?
  - Not a TDP43 'proteinopathy'
- Technical issues
  - Poor study design, variable model, publication bias
  - Translation pathway

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The $SOD1^{G93A}$ model

- ALSTDI tested >70 compounds in 221 studies, 18000 mice
- Identified confounding factors in mixed background (SJLBL6) $SOD1^{G93A}$ transgenic mouse model of ALS
  - Censor non-ALS deaths
  - Litter match control and treatment

- Unable to replicate any published studies
- 'Scott guidelines' (S. Scott, et al. Amyotroph Lateral Scler, 2008. 9(1): p. 4-15.)
- Generic guidelines (A. C. Ludolph, et al. Amyotroph Lateral Scler, 2010. 11(1-2): p. 38-45.)

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ALSTDI replication studies

	Published studies					Retest with ALSTDI study design[1]					
	n		Survival (days)			*n*			Survival (days)		
							Censored[2]				
Drug(s)	Control	Treated	Control	Treated	Lifespan extension	per group at start	Control	Treated	Control	Treated	Lifespan extension
WHI-P131 (27)	24	28	134	200	49.00%	40	34	30	133.9	136.4	1.86%
Celebrex 0.012% (28)	12	12	126	150.2	19.00%	90[3]	88	64	129.8	130.5	0.52%
Celebrex 1500 ppm in chow (21)	27	28	NA	NA	25.00%						
Creatine 2% (28)	12	12	126.1	151.4	19.80%	40	38	39	126	126.9	0.67%
Creatine 2% (29)[4]	6	7	143.7	169.3	17.80%						
Minocycline 50 mpk (33)[5]	7	7	130.3	150.9	15.80%	48*	47	44	135.7	134.9	−0.60%
Ceftriaxone, 200 mpk (36)[6]	20	20	122	135	10.70%	63	59	62	128	129.3	1.02%
Riluzole 0.1 mg/ml in water (~22mpk) (19)	8	8	134	148	10.4%	40	34	35	132.3	134.9	1.96%
Riluzole 24 mpk in chow (20)	11	10	127	140	10.2%						
Riluzole 44 mpk in chow (20)	11	11	127	139	9.4%						
PBA (Sodium phenylbutyrate) at 400 mpk (37)	20	20	125.7	153.2	21.9%	24	24	22	132.6	132.4	−1.75%
Thalidomide, 50 mpk (38)[7]	10	10	130	145	12%						
Thalidomide, 100 mpk (38)	10	12	130	151	16%	24	22	22	134.5	131.9	−1.93%
Thalidomide, 200 mpk						76	73	73	130.2	133	2.20%

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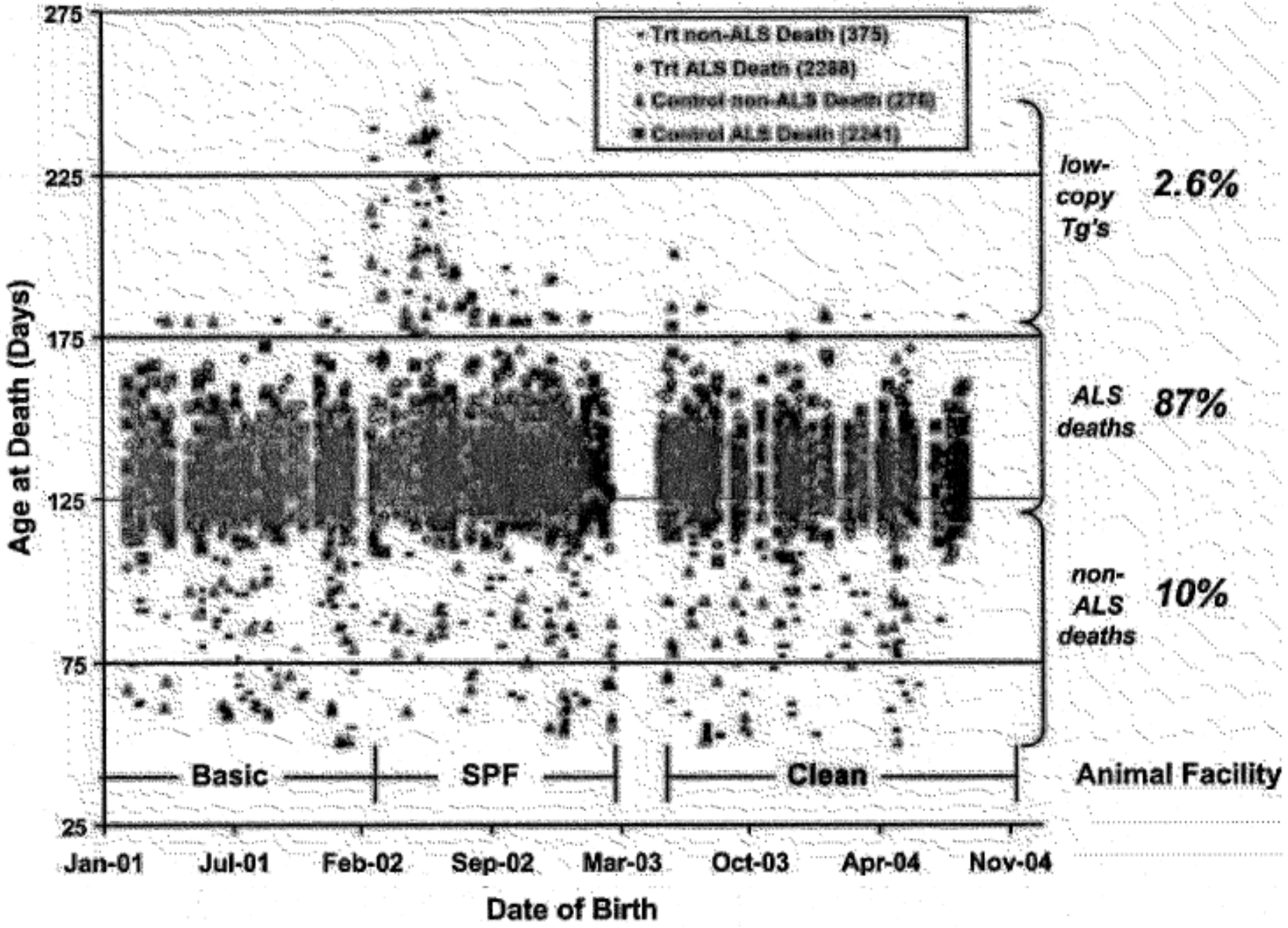
Our approach to the ALS mouse model

- B6SJL-Tg (SOD1-G93A)1Gur/J (stock number 002726)
  - Transgenic for human $SOD1^{G93A}$ gene (~23 copies) driven by endogenous promoter
- Backcrossed onto the C57Bl/6 background (Harlan UK, C57Bl/6 J OlaHsd) for >20 generations
- Inbred line, homogenous genetic background.
- Approximately half the variation of the commonly used mixed genetic background
- Developed a battery of objective robust measures of disease progression
- Protocol designed to avoid common pitfalls found in the majority of published studies
- Mead et al. (2011). "Optimised and Rapid Pre-clinical Screening in the SOD1 Transgenic Mouse Model of Amyotrophic Lateral Sclerosis (ALS)." PLoS One **6(8): e23244.**


Trt non-ALS Death (375)
Trt ALS Death (2288)
Control non-ALS Death (276)
Control ALS Death (2241)
low-copy Tg's
2.6%
ALS deaths
87%
non-ALS deaths
10%
Age at Death (Days)
275
225
175
125
75
25
Basic
SPF
Clean
Animal Facility
Jan-01
Jul-01
Feb-02
Sep-02
Mar-03
Oct-03
Apr-04
Nov-04
Date of Birth

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Early rotarod defect

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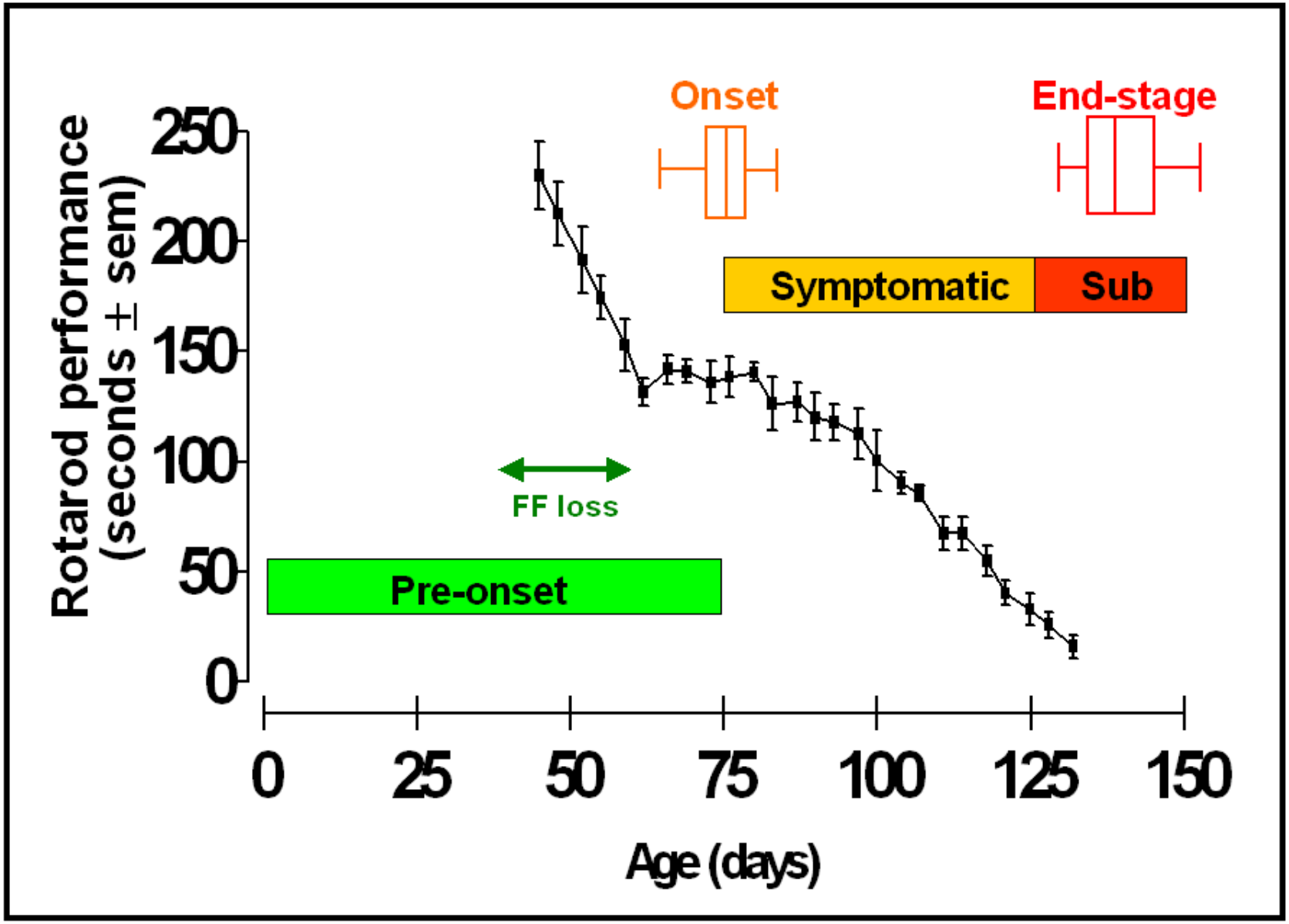

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Muscle pathology

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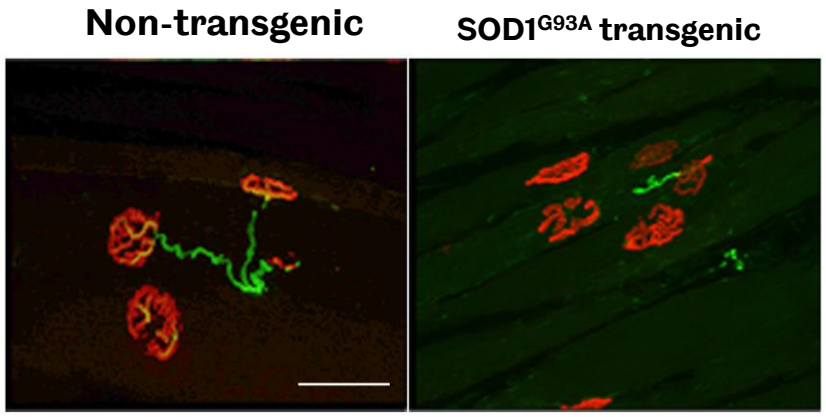

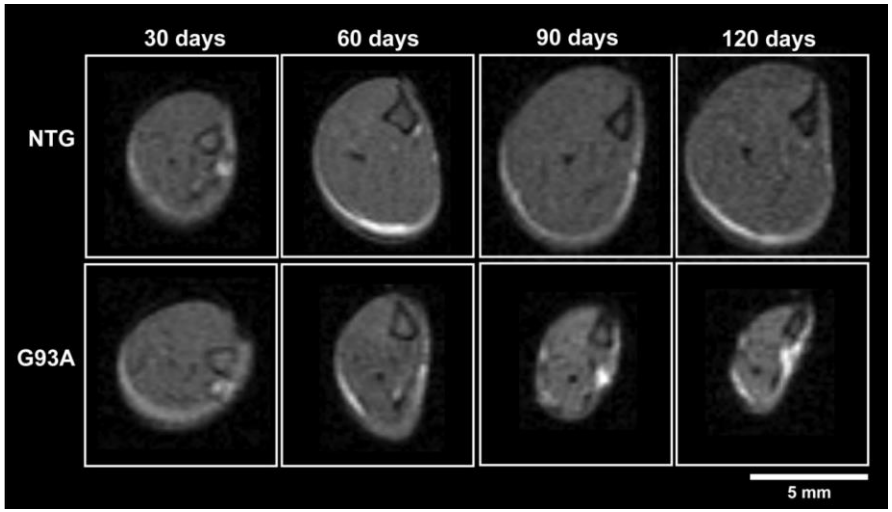

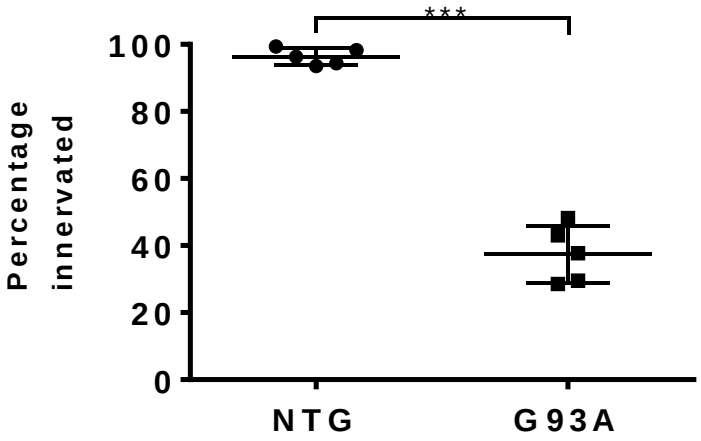

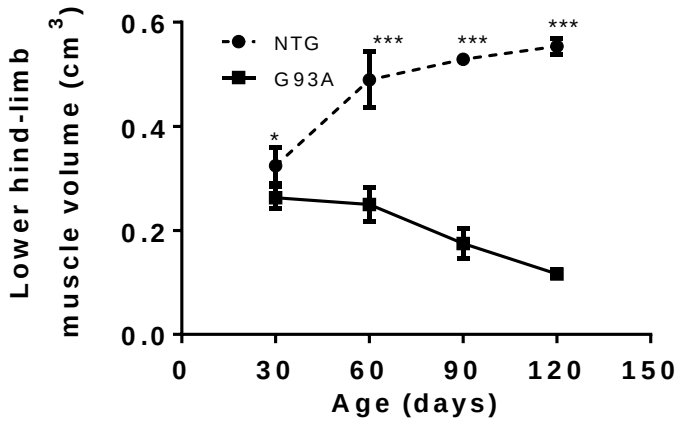

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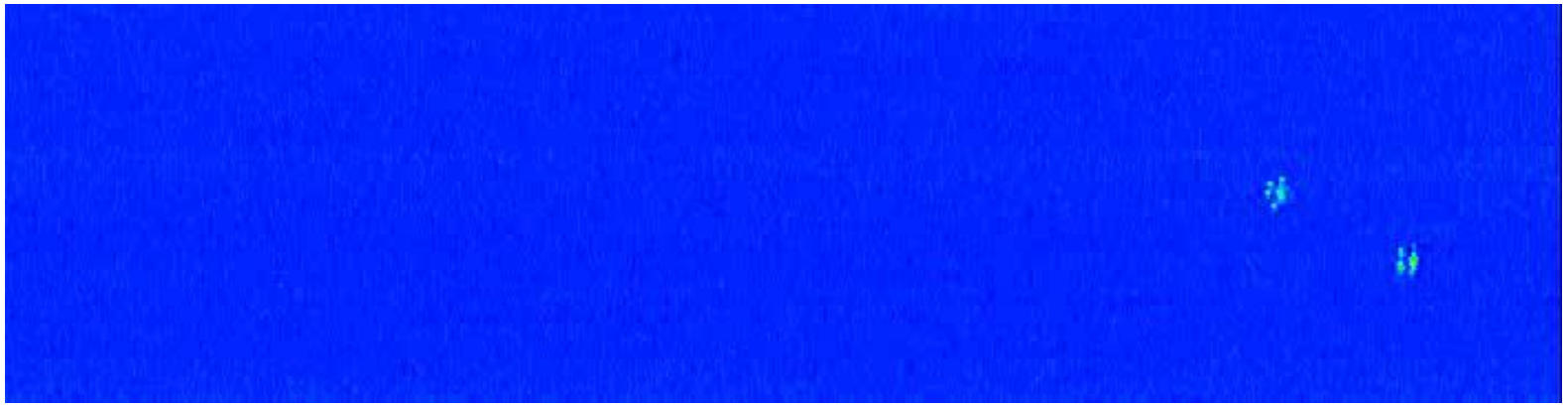
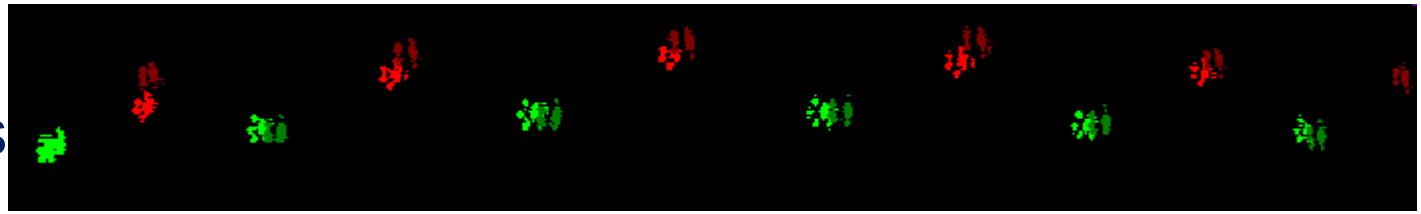
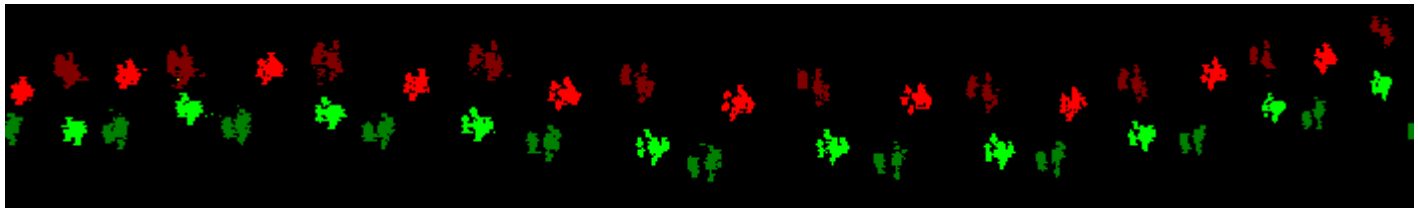


NTG
105 days



G93A
105 days



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Catwalk gait analysis

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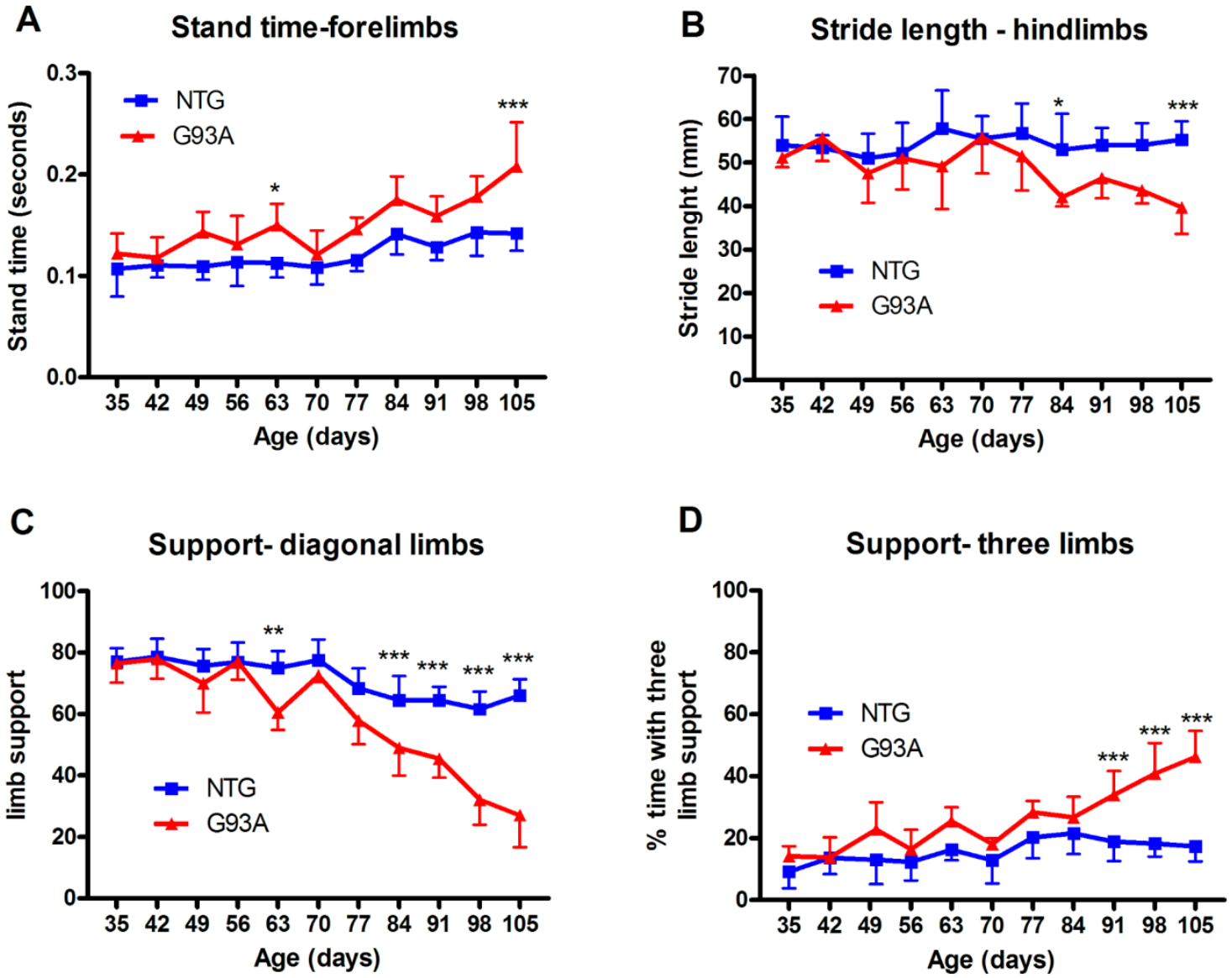

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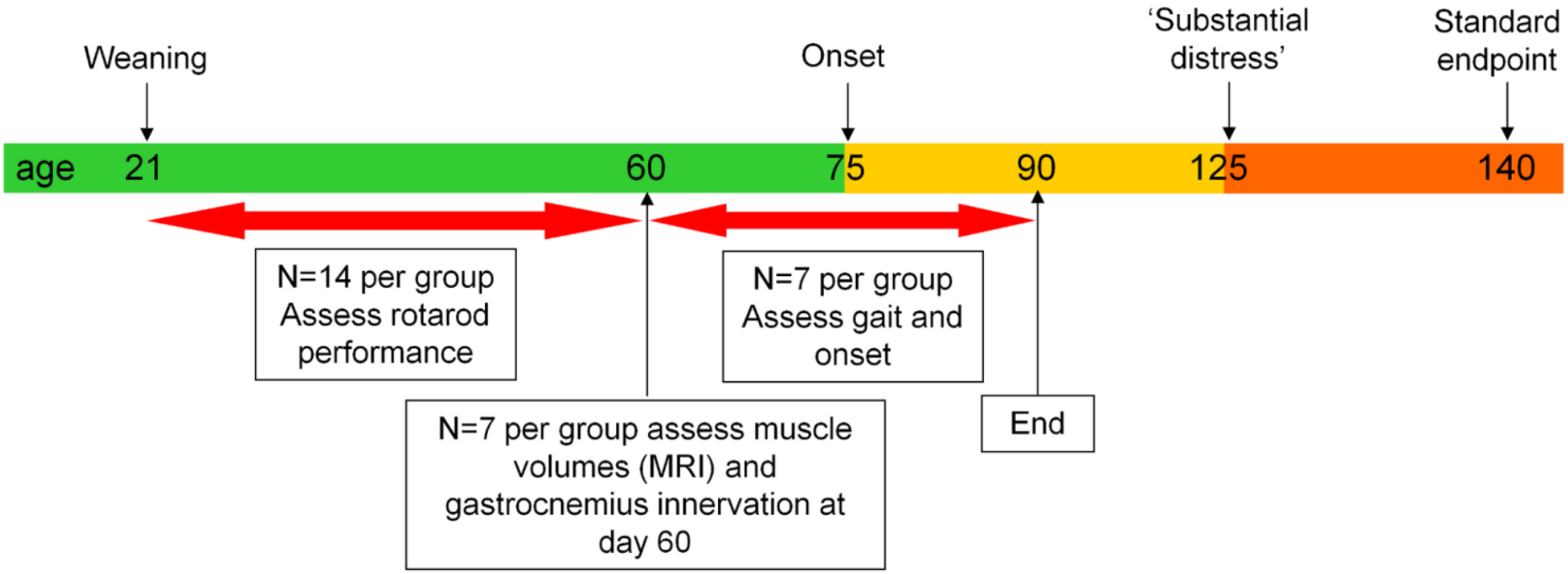
'Rapid' screening in G93A model



Mead et al. (2011). "Optimised and Rapid Pre-clinical Screening in the SOD1 Transgenic Mouse Model of Amyotrophic Lateral Sclerosis (ALS)." PLoS One **6(8): e23244.**

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COULD WE IMPROVE ON THE ROTAROD TEST?

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Fast trac running wheel setup

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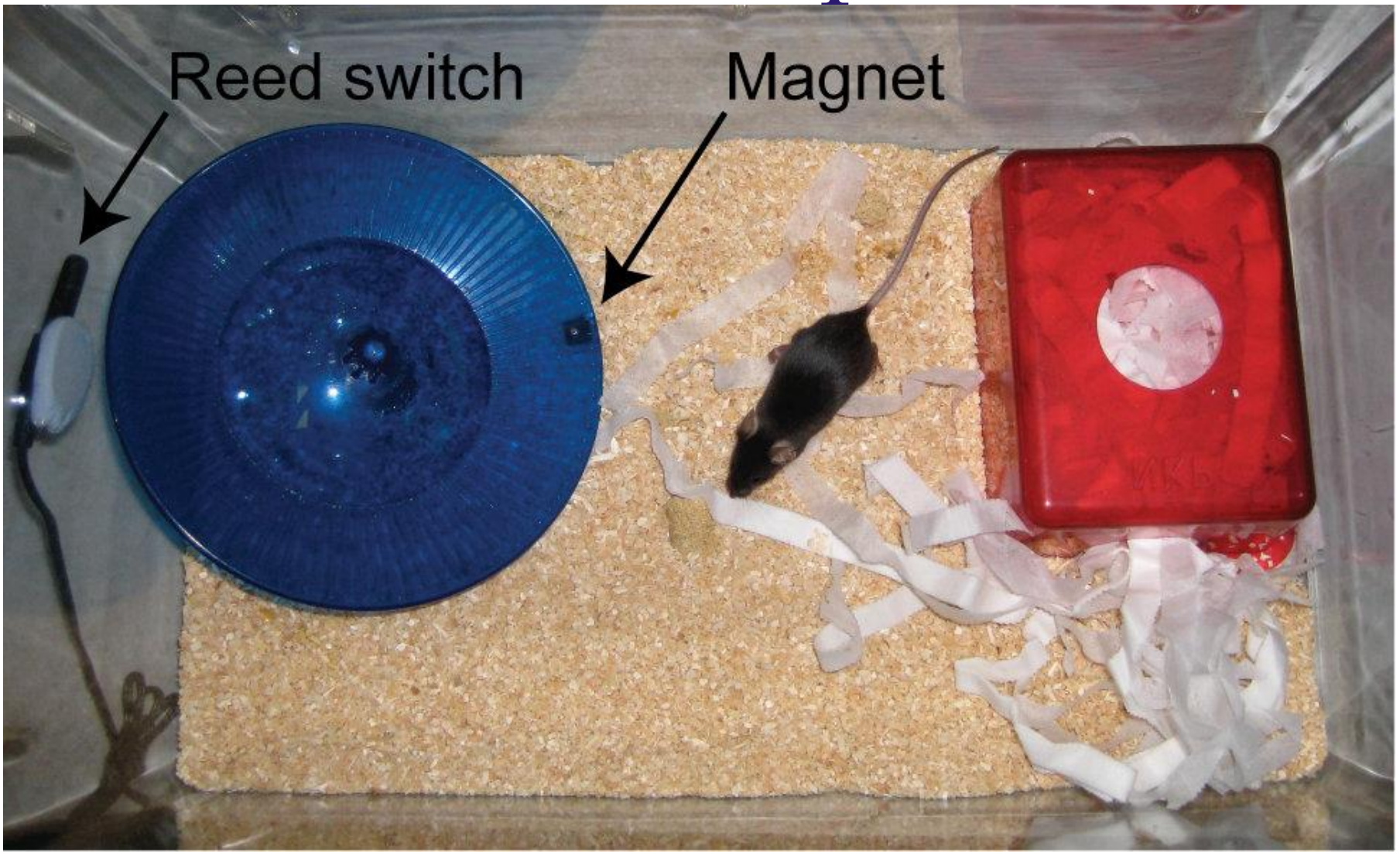
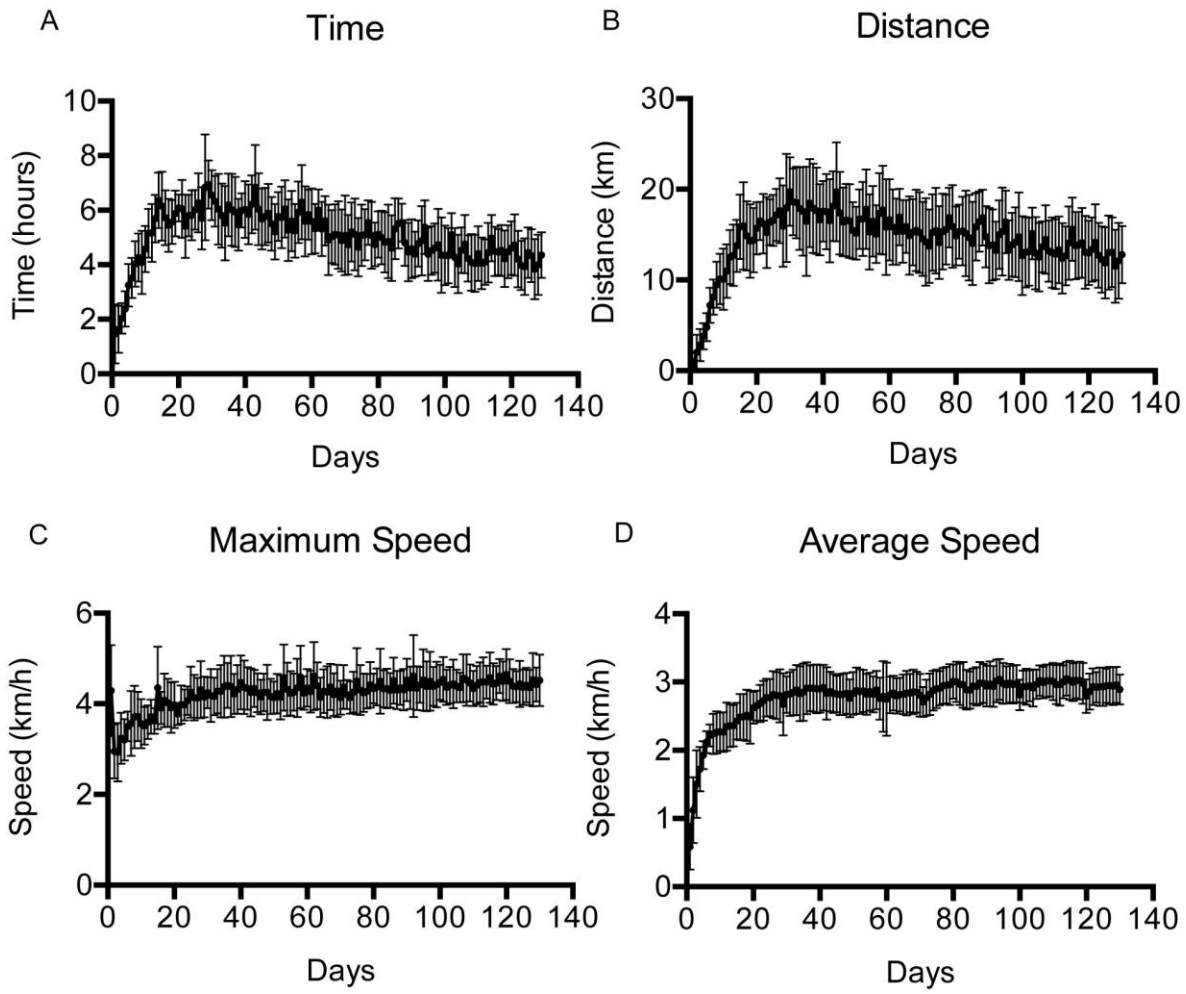


Wild-type mouse data



At peak ~

- 6 hours running time (mean during plateau phase 5.06 ± 0.70 hours/day)
- Average distances of 15-20 km (mean during plateau phase 15.07 ± 1.78 km/day.)

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SOD1^{G93A} data

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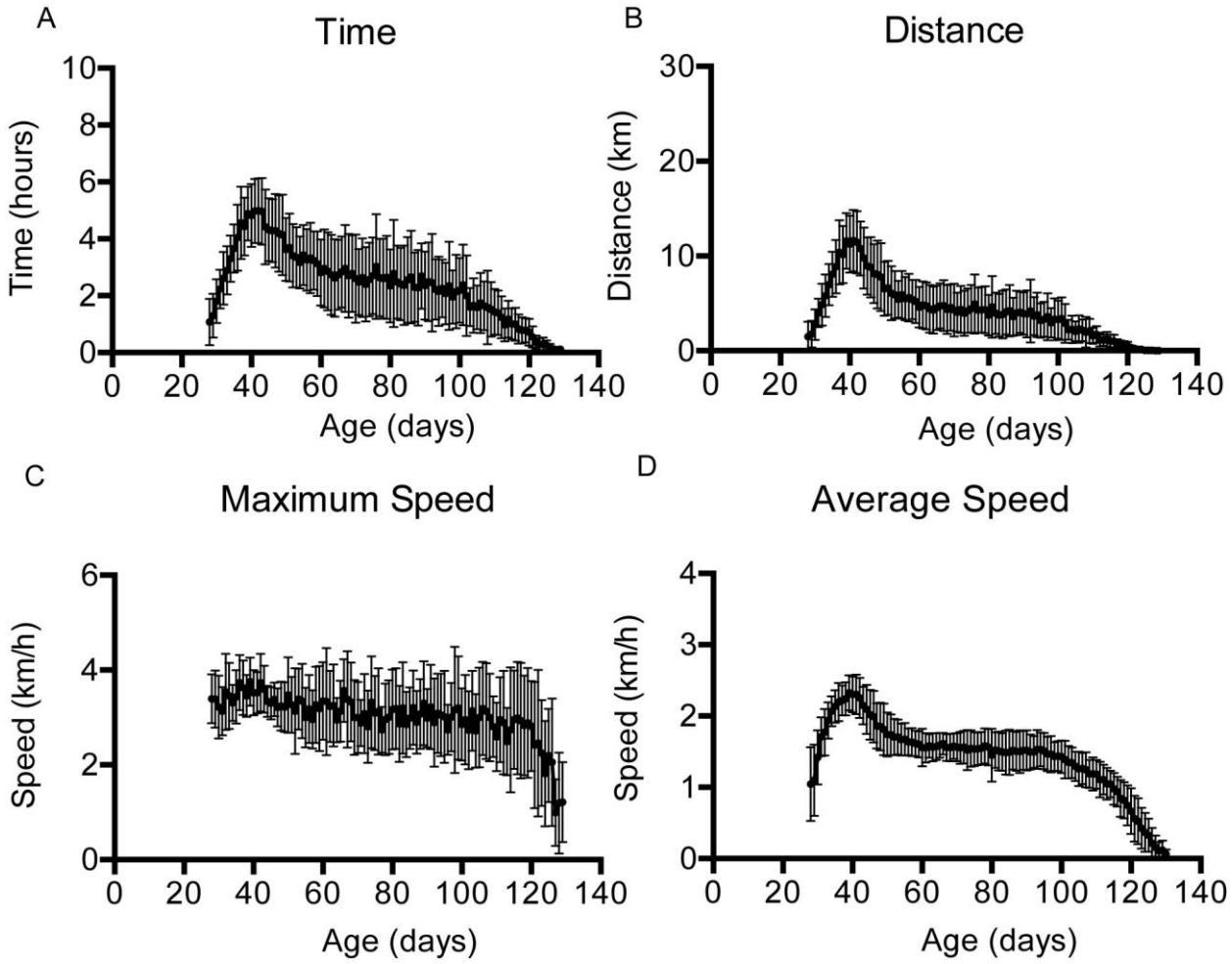
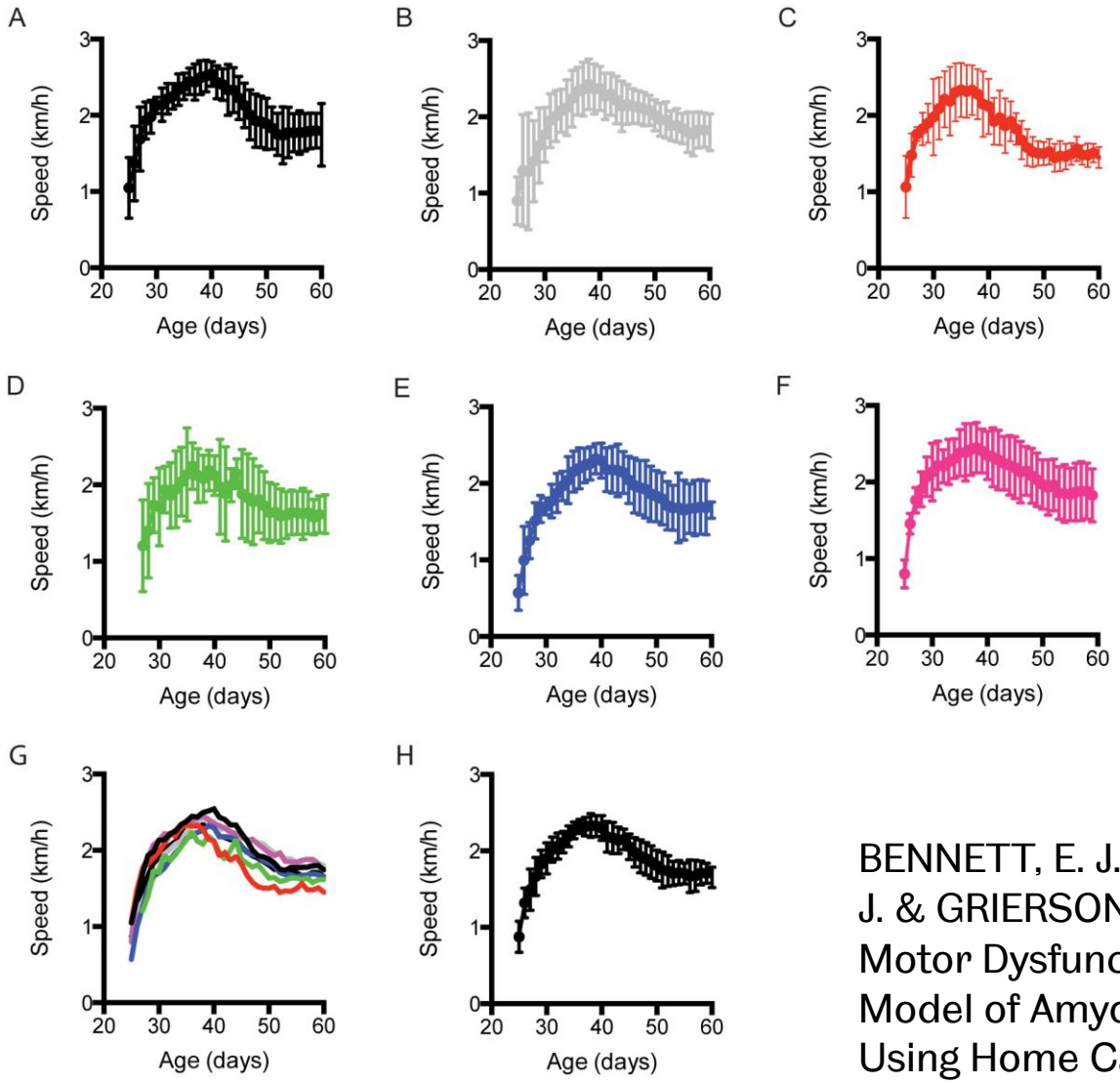


Statistical power analysis for a 10 day extension in time to reach a 20% decline in average speed:

- **Rotarod** test would require **14** mice per group.
- **Fast Trac** methodology would require **5** mice per group.

Reproducibility

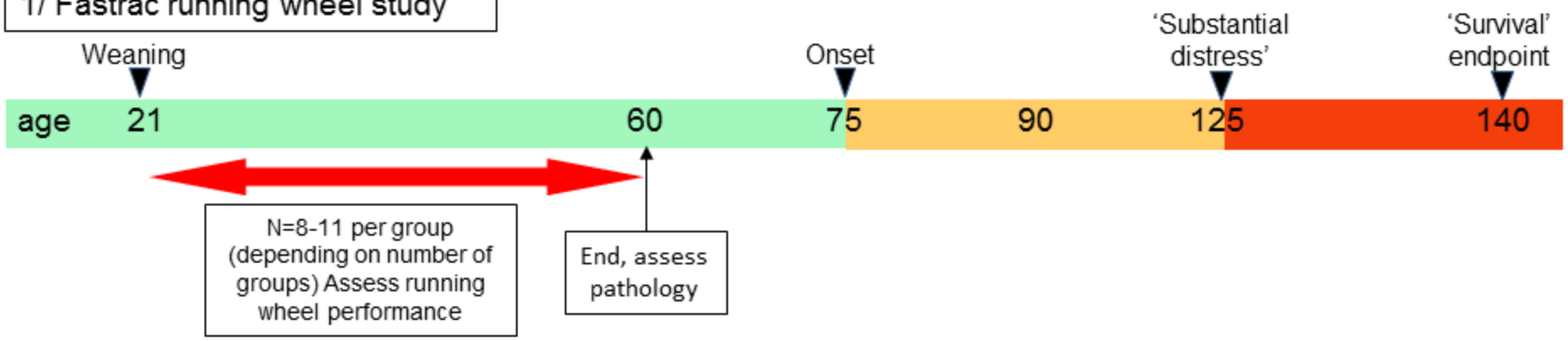


- Control groups from 6 therapeutic studies
- Between study CV for time to reach a 20% decline in average speed is **3.9%.**

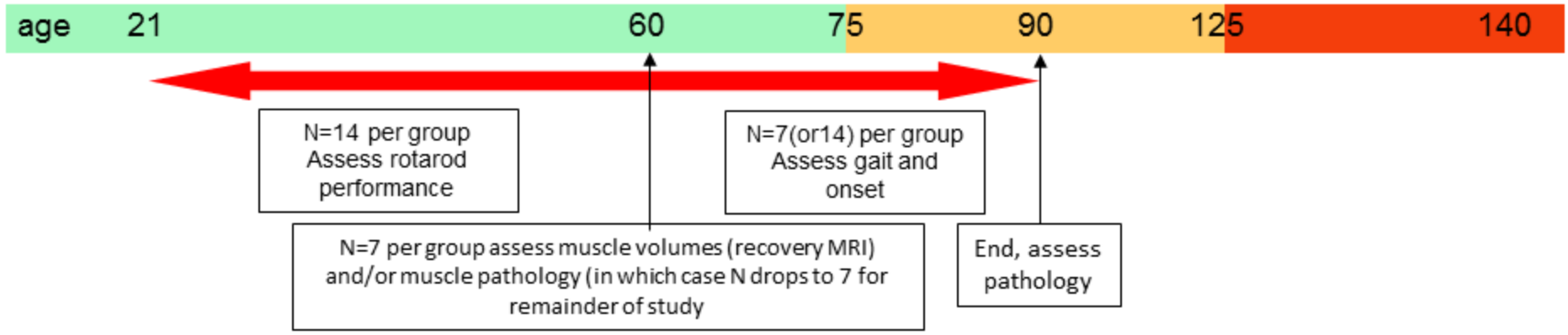
BENNETT, E. J., MEAD, R. J., AZZOUZ, M., SHAW, P. J. & GRIERSON, A. J. 2014. Early Detection of Motor Dysfunction in the SOD1G93A Mouse Model of Amyotrophic Lateral Sclerosis (ALS) Using Home Cage Running Wheels. *PLoS One,* 9**,** e107918.

Screening paradigms in the SOD1^{G93A} mouse model of MND available at SITraN

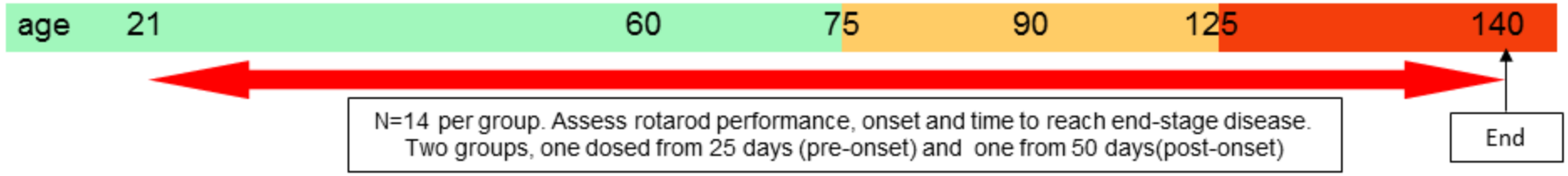
1/ Fastrac running wheel study



2/ Confirmatory study



3/ Survival study



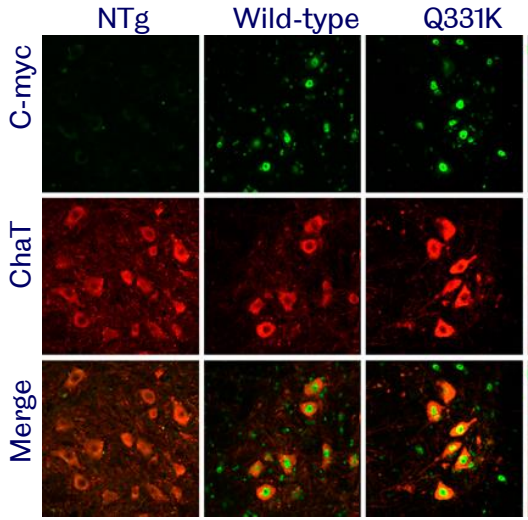
TDP-43 Mouse model

- C-myc tagged human TDP43 (TDP43^{Q331K} and TDP43^{WT}) expressed using a murine prion-promoter, previously reported to drive transgene expression abundantly in the CNS

- Relatively low level of transgene expression, originally designed to match the expression level of the endogenous mouse gene and matching expression in the mutant to that in the wild-type mice

TDP-43^{Q331K} mice show:

- Widespread alterations in gene splicing
- Reduced rotarod, performance
- Reduced grip strength,
- Motor neuron and motor axon loss
- TDP43 shows no signs of mislocalisation
- Disease progression plateaus at 10 months



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The University Of Sheffield.

Preclinical pathway

Pharmacokinetics

- Test dose route and formulations

Pharmacodynamics

- Mechanistic effect of drug in target organ
- Intramuscular tracer injection

'Short term' efficacy

- Efficacy on disease initiation and/or early Progression –POC

'Long term' efficacy

- Efficacy on late disease progression
- Effect on time to reach end-stage

As much a test of the drug as the model

Lack of efficacy data meaningless without exposure data

Many Thanks

Funding

Family of A. Kenneth Snowman and the MND Association, NC3Rs, Wellcome trust, MRC

SITraN
Prof Dame Pamela Shaw
Prof Mimoun Azzouz
Dr Andy Grierson
Dr Adrian Higginbottom

Staff/Students
Dr Ellen Bennett, SOD1 mouse models
Jodie Stephenson, TDP-43 mouse
Stephanie Gentles, BG12 project
Dr Nazia Maroof, AZD1080 project
Matthew Sellwood, KEAP1 project
Heledd Brown-Wright , GPCR project

Preclinical MRI facility (Sheffield)
Aneurin Kennerley
Jason Berwick (Psychology)
Paul Sharp (Psychology)

Aix Marseille Universite´ , France
Claire Sunyach
Brigitte Pettmann