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qMotor, a set of rules for sensitive, robust and quantitative measurements of motor performances in mice

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Abstract

Phenotypic analysis of mouse models of human diseases is essential to understand the underlying disease mechanisms and to develop therapeutics. Many models of neurodegenerative diseases are associated with motor dysfunction, a powerful readout for the disease. We describe here a set of measures to quantitatively monitor early disease onset and progression by rotarod analyses. We named this set of rules qMotor because it enables sensitive, robust and quantitative measurements of motor performance. Results can be obtained in three days. qMotor can be used to assess early disease onset, before paralysis, as well as disease progression in diverse mouse models and can be exploited to define robust and humane experimental endpoints, thereby reducing animal suffering. As an example application, we apply qMotor to SOD1^{G93A} transgenic mice. Early studies with the original transgenic SOD1^{G93A} mice in the hybrid background (B6SJL-Tg(SOD1-G93A)) have been criticized due to high noise in this mixed background and inadequate study designs. We applied qMotor in SOD1^{G93A} transgenic mice in an inbred C57BL/6J background, hereafter called iSOD1^{G93A}, and demonstrate a remarkably robust and consistent phenotype in this line that can be used to evaluate therapeutic approaches. qMotor is a protocol generically applicable to different mouse models.

Introduction

Neurodegenerative diseases are devastating and affect an increasing number of individuals in our aging society. Mouse models, recapitulating key features of human neurodegenerative diseases have been generated and are valuable to study disease mechanisms and potential therapeutics¹. Because no treatments have yet translated into humans to either stop or slow down the progression of these increasingly prevalent neurodegenerative diseases, mouse models have been criticized¹. However, these mouse models develop representative features of the human diseases and the poor translatability of preclinical studies may well be the result of inadequate study designs rather than flaws in the model per se¹.

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Author Contributions

L.M.L. wrote and validated the protocol. I.D. performed the previously published experiments and contributed to the data analyses⁷.

L.M.L. and A.B. wrote the manuscript.

Competing Financial Interests

The authors declare that they have no competing financial interests.

Rationale for the Protocol

Many models of neurodegenerative diseases are associated with motor dysfunction. Thus, assessing motor deficits presents a powerful tool to evaluate potential therapeutic treatments in diverse disease models.

Measures frequently used to detect disease phenotype in SOD1 transgenic mice, such as weight loss or gait analysis, reveal changes at a late stage^{2,3}. Thus, the vast majority of studies conducted to date have used protocols with severe experimental endpoints (paralysis or death) to assess disease onset and progression in SOD1 mice⁴. In this protocol we describe how to implement qMotor, a simple set of measures and rules that enables quantitative and robust assessment of motor performances in transgenic mice thus facilitating the study of neurodegenerative diseases and the assessment of potential therapeutic approaches. We first used qMotor to evaluate the therapeutic efficacy of a therapeutic approach³.

qMotor is a significant improvement over alternative methods. Many study uses measures of such as weight loss, gait analysis, paralysis or death, reveal changes at a late stage and require an larger number of animals^{2,3}. qMotor can be broadly used to detect disease onset before severe paralysis and to conduct robust studies with humane yet robust endpoints, thereby reducing animal suffering and animal numbers, in line with the principle of the 3Rs

Distinguishing skill learning and motor performances by rotarod analyses

qMotor utilises the rotarod test to assess motor performance. In the rotarod test, mice are trained to run on a horizontal rotating rod, and the latency to fall is used as a measure of motor ability. Rotarod analysis can be used for two purposes: to study skill learning⁵ and to assess motor performances⁶. Performance is known to increase over time during multiple rotarod sessions as training progresses and plateaus after a few sessions⁵. This is the result of motor skill learning and does not reflect fitness⁵. Intersession improvement is known to decrease with the number of sessions⁵. After the initial learning period, rotarod analyses measure motor performances⁵. Thus, it is important to avoid the confounding effect of the motor learning phase to allow quantitative assessment of motor performances by rotarod analyses.

Assessing motor deficits by rotarod analyses

Previously, we have used rotarod analyses to demonstrate the therapeutic efficacy of the selective phosphatase inhibitor Sephin1 in two independent mouse models³, using a refined rotarod procedure that we now describe here. We found that Sephin1 prevents the motor and molecular defects in a transgenic mouse model of the demyelinating neuropathy Charcot-Marie-Tooth 1B (CMT-1B) ³. The CMT-1B model, first published in 2006⁷, is a robust model of CMT-1B in the FVB/N background, and has been used in numerous studies. Deletion of serine 63 in myelin protein (P0S63del), one of the most abundant proteins made by Schwann cells in the peripheral nervous system, causes the demyelinating neuropathy CMT-1B in humans, by a gain of toxic property due to the misfolding of the mutant protein⁷. Transgenic mice expressing the mutant P0S63del transgene recapitulates key

features of the human disease⁷. The CMT-1B mice develop motor deficits due to myelination defects as a consequence of the misfolding of myelin P zero⁷. Whilst the CMT-1B mice do not manifest any overt clinical phenotype, repeated rotarod analyses at 4 months of age with 14 CMT-1B mice and 14 wild-type littermates reveal robust deficits in CMT-1B mice^{3,7–9}.

We also previously reported that Sephin1 prevents the motor and molecular defects of the SOD1^{G93A} ALS mouse model. In this study, we found robust phenotypes by rotarod analysis with 4 or 6 animals per group⁷ whilst previous guidelines recommended using a cohort of 24 animals per group to evaluate the phenotype in SOD1 transgenic mice¹⁰. Our previous study³ demonstrated that rotarod analyses are sensitive and enable the detection of a motor phenotype from an early stage of the disease, before the onset of visible motor defects with a small number of animals. Here we describe below the mouse strain we selected for these studies and the detailed protocol, qMotor, we established to generate these results³. This protocol can be used with diverse mouse lines to generate robust results with a reduced number of animals.

Mouse strain selection

To establish the procedure, we recommend testing transgenic mice with motor deficits of different severities together with their wild-type, aged matched, litter mates. Amongst the diverse models of neurodegenerative diseases, the transgenic SOD1^{G93A} mouse model is particularly attractive since it faithfully recapitulates many defining features of amyotrophic lateral sclerosis (ALS): the misfolding of the disease causing protein, motor defects due to motor neuron loss, hind limb paralysis and neuroinflammation^{11,12}.

The original SOD1^{G93A} strain is maintained by crossing transgenic hemizygous male with female C57BL6SJL/J hybrids. This model has been widely criticized because no studies performed in this model have been translated to humans so far^{10,13}. However, because ALS still represents an unmet medical need, and the mouse models recapitulate key features of the human disease, it seems unreasonable to dismiss the potential usefulness of this model.

Methodological limitations of the studies relying on the SOD1^{G93A} line in a mixed background have been highlighted previously and some guidelines have been published to overcome them^{10,13}. However, despite the fact that mixed backgrounds are notorious for introducing major variability, the importance of the mixed background as a confounding factor in animal studies is overlooked and most studies continue to use the SOD1^{G93A} in the mixed C57BL6SJL/J background. To circumvent these limitations, SOD1^{G93A} transgenic mice have been produced in a pure C57BL/6J background (hereafter called iSOD1^{G93A}). This line exhibits an extremely robust disease progression with very little background noise and remarkable consistency in intra- and inter-study in different labs^{2,3}, as long as the transgene copy number remains constant in the colony and in the experimental group. This latter caveat is controlled by analysing the transgene copy number.

Although SOD1 mutations account for only a subset of familial forms of ALS, studying rare forms of a disease may shed light on mechanisms or therapeutic strategies that may be

relevant to more common diseases. Here we provide a robust protocol to monitor the motor deficits in iSOD1^{G93A} mice. This protocol is applicable to diverse mouse models.

In the B6SJL-Tg(SOD1-G93A)1 model, previous guidelines recommended using a cohort of 24 animals in each group (wild-type and transgenic)¹⁰. The robustness of the phenotype in iSOD1^{G93A} line implies that the numbers of animals required for a conclusive study could be significantly reduced². Moreover, previous guidelines used death or extremely severe phenotypes as an endpoint¹⁰. This needs to be revised to comply with the European Union directive on animal research (C., 2010-63-EU, art. 13.3.), which states that “death as an endpoint to a procedure should be avoided as far as possible and replaced by earlier, humane endpoints”. This is in line with the principle of the 3Rs (Replacement, Reduction and Refinement) and with the regulation on the use of Animals in Research.

In contrast to the CMT-1B mice, the iSOD1^{G93A} transgenic mice develop hind limb paralysis with 100% penetrance after 12 weeks^{2,3}. The motor phenotype of the iSOD1^{G93A} transgenic mice is severe, suggesting that it should be possible to reliably and quantitatively measure the motor deficits in iSOD1^{G93A} mice by rotarod analyses with fewer mice than is required to quantify the motor defects in CMT-1B mice, which do not develop any visible symptoms. Moreover, the ability to detect motor deficits in CMT-1B in the absence of visible symptoms suggests that it should be possible to detect the early stage of the disease in iSOD1^{G93A} transgenic mice, before the onset of paralysis observed at 12 weeks in this line³. Detecting motor deficits before paralysis represents an attractive possibility, which could lead to defining humane endpoints, thereby refining protocols and reducing animal suffering.

Overview of qMotor

The essential refinements of the rotarod procedures leading to qMotor consist of increasing the trial duration and increasing the number of trials. This protocol may be longer than previous protocols but generates robust results with fewer animals.

Traditionally, rotarod analyses with SOD1 mice are performed with three trials by recording the latency to fall on an accelerating rotarod (4-40 rpm) with a trial duration of 150-180 seconds¹⁴. In contrast, rotarod analyses with CMT-1B mice are traditionally performed with six sessions of three trials. Each session lasts 900 seconds: 300 seconds of acceleration (4-40 rpm) followed by 600 seconds at 40 rpm^{3,7}. As previously observed⁷, the mice improve their performances during the first three sessions, as training progresses (Fig. 1). After the fifth session, improvements plateau (Fig. 1) and 5. Consequently, the motor deficits of CMT-1B mice are not observed in the first three sessions, as these are confounded by the learning phase, but became apparent after the fourth session⁷ (Fig. 1). Similarly, when looking at the therapeutic benefit of Sephin1, this is not detectable for the first three sessions but is evident and robust after the fourth session in both female and male animals (Fig. 1 and Supplementary Fig. 1). These example results illustrate that repeated rotarod analyses can be used to quantitatively assess motor deficits in absence of a visible phenotype and demonstrates that trial duration and number of trials can have an influence on rotarod tests.

Knowing that we can quantitatively assess the motor deficits of CMT-1B mice in absence of visible motor phenotype, we reasoned that rotarod tests with iSOD1^{G93A} mice could be improved by increasing trial duration and number of trials. In a previous study³, we therefore tested iSOD1^{G93A} mice and wild-type littermates (n=4-6 mice per group) using a refined rotarod procedure consisting of a 3-days trial, with 3 trials a day³. In Fig. 2 we present the different trials of our previous study³. The duration of each trial was set at 300 seconds based on pilot experiments showing that this duration was sufficient to reveal the phenotype of iSOD1^{G93A} mice before the onset of paralysis. We performed rotarod tests with iSOD1^{G93A} mice and wild-type littermates at 60 days of age and noticed, as previously reported⁵, that performances improved with the number of sessions (Fig. 3a). Because previous studies have shown that the improvement in rotarod performances plateaus after 5-6 trials⁵, this learning period must be excluded from experimental tests aimed at assessing motor performances. In a typical experiment, after the learning period, the motor defects in iSOD1^{G93A} mice are robust and consistent in the three trials (Fig. 3a). Here we reanalyzed our previously published study³ by separating genders and found that the phenotype was similarly robust in both genders (Supplementary Fig. 2). Rotarod tests performed on a separate cohort of wild-type and iSOD1^{G93A} mice at 90 days of age confirmed that the motor deficits in iSOD1^{G93A} were recapitulated in both males and females (Supplementary Fig. 3). This establishes a robust method to quantitatively assess motor defects in iSOD1^{G93A} mice with a small number of mice and before the visible paralysis.

Using qMotor to test drug efficacy

The procedure can be used to assess the efficacy of drugs over time. We show results obtained from treating iSOD1^{G93A} mice with Sephin1 over time by rotarod analyses (Fig. 3b). This figure represents the different trials of our previous study³. Four experimental groups were used composed of randomized iSOD1^{G93A} mice and wild-type littermates, treated with Sephin1 (5mg/kg, once a day) or vehicle from 4 weeks of age. As noted above, the first 6 sessions (Day 1 and 2) of the rotarod tests are excluded from analyses aimed at measuring motor performances because of the confounding effect of the learning phase⁵. Rodents retain rotarod skills after they have learned them⁵ (Fig. 3b) so there is no need for additional training periods, even after a month without training. After the learning phase in the first 2 trial days, the subsequent trials are remarkably consistent in all four groups (Fig. 3b). This demonstrates that qMotor is extremely robust to measure motor defects in iSOD1^{G93A} mice and further underscores the robustness and consistency of the motor phenotype in iSOD1^{G93A} mice. The efficacy of drugs can also be tested, for example qMotor was used to test the efficacy of Sephin1 iSOD1^{G93A} mice³. We observed robust differences between the iSOD1^{G93A} mice treated with Sephin1 or vehicle using 4 or 6 mice in each group³. These differences were highly significant on each trial day, day 62, day 90-92 and day 110-112 (Fig. 3b). We reanalysed our published results separating genders and found that similar results were obtained in both genders (Supplementary Fig. 2). These results demonstrate that qMotor revealed motor deficits in iSOD1^{G93A} mice with very little inter-cohort variations. These results highlight the robustness of the method, the predictability of motor deficits in this mouse line, as well as the potency of the treatment.

Materials

REAGENTS

- Mice

We use transgenic mice expressing the mutant SOD1^{G93A} maintained in C57BL/6J. Other mice could be used, as discussed in the Introduction. CAUTION Researchers must comply with national regulations concerning animals and their use.

This procedure is in compliance with the regulation on the use of Animals in Research (UK Animals Scientific Procedures Act of 1986 and the EU Directive 2010/63/EU). Institutional approval for this procedure has been obtained from the Medical Research Council Laboratory of Molecular Biology UK under the project licence number 70/7956 with local ethical approval from the LMB Animal Welfare and Ethical Review committee and also from the Division of Genetics and Cell Biology Italy, San Raffaele Scientific Institute, 20132 Milan, Italy.

- 70% EtOH and tissue wipes

- Non-toxic red ink/pen (CRITICAL use red because mice are not able to see this colour), e.g. Pen Sharpie Permanent Marker Red (VWR, 811-0025)

REAGENT SETUP

Housing and husbandry of experimental animals

Animals must be housed and cared for according to the Home Office Code of Practice for the Housing and Care of Animals used in Scientific Procedures.

We keep mice in specific pathogen free ventilated cages (Tecniplast GM500, Tecniplast) on Lignocel FS14 spruce bedding (IPS, Ltd.) and Enviro-Dri nesting material (LBS) at 19-23°C with 12 h light dark cycle with light from 7.00 a.m. to 7.00 p.m.. We feed our experimental animals with Dietex CRM pellets (Special Diet Services). The maximum number of males housed in individual cage is up to 4 and the maximum number of females is up to 5 in accordance. To monitor health conditions, check all experimental animals visually every day, clean out when soiled and perform a physical health check each week on all mice. Weigh the experimental animals weekly. We provide the experimental animals reaching moderate severity limit with mash (Dietex CRM pellets soaked in water).

Generation of mice

The CMT-1B mice are transgenic for mutant myelin protein zero with deletion of serine 63 (P0S63del) and are maintained in the FVB/N background⁷. We use hemizygous males and females CMT-1B mice and aged-matched wild-type littermates in experiments. SOD1 mutant mice iSOD1^{G93A} are maintained in C57BL/6J. Experimental animals are generated by crossing hemizygous iSOD1^{G93A} males with C57BL/6J females. The disease phenotype is extremely robust in this line, and remarkably similar to the iSOD1^{G93A} C57BL/6J line², when the transgene copy number remains constant. The robustness and penetrance of the

phenotype enables the rapid identification of mice with reduced transgene copy number because this lead to a loss or reduction of phenotype. This was observed once since we obtained this line in 2011.

Genotyping and copy number analysis

Identify mice by ear clipping and retain the ear tissue for extraction of genomic DNA using the TaqMan® Sample-to-SNP™ Kit (ThermoFisher Scientific, 4403081). Genotype CMT-1B mice as described elsewhere⁷. Perform genotyping PCRs on genomic DNA in a 10 µl volume with 500 nmol each of human SOD1 primers IMR002: ctaggccacagaattgaaagatct IMR0043: gtaggtggaaattctagcatcatc IMR0113: catcagccctaattccatctga IMR0114: cgcgactaacaatcaaagtga. Following PCR visualise the products using QIAxcel DNA Fast Analysis Kit (3000) Kit (Qiagen, 929008). The wild-type band is 324 bp and human SOD1^{G93A} is at 236 bp.

Perform transgene copy number analysis by quantitative PCR. We use 12.5 ng cDNA, SYBR® select Master Mix (Applied Biosystems, (Ref 4472908, applied biosystems) on a Corbett Rotor-Gene version 6000 Warrington, UK), using human SOD1^{G93A} (f): gtgtgcgtgctgaaggcgca, SOD1^{G93A} (r): ccaccttgcccaagtcattctgc, in a total volume of 12.5 µl. Compare ddCt values to a reference cDNA sample containing only two copies of human SOD1.

Allocating animals to experimental groups

Sex- and age-matched transgenic male and female and their wild-type littermates should be used in experiments. Litters should be randomized using any randomization software (for example www.random.org) to create cohorts of the placebo and treatment experimental groups.

Sample size

Determine animal cohort numbers using either previous studies⁷ or preferably a priori power calculation on pilot experiments (Fig. S6 in 3).

Drugs

We used Sephin1 (acetate salt; molecular weight: 256.93) once daily (5 mg/kg) or vehicle (sterile water). Sephin1 should be prepared by solubilizing in water (225 mg in 100 ml with sterile water for a final concentration 2.25 mg/ml), followed by sonication (10 min, 5 sec ON; 5 sec OFF). Aliquots can be frozen. Aliquots should be thawed each day prior to treatment. We do not recommended freezing and thawing aliquots. As the average weight of mice (6-7 weeks old) was 22.5 g, 50 µl of Sephin1 or vehicle were daily orally administered to individual mice (for a treatment of 5 mg/kg) between 9.00 and 10.00 a.m. using 1 ml syringe attached to plastic feeding tube (FTP-20-38, Instech Laboratories, Inc). Other drugs should be dissolved as previously determined and appropriate dosage will need to be determined following pharmacokinetic studies and pharmacodynamics analysis.

EQUIPMENT

Accelerating rotarod (UGO BASILE, model number 47600, serial number 0767U08)

CRITICAL: Do not change instruments in between one experiment. Small changes can affect results.

Procedure

Experimental setup

- 1** To setup on each day of testing, keep mice in their home cages and move the cages to the testing room to allow the mice to be acclimatised to the testing room for at least 60 min. **CRITICAL** Timing should be such that tests are performed during the light cycle.
- 2** Tail mark the mice with non-toxic red ink (I, II, III, IIII, IIIII)
CRITICAL STEP Do not mix the genders in the same run. Mice behaviour might be affected when you mix genders. Always start with male animals before females.

Habituation

- 3** For habituation place animal for 1 min on a static rotor and 1 min at constant speed (4 rpm). After at least 10 min repeat this step (Fig. 2a). **CRITICAL STEP** Habituation is only performed once before carrying out the first trial.
- 4** Return the mice to their home cages and return mice to their home environment or proceed with step 6.
- 5** Clean rotarod machine with 70% EtOH between each group of mice.

Learning phase

CRITICAL: Mice remember the motor skills after the initial learning period. Thus, in longitudinal studies, the learning trials are only performed once, before the first test.

- 6** Transfer mice to the testing room at least one hour before testing, keeping mice in their home cages.
- 7** Place mice on the rod so that they run forward and set apparatus to accelerating mode 4 to 40 rpm in 300 s and record the latency to fall. The upper limit of 300 s per trial was found optimal for the iSOD1^{G93A} but may vary between different mouse models.

CRITICAL STEP: The trial duration may differ between different mouse models. In the iSOD1^{G93A} mice used here, the 300 s duration in rotarod tests is such that the average latency to fall of wild-type mice, after the learning period (after the increase in performances between sessions reaches a plateau) is 50 seconds below the maximum trial duration. In pilot experiments, tests can be carried out with mice with and without motor deficits (transgenic mice and wild-type aged matched litter mates in the examples used) on an accelerating rotarod (4-40rpm) for duration 300 s. If repeated trials under these conditions fail to

detect the phenotype, the duration of the trials may be extended to 600 and 900 s, continuing with the constant speed of 40rpm. As shown here, the optimal cut-off time varies between different models but is consistent within the same line with very little intra- or inter cohort variations, as exemplified here in the different experiments presented.

- 8 Clean apparatus after every run with 70% EtOH. **CRITICAL:** For unbiased results machine should be cleaned between each group of mice.
- 9 Repeat steps 7-8 twice, giving at least a 15 minutes interval between trials for each mouse.

CRITICAL STEP: The number of learning trials may be increased if the mice have motor deficits. The learning is considered complete when the increase in performances between each session reaches a plateau.

- 10 Return mice to home cages and home environment overnight.

Testing phase

- 11 Repeat steps 6-9 over three trials performed over three consecutive days. Thus in total, 9 trials should be performed to assess motor performances at a given time (Fig. 2b). All experiments should be carried out at 4-40 rpm in 300 s for iSODG93A and for CMT-1B in 900 s (300 s 4-40 rpm and 600 s 40rpm). Perform trials as described in learning phase with a minimum of 15 min between each trial.

CRITICAL STEP The duration of the entire protocol (learning and testing) depends on the severity of the motor deficits of the mice, which determines the duration of the trial and also on the number of animals. For example, in an experiment with an acceleration time of 300 s per trial, like in iSOD^{G93A} mice, testing one set of mice (5 animals) takes in total one hour on three consecutive days including the resting intervals. For one experiment with a group of 40 mice, the required trials will take a minimum of two hours (running new groups in the resting intervals of previous groups). When testing mice with mild motor deficits, like CMT-1B mice, the duration of the trials may be longer. In the case of CMT-1B mice, the duration of experiments is around 6 hours for 40 mice per day.

Analysis

- 12 Analyse data using two-way repeated-measures ANOVA and Bonferroni post-hoc procedure to correct for multiple comparisons (GraphPad Prism Software). The level of significance can be set at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. No samples, mice or data points should be excluded from the analyses. The first trials corresponding to the learning phase on the rotarod (until the performances reach a plateau) need to be excluded from quantitative analysis to assess motor performances without the confounding effect of the motor learning phases.
- 13 The ARRIVE guidelines must be followed when reporting animal studies 15.

TIMING

Testing one set of mice (5 animals) takes 20 min for habituation (steps 1-5) and 45 min for three trials (step 6-10 or 11) each day. Analysis (steps 12-13) takes 30-60 min. [AU: Need timing information for all steps.]

TROUBLESHOOTING

Mice might cling to the rod instead of falling down. Mice performing two full passive rounds clinging onto the rod, indicates a failure of motor function. When this happens, stop the trial for this mouse by pushing down the lever and record the time. Any excluded mice must be reported.

ANTICIPATED RESULTS

By optimizing trial duration and by repeating rotarod analyses, we find that rotarod measures are extremely robust and enable assessment of motor disease with a reduced number of animals. The motor deficits may not be observed in the first trials but becomes apparent and robust upon repeated trials (Fig. 1 and 3) with a relatively smaller number of animals than other methods. The quantitative nature of qMotor allows for the measurements of drug efficacy in different mouse models (Fig. 1 and Fig 3). Importantly, the sensitivity of this method is such that it allows detection of motor deficits in the absence of visible symptoms (Fig. 1 and Fig 3). qMotor therefore can be used to define humane endpoints in experiments, thereby enabling the refinement of protocols and reducing animal suffering and reducing animal numbers. This method is applicable to many different disease models associated with motor deficits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Editorial Summary

This protocol describes qMotor, a set of rules for rotarod analysis of motor performances in mice. It can be used to assess early disease onset and disease progression in diverse mouse models with a small number of animals.

Tweet

#neurodegeneration, #neurodegenerativedisease, #ALS, #Alzheimers,
#charcotmarietooth, #Parkinsons, #huntingtons disease, #rotarod, #MotorFunction,
#mouse #behaviour #neuroscience, #pharmacology. #ALSTDI, #MND, #highQfoundation, #mousemodel

Figure 1. Rotarod analysis of CMT-1B mice.

Rotarod analysis of 4-month old wild-type or CMT-1B mice of indicated age following oral gavage with Sephin1 (1 mg/kg) or vehicle for 3 months. Data are means of three trials performed on the same day with 14 mice $\pm$ SEM. The learning phase and the testing of motor skills are indicated. No samples, mice or data points were excluded from the analyses. All data shown were collected while conforming to governmental and institutional guidelines for care and use of laboratory animals. *P 0.05; **P 0.01; ***P 0.001; ****P 0.0001; (F(1,29)=22.8, $p < 0.001$). Institutional approval for this procedure has been obtained. Raw data in Supp Table 1.

Figure 2. Workflow of qMotor.

a, Mice are first habituated for 1 minute on a static rotor and 1 minute at constant speed (4 rpm). This habituation is done twice before the first test phase only. **b**, Animals are trained to learn motor skills over three trials performed over two consecutive days with a minimum of 15 min break. **c**, Trained animals are tested by rotarod analysis, usually with three trials a day for three days.

Figure 3. Early motor deficits and Sephin1 efficacy in iSOD1^{G93A} mice.

a, Repeated rotarod analysis (3 trials a day) of wild-type and iSOD1^{G93A} mice (60 days old). Data are means $\pm$ SEM (n=4). **b**, Repeated rotarod analysis of wild-type or iSOD1^{G93A} mice of indicated age (days) treated orally with Sephin1 (5 mg/kg) or vehicle once a day from 28 days of age. Data are means of 3 trials performed on the same day by 4 to 6 mice per group $\pm$ SEM. The learning phase and the testing of motor skills are indicated. No samples, mice or data points were excluded from the analyses. All data shown were collected while conforming to governmental and institutional guidelines for care and use of laboratory animals. *P 0.05; **P 0.01; ***P 0.001; ****P 0.0001; (F(1,14)=12.13, p=0.004). Institutional approval for this procedure has been obtained. Raw data in Supplementary table 2.